Reproduction in Domestic Animals

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Lisbon, Portugal
27 – 29 October 2016

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European Society for Domestic Animal Reproduction
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WILEY Blackwell

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WILEY Reproduction in Domestic Animals

The local organizing Committee is delighted to invite you to join the 20th Annual Conference of the European Society for Domestic Animal Reproduction (ESDAR), jointly with the 13th Conference of the Spanish Association for Animal Reproduction (AERA), in Lisbon, Portugal, from the 27th to the 29th October 2016. We are extremely pleased that, for the first time, AERA has joined ESDAR conference, making this meeting more appealing to the scientific community and to veterinary clinicians in the field of animal reproduction. In addition this year, a satellite workshop on innovations to improve fertility in dairy cows (Joint FECUND-PROLIFIC EU Projects Final Conference) will be also held on the 30th October, at the location of the ESDAR-AERA conference. This will be a great opportunity to attract mainly young researchers to join ESDAR and AERA, to participate in these scientific meetings and to establish future interinstitutional collaborations.

Twenty years have already passed by since the first ESDAR meeting was held in 1997. Since then, each year, ESDAR congresses have contributed to strengthening the scientific knowledge on animal reproduction and collaborations throughout numerous European research laboratories. These meetings besides playing a very important role on personal and scientific growth of young researchers have also contributed to dissemination of knowledge on domestic animal reproduction to veterinary practitioners, to strengthen the link between science and practice. The scientific programme of ESDAR-AERA conference this year includes five plenary lectures by distinguished scientists, ten workshops, eight oral communications sessions and posters presentations that will address issues on different topics on basic and applied science on several animal species.

Lisbon always offers something special to its visitors with its mild climate, bright colours, excellent gastronomy, rich history, diverse culture life and the breathtaking landscape in the surrounding areas. Lisbon is a historic capital, where cultural influences mingle with modern trends and life styles, creating spectacular contrasts. You could use the chance to visit this wonderful city and surroundings, such as Cascais and Sintra, recognized as world heritage sites by UNESCO.

Meeting colleagues and friends from different countries is an asset of this conference. At the conference dinner at the restaurant "Adega do Kais", you will be able to enjoy the company of your friends, as well as music, dance and traditional Portuguese dishes.

You are warmly welcome to this wonderful meeting in the lovely city of Lisbon. We are looking forward to seeing you here!

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

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Local Organizing Committee, ESDAR-AERA 2016

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REVIEW ARTICLE

WILEY Reproduction in Domestic Animals

Ovulation-inducing factor (OIF/NGF) in seminal plasma: a review and update

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The ovulation-inducing effect of seminal plasma was first reported in Bactrian camels over 30 years ago, and the entity responsible was dubbed 'ovulation-inducing factor' (OIF). More recent studies, primarily in llamas and alpacas, characterized the biological and chemical properties of OIF and ultimately identified it as βNGF. This recent discovery has allowed a convergence of knowledge previously separated by discipline and by mechanism; that is, neurobiology and reproductive biology, and autocrine/paracrine vs endocrine. To preserve this link, we have referred to the seminal factor as OIF/NGF. As a highly conserved protein, the implications of discoveries related to OIF/NGF in reproductive tissues extend beyond the camelid species, and results of recent studies show that the presence and function of OIF/NGF in seminal plasma are conserved among species considered to be induced ovulators as well as those considered to be spontaneous ovulators. The abundance of OIF/NGF in seminal plasma and the effects of seminal plasma on ovarian function strongly support the idea of an endocrine mode of action (i.e. systemic distribution with distant target tissues). This review is intended to provide an update on the progress in our understanding of the nature of OIF/NGF in seminal plasma and its effects on reproductive function in the female, including the effects of dose and route of administration, evidence for ovarian effects in other species, tissue sources of OIF/NGF and early findings related to the mechanism of action of OIF.

1 | INTRODUCTION – A BRIEF HISTORY OF OIF

The persistence among species of an elaborate accessory gland system in males and the physiognomy of copulation-induced ovulation in females are two of the most intriguing mysteries in reproductive biology. The existence of these two phenomena has been rationalized as simply evolutionary vestiges of more primitive lineages (Bedford, 2004; Conaway, 1971; Kauffman & Rissman, 2005), but more recent discoveries about an ovulation-inducing factor (OIF) in seminal plasma provide at least one explanation that links the two together.

The discovery of copulation-induced ovulation was reported by Walter Heape more than 100 years ago when he described that *The doe rabbit only permits coition when undergoing oestrus, and if the male is withheld at that time the ripe ova in the ovary degenerate; they are not dehisced from the ovary* (Heape, 1905). Far from being the exception, we now know that every mammalian Order has one or more species displaying induced ovulation (Kauffman & Rissman, 2005), suggesting that the mechanism is not simply a taxonomic relic. The phenomenon of induced ovulation was reported in South American camelids in the late 1960s (England, Foote, Mathewes, Cordozo, & Riera, 1969; San Martin et al., 1968) and in Bactrian and dromedary camels about a decade later (Chen & Yuen, 1979; Musa & Abusineina, 1978; Shalash & Nawito, 1964). Early studies in alpacas and llamas (Fernandez-Baca, Madden, & Novoa, 1970) revealed that ovulation occurred in >95% of females subsequent to mounting and penile intromission compared to <14% in which intromission was not allowed. These results led to the concept that physical stimulation of the genitalia during copulation is the primary trigger for inducing ovulation in these and other species of induced ovulators.

Yet, early studies in alpacas and llamas were not designed to control potential confounding factors that may influence ovulation, and workers in China suggested that some factor in the semen was responsible for eliciting ovulation in Bactrian camels, rather than the mechanical stimulation of copulation. Ovulation occurred after intravaginal (Chen, Yuen, & Pan, 1985; Xu, Wang, Zeng, Jiang, & Gao, 1985) or intramuscular/intrauterine (Pan et al., 1992) administration of Bactrian seminal plasma to female Bactrian camels. Similar to early studies in llamas and alpacas, however, interpretation of results in Bactrian camels was hampered because of a low number of observations and that intravaginal and intrauterine routes of treatment may have confounded the effect of semen itself.

Twenty years after the original discovery in Bactrian camels, the existence of an ovulation-inducing factor (OIF) in seminal plasma was convincingly confirmed in a series of studies involving llamas and alpacas (Adams, Ratto, Huanca, & Singh, 2005) wherein >90% of females ovulated after administration of a single intramuscular dose of seminal plasma (Adams et al., 2005). It is not surprising that the discovery of OIF in seminal plasma was made in species categorized as induced ovulators because factors influencing the occurrence of ovulation can be studied without the confounding effects of spontaneous ovulation. Yet, it is surprising that the discovery was not made sooner, given the abundance of OIF in the seminal plasma of species representative of both induced and spontaneous ovulators (Harper & Thoenen, 1980; Hofmann & Unsicker, 1982; Ratto, Delbaere, Leduc, Pierson, & Adams, 2011).

The following review is intended to provide an update on the progress in our understanding of the nature of OIF in seminal plasma and its effects on reproductive function in the female. We will present results of studies on the endocrine and ovarian response to OIF in the female, the biochemical identity of OIF, the effects of dose and route of administration, evidence for ovarian effects in other species, tissue sources of OIF and early findings related to the mechanism of action of OIF.

2 | CHEMICAL IDENTITY OF OIF IN SEMINAL PLASMA

The initial supposition that OIF is related to the GnRH peptide was reasonable based on its LH-releasing effects on pituitary cells (Paolicchi, Urquieta, Del Valle, & Bustos-Obregon, 1999) and the presence of GnRH immuno-reactivity in human seminal plasma (Izumi, Makino, & Iizuka, 1985; Sokol, Peterson, Heber, & Swerdlof, 1985). Yet, the addition of GnRH antibodies to *in vitro* rat pituitary cell culture did not block the LH-releasing effect of alpaca seminal plasma (Paolicchi et al., 1999). The lack of a validated bioassay to test the effects of various biochemical fractions of seminal plasma hampered early attempts to isolate and purify OIF in the seminal plasma of Bactrian camels (Li & Zhao, 2004; Pan et al., 2001; Zhao, Li, & Chen, 2001). Some suggested that OIF consists of bioactive forms of different molecules ranging from 16 to 54 kDa (Pan et al., 2001), while others suggested that at least two fractions of camel seminal plasma were able to elicit LH secretion from *in vitro* culture of rat pituitary cells (Li & Zhao, 2004).

In a series of experiments designed to determine the chemical identity of OIF, we attempted to ablate its biological activity by using (i) molecular mass cut-off filtration, (ii) treatment with proteinase K, charcoal or heat and (iii) treatment with pronase E (Ratto, Huanca, & Adams, 2010). An *in vivo* llama ovulation bioassay was used to test the various fractions of seminal plasma produced by the treatments. Results documented that OIF is not a steroid, prostaglandin or GnRH; rather, it is a protein that is resistant to heat and enzymatic digestion with proteinase K, and has a molecular mass of approximately 30 kDa (Ratto et al., 2010; Table 1). In a follow-up study, protein fractions of llama seminal plasma were isolated and purified using liquid chromatography and tested using the *in vivo* llama ovulation bioassay (Ratto et al., 2011). One purified protein (fraction C_2) elicited a pre-ovulatory LH surge followed by ovulation and corpus luteum formation in llamas after intramuscular administration (Fig. 1, Table 2).

The identity of the OIF molecule was determined unequivocally in a study designed to test the hypothesis that OIF is a single distinct and widely conserved entity (Ratto et al., 2012). Seminal plasma from llamas and bulls (representative of induced- and spontaneous ovulators) was used. The previously identified protein fraction C_2 was isolated and found to have a molecular mass of 13,221 Da, and tryptic amino acid sequences of it were found to be homologous with human, porcine, bovine and murine sequences of ß-NGF. X-ray diffraction data were used to solve the full sequence and structure of OIF, and this seminal protein was found to be identical to beta-nerve growth factor (ß-NGF; Fig. 2). The same study confirmed that purified OIF had the same biological activity as that of NGF as evidenced by the induction of neurite development and upregulation of trkA in PC_{12} cells *in vitro* – a specific bioassay for NGF. Western blot analysis of llama and bull seminal plasma confirmed immuno-recognition of OIF using polyclonal mouse anti-NGF, and administration of ß-NGF from mouse submandibular glands induced ovulation in llamas. Therefore, we concluded that OIF purified from seminal plasma of llamas is the highly conserved protein ß-NGF (Ratto et al., 2012). At the same time, authors of another independent study also concluded that ß-NGF is a major protein expressed in the seminal plasma of alpacas and is

TABLE 1 Ovulation rate in llamas treated with different fractions of llama seminal plasma (SP) based on molecular mass, or after exposing seminal plasma to various treatments (from Adams & Ratto, 2013)

Whole SP	≥30 kDa	10–30 kDa	5–10 kDa	<5 kDa
9/9[a] (100%)	9/9[a] (100%)	0/9[b] (0%)	0/9[b] (0%)	0/9[b] (0%)
Untreated SP	Charcoal	Heat (65°C)	Proteinase K	Pronase E
16/17 [a] (94%)	7/7 [a] (100%)	7/7 [a] (100%)	7/7 [a] (100%)	0/10[b] (0%)

[a,b]Within rows, values with different superscripts are different ($p < .01$).

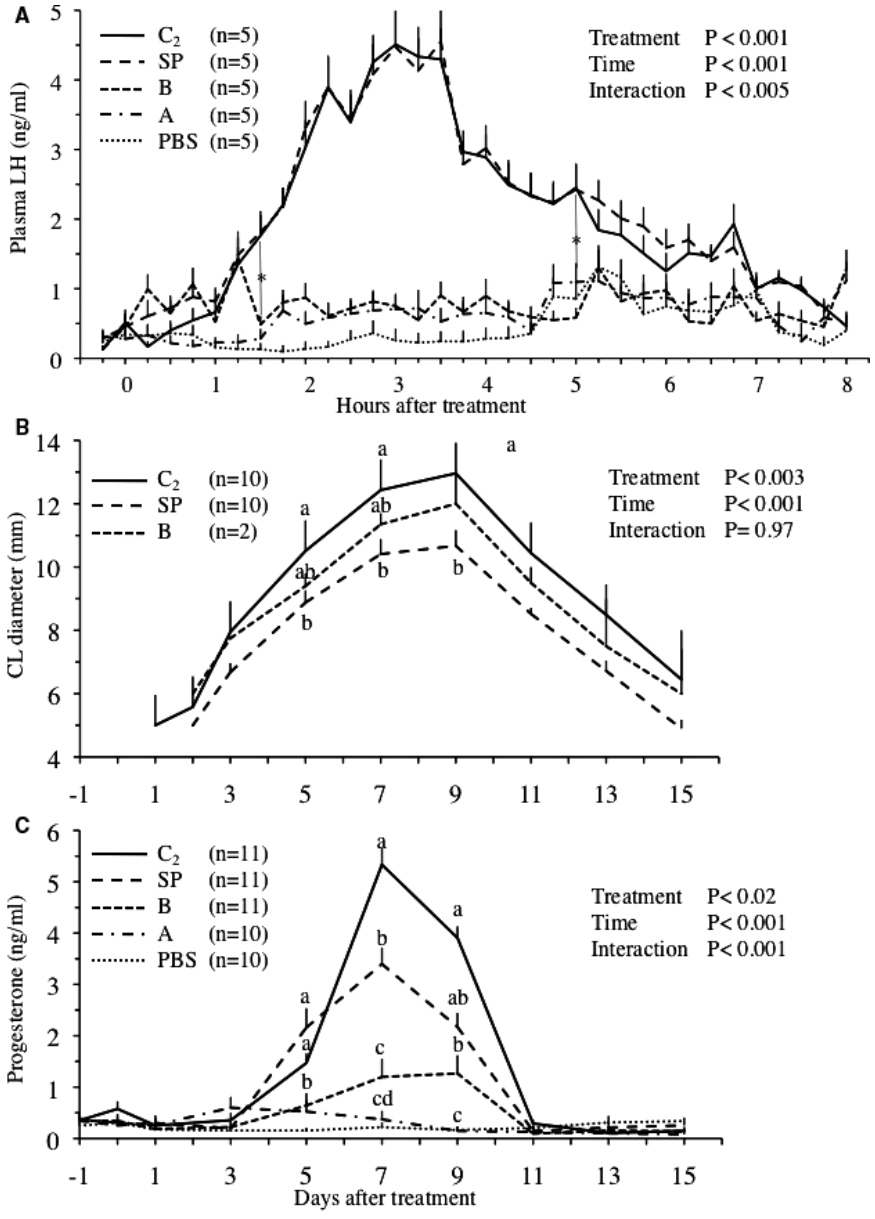

FIGURE 1 Effect of different protein fractions of llama seminal plasma on circulating LH concentration (a), CL diameter (b) and plasma progesterone concentration (c) in llamas (mean ± *SEM*) given whole seminal plasma (SP, positive control), fractions A or B (isolated by hydroxyapatite column chromatography), fraction C_2 (isolated by gel filtration chromatography) or phosphate-buffered saline (PBS, negative control). *Interval during which values in SP and C_2 were higher ($p < .05$) than in other groups. [abcd] Within days, values with no common superscript are different ($p < .05$) (modified from Ratto et al., 2011)

TABLE 2 Effect of protein fractions of llama seminal plasma (SP), isolated by column chromatography, on ovulation and corpus luteum development in llamas (mean ± *SEM*; from Adams & Ratto, 2013)

	Saline	Whole SP	Fraction A	Fraction B	Fraction C_2
Ovulation rate (%)	0/14[a] (0%)	14/15[b] (93%)	0/14[a] (0%)	2/15[a] (13%)	14/15[b] (93%)
Day CL detected[c]	—	2.9 ± 0.1[a]	—	2.5 ± 05[a,b]	2.1 ± 0.2[b]
Maximum CL diameter (mm)	—	11.0 ± 0.4[a]	—	12.0 ± 1.0[a,b]	13.3 ± 0.4[b]
CL diameter on Day 15[c] (mm)	—	4.9 ± 0.2[a]	—	4.5 ± 0.5[a]	6.4 ± 0.5[b]

[a,b]Within rows, values with different superscripts are different ($p < .01$).
[c]Day 0 = day of treatment.

responsible for inducing ovulation (Kershaw-Young, Druart, Vaughan, & Maxwell, 2012).

Nerve growth factor belongs to a family of neurotrophins, all of which exist in nature as homodimers with a molecular mass of 26–27 kDa (Kolbeck, Jungbluth, & Barde, 1994). Originally discovered in mouse sarcoma, cobra venom and mandibular salivary glands of adult mice, NGF has been characterized classically by its role in promoting survival and growth of sensory (dorsal root) and sympathetic neurones, and cells of the adrenal medulla (Angeletti & Bradshaw, 1971). Yet, NGF has subsequently been identified in a variety of non-neuronal cells including tissues of both male and female reproductive organs. Early purification experiments revealed that bovine seminal plasma

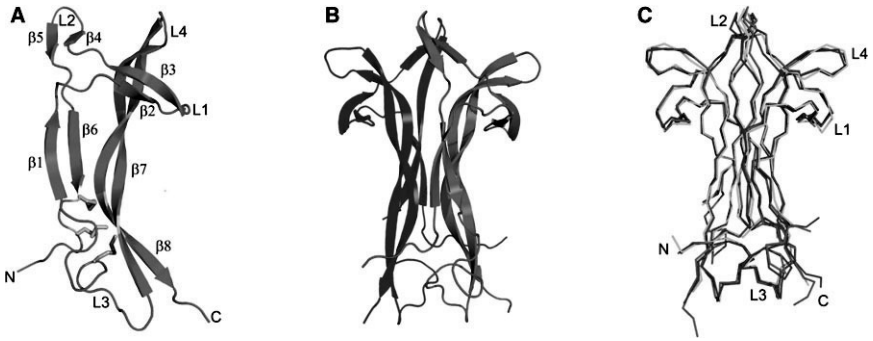

FIGURE 2 Protein structure of ovulation-inducing factor (OIF) from seminal plasma. (a) Monomer of OIF. β-Strands are labelled (β1–β8). Loops are labelled L1–L4. The three disulphide bridges are shown in stick representation. (b) Biological dimer of OIF, rotated 90° with respect to figure (a). The OIF monomers are coloured red and blue. (c) Superpositions of OIF (blue) on mouse NGF (red) and human NGF (green) revealing structural similarity between OIF and NGF. The loops of one of the two monomers are labelled L1–L4 (from Ratto et al., 2012)

is a rich source of NGF (Harper, Glanville, & Thoenen, 1982) and is likely produced primarily by the vesicular glands (Hofmann & Unsicker, 1982). It has also been detected in the prostate gland of guinea pigs, rabbits and bulls (Harper & Thoenen, 1980; Harper et al., 1979, 1982). Because of the existing body of literature in which the term OIF has been used, we have adopted the use of term *OIF/NGF* (Ratto et al., 2012) to designate that of seminal plasma origin and to provide a bridge between the bodies of knowledge pertaining to reproductive biology and neurobiology.

3 | ENDOCRINE AND OVARIAN EFFECTS OF OIF/NGF

3.1 | LH release and ovulation

The effects of intramuscular and intrauterine administration of seminal plasma were examined in a series of experiments in alpacas and llamas to document the role of seminal plasma on ovulation in females of the same species and to determine whether the route of action on the ovary is local or systemic (Adams et al., 2005; Ratto, Huanca, Singh, & Adams, 2005; Table 3). Collectively over four separate experiments, intramuscular administration of seminal plasma (equivalent to one-fourth to half of an ejaculate) resulted in ovulation in 33 of 35 (94%) females compared to 0 of 35 (0%) given saline. To determine whether ovulation induced by treatment with seminal plasma was associated with a pre-ovulatory surge in circulating concentrations of LH, blood samples were collected frequently from female llamas for 8 hr after treatment (Adams et al., 2005; Fig. 3). The timing of the LH surge in response to seminal plasma treatment was similar to that reported after natural mating (i.e. it began within 30 min of treatment and was maximal by 2 hr; Bravo, Fowler, Stabenfeldt, & Lasley, 1990; Bravo, Stabenfeldt, Lasley, & Fowler, 1991) and consistent with that reported in Bactrian camels (Xu et al., 1985). Yet, the duration of the LH surge was prolonged after treatment with seminal plasma compared to GnRH treatment; that is, LH concentrations had not yet returned to basal levels by 8 hr (Fig. 3). By ultrasonographic examination every 4 hr, ovulations were detected 29.3 ± 0.7 hr after treatment with seminal plasma (Adams et al., 2005), similar to the interval after natural mating or treatment with GnRH or LH (30.0 ± 0.5, 29.3 ± 0.6, 29.3 ± 0.7 hr, respectively; Ratto, Huanca, Singh, & Adams, 2006).

Results supported the concept of an endocrine route of action, but paradoxically, none (0 of 12) of the alpacas ovulated after being given seminal plasma by transcervical intrauterine deposition in the initial study (Adams et al., 2005). This led to a subsequent study to test the hypothesis that differences are due to attenuated absorption of OIF from the genital mucosa compared to intramuscular administration. As copulation in camelids is a prolonged event (30–50 min; Bravo et al., 1990; San Martin et al., 1968) and ejaculation is intrauterine, a normal sequelae of copulation is acute, transient inflammation of the endometrium as a result of repeated abrasion by the

TABLE 3 The ovulatory effect of seminal plasma administered intramuscularly or by intrauterine infusion with or without endometrial curettage

	Intramuscular		Intrauterine		Intrauterine with curettage	
Ovulation rate	**Seminal plasma**	**Saline**	**Seminal plasma**	**Saline**	**Seminal plasma**	**Saline**
Alpacas (Adams et al., 2005)	13/14 [a] (93%)	0/14[b] (0%)	0/12[b] (0%)	0/12[b] (0%)	–	–
Alpacas (Ratto et al., 2005)[e]	14/15[a] (93%)	0/15[c] (0%)	7/17[b] (41%)	0/15[c] (0%)	10/15[a,b] (67%)	0/15[c] (0%)
Llamas (Adams et al., 2005)	6/6 [a] (100%)	0/6 [b] (0%)	–	–	–	–
Total	33/35 [a] (94%)	0/35 [d] (0%)	7/29 [b,e] (24%)	0/27 [d] (0%)	10/15 [c] (67%)	0/15 [d] (0%)

[a,b,c,d]Within rows, proportions with different superscripts are different ($p < .05$).
[e]Dose of seminal plasma was twice that used in Adams et al., 2005 (i.e. 2 ml vs 1 ml).

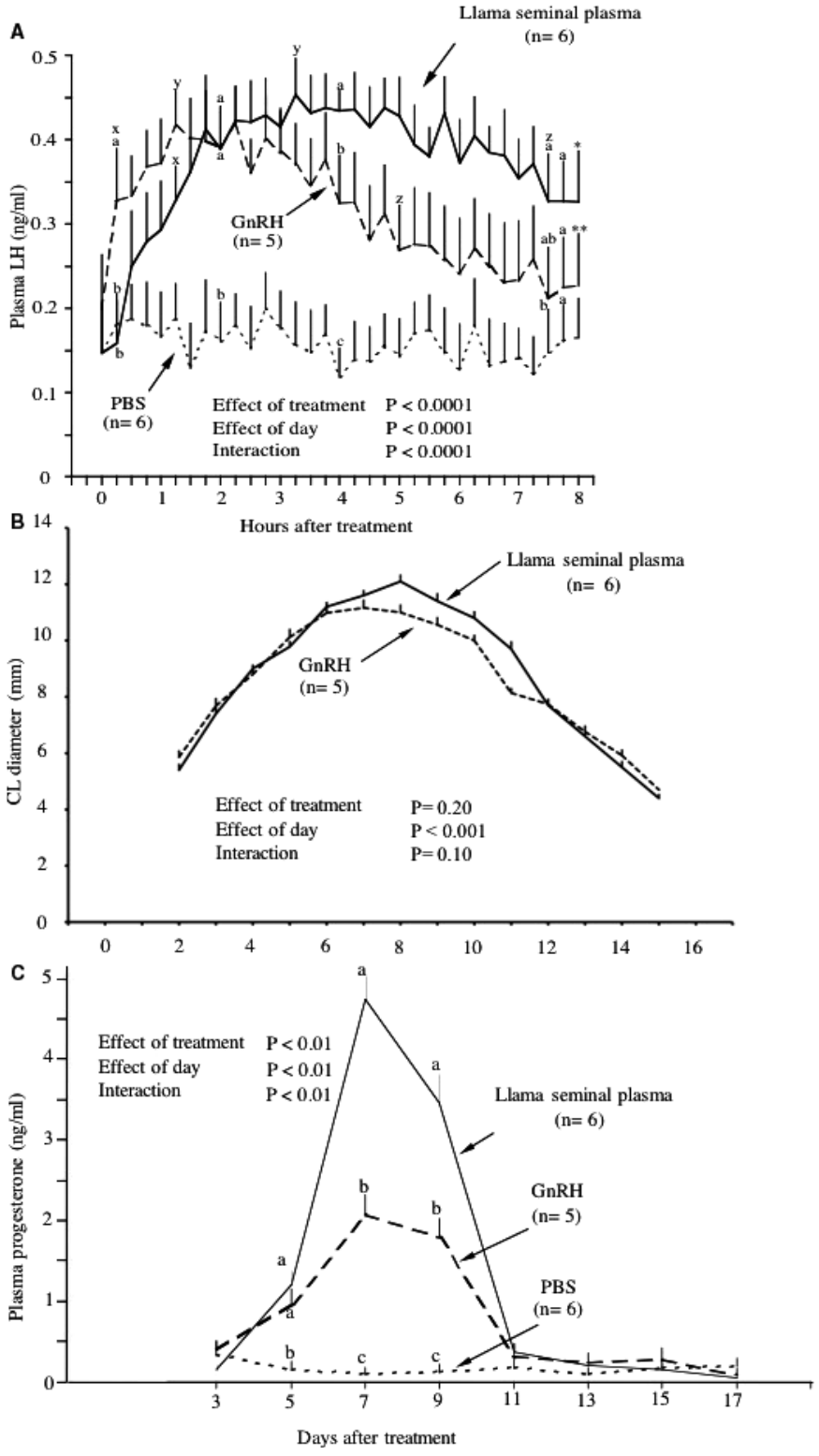

FIGURE 3 Plasma LH concentration (a), corpus luteum diameter (b) and plasma progesterone concentration (c) in female llamas (mean ± *SEM*) after intramuscular treatment with llama seminal plasma, GnRH or phosphate-buffered saline (PBS) (modified from Adams et al., 2005). [abc] On a given day, values with no common superscript are different among groups ($p < .05$). [xyz] Within group, the first increase, the maximum and the first decrease from maximum concentration ($p < .05$). * Within group, the last value is higher than the pre-treatment value ($p < .05$). ** Within group, the last value is not different from the pre-treatment value ($p = .9$)

penis (Bravo, Moscoso, Ordonez, & Alarcon, 1996). A follow-up study involved twice the dose of seminal plasma and intrauterine treatment with endometrial curettage (to simulate the abrasive effects of copulation; Ratto et al., 2005). In contrast to the first study in which no ovulations were detected after intrauterine administration of seminal plasma, 41% of females ovulated in the no-curettage group and 67% ovulated in the curettage group (Table 3). Importantly, no ovulations (0 of 42) occurred after administration of saline by the intrauterine route with or without curettage (Table 3). The greater effectiveness of seminal plasma when given intramuscularly than by the intrauterine route is consistent with that of GnRH where, in rabbits, the ovulation induction dose was 10 to 20 times higher when given intravaginally than intramuscularly (Quintela et al., 2004; Rebollar et al., 2012; Viudes-de-Castro, Lavara, Marco-Jiménez, Cortell, & Vicente, 2007).

Our interpretation of these findings is that the ovulatory response to seminal plasma in llamas and alpacas is a function of the degree of absorption of a seminal factor from the genital mucosa into circulation (i.e. systemic dose) and not a response to physical stimulation of the tubular genitalia itself.

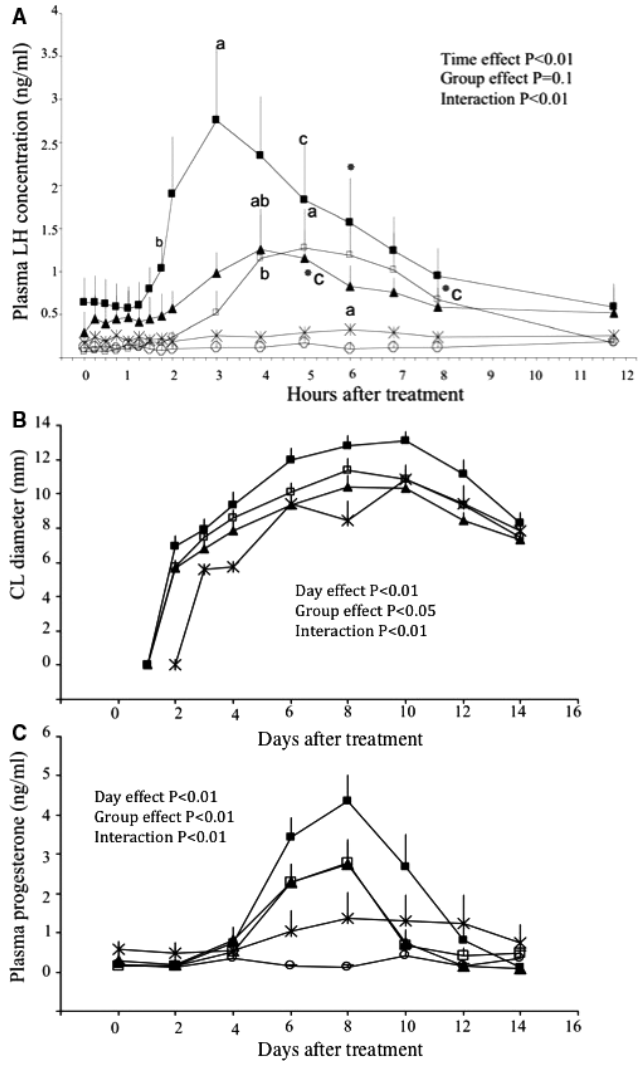

FIGURE 4 Plasma LH concentration (a), CL diameter (b) and plasma progesterone concentration (c) in llamas (mean ± *SEM*) given a single intramuscular dose of OIF (60 μg *, 125 μg □, 250 μg ▲, 500 μg ■) or PBS (○) (modified from Tanco et al., 2011). [abc] Within group, the maximum concentration, the first increase and the first decrease from maximum ($p < .05$).* Within group, the last value higher than pre-treatment levels ($p < .05$)

3.2 | Dose-related effects of OIF/NGF

If the ovulatory response is a function of the degree of absorption of a seminal factor from the genital mucosa into circulation, then the female's response should be dose-dependent. To confirm a dose–response relationship between OIF and ovulation, and to determine whether the dose is physiologically relevant in terms of the proportion present in a normal ejaculate, female llamas were given a single intramuscular dose of 500, 250, 125 or 60 μg of purified OIF (representative of the amount present in 1/25th to 1/200th of a normal ejaculate). A clear dose–response relationship was observed in circulating concentrations of LH, the incidence of ovulation, CL diameter and plasma progesterone concentrations (Fig. 4, Table 4). A dose-related effect on ovulation rate in alpacas was confirmed in a later study using recombinant human NGF (Stuart et al., 2015). Interestingly, a similar dose-related response was observed with GnRH treatment (Silva, Recabarren, Recabarren, Adams, & Ratto, 2012). A single intramuscular dose of 50, 25, 12.5 or 6.25 μg of a GnRH analogue in llamas resulted in a progressive decrease in the magnitude of the pre-ovulatory LH surge as well as in the ovulatory response (Fig. 5), although no effect on the form and function of the CL was detected. We conclude that OIF has a dose-dependent effect on the ovulatory mechanism and that this effect is evident at physiologically relevant doses; that is, as little as 1/100th that present in an ejaculate.

In a direct comparison of different routes of administration, results of a recent study confirm that the dose of OIF required to elicit pituitary and ovarian responses is higher when administered by intrauterine infusion than by intramuscular or intravenous routes (Silva, Fernández, Ulloa-Leal, Adams, & Ratto, 2015). Female llamas treated with 2 mg OIF intramuscularly or intravenously developed a similar surge in plasma LH concentration and ovulation rates (86 and 100%, respectively), but those treated by intrauterine deposition had no LH response and no ovulations. Increasing the intrauterine dose to that equivalent to total amount present in an average llama ejaculate (i.e. 5 ml seminal plasma or 20 mg of OIF), however, induced a surge in circulating LH concentration comparable to that obtained with a lower dose administered intramuscularly or intravenously, and a 100% ovulation rate.

TABLE 4 Effect of dose of purified OIF on ovulation and CL development in llamas (mean ± *SEM*; Day 0 = day of treatment) (modified from Tanco et al., 2011)

Group	Saline	60 μg	125 μg	250 μg	500 μg
Proportion that ovulated	$0/10^{a}$ (0%)	$3/10^{a}$ (30%)	$7/10^{b}$ (70%)	$9/10^{b}$ (90%)	$9/10^{b}$ (90%)
Day of 1st detection of CL	—	3.3 ± 0.3^{a}	2.3 ± 0.2^{b}	2.5 ± 0.2^{b}	2.1 ± 0.1^{b}
Maximum CL diameter (mm)	—	10.9 ± 1.0^{a}	$11.6 \pm 0.7^{a,b}$	10.8 ± 0.7^{a}	13.5 ± 0.5^{b}
CL diameter on Day 8 (mm)	—	8.5 ± 2.0^{a}	$11.3 \pm 0.8^{a,b}$	$10.4 \pm 0.7^{a,b}$	12.8 ± 0.6^{b}

[a,b]Within rows, values with different superscripts are different ($p < .05$).

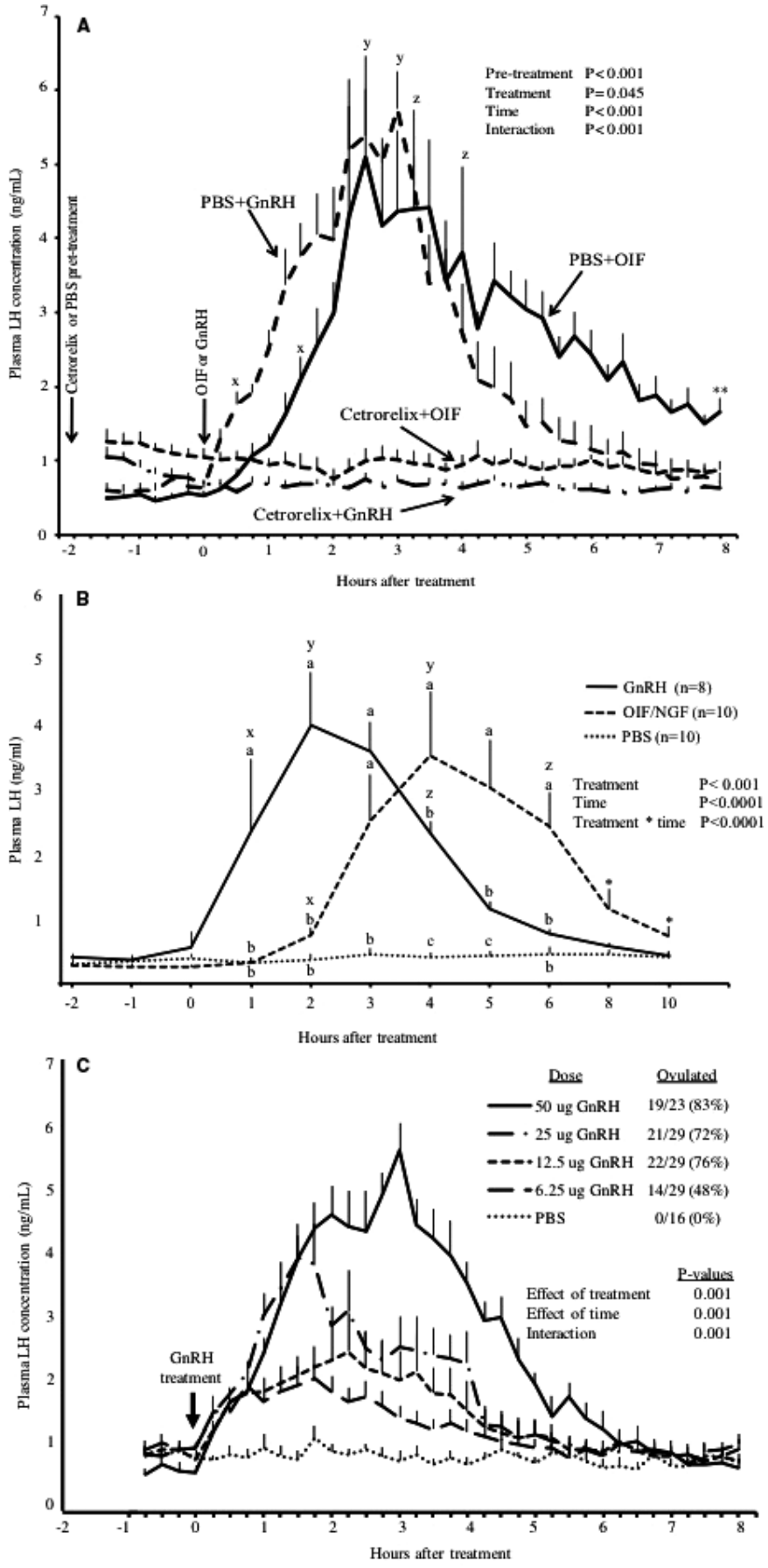

FIGURE 5 Plasma LH concentrations (mean ± *SEM*) in llamas after treatment with (a) GnRH or OIF/NGF with or without pre-treatment with cetrorelix (GnRH antagonist; modified from Silva Niñoet al., 2011; Silva Smulders et al. 2011), (b) GnRH, OIF/NGF or phosphate-buffered saline (PBS; from Ulloa-Leal et al., 2014) and (c) 50, 25, 12.5 or 6.25 μg of GnRH, or 0.5 ml PBS (modified from Silva, Colazo et al., 2012; Silva, Recabarren et al, 2012). [abc]For a given time, values with no common superscript are significantly different among groups. [xyz] Within group, the first significant increase, maximum and first significant decrease from maximum concentration. * OIF/NGF group tended to be higher than GnRH. ** The last value is significantly higher than the pre-treatment value

3.3 | Luteotrophic effect

Pregnancy is dependent on a functional corpus luteum (CL) throughout gestation in camelids (Al-Eknah, Homeida, Ramadan, Al-Modhi, & Al-Busadah, 2001; Bravo, Bazab, Troedsson, Villalta, & Garnica, 1996). Rescue of the CL from luteolysis between 8 and 10 days after ovulation has been implicated in maternal recognition of pregnancy in llamas (Adams, Sumar, & Ginther, 1991), and the ability of the CL to respond to early pregnancy signals may be associated with early luteogenic events. Perhaps as surprising as the ovulation-inducing effect of seminal plasma discovered in our initial study was the apparent positive effect it had on the ensuing CL (Adams et al., 2005). Female llamas treated intramuscularly with a conservative dose of homologous seminal plasma developed a CL that tended to be larger, regressed later and produced more than twice as much progesterone than CL resulting from GnRH-induced ovulation (Fig. 3). In contrast, a luteotrophic effect was not detected in alpacas treated with recombinant human NGF (Kershaw-Young et al., 2012; Stuart et al., 2015).

Results of the initial study provided rationale for the hypothesis that the luteotrophic effect is a result of the LH secretion pattern elicited by seminal plasma. The surge in plasma LH concentration triggered by seminal plasma was sustained beyond the 8-hr sampling period in OIF-treated animals, whereas LH concentrations were basal by 6 hr after GnRH treatment (Fig. 3). The sustained LH release and luteotrophic effects of seminal plasma have been confirmed in several subsequent studies using OIF isolated and purified from the seminal plasma of llamas (Figs 4 and 5; Fernandez et al., 2014; Ratto et al., 2011; Silva, Smulders et al., 2011; Silva et al.,2014 Tanco, Ratto, Lazzarotto, & Adams, 2011; Ulloa-Leal, Bogle, Adams, & Ratto, 2014). A similar relationship between LH secretion and luteogenesis has been described in primates and laboratory species (Bomsel-Helmreich, Huyen, & Durand-Fasselin, 1989; Chandrasekher et al., 1994; Ishikawa, 1992; Peluso, 1990).

The CL receives more blood per unit of tissue than any other organ of the body (Wiltbank, Dysko, Gallagher, & Keyes, 1988); hence, angiogenesis plays a vital role during CL formation. In a study designed to determine whether the luteotrophic mechanism is related to vascular perfusion of the developing CL in llamas, blood flow was assessed by power-flow Doppler ultrasonography every 4 hr after treatment with OIF/NGF or GnRH (Ulloa-Leal et al., 2014). Compared to GnRH treatment, llamas treated with OIF/NGF had greater vascular flow to the pre-ovulatory follicle 4 hr after treatment ($p < .001$), and greater vascular flow to the CL and greater plasma progesterone concentrations 6 days after treatment ($p < .001$; Fig. 6). In addition to a dose-dependent effect (Tanco et al., 2011), the luteotrophic effect of OIF/NGF was influenced by the timing of treatment. Two doses of OIF/NGF, given before and at the time of ovulation, induced the development of larger CL with greater vascularization and that produced more progesterone than CL induced by a single pre-ovulatory dose (Fernandez et al., 2014).

The role of OIF/NGF directly at the level of the ovary, however, remains an open question, particularly in the light of the observation that NGF stimulated progesterone secretion from mid-luteal stage bovine CL *in vitro* cell culture (Miyamoto, Okuda, Schweigert, & Schams, 1992). In this regard, both camelid and bovine seminal plasma were found to be luteotrophic in cattle (a spontaneous ovulator) despite that no measurable increase in plasma LH concentrations was detected (Tanco, Van Steelandt, Ratto, & Adams, 2012; Tribulo, Bogle, Mapletoft, & Adams, 2015). The most recent data in cattle suggest that the luteotrophic effect of OIF/NGF is mediated, in whole or in part, directly at the level of the ovary through interaction with trkA receptors on granulosa and theca cells of the dominant follicle and developing CL (Fig. 7, Carrasco, Singh, & Adams, 2016).

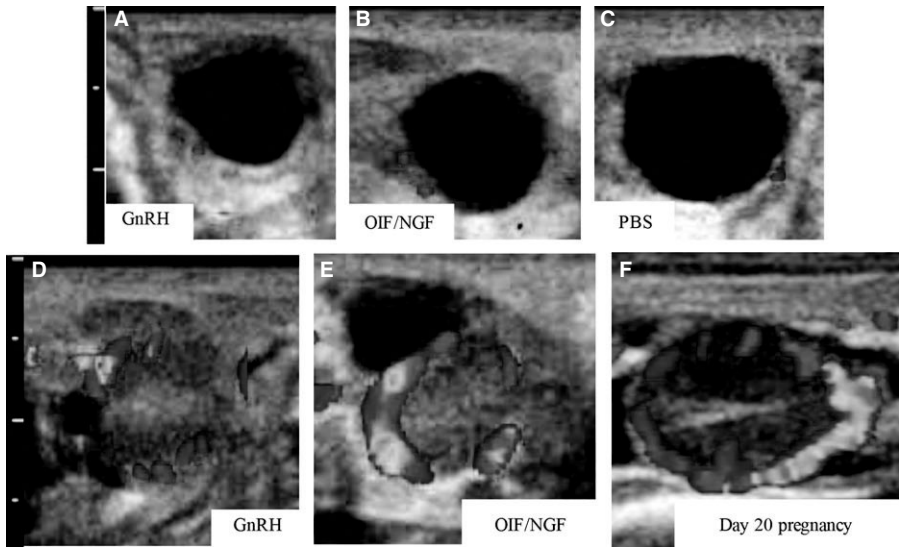

FIGURE 6 Doppler power-flow images of the pre-ovulatory follicle 4 hr after GnRH, OIF/NGF or PBS treatment (a, b, c) and of the CL 6 days after GnRH or OIF/NGF treatment (d, e) and on Day 20 of pregnancy in llamas (f) (modified from Ulloa-Leal et al., 2014 and Bogle, 2016)

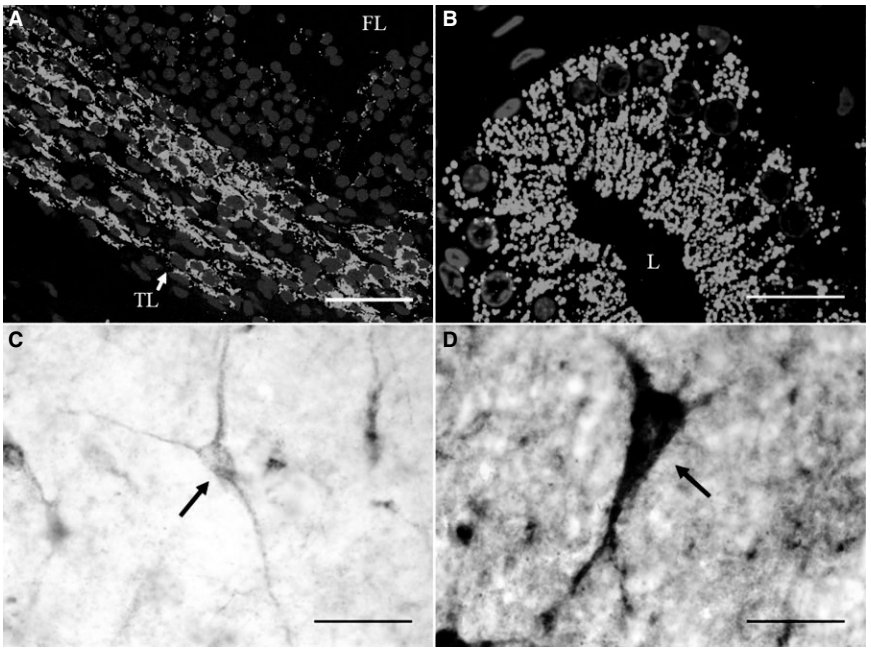

FIGURE 7 Detection of NGF and its receptor (trkA) in bovine and llama tissues. (a) TrkA immuno-reactivity (green) in a pre-ovulatory follicle of a cow (blue = nucleus counterstain). The theca layer (TL) displays a high degree of immuno-reactivity. FL: Follicular lumen. Scale bar 50 microns (b). Immuno-detection of NGF (green) in a semi-thin section of llama prostate. The cytoplasm of prostatic cells is densely populated by immuno-reactive secretory granules (red = nucleus counterstain). L: Lumen. Scale bar 10 microns. (c, d) Presence of NGF (c) and trkA (d) immuno-reactive neurones (arrows) in the llama hypothalamus. Scale bar 50 microns. (Carrasco & Adams, unpublished)

4 | MECHANISM OF ACTION

Classically, ovulation in mammals involves pulsatile secretion of GnRH from the hypothalamus into the hypophyseal portal system, followed by the release of LH from the anterior pituitary into systemic circulation (Karsch, 1987). While it is clear that the ovulatory effect of OIF/NGF in seminal plasma is mediated through a surge release of LH into circulation, it is not clear whether the site of action is solely at the level of the hypothalamus or also involves the pituitary gland. In an elegant study to determine the site of action of OIF/NGF, pre-treatment of llamas with a GnRH antagonist (cetrorelix) ablated the effects of OIF (i.e. blocked LH release and ovulation), suggesting a direct or indirect effect of OIF on GnRH neurones in the hypothalamus (Fig. 5; Silva, Niño et al., 2011). As ovarian steroids exert a modulatory effect on the mediobasal hypothalamic neurones to influence GnRH pulse secretion (Caraty et al., 1998; Terasawa et al., 2010), a study was carried out to test the hypothalamic pathway by examining the response to OIF/NGF treatment in ovariectomized and oestradiol-treated llamas (Silva, Recabarren et al., 2012). The LH response to OIF/NGF treatment was muted in ovariectomized llamas and was partially restored by pre-treatment with oestradiol, consistent with the hypothesis that the pathway of OIF/NGF involves the hypothalamus.

Results of *in vitro* studies, however, document that OIF/NGF also has a direct effect on pituitary gonadotrophs. Treatment of primary cultures of llama and bovine anterior pituitary cells induced LH secretion, and its magnitude increased with treatment dose (Bogle, Ratto, & Adams, 2012). This is consistent with earlier studies in which the addition of purified OIF or seminal plasma from Bactrian camels or alpacas to a primary culture of rat pituitary cells induced secretion of LH (Paolicchi et al., 1999; Zhao et al., 2001).

Presumably the effect of OIF/NGF on the hypothalamo-pituitary-ovarian axis is brought about by binding with specific and non-specific receptors, trkA and p75, respectively. If so, then determining the location and temporal expression of these receptors will aid in our understanding of the mechanism by which OIF/NGF elicits LH release. The neural pathways involved in the activation of GnRH neurones in induced ovulators are poorly understood, and no studies have been reported in camelids. Initial studies of the pattern of distribution of GnRH neurones in the hypothalamus of llamas revealed that over 60% of GnRH neurones were located in the anterior and medio-basal hypothalamus on the lateral aspects of the third ventricle, but were scattered widely rather than in focal accumulations or nuclei (Fig. 7, Carrasco, 2016). The proximity between the cerebral ventricle and GnRH neurones suggests a potential route for OIF/NGF to stimulate the pre-ovulatory secretion of GnRH/LH, that is, via the cerebrospinal fluid. Yet, this route implies that circulating OIF/NGF crosses the blood–brain barrier, a matter of some controversy (Frieden et al., 1993; Pan, Banks, & Kastin, 1998). With over 100 amino acids and a molecular mass of 26 kDa, the OIF/NGF molecule is too big to diffuse passively through the blood–brain barrier (Banks, 2009) and therefore would require an active transport mechanism. In an initial attempt to bio-track exogenous OIF/NGF, we were able to detect the biotinylated molecule in the cerebrospinal fluid in rabbits (Berland et al., 2013), but could not replicate these results in llamas.

Receptors for NGF have been identified within the hypothalamus of rats (Gibbs & Plaff, 1994), but we have only begun to examine their distribution in camelids (Fig. 7; Carrasco, 2016). Studies are needed in induced ovulators to determine whether this seminal protein that is absorbed into circulation from the uterine lumen passes through the blood–brain barrier and whether its receptors are co-localized with GnRH neurones in the hypothalamus. It is interesting to note that among studies, the LH surge elicited by exogenous OIF/NGF begins and peaks 1–2 hr after that elicited by exogenous GnRH (Figs 3–5). The relatively delayed response with OIF/NGF may reflect an intermediate step (i.e. a different cell type) in the pathway required to elicit

GnRH/LH release. It is also notable that magnitude of the LH surge was similarly dose-dependent subsequent to treatment with the two peptides (Silva, Colazo, & Ratto, 2012; Tanco et al., 2011).

Other possible pathways include direct action on GnRH neurone terminals outside the blood–brain barrier or simply a direct action on the pituitary gonadotrophs themselves. The presence of NGF has been detected in the 75% of LH-containing gonadotroph cells and 44% of those cells expressed the high-affinity NGF receptor trkA in rat anterior pituitary cells (Patterson & Childs, 1994) suggesting a functional link between NGF and LH secretion in gonadotrophs that has not yet been elucidated. Alternatively, some suggest that β1 tanycytes participate in the pulsatile release of GnRH into the portal blood (Rodriguez et al., 2005). Most GnRH nerve fibres and their endings are concentrated in the lateral regions of the median eminence and are separated from the perivascular space by a continuous cuff formed by tanycytes, a unique cell type lining the floor of the third ventricle (Rodriguez, Gonzalez, & Delannoy, 1979). Perhaps, OIF/NGF within the median eminence induces the secretion of molecules that control the transient and cyclic release at the GnRH terminals (Prevot, 2002). Another hypothesis involves the organum vasculosum of the lamina terminalis (OVLT) whose complex arrangement of GnRH neurone dendrites in the rostral preoptic area is putatively outside of the blood–brain barrier and therefore able to directly sense molecules travelling in the systemic blood (Herde, Geist, Campbel, & Herbison, 2011; Rodriguez, Blazquez, & Guerra, 2010).

5 | OVARIAN EFFECTS IN OTHER SPECIES

5.1 | Other induced ovulators

OIF/NGF has been recently detected in dromedary seminal plasma by Western blot and chromatography (Kumar et al., 2013) and proteomic techniques (Druart et al., 2013); however, there are no reports on the effects of purified OIF/NGF on ovarian function in old world camelids. Outside the Family Camelidae, rabbits and koalas are the only induced ovulators in which semen-induced ovulation has been examined. In koalas, the presence of a seminal factor was inferred from the observation that four of nine females ovulated after urogenital sinus deposition of semen from conspecific males (Johnston, O'Callaghan, Nilsson, Tzipori, & Curlewis, 2004). Follow-up studies have not been carried out in koalas to confirm the effect or the identity of the seminal factor as OIF/NGF.

Despite that rabbits are the most widely studied induced ovulator and the first species to be identified as such more than 100 year ago, the principal stimulus responsible for triggering ovulation in rabbits is still unclear. Recently, OIF/NGF has been found in high concentration in rabbit seminal plasma (Casares-Crespo, Talaván, & Viudes-de-Castro, 2016; Maranesi et al., 2015; Silva, Niño et al., 2011), but intramuscular administration of neither rabbit nor llama seminal plasma-induced ovulation in receptive does (Cervantes, Palomino, & Adams, 2015; Silva, Niño et al., 2011). Seminal plasma treatment was, however, associated with an increase in the number of antral follicles and the presence of large haemorrhagic anovulatory follicles (Silva, Niño et al., 2011), similar to that observed after ovarian superstimulation of does with eCG (Garcia-Ximenez & Vicente, 1990; Mehaisen, Vicente, Lavara, & Viuedes-de-Castro, 2005). Seminal plasma treatment, however, did not elicit a detectable increase in plasma LH concentration in rabbits (Cervantes et al., 2015). The role of other behavioural/physical stimuli was readily apparent in the latter study wherein the incidence of ovulation was equally high (i.e. >80%) among group-housed does treated with GnRH, seminal plasma or saline. In does housed individually, however, ovulation occurred only in the positive control group (GnRH). Results of another recent study suggest that semen may augment a primary somatosensory stimulus to trigger LH release and ovulation because artificial insemination of rabbits with raw semen, saline or raw semen after lumbar epidural anaesthesia resulted in ovulation in 8 of 8, 3 of 8 and 0 of 8 rabbits (Rebollar et al., 2012).

5.2 | Spontaneous ovulators

The presence of an operative OIF/NGF system controlling ovarian function may not be restricted to species traditionally classified as induced ovulators. In cattle and pigs, coital activity was associated with enhanced LH secretion and a higher ovulation rate (Jochle, 1975; Marion, Smith, Wiley, & Barrett, 1950; Odhiambo et al., 2009; Signoret, de Mesnil du Buisson, & Mauleon, 1972). Furthermore, a pronase-sensitive protein in the seminal plasma of pigs accelerated ovulation in gilts after intrauterine deposition (Waberski et al., 1995), and studies on human seminal plasma suggest the presence of a GnRH-like substance (Izumi et al., 1985; Sokol et al., 1985).

In rats, upregulation of trkA and NGF mRNA was detected in granulosa and theca cells, respectively, at the first pre-ovulatory surge of gonadotropins at the time of puberty, and the use of NGF antibodies or a trkA blocker at the time of the pre-ovulatory LH surge inhibited ovulation (Dissen et al., 1996, 2000). When a superstimulated pre-pubertal mouse model was used to determine the functional role of OIF/NGF in spontaneous ovulators (Bogle, Ratto, & Adams, 2011), llama seminal plasma not only induced more mice to ovulate, but more ovulations per mouse than in negative controls, and the effect was nearly as potent as the positive controls given hCG.

In cattle, the effects of treatment with purified OIF/NGF from llama seminal plasma were examined using pre-pubertal heifers; for the same reason, pre-pubertal mice were used – to minimize the confounding effect of spontaneous ovulation (Tanco et al., 2012). Contrary to the effect seen in mice, purified OIF/NGF did not induce ovulation in heifers. It did, however, hasten both the regression of the extant dominant follicle and the emergence of a new follicular wave, suggesting a role in controlling follicular wave dynamics through a suppressive effect on the dominant follicle. Purified OIF/NGF did not induce ovulation in sexually mature heifers (Tanco et al., 2012), but bovine seminal plasma treatment resulted in significantly more synchronous ovulation in heifers treated with LH and also had a luteotrophic effect (Tribulo et al., 2015).

TABLE 5 OIF/NGF immuno-reactivity in the epithelium and lumen of male accessory sex glands (modified from Bogle, 2016; and Maranesi et al., 2015)

Tissue	Llama	Rabbit	Rat	Bull	Bison	Elk	Deer
Ampulla	–	nd	–	+++	+++	++	±
Prostate (body)	+++	+++	–	++	–	–	++
Prostate (disseminate)	+++	nd	na	nd	–	nd	nd
Coagulating gland	na	na	+++	na	na	na	na
Vesicular gland	na	++	–	+++	++	–	–
Bulbourethral gland	±	nd	–	–	–	nd	+

Relative OIF/NGF staining intensities were graded: +++ (very strong), ++ (strong), + (moderate to weak), ± (faint), – (absent), na (not applicable) and nd (not determined).

6 | SOURCE AND ABUNDANCE OF OIF/NGF IN THE MALE

The initial discovery of NGF in the prostate of the guinea pig (Harper et al., 1979) prompted a search for the source and relative abundance of NGF in the male accessory glands of other species. In a comparison of the male genital glands of the mouse, rat, guinea pig, hamster, rabbit, human and bull, only the prostate glands of the guinea pig, the rabbit and bull were found to contain NGF and that of the guinea pig contained the highest concentrations (Harper & Thoenen, 1980). The later discovery that OIF is NGF led to renewed interest in the source of the molecule in the male and its role in seminal plasma. A recent comparison of five different species revealed that OIF/NGF is present in at least one male accessory gland in all species (Table 5; Bogle, 2016). The principal source of OIF/NGF appears to be the prostate gland in llamas, rabbits, guinea pigs and white-tailed deer and the ampullae and vesicular glands in cattle and bison.

Of species examined to date, the concentration of OIF/NGF in seminal plasma appears to be highest in camelids and rabbits, based on response to *in vivo* bioassay (ovulation), *in vitro* bioassay (PC_{12} cells) and immunoassay (ELISA, RIA, immuno-histochemistry; Bogle, 2016; Tribulo et al., 2015; Silva, Niño et al., 2011; Maranesi et al., 2015; Casares-Crespo et al., 2016). In llamas treated with similar volumes of seminal plasma, the ovulation rate was 90–100% using camelid or rabbit seminal plasma (Adams et al., 2005; Silva, Niño et al., 2011), 26% using bull semen (Ratto, Huanca, Singh, & Adams, 2006), 29% using stallion semen and 18% using boar semen (Bogle et al., 2011). The presence of OIF/NGF in the seminal plasma of bulls, stallions and boars was subsequently confirmed using proteomic characterization (Druart et al., 2013). The concentration of OIF/NGF in bovine seminal plasma was found to be only 10 to 20% that of llama seminal plasma, and treatment of female llamas with a dose of bovine seminal plasma equivalent to the dose of OIF/NGF in llama seminal plasma resulted in an ovulation rate similar to that induced by llama seminal plasma (Tribulo et al., 2015).

7 | CONCLUSIONS

Nearly 30 years after the observation of an ovulation-inducing effect of seminal plasma was made in Bactrian camels, the protein, dubbed OIF, was identified as βNGF. As a highly conserved protein, the implications of discoveries related to OIF/NGF in reproductive tissues extend beyond the camelid species; the presence and function of an OIF/NGF system in the reproductive tract have now been documented in many species including both induced and spontaneous ovulators. The abundance of OIF/NGF in seminal plasma and the effects of seminal plasma on ovarian function strongly support the idea of an endocrine mode of action (i.e. systemic distribution with distant target tissues). The ovulatory response to seminal plasma in llamas and alpacas is brought about by a surge in circulating concentrations of LH and is a function of the degree of absorption of a seminal factor from the genital mucosa into circulation (i.e. systemic dose) and not a response to physical stimulation of the tubular genitalia itself. The mechanism and sites of action of OIF/NGF in the hypothalamo–pituitary–gonadal axis are as yet unclear, but may well involve tissue receptors at all three levels. The luteotrophic effect of OIF/NGF exemplies a multilayer mechanism because the effect has been associated with both the magnitude of LH release (central mechanism) and temporal changes in the expression of specific receptors in ovarian follicles (local mechanism). The greatest concentrations of OIF/NGF have been measured in the seminal plasma of camelids and rabbits, but it has been identified in the glandular tissue of least one of the male accessory glands in all species examined to date. Further characterization of OIF/NGF and tools to measure it are needed to determine the its relative prevalence and functional role among species and to permit test of the hypothesis that some as yet unexplained causes of infertility are based on alterations in the sensitivity to, or abundance of, this molecule.

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CONFLICT OF INTEREST

There is no conflict of interest that could compromise the impartiality of the research reported.

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ORIGINAL ARTICLE

WILEY Reproduction in Domestic Animals

Generation of human organs in pigs via interspecies blastocyst complementation

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Contents

More than eighteen years have passed since the first derivation of human embryonic stem cells (ESCs), but their clinical use is still met with several challenges, such as ethical concerns regarding the need of human embryos, tissue rejection after transplantation and tumour formation. The generation of human induced pluripotent stem cells (iPSCs) enables the access to patient-derived pluripotent stem cells (PSCs) and opens the door for personalized medicine as tissues/organs can potentially be generated from the same genetic background as the patient recipients, thus avoiding immune rejections or complication of immunosuppression strategies. In this regard, successful replacement, or augmentation, of the function of damaged tissue by patient-derived differentiated stem cells provides a promising cell replacement therapy for many devastating human diseases. Although human iPSCs can proliferate unlimitedly in culture and harbour the potential to generate all cell types in the adult body, currently, the functionality of differentiated cells is limited. An alternative strategy to realize the full potential of human iPSC for regenerative medicine is the in vivo tissue generation in large animal species via interspecies blastocyst complementation. As this technology is still in its infancy and there remains more questions than answers, thus in this review, we mainly focus the discussion on the conceptual framework, the emerging technologies and recent advances involved with interspecies blastocyst complementation, and will refer the readers to other more in-depth reviews on dynamic pluripotent stem cell states, genome editing and interspecies chimeras. Likewise, other emerging alternatives to combat the growing shortage of human organs, such as xenotransplantation or tissue engineering, topics that has been extensively reviewed, will not be covered here.

1 | INTRODUCTION

Human pluripotent stem cells (hPSCs) can be derived from preimplantation blastocysts (Thomson et al., 1998) or generated through nuclear reprogramming such as somatic cell nuclear transfer (SCNT) (Tachibana et al., 2013; Wakayama, Perry, Zuccotti, Johnson, & Yanagimachi, 1998) and transcription factor-induced pluripotent stem cells or iPSCs (Aasen et al., 2008; Takahashi et al., 2007; Yu et al., 2007). Two characteristic properties of hPSCs, the unlimited proliferative capability in culture and the ability to differentiate into all cell types in the adult body, hold great potential to provide unlimited source material for cell-based therapies. In addition, patient-specific SCNT PSCs or iPSCs are also important for the advancement of personalized medicine. To realize their potential, more efforts have been

dedicated to the development of strategies for generating mature and functional cells and tissues for transplantation from hPSCs. Although some therapies are already in advanced clinical trials, to date, no hPSCs-based therapy is available in the clinic.

Current strategies for obtaining cells and tissues from hPSCs are largely based on in vitro differentiation, although effective on many fronts, have several limitations: (i) in vitro differentiation is not synchronized and there still remain undifferentiated hPSCs present in the differentiation cultures, which will raise safety concerns as they are prone to generate teratomas; (ii) different hPSC lines are known to have variable efficiency in differentiating towards a specific lineage (Osafune et al., 2008); (iii) cells differentiated from hPSCs in vitro are mostly immature cell types similar to cells of foetal or neonatal origin (Hrvatin et al., 2014); (iv) large-scale production of hPSCs derivatives is still not in place for many lineages; (v) current differentiation protocols are not amenable for the generation of three-dimensional transplantable tissues and organs. To overcome these problems, better and more efficient protocols, strategies to functionally mature differentiated cells and scaling up differentiation methods are needed.

Through millions of years of evolution nature has established robust developmental programmes for each living organism, and some of these developmental processes are well conserved among species. By taking advantages of these conserved developmental programmes, we may consider differentiating hPSCs in an in vivo environment of an animal host. Complex tissues and organs are routinely formed in vivo. In vivo differentiation is highly synchronized and guided by spatiotemporal dynamic developmental signals; embryonic cells know exactly where to go and what to become. Uncommitted cells are normally eliminated during the fast-paced and efficient developmental processes. In addition, in vivo differentiation likely will yield functional and mature cell types suitable for transplantation. Although this is an exciting possibility, two major challenges have to be overcome for targeted in vivo tissue and organ generation using hPSCs: first, we need to have a robust protocol for the generation and propagation of chimeric-competent hPSCs; second, we need to solve the issue of stochastic chimeric contribution of PSCs. The goal of this review is to examine the recent advances in the field of interspecies chimeric complementation, highlighting the two main approaches: zygote genome editing and pluripotent stem cells. Other strategies for the generation of transplantable organs such as xenotransplantation, tissue engineering and 3D printing, which have been extensively reviewed (Badylak, Weiss, Caplan, & Macchiarini, 2012; Murphy & Atala, 2014; Yang & Sykes, 2007), will not be discussed here.

2 | BLASTOCYST COMPLEMENTATION

Mammalian development is a highly regulated process for the precise generation of tissues and organs that provide vital life support for an adult organism. Organ and tissue generation is the result of seamless coordination of intrinsic genetic programme and extrinsic niche factors during different stages of development. Embryonic cells, starting from a single zygote, follow a comprehensive blueprint to turn on specific genetic programmes for lineage specification and tissue formation. The derivation of germline-competent embryonic stem cells (ESCs) and development of gene-targeting technologies have facilitated the generation of thousands of mouse genetic models and consequently help us gain a deeper understanding of genetic principles underlying embryogenesis. Now we know genetic programmes are governing many aspects of tissue and organ formation, from specification to maturation. Some of these genetic programmes, if disrupted and despite the existence of intact developmental niche factors, can lead to the generation of embryos, foetuses or neonates lacking entire tissues or organs. For example, *Pdx1* knockout mice are deficient in pancreatic development. *Pdx1* knockout mouse neonates lack entire pancreases and die soon after birth (Offield et al., 1996); mice lacking *Sal1* gene cannot survive long after birth due to kidney agenesis (Nishinakamura et al., 2001); *Runx1* knockouts are embryonic lethal at approximately E12.5 that is characterized by a complete absence of definitive haematopoiesis (Van Deursen, Hiebert, Grosveld, & Downing, 1996; Wang et al., 1996); *Nkx2.5* deficiency in mice leads to embryonic lethality around E10.5 with retarded cardiac development (Lyons et al., 1995).

Via gene knockouts or other genetic strategies differentiation capabilities can be disabled in lineage progenitors, thereby preventing them from contributing to tissue and organ generation. Meanwhile, the extrinsic niche factors necessary for tissue and organ formation remain intact. In this regard, the developmental niche is considered "empty" due to lack of commitment from progenitor cells. The chimeric capability of donor wild-type PSCs can thus be harnessed to "fill" these empty niches, and as a result, the generated organ will mostly consist of donor cells. This approach is often referred to as blastocyst complementation, named so largely because donor cells are typically delivered into the host at the blastocyst stage, and was first introduced in 1993 in a study by Chen et al. (Chen, Lansford, Stewart, Young, & Alt, 1993) where the authors used wild-type mouse ESCs (mESCs) to complement Rag2-deficient recipient mouse blastocysts. Rag2 knockout mice lack the development of T and B lymphocytes and thus successfully complemented animal by wild-type mESCs will have T and B lymphocytes of exclusive donor origin. Later, blastocyst complementation has also been applied for the study of lens development. When wild-type mESCs were used for the complementation of homozygous aphakia mutant mouse strain, normal lens were generated; in contrast, Rb-deficient mESCs generated an aberrant lens phenotype (Liégeois, Horner, & DePinho, 1996). Blastocyst complementation was first used for organ generation in 2007 by Douglas Melton's group (Stanger, Tanaka, & Melton, 2007). In this study, the authors used wild-type mESCs to complement mouse blastocysts deficient in *Pdx1*, a key gene in the pancreas development. As a result, the donor mESCs populated the entire pancreatic epithelium in the *Pdx1*-deficient host (Stanger et al., 2007). In another study, Espejel et al. complemented *Fah*-deficient blastocyst with wild-type mouse iPSCs (miPSCs) to demonstrate that donor miPSCs could contribute to normal hepatocytes differentiation in *Fah*-deficient host independent of cell fusion (Espejel et al., 2010).

A landmark paper from Hiromitsu Nakauchi's group in 2010 demonstrated the feasibility of using blastocyst complementation for the xeno-generation of organs (Kobayashi et al., 2010). In their study, unlike Stanger et al., the author used rat PSCs to complement *Pdx1*-deficient mouse blastocysts. Interestingly, rat PSCs could successfully chimerize mouse development and generated viable rat-mouse interspecies chimeras. Importantly, rat PSCs could also generate an entire rat pancreatic epithelium inside the *Pdx1*-null mouse hosts. The generated rat pancreases expressed proper molecular markers and were functional as evidenced by their ability to maintain normal serum glucose levels in adult chimeras. Later, as a second successful attempt for using rat-mouse complementation system for organ generation, Isotani et al. complemented blastocysts from nude mouse that lack thymus with rat ESCs and obtained a functional rat thymus in nude mouse host (Isotani, Hatayama, Kaseda, Ikawa, & Okabe, 2011). In another attempt, Usui et al. tested blastocyst complementation for the generation of kidneys. In this study, however, the authors found that unlike mouse PSCs rat PSCs could not complement kidney agenesis defect of mouse *Sal1* knockout embryos, suggesting key molecules involved in the interaction between mesenchyme and the ureteric buds during kidney development are not conserved between the two species (Usui et al., 2012).

The success of rat-mouse organ complementation also raises the intriguing possibility to generate functional human organs in host species other than humans. Considering its resemblance to humans in anatomy, physiology, organ size, genomic similarity and cell cycle characteristics, pig constitutes a good candidate for such purpose. Pig genome has been recently sequenced (Groenen et al., 2012), and SCNT is also available in the pig (Park et al., 2001; Lai 2002), which have made the pig one of the most popular large animal models in biomedical research (Prather, Lorson, Ross, Whyte, & Walters, 2013). Recently, rapid evolving field of genome editing has also embraced pig (Carlson et al., 2012; Hai, Teng, Guo, Li, & Zhou, 2014; Hauschild et al., 2011; Wang et al., 2015; Whitworth et al., 2014; Whyte & Prather, 2012). By combining SCNT and genome editing, a number of useful pig models for human diseases have been created, such as cystic fibrosis (Rogers et al., 2008), diabetes (Renner et al., 2010; Umeyama et al., 2009), Alzheimer's disease (Kragh et al., 2009), retinitis pigmentosa (Petters et al., 1997; Ross et al., 2012) and spinal muscular atrophy (Lorson et al., 2011). Most recently, Hiromitsu Nakauchi's group has also demonstrated the feasibility of organ generation using blastocyst complementation in pig (Matsunari et al., 2013). To obtain organ-disabled hosts, the authors cloned fibroblasts expressing a transgene *Hes1* under the *Pdx1* promoter (*Pdx1-Hes1*). *Pdx1-Hes1* transgene expression suppresses pancreatic programme thus cloned embryos' pancreatic development was disabled, similar to *Pdx1* knockout, which resulted in the creation of apancreatic pig. *Pdx1-Hes1* embryos were thus used as recipients for the complementation with wild-type donor cells. In contrast to rodents, there is lack of chimeric-competent pig ESCs/iPSCs. Instead, the authors cloned fibroblasts expressing huKO fluorescent protein and used their blastomeres as donor cells to complement the *Pdx1-Hes1* embryos via aggregation chimera formation. As expected, huKO blastomeres could successfully contribute to chimera formation in *Pdx1-Hes1* host. Importantly, huKO blastomeres could generate entire pancreatic epithelium in *Pdx1-Hes1* foetuses. Moreover, chimeric pigs generated by complementation could grow into adulthood with functional pancreases.

3 | PROGRAMMABLE NUCLEASE-BASED GENOME EDITING

Genome editing with programmable nucleases including ZFNs, TALENs and CAS has spawned a new revolution in biomedical research. These nucleases can recognize and target-specific DNA sequences. Once bind to DNA, they can generate double-strand breaks (DSBs) and the repair of which depends on two main cellular DSBs repair pathways: the error-prone non-homologous end joining (NHEJ) and error-free homology-directed repair (HDR). Unlike HDR, NHEJ is high efficient and is active in all cell cycles. NHEJ mostly produces indels in the genome that will lead to loss-of-function of gene(s) and is useful for the generation of gene knockout(s). Importantly, NHEJ-based gene knockout strategy proves to be highly efficient when combined with programmable nucleases in the zygote and has been successfully used for the creation of a variety of knockout animals in many species including mouse, rat, pig, sheep, cow and non-human primates (Geurts et al., 2009; Hauschild et al., 2011; Sung et al., 2013; Wang et al., 2013; Hai et al., 2014; Niu et al., 2014; Liu et al., 2014). Thus, zygote genome editing potentially can obviate the need for pre-existing gene knockout strains and serve as a robust platform for interspecies blastocyst complementation. For detailed discussion on programmable nuclease-based genome editing technologies, the readers are referred to a couple of excellent recent reviews (Doudna & Charpentier, 2014; Gaj, Gersbach, & Barbas, 2013).

4 | PLURIPOTENT STEM CELLS

To generate human organs in pigs using blastocyst complementation, another key to success is the right human PSCs that can contribute to the early pig development for the generation of chimeric human–pig embryos. Of note is that the successful capturing and culturing authentic rat PSCs (Buehr et al., 2008; Li et al., 2008) was the key to success for interspecific blastocyst complementation in rodents. Although we have gained considerable mechanistic understanding of pluripotency, the majority of information was derived from rodent studies and we have yet to achieve the derivation of chimeric- and germline-competent PSCs from species other than rodents.

Mouse ESCs were the first pluripotent cell type captured from a developing embryo (Evans & Kaufman, 1981; Martin, 1981). Initially, mESCs were derived and cultured on mitotically inactivated mouse embryonic fibroblasts (MEFs) in the presence of serum. Better understanding of the self-renewal of mESCs led to the refinement of culture parameters and identified LIF and BMP4 as key factors to maintain mESCs identity. Further studies identified a ground state culture composed of two chemical inhibitors: CHIR99021, a GSK3 inhibitor

that activates the canonical Wnt signalling pathway and PD0325901; FGF/Erk inhibitor that enabled efficient derivation of mESCs from a variety of non-permissive strains and the capturing of authentic ESCs from rat (Buehr et al., 2008; Li et al., 2008; Ying et al., 2008). ESCs are well known for two defining properties: self-renewal and pluripotency. Self-renewal confers ESCs the ability to proliferate indefinitely in culture, thereby providing unlimited amount of cells for a variety of downstream applications. Pluripotency refers to the ability of ESCs to differentiate into all adult cell types in vivo after them being injected back to early developing embryos. ESCs can also contribute to the germline in a chimeric animal and thus have enabled the generation of thousands of transgenic animal models that have enhanced our molecular and genetic understanding of various biological processes during development and provide us with novel insights into human diseases.

The first human ESC line was derived in 1998 by Thomson and colleagues (Thomson et al., 1998). Like mESCs, human ESCs (hESCs) were derived from ICMs of pre-implantation blastocysts. Surprisingly, however, culture conditions for mESCs and hESCs are quite different. hESCs were typically cultured in bFGF/Activin-A-containing medium (FA), and FA medium leads to differentiation of mESCs. Likewise, hESCs could not be stably maintained in mESC culture conditions. Beside cell culture parameters, there are other notable differences between hESCs and mESCs: (i) hESC colonies appear more flatter than mESCs; (ii) self-renewal of hESCs is dependent on bFGF/TGF-β signalling pathways, while LIF/BMP signalling pathways are important for mESCs; (iii) in sharp contrast to mESCs, hESCs survive poorly after trypsinization, indicating a low single-cell cloning efficiency; (iv) female hESCs retain one active and one inactivated copy of X chromosome (XaXi), while both X chromosomes are active in mESCs (XaXa). mESCs can generate germline chimeras, passing a stringent pluripotency test. In this context, however, hESCs cannot be tested for their chimeric competency due to ethical considerations, thus leaving the door open whether hESCs truly represent a genuine ESC line from the human blastocysts. A recent study demonstrated that rhesus macaque ESCs grown in hESC culture were incapable of generating chimeras after being injected into rhesus blastocysts followed by embryo transfer to surrogate females, suggesting hESCs in conventional culture condition are not chimeric-competent (Tachibana et al., 2012).

These differences between hESCs and mESCs were first attributed to species-specific divergence of pluripotency programme. The derivation of another PSC type, the epiblast stem cells (EpiSCs), from post-implantation rodent embryos using hESC-like cultures in 2007 suggests it is not as simple as species differences and hESCs likely resemble post-implantation epiblasts (Brons et al., 2007; Tesar et al., 2007). EpiSCs could be derived from diverse stages of post-implantation epiblasts (Kojima et al., 2014; Wu et al., 2015). Like hESCs, EpiSCs also grow as "flattened" colonies, have low single-cell cloning efficiency and require FGF/TGF-β signalling activation for self-renewal. With modification of derivation method, EpiSCs could also be directly derived from ICMs of blastocysts, further solidify the idea that hESCs are likely in vitro counterpart of post-implantation epiblast cells (Najm et al., 2011). Isolation of EpiSCs has also contributed to the realization that there are different phases of pluripotency during development, which can be captured in culture in at least two distinct pluripotent states: "naive" and "primed," respectively (Nichols & Smith, 2009). Among other differences, the chimeric competency of naive vs. primed PSCs is particularly intriguing. Naïve cells, such as mESCs, can efficiently contribute to germline-competent chimeras following their injection into host blastocysts, while primed EpiSCs rarely do (Kim et al., 2013). On the contrary, when grafted back to stage-matched tissue, the post-implantation epiblasts, EpiSCs could efficiently generate ex vivo chimeric embryos with contribution to all three primary germ layers: mesoderm, endoderm and ectoderm, as well as the primordial germ cells (Huang, Osorno, Tsakiridis, & Wilson, 2012; Kojima et al., 2014; Wu et al., 2015). In this regard, mESCs failed integrating to post-implantation embryos upon grafting (Huang et al., 2012).

Several practical advantages, such as high single-cell cloning efficiency, higher developmental potency and ease with gene targeting associated with naïve state PSCs, have fuelled the search for culture conditions that can stabilize naïve human PSCs. First naive hESCs were stabilized with transgenes including Oct4, Klf4 and Klf2 (Hanna et al., 2010). Following this initial work, a series of recent studies, using different strategies and culture conditions, have claimed the generation of naïve hPSCs from different sources: through de novo derivation from human blastocysts, via nuclear reprogramming or conversion from existing primed hPSCs (Chan et al., 2013; Gafni et al., 2013; Guo, von Meyenn, Santos, Chen, & Reik, 2016; Takashima et al., 2014; Theunissen et al., 2014; Wang et al., 2014; Ware et al., 2014). hESCs grown in these culture showed some features reminiscent of mESCs, such as elevated single-cell cloning efficiency, higher HDR efficiency, hypomethylated genome, bivalent metabolic pathways, expression of naïve signature genes and faster growth kinetics (Wu & Belmonte, 2015a, 2015b). Moreover, cynomolgus monkey ESCs cultured using a modified naïve hESC culture could successfully generate chimeric foetuses, albeit at low efficiency, providing support for the chimeric potential of primate PSCs in naïve culture (Chen et al., 2015). While these studies are informative, the issue of whether a naïve state analogous to mouse truly exists in humans remains unsettled. Besides, there is lack of functional test for human naivety and most of the characterization was carried out at molecular levels. For now, it seems that global comparisons of cultured naïve hPSCs with the ICMs of the human blastocyst will provide the strongest support. It should be noted that there might exist multiple naïve-like states, which explains the diverse culture conditions used for stabilizing naïve hPSCs.

One of the most desirable features of naïve hPSCs is their potential to contribute to interspecies chimeras, which is required for successful blastocyst complementation to generate human organs in animal hosts. Also, interspecies chimeras with naïve hPSCs may provide a system for in vivo disease modelling and drug screening (Wu & Belmonte, 2015a, 2015b, 2016). A previous study showed that primed hESCs could not efficiently contribute to human–mouse chimeric embryos following their injection into the mouse blastocysts (James, Noggle, Swigut, & Brivanlou, 2006). To test whether naïve hPSCs are more efficient in the generation of human–mouse

chimeric embryos, Gafni et al. injected NHSM-cultured naïve hPSCs to mouse blastocysts followed by embryo transfer (Gafni et al., 2013). Interestingly, the authors observed robust chimeric contribution of naïve hESCs in mouse embryos from E8.5–E10.5 developmental stages. Yet, chimeric contribution of human naïve PSCs was not observed by Jaenisch's group. Theunissen et al. used NHSM- and 5iLA-cultured cells and did not detect any human cells in post-implantation E10.5 embryos (Theunissen et al., 2014). A follow-up study by the same group with a more sensitive assay based on the detection of human mitochondrial DNA, however, did detect chimeric contribution, although with limited efficiency, of human naïve PSCs in E10.5 mouse embryos (Theunissen et al., 2016). Several possibilities likely account for this discrepancy: (i) cell injection timing, number and embryo culture may vary among labs; (ii) embryo handling and injection techniques are likely different among researchers; (iii) imaging methods used for fluorescent signals detection in low-grade chimeras. Regardless, human cells detected in the cross-species chimeric embryos by both Gafni et al. and Theunissen et al. were not analysed with lineage-specific markers thus are unclear whether they are properly differentiated. These results suggest that generation of interspecific human–mouse chimeras with naïve hPSCs is inefficient. This inefficiency of intermixing human and mouse cells in early development is likely a result of divergent early developmental processes between primate and rodent; for example, epiblast forms an egg-cylinder shape in rodents but assumes a bilaminar embryonic disc in primates.

In addition to naïve and primed states, there may exist other pluripotent states that can be stabilized with either transgenes or different culture conditions (Wu & Belmonte, 2014; Wu et al., 2015). We have recently uncovered a novel primed pluripotent state that confers interspecific chimeric competency between human and mouse after grafting human cells to gastrulating mouse epiblast (Wu et al., 2015). Human cells were found efficiently incorporated into the posterior part of the developing mouse embryo and contributed to three primary germ lineages. This raises an interesting possibility of "epiblast complementation" for the generation of early human progenitors ex vivo.

5 | CONCLUSIONS

The combination of zygote genome editing and chimeric-competent hPSCs offers an attractive platform for realizing hPSCs' full potential towards generating transplantable organs. If successful, this approach will lead to a paradigm shift in regenerative medicine and will help to overcome the shortage of organ donors. However, the inefficiency of existing naïve hPSCs to contribute to chimeric formation in mouse is casting doubts whether this will be a viable approach. It remains to be seen whether the inefficiency of naïve hPSCs in chimeric contribution observed in mouse is also true with a large animal species such as the pig. Future studies on improving chimeric efficiency of hPSCs in an animal host are warranted to turn the dream of xeno-human organ generation into reality.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

J.W. drafted the review and all the other authors helped editing and writing the review.

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REVIEW ARTICLE

WILEY Reproduction in Domestic Animals

Placental metabolism: substrate requirements and the response to stress

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Contents

The placenta is a dynamic, metabolically active organ with significant nutrient and energy requirements for growth, nutrient transfer and protein synthesis. It uses a range of substrates to meet its energy needs and has a higher rate of oxygen (O_2) consumption than many other foetal and adult tissues. Placental metabolism varies with species and alters in response to a range of nutritional and endocrine signals of adverse environmental conditions. The placenta integrates these signals and adapts its metabolic phenotype to help maintain pregnancy and to optimize offspring fitness by diversifying the sources of carbon and nitrogen available for energy production, hormone synthesis and foeto-placental growth. The metabolic response of the placenta to adversity depends on the nature, severity and duration of the stressful challenge and on whether the insult is maternal, placental or foetal in origin. This review examines placental metabolism and its response to stresses common in pregnancy with particular emphasis on farm species like the sheep. It also considers the consequences of changes in placental metabolism for the supply of O_2 and nutrients to the foetus.

1 | INTRODUCTION

As the interface between the mother and foetus, the placenta has multiple functions important to the successful outcome of pregnancy. It transports O_2, nutrients, ions and key micronutrients from mother to foetus as well as wastes, such as carbon dioxide (CO_2) and urea, in the opposite direction (Sibley, Glazier, & D'Souza, 1997). The placenta also converts nutrients that it receives to other forms to provide alternative substrates for foeto-placental metabolism and growth (Fig. 1). In addition, the placenta produces hormones and growth factors that are released into the maternal and foetal circulations (Burton & Fowden, 2012). These have key roles in the maintenance of uterine quiescence and the maternal physiological adaptations to pregnancy that are essential for meeting the increasing nutrient demands of the growing foetus. Finally, the placenta acts as a barrier restricting access of maternal hormones and xenobiotics to the foetus by enzymatic inactivation or transporting them back into the maternal circulation. Consequently, the placenta is a metabolically active organ with significant nutrient and energy requirements.

The placenta uses a range of substrates to meet its energy needs and has a higher rate of oxygen (O_2) consumption than either the adult or the foetus (Hay, 1991). Placental metabolism varies with species and alters in response to the foetal nutrient demands for growth with increasing gestational age (Fowden, 1997; Pere, 2003). It is also responsive to homoeostatic challenges that evoke stress responses in both the mother and foetus (Sferruzzi-Perri & Camm, 2016; Vaughan, Sferruzzi-Perri, Coan, & Fowden, 2012). The mother signals adverse changes in the general environment, such as scarcity or excess of nutrients, low oxygen availability or extremes of temperature, as well as physiological changes in the individual, like the availability of fuel reserves and the degree of glycaemic control and physical activity (Fowden, Ward, Wooding, & Forhead, 2010; Fowden, Forhead, Sferruzzi-Perri, Burton, & Vaughan, 2015; Gaccioli, Lager, Powell, & Jansson, 2013;: Sferruzzi-Perri & Camm, 2016). The foetus signals mismatches between its supply and demand for nutrients in relation to its mass, genotype and degree of maturation (Burton & Fowden, 2012; Vaughan et al., 2012). In many circumstances, the signalling is via stress and other metabolic hormones, like the glucocorticoids, catecholamines, leptin and insulin (Fowden et al., 2015).

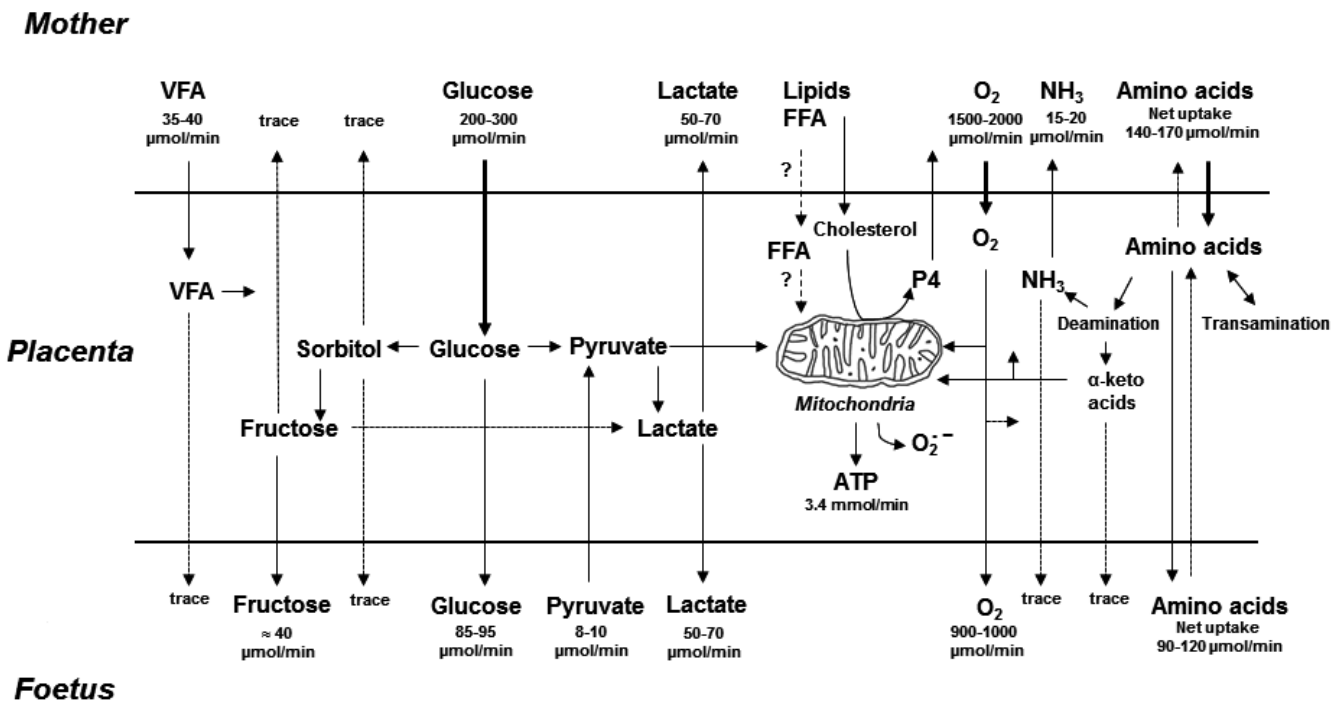

FIGURE 1 Schematic diagram showing the rates of transport of oxygen and nutrients from the uterine circulation and into the umbilical circulation in pregnant sheep with a foetus of an average weight of 3 kg with a 300-g placenta (complete placentomes) at approximately 80–90% of gestation. Solid lines = Major routes of metabolism. Dashed lines = Minor routes of metabolism Trace = ≤5 µmol/min. VFA = Volatile fatty acids. FFA = Free fatty acids. NH_3 = Ammonia. Data from Sparks et al., 1982; Meznarich et al., 1987; Smeaton et al., 1989; Hay et al., 1990; Carver & Hay, 1995; Chung et al., 1988; Teng, Tjoa, Fennessey, Wilkening, & Battaglia, 2002; Regnault et al., 2007, 2010; Fowden & Forhead, 2011; Vaughan et al., 2016

The stresses experienced during pregnancy may be chronic or acute depending on their origin. Chronic stress induced early in pregnancy often reduces foeto-placental growth, whereas more acute stresses tend to alter availability of specific nutrients and hormones transiently without major effects on intrauterine growth (Fowden, Ward, Wooding, Forhead, & Constancia, 2006). In farm animals and other species, there are also changes in placental morphology and nutrient transport capacity in response to stressful conditions including undernutrition, hypoxia and manipulations of dietary composition, maternal adiposity, glucose availability and glucocorticoid concentrations (Fowden et al., 2010, 2015; Gaccioli et al., 2013). However, much less is known about the effects of these stresses on placental metabolism *per se*. This review, therefore, examines placental metabolism and its response to stresses common during pregnancy with particular emphasis on farm animals like the sheep. It also discusses the consequences of changes in placental metabolism for the supply of O_2 and nutrients to the foetus. It does not consider the effects of stressful conditions on placental growth and development as these topics have been reviewed recently (Fowden et al., 2015; Gaccioli et al., 2013; Sferruzzi-Perri & Camm, 2016).

2 | OXYGEN

2.1 | Normal requirements

Like most tissues, aerobic respiration is the main source of placental ATP during normal conditions. In the farm animals studied to date, the respiratory rate of the combined uteroplacental tissues is higher per kg total tissue than that seen per kg of foetus (Fowden, Forhead, Silver, & Macdonald, 1997). As most of this O_2 consumption is placental rather than myometrial (Hay, 1991), rates of O_2 consumption per kg placenta are at least three- to fourfold higher than those per kg of foetus as a whole (Table 1) and similar to that of the foetal brain (Hay, 2006). Oxygen consumption rates calculated per kg placenta are of the same order of magnitude in different species with an epitheliochorial placenta and similar to those of the haemochorial human placenta (Table 1; Hay, 2006). Of the O_2 consumed by the ovine placenta, approximately 70–75% is used to generate ATP by mitochondrial oxidative phosphorylation using a variety of substrates including carbohydrates, amino acids, probably certain volatile fatty acids (VFA) and possibly also some free fatty acids (Fig. 1). The majority of this ATP is used for protein synthesis and active transport processes (Carter, 2000). Oxygen is also used in placental mitochondria without generating ATP through proton leak and superoxide production along the electron transport system and for synthesis of progesterone or other steroids (Fig. 1). These processes account for another 15–20% of the O_2 consumed by the ovine placenta, while the remaining 10–15% is non-mitochondrial due to cellular oxidative reactions unrelated to energy production (Carter, 2000).

Oxygen consumption by the combined uteroplacental tissues increases by 25–50% between mid-gestation and late gestation in sheep and horses but not in pigs when expressed per kg wet weight (Bell, Kennaugh, Battaglia, Makowski, & Meschia, 1986; Fowden,

TABLE 1 Average weight-specific rates of consumption (or production[a]) of oxygen, glucose and lactate by the placenta (calculated using uteroplacental values expressed per unit of total weight of whole placentomes) and foetus (using body weight) of different species during late gestation (≥80% gestation)

	Placental consumption or production μmol/min/kg placenta			Foetal umbilical uptake μmol/min/kg foetus		
	Oxygen	Glucose	Lactate[a]	Oxygen	Glucose	Lactate
Sheep	1700	350	250	310	30	30
Cow	1200	270	160	300	30	30
Pig	1070	200	250	340	40	40
Horse	1900	400	50	290	40	10

Data derived from Comline & Silver, 1976; Reynolds et al., 1985; Ferrell, 1991; Fowden & Silver, 1995; DiGiacomo & Hay, 1990; Fowden et al., 1997; Fowden, Forhead et al., 2000; Fowden, Taylor et al. 2000 Aldoretta & Hay, 1999.

Forhead, White, & Taylor, 2000; Fowden, Taylor, White, & Forhead, 2000; Molina, Meschia, Battaglia, & Hay, 1991; Reynolds, Ford, & Ferrell, 1985). In contrast, when values are calculated per gram dry weight of placenta alone, there is little change in placental O_2 consumption during the second-half of gestation in sheep (Vatnick & Bell, 1992). However, growth rates of the placenta and foetus differ over this period of gestation and also show wide species variation (Wooding & Burton, 2008). Consequently, the amount of O_2 consumed by the uteroplacental tissues as a proportion of the total uterine O_2 uptake alters with gestational age depending on the species. For instance, in sheep, the percentage of uterine O_2 uptake used by the uteroplacental tissues decreases from 80% to 40–45% between mid-gestation and late gestation, whereas, in horses and pigs, this percentage remains at 55% and 65%, respectively, throughout the second-half of gestation (Bell et al., 1986; Fowden, Forhead et al., 2000; Fowden, Taylor et al. 2000; Molina et al., 1991; Reynolds et al., 1985).

2.2 | Response to stress

Absolute rates of uteroplacental O_2 consumption vary with placental weight and are often reduced in response to chronic stresses such as hyperthermia and hypoglycaemia that compromise placental growth from early in development (Carter, 2000; Carver & Hay, 1995; Thureen, Trembler, Meschia, Makowski, & Wilkening, 1992). However, when all the data available for pregnant sheep in late gestation are summarized, rates of O_2 consumption calculated per kg of placenta are relatively unaffected by the range of acute and chronic stresses studied to date (Fig. 2). It is only when placental growth is severely compromised by carunclectomy before pregnancy that O_2 consumption per kg placenta is reduced (Fig. 2). Even when uterine O_2 delivery is reduced by 50% by maternal anaemia, uteroplacental O_2 consumption is maintained by increasing O_2 extraction (Delpapa, Edelstone, Milley, & Balsan, 1992). Normal rates of uteroplacental consumption and umbilical uptake of O_2 are also sustained in a similar manner when uterine O_2 delivery is reduced for 24 h by restricting uterine blood flow (Carter, 2000; Hooper, Walker, & Harding, 1995). In contrast, when O_2 availability is reduced chronically by pregnancy at high altitude, O_2 consumption per unit weight of human placenta appears to decline relative to sea-level values (Illsley Caniggia, & Zamudio, 2010). Collectively, these findings suggest that the rate of placental respiration varies little with

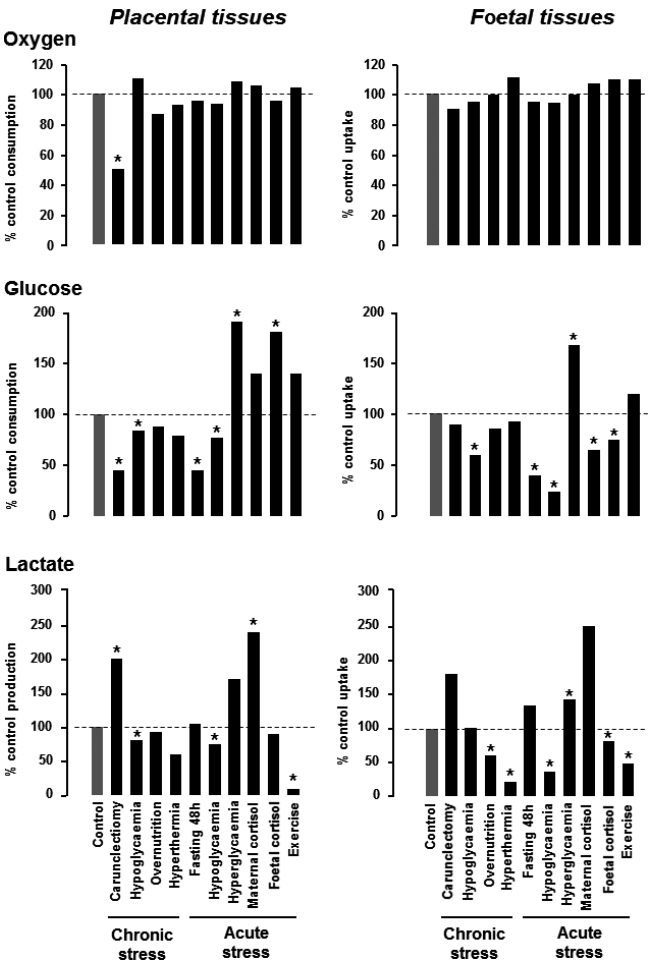

FIGURE 2 Rates of placental consumption or production (calculated from uteroplacental measurements and expressed per kg total whole placentomes) and of foetal umbilical uptake (per kg foetus) of oxygen, glucose and lactate in sheep in late gestation in response to a range of acute and chronic stresses presented as a percentage of the normal control values. * significantly different from control animals as identified in the individual studies. Data derived from Chandler et al., 1985; Owens, Falconer, & Robinson, 1987a; Owens et al., 1987b; DiGiacomo & Hay, 1990; Carver & Hay, 1995; Thureen et al., 1992; Aldoretta et al., 1994; Aldoretta & Hay, 1999; Carver et al., 1997; Wallace, Bourke, Aitken, Leitch, & Hay, 2001; Ward et al., 2004; Regnault et al., 2007; Limesand, Rozance, Brown, & Hay, 2009; Fowden & Forhead, 2011; Vaughan et al., 2016

nutritional stresses but may adapt when the O_2 supply is restricted chronically by hypoxia or low uterine blood flow.

To date, little is known about placental energetics or mitochondrial function during stressful conditions in farm animals. In the human and rodent placenta, both nutritional and hypoxic stresses alter mitochondrial function. More specifically, there are changes in mitochondrial biogenesis, morphology, apoptosis and abundance of electron transport complexes and uncoupling proteins during common pregnancy stresses including maternal diabetes, obesity, pre-eclampsia, calorie restriction, protein deprivation and high-altitude hypoxia (Belkacemi, Desai, Nelson Michael, & Ross Michael, 2011; Chiaratti et al., 2015; Colleoni et al., 2013; Hastie & Lappas, 2014; Hercules, Esquisatto, Moraes, Amaral, & Catisti, 2013; Mando et al., 2014; Mayeur et al., 2013). Increased abundance of uncoupling protein-2 has also been observed in the ovine placenta at mid-gestation and late gestation of ewes undernourished during early pregnancy (Gnanalingham et al., 2007). These mitochondrial changes are likely to affect the efficiency of ATP production and superoxide generation with wider implications for placental function (Fig. 1). Certainly in humans and mice, nutritional and hypoxic stimuli alter placental ATP content (Chiaratti et al., 2015; Tissot van Patot et al., 2010). Consequently, even though placental O_2 consumption is maintained during many stressful conditions (Fig. 2), there may be changes in placental energetics and consumption of oxidative substrates that affect foetal delivery of nutrients and O_2. Indeed, rates of umbilical O_2 uptake per kg sheep foetus vary little in response to acute and chronic stresses, which indicates that the foetus grows primarily in relation to its overall O_2 availability (Fig. 2).

3 | CARBOHYDRATES

3.1 | Normal requirements

In all farm animals studied to date, the main carbohydrate used by the uteroplacental tissues is glucose (Fig. 1). Its primary source in normal conditions is the mother. Glucose crosses the placenta by facilitated diffusion down a materno-foetal glucose concentration gradient using glucose transporters (GLUTs). However, when this gradient is abolished experimentally in sheep, uteroplacental glucose consumption remains at 80% of normal values by deriving glucose from the foetal circulation (Simmons, Battaglia, & Meschia, 1979). Two GLUT isoforms, GLUT1 and GLUT3, have been detected in ruminant and equine placenta and are used sequentially in transplacental glucose transfer (Wooding & Burton, 2008). GLUT 8 has also been identified in the ovine placenta and may be involved in transporting glucose across the foetal facing membranes (Limesand, Regnault, & Hay, 2005).

In late gestation, foetal and placental rates of glucose consumption calculated by kg tissue vary between species but are consistently five- to 10-fold higher in the placenta than foetus (Table 1). Consistent with the lower rates of foetal glucose uptake in sheep and cows in late gestation (Table 1), the cotyledonary epitheliochorial placenta of these ruminants appears to use a greater proportion of the glucose taken up from the uterine circulation (55–85%) than the diffuse epitheliochorial placenta of horses and pigs (25–50%, Fowden, 1997). Glucose consumption per kg of combined uteroplacental tissues increases between mid-gestation and late gestation in sheep but decrease over the last-third of gestation in the horse, although, in both species, the percentage of total uterine glucose uptake used by the uteroplacental tissues is less near term than earlier in gestation (Bell et al., 1986; Fowden, Forhead et al., 2000; Fowden, Taylor et al. 2000; Molina et al., 1991).

In sheep, the glucose consumed by the uteroplacental tissues is known to be used for oxidative phosphorylation and synthesis of polyols, other sugars and carbohydrates (Fig. 1). Measurements made with tracer glucose indicate that, of the glucose used by the uteroplacental tissues, 15–20% is oxidized to CO_2, approximately 30% is converted to lactate via glycolysis and 5–10% is metabolized to fructose via sorbitol (Aldoretta & Hay, 1999). The remaining 40–50% of the glucose carbon is unaccounted for but may contribute to the short-term turnover of amino acids, glycerol and keto acids and/or to the synthesis of substances with longer turnover times such as glycosaminoglycans, proteins and lipids (Aldoretta & Hay, 1999; Kim, Song, Wu, & Bazer, 2012). Some of the lactate and fructose produced by the ovine placenta may also be used oxidatively for ATP generation, which, together with glucose, could account for up to 50% of the normal rate of uteroplacental O_2 consumption (Meznarich, Hay, Sparks, Meschia, & Battagla, 1987; Sparks, Hay, Bonds, Meschia, & Battaglia, 1982). However, the majority of the lactate and fructose produced by the ovine placenta in late gestation appears to be transported into either the umbilical and/or uterine circulations (Fig. 1). GLUT8 may be responsible for fructose transport but little is known about placental expression of the monocarboxylate transporters (MCTs) that transport lactate in any farm animal (Limesand et al., 2005). Two MCT isoforms, MCT1 and MCT4, have been identified in human and mouse placenta with species-specific polarized expression on maternal and foetal facing membranes indicative of different transport kinetics at the two surfaces (Nagai, Takebe, Nio-Kobayashi, Takahashi-Iwanaga, & Iwanaga, 2010; Settle et al., 2004).

Fructose is also detected in high concentrations in foetal pigs, cows and horses but whether the placenta produces fructose and releases it into the foetal circulation in late gestation in these species remains unclear (Silver, 1984). Porcine trophectoderm cells can use fructose *in vitro* to synthesize glycosaminoglycans such as hyaluronic acid and the ovine placenta uses small amounts of fructose oxidatively and to produce lactate *in vivo*, although little is known about these metabolic processes in other species (Kim et al., 2012; Meznarich et al., 1987). In contrast, lactate production by the uteroplacental tissues has also been observed in pigs, horses and cows (Table 1). In the ovine and bovine placenta, net production of lactate appears to be derived solely from glucose and varies directly with the rate of uteroplacental glucose consumption in normal conditions (Aldoretta, Carver, & Hay, 1994; Aldoretta & Hay, 1999; Comline & Silver, 1976). Uteroplacental lactate production per unit weight of total tissue increases between mid-gestation and late gestation in sheep and horses in association with changes in its relative distribution between the uterine and umbilical circulations (Bell et al., 1986; Fowden, Forhead et al., 2000; Fowden, Taylor et al. 2000; Sparks

et al., 1982). In sheep at mid-gestation, uteroplacental lactate production is low and distributed almost entirely into the uterine circulation, whereas, by late gestation, production is three- to fourfold higher per unit weight and distributed equally into the foetal and maternal circulations (Bell et al., 1986; Fig. 1). In horses, uteroplacental lactate production is undetectable at mid-gestation while near term, it occurs at a significant rate and is distributed solely to the foetus (Fowden, Forhead et al., 2000; Fowden, Taylor et al. 2000). Similarly, in cows near term, the majority of lactate produced by the uteroplacental tissues is released into the umbilical circulation, although absolute rates of production vary with breed (Comline & Silver, 1976; Ferrell, 1991). Like cows, uteroplacental lactate production in pigs appears to be delivered primarily to the foetus near term and makes a greater contribution to the daily foetal carbon requirement in pigs than other farm animals (Fowden Forhead, Silver & Macdonald 1997). The mechanisms involved in these ontogenic changes in uteroplacental production and distribution of lactate remain unknown but may involve alterations in cell types or cellular O_2 availability within the placental tissues and, possibly, a switch from oxidative to more glycolytic metabolism of glucose towards term. However, as lactate and fructose can both be utilized by foeto-placental tissues (Meznarich et al., 1987; Sparks et al., 1982), their placental production provides alternative sources of carbon for foetal metabolism and growth, which may be beneficial in stressful conditions.

3.2 | Response to stress

During nutritional stresses, uteroplacental consumption and umbilical uptake of glucose alter largely in line with the changes in maternal glycaemia and the transplacental glucose concentration gradient (Hay, 2006). In sheep, stresses which produce maternal hypoglycaemia, therefore, tend to reduce glucose consumption calculated per kg placenta while, conversely, maternal hyperglycaemia increases these rates (Fig. 2). During maternal hypoglycaemia lasting two to seven days, percentage distribution of uterine glucose uptake between ovine uteroplacental and foetal tissues does not alter and both share equally in the reduced glucose availability (Fowden & Forhead, 2011; Hay, Molina, DiGiacomo, & Meschia, 1990; Hay, Sparks, Wilkening, Battaglia, & Meschia, 1983). However, as maternal hypoglycaemia becomes prolonged, the uteroplacental tissues appear to use proportionally more of the uterine glucose uptake than in normoglycaemic conditions (Carver & Hay, 1995; Hay et al., 1983). The relationship between uteroplacental glucose consumption and maternal glucose levels is, therefore, more complex during stressful than normal conditions. Particularly in late gestation, foetal sheep can activate glucogenesis when hypoglycaemic or hypercortisolaemic, which raises their glucose levels independently of the maternal concentrations (DiGiacomo & Hay, 1990; Houin et al., 2015; Ward, Wooding, & Fowden, 2004). This has consequences for the transplacental glucose concentration gradient and carbon fluxes from the placenta to foetus and vice versa (DiGiacomo & Hay, 1990). Indeed, net uteroplacental glucose consumption varies directly with the foetal glucose concentration when foetal glucose levels are manipulated experimentally independently of the mother (Hay et al., 1990; Thureen et al., 1992; Ward et al., 2004).

Chronic stresses that reduce placental growth such as hyperthermia and hypoglycaemia alter the glucose transport capacity of the ovine placenta at any given transplacental gradient, which suggests that other morphological and/or functional factors are influencing placental fluxes and consumption of glucose in these circumstances (Fowden et al., 2010). Certainly, placental GLUT expression is affected by longer term variations in maternal glycaemia with decreases in GLUT1 abundance in hypoglycaemia and hyperthermic conditions, and in both GLUT1 and GLUT 3 abundance in response to maternal hyperglycaemia in ewes (Das, He, Ehrhardt, Hay, & Devaskar, 2000; Das, Sadiq, Soares, Hay, & Devaskar, 1998; Ma et al., 2011; Zhu, Ma, Long, Du, & Ford, 2010). Similar changes in the glucose transport capacity associated with altered GLUT expression are observed in the small placenta of carunclectomized ewes and in the mouse placenta after maternal undernutrition and other dietary manipulations (Owens, Falconer, & Robinson, 1987b; Vaughan et al., 2012).

During uterine artery constriction, ovine uteroplacental tissues use less glucose, which sustains umbilical glucose uptake in the face of the reduced uterine glucose delivery (Hooper et al., 1995). As placental O_2 consumption is maintained in these and other stressful conditions in which placental glucose consumption is reduced (Fig. 2), the ovine placenta must switch from glucose to other oxidative substrates to maintain its respiratory rate (Fig. 1). In contrast, when O_2 availability is reduced at high altitude, the human placenta uses 60% more glucose and 20% less O_2 than at sea level (Illsley & Caniggia, 2010). The hypoxic human placenta, therefore, appears to switch from oxidative phosphorylation of glucose to a greater dependence on glycolysis to meet its ATP requirements, thereby sparing O_2 but reducing glucose availability for foetal delivery.

In sheep, placental production and umbilical uptake of lactate appear to parallel placental glucose consumption during most stressful conditions (Fig. 2). However, in the small placenta of carunclectomized ewes, uteroplacental lactate production exceeds the rate of uteroplacental glucose consumption, so there must be other carbon sources for lactate synthesis and/or oxidative phosphorylation in these animals (Owens et al., 1987b). Similarly, when either maternal or foetal cortisol levels are raised, placental lactate production appears to vary independently of placental glucose consumption (Fig. 2). With cortisol overexposure from the foetus, uteroplacental lactate production is unaffected despite increased uteroplacental glucose consumption, whereas, when cortisol is infused maternally, uteroplacental lactate production increases without a significant rise in uteroplacental glucose consumption (Vaughan, Davies, Ward, De Blasio, & Fowden, 2016; Ward et al., 2004). Thus, lactate production by ovine uteroplacental tissues is regulated dynamically and is responsive to foetal and maternal environmental cues. Indeed, the ovine placenta can switch rapidly from net production to net consumption of lactate during exercise and from releasing lactate into the foetus to clearing it from the foetal circulation within 4 hr of uterine artery restriction (Chandler, Leury, Bird, & Bell, 1985; Hooper et al., 1995).

4 | AMINO ACIDS

4.1 | Normal requirements

The placenta transports, utilizes, produces and interconverts amino acids. The ovine placenta has a high rate of protein synthesis and, given the changes that occur in placental morphology over the second-half of gestation, its rate of protein turnover is also likely to be high (Bell & Ehrhardt, 2002; Vatnick & Bell, 1992; Wooding & Burton, 2008). In sheep, all nine essential amino acids that cannot be synthesized *de novo* and most of the other amino acids needed for protein synthesis are taken up from the uterine circulation against their concentration gradients using energy-dependent active transport (Bell & Ehrhardt, 2002). In late gestation, foetal concentrations of most amino acids are also higher than those of the mother in cows and pigs although not consistently in the horse (Ashworth, Nwagwu, & McArdle, 2013; Silver, Fowden, Taylor, Knox, & Hill, 1994; Zicker, Vivrette, & Rogers, 1994). There are also breed differences in foetal and maternal amino acid profiles and in foetal to maternal concentration ratios for specific amino acids in sheep, pigs and horses, which may relate, in some instances, to differences in nutrition (Ashworth, Dwyer, McIlvaney, Wekman, & Rooke, 2011; Ashworth et al., 2013; Jobgen et al., 2008; Kwon et al., 2004; Silver et al., 1994; Wu, Pond, Ott, & Bazer, 1998; Zicker et al., 1994). In addition, foetal to maternal concentration amino acid ratios may change with increasing gestational age in pigs and horses (Ashworth et al., 2013; Silver et al., 1994; Wu et al., 1998; Zicker et al., 1994).

Although net amino acid transport is from mother to foetus for most amino acids, significant bidirectional fluxes have been observed across ovine placental membranes using labelled amino acid tracers (Battaglia, 2002). For three amino acids (glutamate, aspartate and serine), there is no net uteroplacental uptake from the ovine uterine circulation (Regnault, de Vrijer, & Battaglia, 2002). Instead, the uteroplacental tissues derive these amino acids from the foetal circulation. Multiple amino acid transporter systems have been identified in the ovine placenta, which differ in their amino acid specificity, sodium dependence and localization within the placental barrier (Regnault et al., 2002; Wooding & Burton, 2008). Amino acid specificity of the transporter systems overlaps for some amino acids, so there is competition between these amino acids for uteroplacental uptake and transplacental transport, which depends on their concentrations in the maternal circulation (Regnault et al., 2002).

The ovine placenta is a net consumer of glutamate, serine and three branched-chain amino acids (BCAA), valine, leucine and isoleucine, and also releases glutamine, methionine and glycine into the foetus in excess of the uterine uptakes (Chung, Teng, Timmerman, Meschia, & Battaglia, 1998). Thus, significant catabolism and/or transamination of amino acids occurs within the ovine placenta, which leads to the production of ammonia and α-keto acids (Fig. 1). The ammonia is released primarily into the uterine circulation but can also be used to synthesis other amino acids such as glutamate (Liechty, Kelley, & Lemons, 1991). The α-keto derivatives may be oxidized to produce ATP, released into the foetal circulation or metabolized into amino acids and other substances, such as fatty acids, proteins and peptides that are, in turn, metabolized or secreted by the placenta (Fig. 1). Placental mitochondria have been shown to use several amino acids for oxidative phosphorylation *in vitro* and glutamate is oxidized at high rates by the ovine placenta *in vivo* in late gestation (Moores et al., 1994; : Battaglia, 2002). Given its large placental uptake and synthesis *in utero* from BCAA (Battaglia & Regnault, 2001), glutamate is likely to be quantitatively the most important fuel amongst the amino acids. If complete, its oxidation would account for 10% of the uteroplacental O_2 consumption and provide NADPH for placental steroidogenesis, lipogenesis and nucleoside production.

In addition to oxidation, 6% of the glutamate taken up by the ovine placenta is converted to glutamine, which is then released into the foetal circulation in amounts exceeding its uterine uptake (Moores et al., 1994). Glutamine is also synthesized from BCAA and glutamate by the porcine and equine placenta (Manso Filho, Costa, Wu, McKeever, & Watford, 2009; Self et al., 2004). It is used for foeto-placental synthesis of protein and glycosaminoglycans and is re-converted back to glutamate by foetal ovine liver (Battaglia, 2000; Kim et al., 2012). There is also significant metabolic interconversion of alanine, pyruvate and lactate in the ovine placenta without net uteroplacental alanine consumption (Timmerman et al., 1998). Alanine derived from the maternal circulation is, therefore, exchanged for endogenously produced alanine with the result that net umbilical uptake of alanine is derived from placental transamination and protein turnover with only a small fraction coming from direct transplacental flux (Timmerman et al., 1998). Similarly, serine taken up from both circulations is metabolized to glycine in the ovine placenta, which results in significant umbilical glycine uptake without net uterine uptake (Geddie et al., 1996; Regnault et al., 2002). In addition, the methylenetetrahydrofolate produced by conversion of serine to glycine can be used in purine synthesis or for remethylation of homocysteine to methionine. If homocysteine is taken up from the uterine circulation, this metabolic pathway may also account for the umbilical uptake of methionine in the sheep foetus. Placental amino acid metabolism is, therefore, complex and involves metabolic cycling between the maternal, placental and foetal compartments with important consequences for the amounts and composition of the amino acids delivered to the foetus.

4.2 | Response to stress

In farm animals, foetal and maternal amino acid concentrations are affected by a wide range of stressful conditions including heat stress, undernutrition, hypoglycaemia, protein deprivation and glucocorticoid administration (Ashworth et al., 2011, 2013; Kwon et al., 2004; Regnault et al., 2013; Schaefer, Krishnamurti, Heindze, & Gopinath, 1984; Silver et al., 1994; Timmerman et al., 2000; Wu et al., 1998). For instance, maternal undernutrition influences maternal and foetal amino acid profiles, reduces specific amino acid concentrations and alters the foetal to maternal concentration ratios for specific amino acids in sheep, pigs and horses, which suggests that placental amino acid transport or competition amongst the amino acids for the transporters and/or foeto-placental amino acid metabolism are altered in these circumstances (Ashworth et al., 2011; Kwon et al., 2004;

Schaefer et al., 1984; Silver et al., 1994). In sheep, these changes persist after restoration of normal nutrition which indicates that foeto-placental amino acid metabolism may be permanently altered by nutritional stress earlier in gestation (Kwon et al., 2004). Certainly, undernutrition of pregnant ewes for seven days leads to increased placental BCAA utilization and ammonia production, indicative of increased placental amino acid deamination (Liechty et al., 1991). There are also reductions in the umbilical uptake, transplacental flux and foeto-placental back flux of leucine and threonine after heat stress, even when the lower placental weight is taken into account (Anderson, Fennessey, Meschia, Wilkening, & Battaglia, 1997; Ross, Fennessey, Wilkening, Battaglia, & Meschia, 1996). Similarly, umbilical leucine uptake per kg foetus is less during prolonged maternal hypoglycaemia and coupled with a trend for greater percentage utilization of the uterine uptake by the uteroplacental tissues (Carver et al., 1997). In addition, both maternal undernutrition and foetal dexamethasone administration reduce foetal glutamate concentrations and placental glutamate uptake from the foetal circulation, which indicates that ovine placental–foetal amino acid cycling is also responsive to environmental conditions during late gestation (Houin et al., 2015; Liechty et al., 1991; Schaefer et al., 1984; Timmerman et al., 2000). Reduced placental uptake and metabolism of glutamate may also lower NADPH availability consistent with the decrease in progesterone synthesis seen when foetal glucocorticoids rise in late gestation (Silver, 1984; Timmerman et al., 2000). Similar changes in amino acid cycling between the foetal and placental compartments are also seen in response to manipulation of other foetal hormone concentrations (Teng, Battaglia, Meschia, Narkewicz, & Wilkening, 2001).

When availability of single amino acids is increased experimentally in pregnant ewes, their umbilical uptake and placental utilization is increased significantly, probably at the expense of other amino acids using the same transporters (Battaglia, 2002; Thureen, Baron, Fennessey, & Hay, 2002; Timmerman et al., 1998). Similarly, maternal BCAA infusion increases their umbilical uptake and uteroplacental utilization by deamination as indicated by the increased uteroplacental production of ammonia (Jozwik, Teng, Battaglia, & Meschia, 1999; Jozwik et al., 2001). However, when mixtures of amino acids are infused, umbilical uptake may increase, decrease or be unaffected depending on the specific amino acid due to competitive inhibition by the other amino acids for the different transporter systems in the ovine placenta (Battaglia, 2002). Collectively, these findings suggest that placental amino acid metabolism and transport adapt to environmental stresses in farm animals with implications for foetal growth as seen in humans and rodents (Day et al., 2015; Gaccioli et al., 2013; Lewis et al., 2013; Vaughan et al., 2012).

5 | LIPID, FATTY ACID AND VOLATILE FATTY ACIDS

5.1 | Normal requirements

Although lipids and free fatty acids (FFA) are required for growth and development of foeto-placental tissues, the epitheliochorial placenta of ruminants, pigs and horses appears to be relatively impermeable to these substances compared to the human and rodent haemochorial placenta (Herrera & Ortega-Senovilla, 2014). Both the uterine arteriovenous and the umbilical venous-arterial concentration differences in fatty acids are negligible in sheep, cows and horses during late gestation (Elphick, Hull, & Broughton Pipkin, 1979; James, Meschia, & Battaglia, 1971; Stammers, Silver, & Fowden, 1988). There is also little evidence for transfer of labelled short- or long-chain fatty acids across the ovine placenta, despite the presence of fatty acid transporters in the placentomes at mid-gestation and late gestation (Elphick et al., 1979; Leat & Harrison, 1980; Ma et al., 2011; Zhu et al., 2010). However, in sheep and horses, the placenta does appear to hydrolyse esterified lipids and to desaturate and elongate fatty acids, including the essential C18 fatty acids, which, together with placental synthesis of lipids from glucose and keto acids, may provide an adequate supply of essential lipids and fatty acids to the foeto-placental tissues (Bell & Ehrhardt, 2002; Stammers et al., 1988).

In sheep and cows, rumen fermentation leads to significant amounts of acetate and other volatile fatty acids (VFA), such as β-hydroxybutyrate and acetoacetate, in the maternal circulation. Although these substances are taken up by the uterus in relatively small amounts compared to other nutrients, they are utilized by the uteroplacental tissues and transported to the foetus (Fig. 1). In both sheep and cows, rates of VFA consumption are higher per kg of placental than foetal tissues (Carver & Hay, 1995; Comline & Silver, 1976). In sheep, the β-hydroxybutyrate taken up from the uterine circulation is utilized almost entirely within the uteroplacental tissues with no significant onward transfer to foetus, whereas uterine acetoacetate uptake is distributed equally between the uteroplacental and foetal tissues, although the uterus takes up significantly less acetoacetate than β-hydroxybutyrate (Carver & Hay, 1995; Smeaton, Owens, Kind, & Robinson, 1989). In cows near term, there is significant uteroplacental consumption of acetate at rates eight- to 10-fold higher than those of the foetus when expressed per unit weight (Comline & Silver, 1976). The fate of the VFA used *in utero* remains unknown but may involve oxidative phosphorylation to generate ATP and/or synthesis into steroids and fatty acids (Christie & Noble, 1982; Dhand, Mk, Shepherd, Smith, & Varnam, 1970; Miodovnik et al., 1982).

5.2 | Response to stress

Compared to carbohydrates and amino acids, much less is known about the effects of adverse conditions on the placental metabolism and transport of lipids, FFA and VFA in farm animals. In sheep and horses, there are changes in the lipid and FFA profiles of foetal and maternal plasma in response to maternal undernutrition, which may be related, in part, to altered placental lipid metabolism (Stammers, Hull, Silver, & Fowden, 1995; Stammers et al., 1988). Certainly, in both these species, maternal hypoglycaemia induced by short-term fasting or insulin infusion is associated with increased uteroplacental synthesis and release of prostaglandins, which are hormones derived from arachidonic acid through phospholipid metabolism (Fowden & Silver, 1983; Silver & Fowden, 1982). This has led to the suggestion

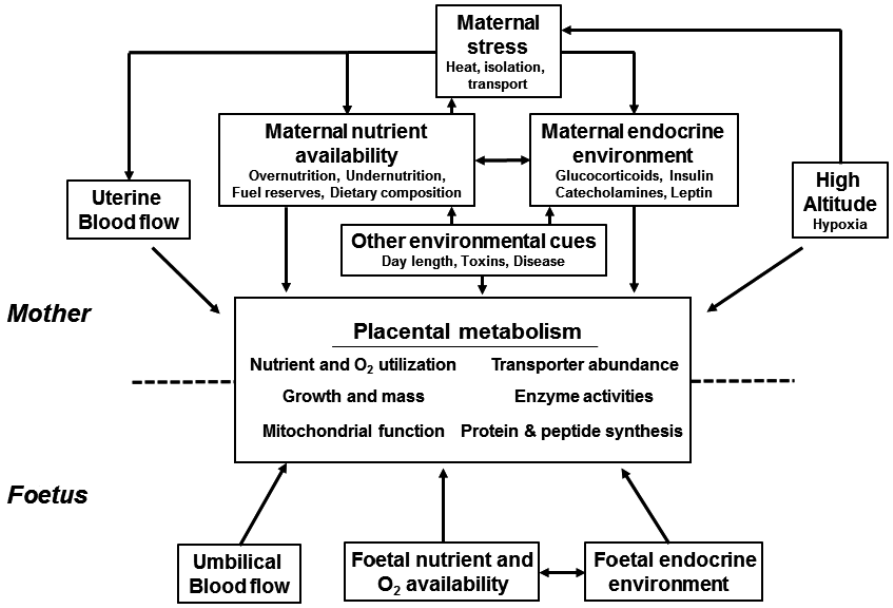

FIGURE 3 Schematic diagram of the stressful and other environmental factors in the mother and foetus influencing placental metabolism during late gestation showing the known placental processes affected by environmental changes

that the placenta may switch from glucose to a greater use of lipids as metabolic fuels when glucose availability is limited, thereby increasing the supply of precursors for prostaglandin synthesis (Fowden, Ralph, & Silver, 1994). This is consistent with the increase in fatty acid transporters seen in the ovine placenta during undernutrition (Ma et al., 2011). Similar increases in placental lipid metabolism are believed to occur in the human and rodent placenta during intrauterine growth restriction (Cetin & Alvino, 2009; Herrera & Ortega-Senovilla, 2014).

Foetal VFA concentrations have been shown to vary naturally with maternal concentrations in sheep and cows but little is known about the factors regulating placental VFA metabolism and transport in adverse conditions (Comline & Silver, 1976). Infusion of β-hydroxybutyrate into pregnant ewes increases its foetal concentration and causes foetal lactacidaemia and hypoxaemia (Miodovnik et al., 1982). Prolonged maternal undernutrition also increases maternal concentrations and uterine uptake of β-hydroxybutyrate through increased maternal fat utilization, which may provide the placenta with alterative oxidative substrates to glucose (Chandler et al., 1985). In contrast, prolonged insulin-induced maternal hypoglycaemia leads to decreased uterine uptake and uteroplacental utilization of both β-hydroxybutyrate and acetoacetate in the absence of changes in the maternal or foetal concentrations (Carver & Hay, 1995). Taken together, these findings suggest that VFA metabolism by the ruminant placenta is responsive to environmental stresses but is determined largely by maternal nutrient availability.

6 | CONCLUSIONS

The placenta is a metabolically labile organ that is responsive to a range of interdependent nutritional and endocrine signals of adversity (Fig. 3). It integrates these multiple signals and adapts its metabolic phenotype accordingly to maintain pregnancy and maximize the chances of foetal survival *in utero*. The metabolic response of the placenta depends on the nature, severity and duration of the stressful challenge and also on whether signals of stress are maternal, placental or foetal in origin (Fig. 3). By diversifying the sources of carbon and nitrogen available to the foetus, the metabolic responsiveness of the placenta also helps to optimize offspring fitness for the prevailing environmental conditions and, thus, improves the likelihood of the offspring reaching reproductive age.

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REVIEW ARTICLE

WILEY Reproduction in Domestic Animals

Parturition effects on reproductive health in the gilt and sow

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Contents

In this review, we address significant characteristics of parturition in the pig and their connection to post-partum reproductive health and fertility. We discuss the normal physiology and behaviour around parturition and the effect of the second phase (expulsion of foetuses) on the third phase of parturition (expulsion of foetal membranes). In addition, we intend to cover retained placenta, and the connection to post-partum uterine health and fertility in the contemporary prolific sow. We also explore factors that support successful parturition or can cause potential problems. Successful parturition in the pig includes the possibility to express adequate maternal behaviour, rapid expulsion of the piglets, complete expulsion of the placenta, neonatal activity and colostrum intake. Abnormal incidents during any phase of parturition can cause subsequent problems. Duration of the expulsion phase of foetuses can be used as a simple measure of whether parturition is considered successful. Prolonged parturition can impair health of the sow and piglet and fertility after weaning. New insights, such as adding more fibre to sow diets during pregnancy, and especially during the period prior to farrowing, may prevent constipation, increase water intake of the sow around parturition and increase milk intake and performance of piglets. Maternal characteristics, including maternal behaviour, ease of parturition, colostrum production and piglet quality parameters, may be utilized to improve success rate of reproductive management during farrowing and early lactation. Additionally, we share some of the recent developments in methods, including ultrasonography in evaluation of post-partum uterine health. In conclusion, successful farrowing is of the greatest importance for reproductive health of the sow and survival of the piglets. We suggest connections exist among prolonged farrowing and yield of colostrum, retained placenta, development of PDS, and impaired involution of the uterus and reduced subsequent fertility.

1 | INTRODUCTION

Reproductive health of the gilt and sow can be defined as the ability to successfully undergo the physiological challenges of the oestrous cycle, mating, pregnancy and lactation. This implies adequate anatomical and physiological resources to complete the challenge of the reproductive cycle without compromising physiological and behavioural needs of the dam or the neonate. Along with increasing litter size, there appears to be demand for welfare-friendly housing systems, which may present a challenge to the producer in terms of reproductive performance of the herd (Einarsson et al., 2014; Kemp & Soede, 2012; van Nieuwamerongen, Bolhuis, van der Peet-Schwering, & Soede, 2014). There are major setbacks associated with increasing litter sizes that are evident at farrowing, weaning and after weaning, when the foundations of the subsequent pregnancy are laid (Algers & Uvnäs-Moberg, 2007; Martineau, Farmer, & Peltoniemi, 2012). This study addresses some of the key reproductive health problems of the modern hyperprolific sow and her numerous offspring. The problems are encountered at farrowing, early lactation, weaning and beyond, and the problems appear interconnected. When normal physiology and behaviour are compromised around farrowing, not only does the neonate struggle for adequate colostrum. Also, the dam encounters

significant problems with expulsion of placental remnants, involution of the uterus and resumption of ovarian activity. The aim of the review therefore was to discuss current understanding of the relationship between the physiology of farrowing, reproductive health and fertility of the gilt and sow.

2 | PHYSIOLOGY AND BEHAVIOUR OF THE SOW AROUND FARROWING

During early pregnancy, after implantation of the embryos and developmental activity of the *corpora lutea* (CL), high and stable concentrations of progesterone dominate the hormonal pattern (Meulen, Helmond, Oudenaarden, & Van der Meulen, 1988). This ensures the progress of pregnancy, and for almost the whole pregnancy, the circulating concentrations of progesterone remain high. One of the key functions of progesterone is to inhibit contractions of the myometrium during pregnancy. At approximately 24–48 hr before the beginning of parturition, activation of the pituitary adrenal axis of the foetuses heralds the end of CL of pregnancy associated with the quick drop in progesterone concentration. At this time, progesterone is metabolized into estradiol. The removal of this hormonal quiescence factor blocking uterine contractions also known as "progesterone block" by activation of the pituitary adrenal axis is supported by peaking prostaglandin $F_{2\alpha}$ concentration of uterine origin. The change in the steroid balance activates oxytocin receptors, and thereby myometrial activity, as increasing oxytocin concentrations bind to their receptors (Taverne & Noakes, 2009; Taverne & van den Weijden, 2008). Prolactin concentration gradually increases prior to farrowing (Anderson, 2000; Ellendorff et al., 1979; Kindahl, Alonso, Cort, & Einarsson, 1982; Osterlundh, Holst, & Magnusson, 1998). The activation of neural receptors by suckling piglets at the mammary gland stimulates oxytocin secretion from the posterior pituitary and prolactin, growth hormone, ACTH and thyroid-stimulating hormone from the anterior pituitary. These hormones promote milk production mainly through promoting milk synthesis in the mammary tissue and let down into the alveoli and lactiferous ducts of the udder (Martineau et al., 2012).

The first stage of farrowing includes dilatation of the cervix, a complex biochemical process involving cytokines, prostaglandins, peptide (relaxin) and steroid hormones (Taverne & Noakes, 2009). A concomitant increase in well-coordinated myometrial contractions is characterized as a response to the increased oxytocin activity in the presence of the changed steroid environment. The second stage of labour in the sow involves straining of the dam in recumbent posture, rupture of the allantochorionic sac and expulsion of foetuses. In the modern sow, the average duration of this stage appears to be approximately 4 hr with a 20-min interval between foetuses born (van Dijk, van Rens, van der Lende, & Taverne, 2005; Gu et al., 2011; Oliviero, Heinonen, Valros, Hälli, & Peltoniemi, 2008). During the third stage of farrowing, uterine contractions persist in a peristaltic manner, high in frequency but lower in amplitude as compared with the second stage. During this stage, the foetal membranes are expulsed in a process that should take no longer than 4 hr (Taverne & Noakes, 2009). Retention of the placenta can be classified as (i) primary placenta retention—no placenta was expelled within the observation of 24 hr after the last piglet, (ii) primary partial placenta retention—some placenta was expelled during parturition and no placenta is expelled after the last piglet within the observation of 24 hr after the last piglet and (iii) secondary placenta retention—expulsion of the last placenta more than 4 hr after expulsion of the last piglet.

Sows approaching farrowing isolate themselves from the rest of the group at approximately 24 hr prior to farrowing and have an innate need to build a nest where the litter is born (Jensen, 1986; Jensen & Redbo, 1987). This nest-building behaviour is characterized by collecting branches, leaves or grass and by rooting and pawing activity (Jensen & Redbo, 1987). The behaviour starts within 24 hr before the birth of the first piglet, is expressed with highest activity 3–8 hr before the birth of the first piglet and should gradually end prior to the birth of the first piglet (Hartsock & Barczewski, 1997; Westin, 2014). It has been argued that the need of the sow to build a nest might have disappeared during domestication, breeding and confinement of sows in modern farrowing crates, but an increasing body of evidence suggests that the modern sow has just as much of need and ability for nest building as her ancestor, the European wild boar (Algers & Uvnäs-Moberg, 2007; Gustafsson, Jensen, de Jonge, Illman, & Špinka, 1999; Oliviero, Heinonen et al., 2008).

Modern housing systems have promoted the confinement of sows in crates during farrowing, whereby the sow is allowed very limited movement and bedding, and nest-building substrate is often absent or very limited as well (Cronin, Simpson, & Hemsworth, 1996; Edwards & Fraser, 1997; Gu et al., 2011; Jensen et al., 1997; Thodberg, Jensen, Herskin, & Jørgensen, 1999; Vestergaard & Hansen, 1984). The restricted movement of the sow by confinement in a cage has been motivated by reduced mortality of the neonatal piglets (Hales, Moustsen, Nielsen, & Hansen, 2014; Hansen & Curtis, 1980). In these conditions, the nest-building behaviour as triggered by endogenous hormonal activity cannot be properly expressed. In the absence of a nest-building substrate, confined sows express prolonged and unsuccessful nest-building behaviour (Damm, Vestergaard, Schrøder-Petersen, & Ladewig, 2000; Damm, Lisborg, Vestergaard, & Vanicek, 2003; Yun et al., 2014; Yun, Swan, Oliviero, Peltoniemi, & Valros, 2015). The stress is characterized by lower oxytocin concentration during the process of parturition, behavioural abnormalities and maintenance of cortisol concentration at a high level after parturition (Jarvis, Reed, Lawrence, Calvert, & Stevenson, 2002; Lawrence et al., 1994; Oliviero, Pastel et al., 2008). Prolonged duration of farrowing is considered as one of the complications of the confinement stress (Oliviero, Heinonen, Valros, & Peltoniemi, 2010).

The lack of opportunity to express appropriate nest-building behaviour can lead to increases in circulating cortisol and ACTH (Jarvis et al., 1997), which indicate a stressful condition. Gustafsson et al. (1999) reported that domestic sows were able to build nests identical to those of wild boars, even after several previous farrowing experiences in confined crates without bedding. This innate behaviour is therefore a clear indication of impending farrowing and is present regardless of housing or availability of bedding material.

One of the triggering factors for nest-building behaviour is a rise in prolactin level (Castrén, Algers, De Passillé, Rushen, & Uvnäs-Moberg, 1994), induced by a decrease in progesterone and an increase in prostaglandins (Algers & Uvnäs-Moberg, 2007). Increase in oxytocin secretion in a rapid pulsatile manner, in turn, appears to stop nest-building behaviour a few hours prior to the birth of the first piglet. Prolonged nest-building behaviour, which may still continue during parturition, is considered abnormal and indicative of problems in the hormonal processes of parturition (Algers & Uvnäs-Moberg, 2007).

In conclusion, the onset of parturition with adequate nest-building behaviour of the sow is under strict endogenous hormonal control. The benefits of using pens and enrichment for free farrowing, making nest building possible, are significant and support normal reproductive physiology of the gilt and sow and support their behaviour prior to and during farrowing.

3 | EFFECT OF PARTURITION AND NEST-BUILDING BEHAVIOUR IN EARLY LACTATION

The mammary gland develops during the last third of gestation, with the accumulation of colostrum brought on by the initiation of farrowing and let down already 12 hr before the birth of the first piglet. This process is guided by pituitary peptide hormonal activity, including prolactin and oxytocin (Martineau et al., 2012). The first milk ejections can therefore appear before farrowing starts or at latest when it is completed (Algers & Uvnäs-Moberg, 2007). During the first hours of parturition, colostrum is readily available almost continuously, but any further release of colostrum requires suckling stimulus and a release of oxytocin (Fraser, 1984). This early milk ejection is favourable for prompt and adequate support to the newborn piglets, and it is strictly correlated with the pre-partum behaviour of the sow, driven by decrease in blood progesterone and increase in circulating prolactin and oxytocin (Algers & Uvnäs-Moberg, 2007). Maternal behaviour in free-ranging sows is normally exercised in a nest isolated from the rest of the group that the sow has built during the pre-parturient period (Jarvis, Reed, Lawrence, Calvert, & Stevenson, 2004). Some hours before farrowing sows show natural nest-building behaviour, such as foraging, rooting and pawing, expressing the desire to build a shelter for protecting their offspring. However, the possibility to perform these natural activities in farrowing crates is limited because of lack of space, material or both. Provision of a biologically relevant stimulus, such as straw, positively affects not only the nest-building behaviour prior to farrowing, but also the nursing and suckling behaviour of sows and piglets. This reduces occasions when sows terminate suckling and frequency of foreleg rowing. It also increases the time the piglets spend suckling and triggers an earlier development of suckling behaviour, which are advantageous for early milk intake by piglets, including the piglets with low birthweight (Herskin, Jensen, & Thodberg, 1999; Loisel, Farmer, Ramaekers, & Quesnel, 2014). Farrowing crates appear to prohibit interactions between the sow and her piglets to some extent, and the provision of space during parturition could facilitate the performance of maternal behaviour. A balanced hormonal pattern at farrowing appears to be important not only for parturition, but it might continue during early lactation. Unlike cows, sows have no teat cisternae, which is why a piglet cannot obtain milk without there being an increase in the intramammary pressure mediated by oxytocin release (Algers & Uvnäs-Moberg, 2007). Sows kept in farrowing crates with no nest-building material tended to have lower oxytocin concentrations during farrowing than sows kept in loose-housed pens with abundant nest-building materials (Oliviero, Heinonen et al., 2008). In the same study, the sows with lower oxytocin concentration had also longer duration of farrowing, which was in accordance with a previous study where sows with long farrowing times had lower basal and lower peak levels of oxytocin during farrowing in comparison with sows with short farrowing times (Castrén, Algers, De Passillé, Rushen, & Uvnäs-Moberg, 1993). Recent results show that events affecting the sow immediately before and during farrowing can also affect early lactation. A plentiful supply of nesting materials prior to parturition leads to an increase in sow plasma oxytocin and prolactin concentrations from 3 days prior to parturition until 7 days post-partum (Yun et al., 2013). This indicates a potential association between nest-building possibilities induced by abundant nesting materials and circulating oxytocin and prolactin concentrations in peripartal sows. In this study, however, sows housed in loose pens with limited nesting materials did not have higher oxytocin concentrations than sows in confined farrowing crates with an equally limited amount of nesting materials. This indicates that abundant nesting materials may make a greater contribution to increased oxytocin concentrations than non-confinement in loose-housed pens (Yun et al., 2013). In the same study, farrowing housing in crates required extra udder stimulation by the piglets to obtain milk in the early lactation period (Yun et al., 2013). This prolonged udder massage might disturb the sow because it cannot be avoided in the crate, likely resulting in poor welfare because of inadequate coping mechanisms of the sow to such prolonged stress caused by the piglets. Indeed, Oliviero, Heinonen et al. (2008) found a higher concentration of salivary cortisol of sows in crates, when compared with sows in pens for loose housing. He suggested that this was caused by the sows having difficulties in denying their offspring from nursing (Fig. 1). When sows are provided with abundant nesting material, they show a greater incidence of careful pre-lying behaviour and appear to perform better maternal behaviour in order to care for their offspring (Yun et al., 2013). Therefore, provision of adequate space and nesting material before parturition can improve sow welfare by providing opportunities for sows to express their normal behaviour, reduce farrowing duration and stillbirth rate, and also reduce potential stresses during the early nursing period. This last progressive effect is of great importance because the conclusion of farrowing is not the terminal point of a successful parturition. At this point, if the sow is unable to produce an adequate amount of colostrum to satisfy the newborn piglets, it will dramatically affect their survival. The influence of the litter on the colostrum production seems smaller than it would be during the rest of the lactation. Piglets fed colostrum *ad libitum* were able to take in up to 450 g/kg birthweight, which is double what piglets can consume in normal conditions (Devillers, Van Milgen,

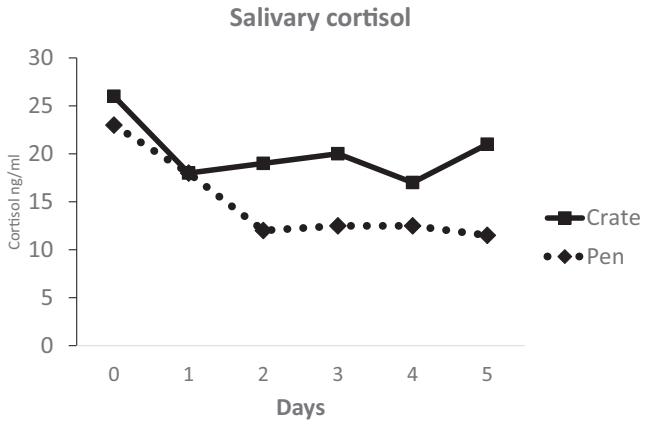

FIGURE 1 Salivary cortisol average concentration in the pen-housed sows and in the crate-housed sows. Day 0 is the farrowing day. (Oliviero, Heinonen et al., 2008)

Prunier, & Le Dividich, 2004). Colostrum yield averages approximately 3.5 kg, but there is substantial variation among sows, with a range from 1 to 6 kg (Devillers, Farmer, Le Dividich, & Prunier, 2007; Quesnel, 2011). Colostrum yield is less correlated to litter size than the birthweight (Devillers et al., 2007; Quesnel, 2011). Colostrum is freely available during parturition, and regular suckling by piglets is necessary to maintain its secretion in the first 24-hr post-partum and beyond (Theil et al., 2006). Therefore, the availability of colostrum is more connected to the vitality of piglets and mostly with the capacity of the sow to produce colostrum (Quesnel, Farmer, & Theil, 2015). Foisnet, Farmer, David, and Quesnel (2010) established that gilts producing a low yield of colostrum (1.1 kg) had greater concentrations of progesterone and lower concentrations of prolactin before farrowing, and at the onset of farrowing, compared with the high-producing gilts (3.9 kg). This was confirmed by Quesnel et al. (2015), where a low ratio of prolactin to progesterone before and during farrowing affected colostrogenesis and piglet survival negatively. In several studies, there was no clear-cut effect of duration of parturition on colostrum yield established (Declerck, Dewul, Piepers, & Decaluwé, 2015; Devillers et al., 2004; Foisnet et al., 2010; Quesnel, 2011). Therefore, instead of a prolonged duration of farrowing, a low colostrum yield may be attributed to hormonal dysfunction. In conclusion, promoting better nest-building behaviour before farrowing might be beneficial not only for better progression of parturition but also to promote adequate colostrum yield.

4 | EFFECT OF FARROWING DURATION ON PLACENTA EXPULSION

The second phase, foetal expulsion, is followed by the last phase of parturition, detachment and expulsion of the placentae. The placenta is supposed to be expulsed within 4 hr after the birth of the last piglet (Taverne & Noakes, 2009). Surprisingly, the duration of placenta expulsion has only been reported twice, once in sows (Jones, 1966) and once in gilts (van Rens & van der Lende, 2004). Jones (1966) reported a duration of 4 hr and van Rens and van der Lende (2004) of approximately 2.5 hr. One of our field studies in sows (Björkman et al., unpublished) showed an average duration of placenta expulsion of 4.5 hr. In the study of van Rens and van der Lende (2004), gilts had an average farrowing duration of 2 hr and an increase in duration of farrowing was significantly associated with an increase in duration of placenta expulsion. In our study (Björkman et al., unpublished), a similar association was found, but only in sows with a farrowing duration of <7 hr. Interestingly, duration of farrowing and placenta expulsion obeyed a quadratic rather than a linear relationship. In sows with a short farrowing duration (<7 hr), placenta expulsion duration increased with increasing farrowing duration, whereas in sows with a prolonged farrowing duration (>7 hr), placenta expulsion duration decreased with increasing farrowing duration. Furthermore, we observed sows that had a partial primary placenta retention (3% of 149 sows) and sows that had a complete primary placenta retention (3% of 149 sows). Sows with partial primary retention had an average farrowing duration of 11 hr, and sows with total primary retention had an average farrowing duration of 17 hr (Björkman, Peltoniemi, Oliviero, & Soede, 2015). The observation suggests that sows with extremely prolonged farrowing duration have reduced placenta expulsion. Secondary uterine inertia, which causes insufficient expulsive force of the uterus, could be one explanation, which is supported by Oliviero, Heinonen et al. (2008), who showed that increased farrowing duration is associated with decreased oxytocin levels. In addition, this is supported by the observation that moderate use of oxytocin injections towards the end of the second phase of the parturition had a significant effect in reducing the duration of placenta expulsion in sows with short farrowing duration (n = 68). On the other hand, oxytocin administration increased the duration of placenta expulsion in sows with prolonged farrowing duration (n = 74 Björkman et al., unpublished). Sows that received oxytocin and with farrowing durations of approximately 20 hr were able to expel their placenta after the last piglet, whereas sows with the same farrowing duration that received no oxytocin experienced primary placenta retention (Björkman et al., unpublished).

Thus, the primary placenta retention seems to be linked with a long farrowing duration. The secondary placenta retention, on the other hand, seems to be linked to a short farrowing duration. In our study, 6% of the sows expelled the last placenta only after 12 hr, 37% after 4–12 hr and only 51% within 4 hr after the birth of the last piglet (Björkman, Peltoniemi et al., 2015). These sows had farrowing durations of 4.5, 6 and 7 hr, respectively.

Another interesting and highly significant difference between sows with short and prolonged farrowing durations is the onset of placenta expulsion. In sows with a short farrowing duration, placenta expulsion starts on average one hour after the birth of the last piglet. In contrast, in sows with prolonged farrowing duration, placenta expulsion starts on average one hour before the birth of the last piglet (Björkman et al., unpublished). This information could be used on the farm as an indicator of a sow at risk of experiencing prolonged farrowing. If piglets are born after expulsion of placenta, it is likely that the sow has been in labour for more than 7 hr. Therefore, oxytocin might be used to support

the placenta expulsion and post-partum care of the sow, to prevent post-partum diseases and reduced subsequent fertility.

In conclusion, farrowing duration seems to have an impact on placenta expulsion. In gilts, a linear correlation was established (van Rens & van der Lende, 2004), whereas in sows, a quadratic relationship pertained (Björkman et al., unpublished). This suggests that sows with prolonged farrowing duration might suffer from secondary uterine inertia. Furthermore, oxytocin has a strongly positive effect on placenta expulsion. If sows with extremely long farrowing durations are treated with oxytocin, they seem still to be able to expel the placenta. However, sows that are not treated with oxytocin might undergo primary placenta retention.

5 | EFFECT OF PARTURITION ON POST-PARTUM UTERINE HEALTH AND POST-PARTUM DYSGALACTIA SYNDROME

Post-partum dysgalactia syndrome (PDS) is characterized by insufficient colostrum and milk production during the first days after farrowing (Martineau et al., 2012). It is considered to be a multifactorial disease involving different pathways, one of which is mediated by endotoxins produced by bacteria in the gut, bladder, mammary gland or uterus (Martineau et al., 2012).

The effect of parturition on the occurrence of PDS has rarely been investigated. In a recent study by Tummaruk and Sang-Gassanee (2013), the percentage of sows with fever during the first 24-hr post-partum increased from 40 to 100% when the farrowing duration increased from <2 to 4–8 hr. This finding suggests that good farrowing management and quick piglet expulsion are important for the post-partum health status of the sow. There is little information available about whether and how parturition affects post-partum disorders. There seems to be a connection between prolonged farrowing duration and post-partum disorders such as PDS; for example, both have common risk factors: constipation (Hermansson, Einarsson, Larsson, & Backstrom, 1978; Martineau, Smith, & Doize, 1992; Oliviero, Kokkonen, Heinonen, Sankari, & Peltoniemi, 2009); increased back fat (Göransson, 1989; Oliviero et al., 2009); no farrowing supervision (Papadopoulos, Vanderhaege, Janssens, Dewulf, & Maes, 2010; Björkman et al., unpublished); restricted space in crates at farrowing (Bäckström, Morkoc, Connor, Larson, & Price, 1984; Cariolet, 1991; Oliviero, Heinonen et al., 2008); and farrowing induction (Papadopoulos et al., 2010; Smith, 1982). In the study by Tummaruk and Sang-Gassanee (2013), a connection between farrowing duration and post-partum fever was established, but not for farrowing duration and PDS. However, PDS was defined according to the presence of udder inflammation and/or agalactia. Thus, only a small proportion of the complex clinical signs (Martineau et al., 2012) has been investigated. To the knowledge of the authors, no study to date has investigated the impact of farrowing duration on uterine health and occurrence of endometritis, which plays an important role in the development of PDS (Martineau et al., 2012). It is feasible to assume that prolonged parturition affects uterine health. During prolonged parturition, the physical barrier against bacterial invasion, the cervix, is open for a long time. As discussed above, prolonged duration can cause placental retention (Björkman et al., unpublished). The placenta represents a perfect medium for ascending bacteria. Furthermore, prolonged farrowing duration seems to be one of the causes of secondary uterine inertia, which is due to exhaustion of uterine muscles and not due to ability to contract (primary uterine inertia). In prolonged parturition, the uterine clearance appears therefore to be compromised (Oliviero, Heinonen et al., 2008). Therefore, our research group made an effort to assess the uterus after parturition (Björkman, Oliviero, & Peltoniemi, 2015). The assessment was performed 3 days *post-partum* using transabdominal ultrasonography (Fig. 2), and the image area of the uterus was categorized as small (n = 61; Fig. 2) or enlarged (n = 46; Fig. 3). Transabdominal ultrasound examination appears to be the only reliable method (Kauffold et al., 2005) because sows with uterine

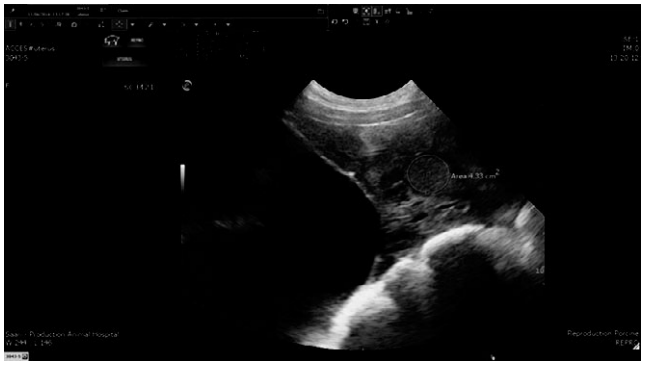

FIGURE 2 Representative image of a sow with a uterine horn already well in the process of involution 3 days post-partum (p.p.). The normal range of the image area between 2 and 7 days p.p. was 4–3 cm^2, respectively (Björkman, Oliviero et al., 2015). This sow had a successful farrowing, no dystocia nor birth help

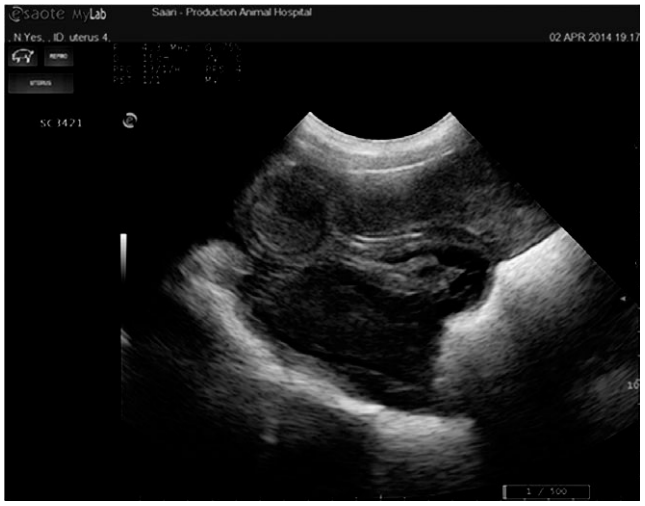

FIGURE 3 Representative image of a sow with a delayed involution of the uterine horn 3 days post-partum. This sow had an unsuccessful farrowing of more than 5 hr. The normal range of the image area between 2 and 7 days p.p. was 3–4 cm^2, respectively (Björkman, Oliviero et al., 2015)

inflammation, such as endometritis, may not exhibit clinical signs such as vaginal discharge (Dalin, Gidlund, & Eliasson-Selling, 1997; de Winter, Verdonck, de Kruif, Devriese, & Haesebrouck, 1995). We investigated the correlation between farrowing duration, dystocia (time between two piglets more than 60 min), placenta expulsion duration, total birth duration (time between expulsion of the first piglet and the last placenta), onset of placenta expulsion, primary placenta retention and the effect of application of birth help or oxytocin on size of the uterus. We established significant correlations between farrowing duration, dystocia, total birth duration, application of birth help, primary placenta retention and enlarged uterus. Sows with an enlarged uterus had, on average, 2 hr longer farrowing durations and 2 hr longer total birth durations. Furthermore, sows with an enlarged uterus more often experienced dystocia and needed birth help. All sows with primary placenta retention had an enlarged uterus (Björkman, Oliviero et al., 2015). Interestingly, oxytocin application had a tendency to decrease uterus size. Furthermore, we found a connection between the presence of fluid (Fig. 4) in the uterine lumen and the application of birth help, as well as presence of dense structures in the uterus (Fig. 5) and primary placenta retention. In addition, we observed a connection between enlarged uterus and heterogeneous echotexture of the uterus (Fig. 3). The same connection was reported earlier in mated sows that failed to conceive and was linked to uterine oedema and inflammation (Kauffold et al., 2005). Fluid accumulation, such as pus, has been associated with uterine inflammation such as endometritis (Martinez, Vazquez, Roca, & Ruiz, 1993), metritis (Botero, Martinat-Botte′, & Bariteau, 1986) and pyometra (Knox & Althouse, 1999).

In conclusion, endometritis is considered to be a factor that affects the occurrence of PDS. Only sporadic studies concerning the impact of farrowing on post-partum health (Tummaruk & Sang-Gassanee, 2013) and ultrasonographic investigation focussing on uterine disorders (Kauffold et al., 2005) have been performed. To our knowledge, no study has investigated the effect of farrowing on uterine health. Ultrasonographic investigation seems to be the method of choice. We demonstrated a connection between increased size of uterus and prolonged farrowing duration, total birth duration, birth help and primary placenta retention. Furthermore, birth help has been linked to fluid accumulation and primary placenta retention to dense structures inside the uterus. Kauffold et al. (2005) demonstrated that increased size of the uterus represents uterine oedema caused by inflammation. It therefore seems more than likely that prolonged farrowing duration impairs uterine health.

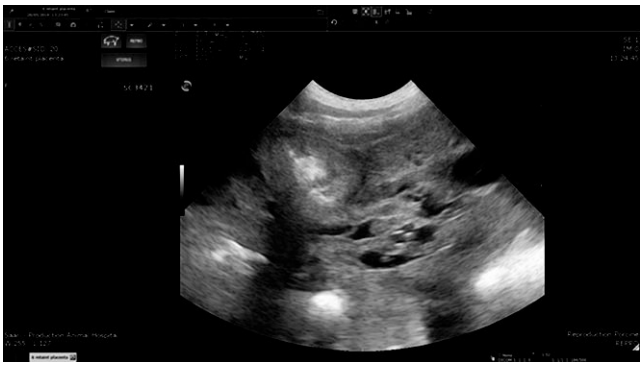

FIGURE 4 A representative image of a post-partum uterine horn of a sow after prolonged parturition (21 hr) and retained placenta. The structures with increased density/heterogeneity are indicative of retained placenta

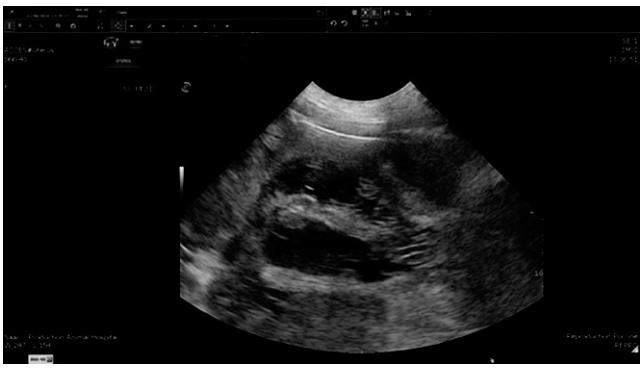

FIGURE 5 A representative image of a sow after prolonged farrowing (>5 hr) and repeated dystocia (four times) and birth help (manual removal of piglets). The uterine horn has a high degree of heterogeneity and some fluid visible within the lumen

6 | EFFECT OF FARROWING DURATION AND PDS ON SOW FERTILITY

The complex interactions among environmental effects on the reproductive physiology of sows at farrowing seem to extend beyond lactation to the subsequent fertility of the sow. Recent findings demonstrate that sows with long farrowing duration (>300 min) have a higher repeat breeding rate (Fig. 6; Oliviero, Kothe, Heinonen, Valros, & Peltoniemi, 2013). This finding could provide valuable information for sow reproductive management. There is no clear explanation yet for this interaction. In free-ranging domestic pigs kept in a seminatural environment, weaning of the litter is a slow and gradual process, taking between 91 and 126 days post-partum (Jensen & Recén, 1989). In contrast, in commercial piggeries, the lactation period is relatively short, usually between 18 and 28 days, and the weaning-to-oestrous interval is, on average, 5 days. The interval between parturition and subsequent insemination is therefore much shorter than under natural conditions, which may allow factors that negatively influence the physiology of parturition to interfere at post-weaning oestrus. A complete involution of the uterus takes approximately 14–21 days (Graves, Lauderdale, Kirkpatrick, First, & Casida, 1967; Hays, Krug, Cromwell, Dutt, & Kratzer, 1978; Palmer, Teague, & Venzke, 1965a,b; Svajgr, Hays, Cromwell, & Dutt, 1974). This is supported by the fact that histological involution of the uterus is completed within 3 weeks of farrowing (Belstra, Flowers, Croom, DeGroot, & See, 2005), which underlines the need to prolong the process of weaning beyond day 21 of lactation. As discussed in the previous section, prolonged farrowing duration seems to affect uterine health negatively and increase post-partum uterus size. Increased size of the uterus can be considered as

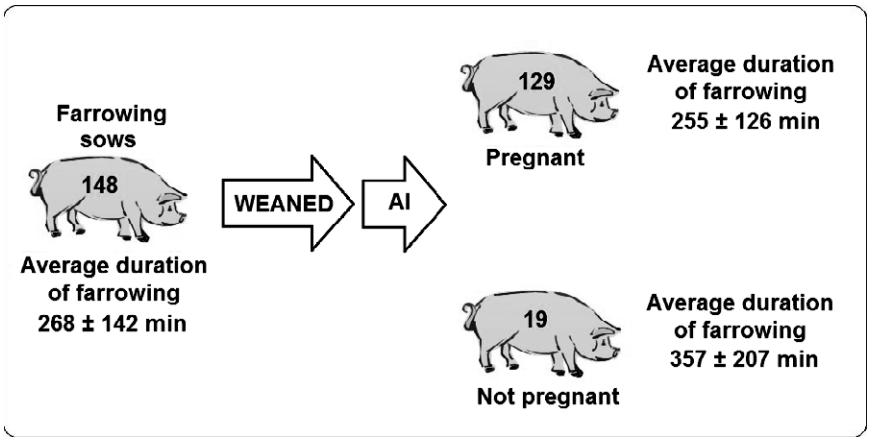

FIGURE 6 Sows that failed to become pregnant at first insemination were having a previous duration of farrowing of 100 min longer than those who became pregnant (Oliviero et al., 2013)

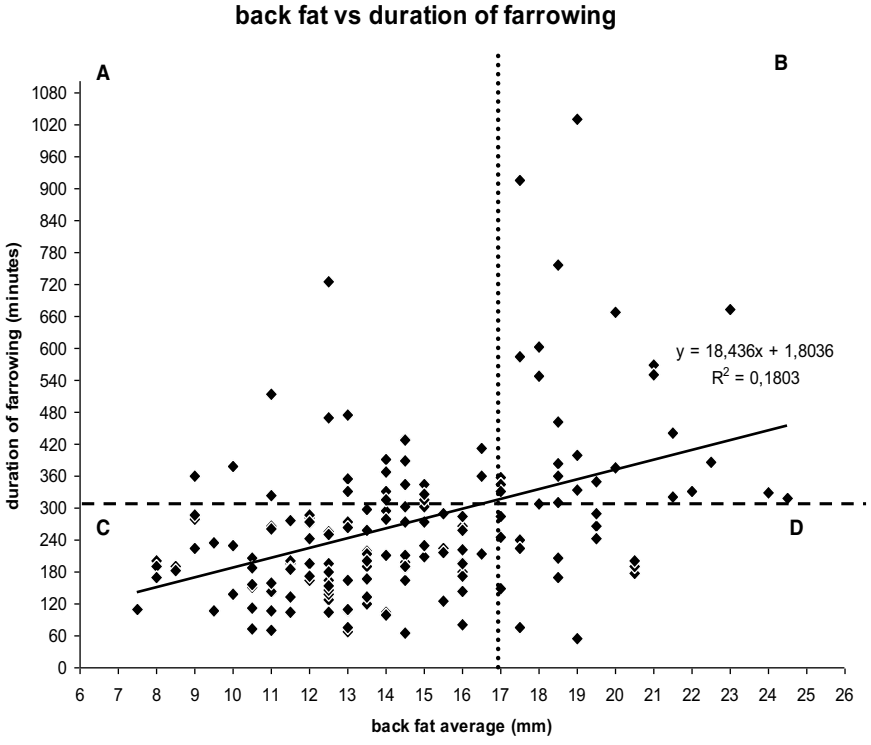

FIGURE 7 Individual sows plotted according to back-fat average and the duration of farrowing. The horizontal dashed line distinguishes prolonged farrowings (>300 min; areas a and b). The vertical dotted line distinguishes the fatter sows (areas b and d). The solid regression line represents a positive relationship between the back-fat average and the duration of farrowing (Oliviero et al., 2010)

swelling due to inflammation (Kauffold et al., 2005). Thus, it may cause, as in other species, that is in beef heifer and dairy cows (Heppelmann et al., 2015; Savc, Kenny, & Beltman, 2016), prolonged uterine involution and thereby interfere with subsequent fertility.

PDS represents another explanation for how prolonged farrowing duration may affect fertility. In a study utilizing a large German database, it was shown that low parity sows suffered more PDS-like symptoms than older sows, with an overall prevalence of approximately 30% (Hoy, 2006). The number of sows not cycling after weaning increased after PDS, the weaning-to-oestrous interval was prolonged, and the repeat breeding rate increased by approximately 4%. In addition, the abortion rate increased as well as the overall mortality of sows (Hoy, 2006). In conclusion, there is at least some evidence to suggest that PDS reduces fertility, not only in terms of reproductive health of the sow and her current neonatal litter, but also subsequent fertility after weaning.

However, further research is needed on uterine involution, follicular development and CL function after breeding. For investigating CL function, we have for the first time described a minimally invasive method that allows tissue collection from the CL. This method, a transvaginal ultrasound-guided biopsy, is rapid, can be performed by one person and has no major effects on the sow's reproductive performance. It can be applied on the farm to study effects on luteal function of housing, management and nutrition (Björkman et al., 2016).

7 | FEEDING THE SOW FOR EASE OF FARROWING

During late pregnancy, one common practice in feeding sows aims to reduce the amount of feed offered and increase the energy of the ration in preparation for the upcoming metabolic changeover into

lactation and farrowing (Farmer, Palin, & Martel-Kennes, 2014; Le Cozler, Beaumal, Neil, David, & Dourmand, 1999). Such concentrated diets usually contain a more limited amount of fibre than do standard gestation diets. This practice aims mainly to ensure that sows receive enough energy during late pregnancy to satisfy upcoming milk production (Decaluwé et al., 2014; Einarsson & Rojkittikhun, 1993). High back-fat values were associated with increased duration of farrowing (Oliviero et al., 2010). In Fig. 7, all the sows in areas C and D had normal durations of farrowing (<300 min), whereas those in areas A and B had farrowings longer than normal (>300 min). Most of the fatter sows (>17 mm of back fat) were in area B, whereas most of the thinner sows (<17 mm of back fat) were in area C. These findings reveal that over condition in the very last part of pregnancy is not recommendable because it could negatively affect the farrowing process and survival of the offspring.

However, there are reports that reducing the volume and fibre content of sow feeds can have negative effects, including increased stereotypic behaviour (Ramonet, Meunier-Salaun, & Dourmad, 1999), the development of gastric ulcers and constipation (Lee & Close, 1987). As sows approach farrowing, mild constipation is common because the intestine is less active approaching parturition (Kamphues, Tabeling, & Schwier, 2000). In addition, water absorption from the intestine increases due to the request for water due to initiation of milk production (Mroz, Jongbloed, Lenis, & Vreman, 1995). Therefore, offering feed low in volume and fibre can worsen constipation and negatively affect milk production. In addition, constipated sows showed higher rates of mastitis than those not constipated, with evidence of a direct effect of constipation on udder health (Hermansson et al., 1978; Persson, 1996). Constipation may also be uncomfortable for the sows, thus impacting their welfare.

Oliviero et al. (2009) found that when sows were fed 7% crude fibre in late pregnancy, their constipation was less severe and they recovered proper intestinal activity faster than those sows fed diets with only 3.8% crude fibre. During the period from 5 days before to 5 days after farrowing, the sows fed a 7% crude fibre diet had an average higher faecal score (less constipation), compared with sows fed a 3.8% crude fibre diet (Fig. 8). The same study revealed that 22% of the sows fed the low fibre diet had extremely severe constipation (more than 5 consecutive days without producing faeces), whereas only 5% of the sows fed the high fibre diet had this condition (Fig. 9). The average individual daily water consumption was also higher in the high fibre group (29.8 ± 4.9 L) than in the low fibre group (20.2 ± 3.3 L) (Fig. 10). Moreover, a high fibre diet fed in late pregnancy until farrowing seemed to improve growth rate of piglets (Oliviero, Heinonen et al., 2008; Quesnel et al., 2009), indicating a positive effect of high fibre diet before farrowing on piglet performance (Oliviero et al., 2009).

Regarding the correlation between faecal score and the duration of farrowing, the average constipation index score was 2 ± 0.6 (range from 0.3 to 3) and the lower constipation index scores (showing more constipation) were negatively related with duration of farrowing (Fig. 11). In conclusion, cases of severe constipation at farrowing can be avoided by increasing the amount of dietary fibre during the

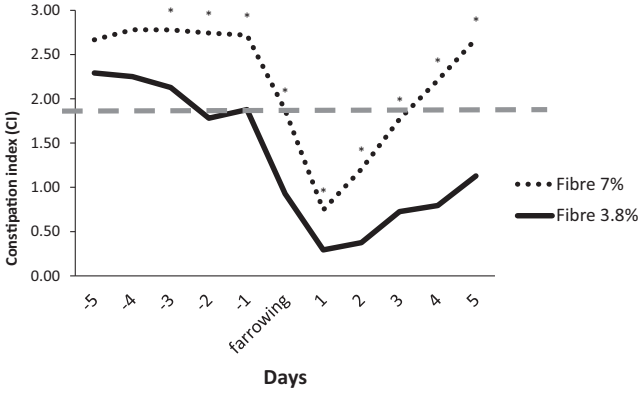

FIGURE 8 Intestinal activity, expressed as constipation index (CI), of sows fed isocaloric diets differing only in crude fibre content. Lower CI values indicate greater constipation state. Normal intestinal activity is considered above the dashed line, CI >1.9 (Oliviero et al., 2009). Significant difference ($p < .05$) is marked with an asterisk

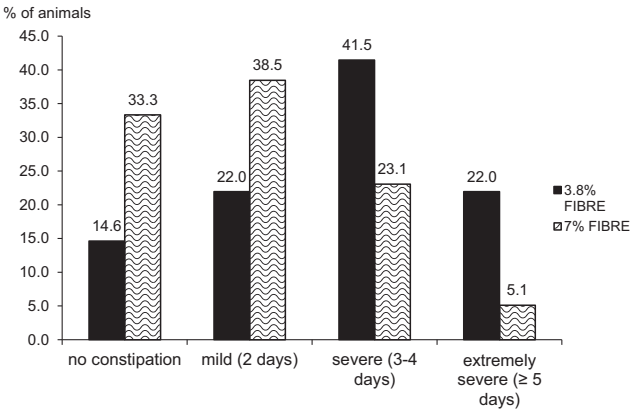

FIGURE 9 Incidence of different degrees of constipation in the sows fed a 7% fibre diet ($n = 40$) and a 3.8% fibre diet ($n = 41$) during the observational period (from five days before to five days after farrowing). Each category considers consecutive days of absence of faeces (Oliviero et al., 2009). Significant difference ($p < .05$) is marked with an asterisk

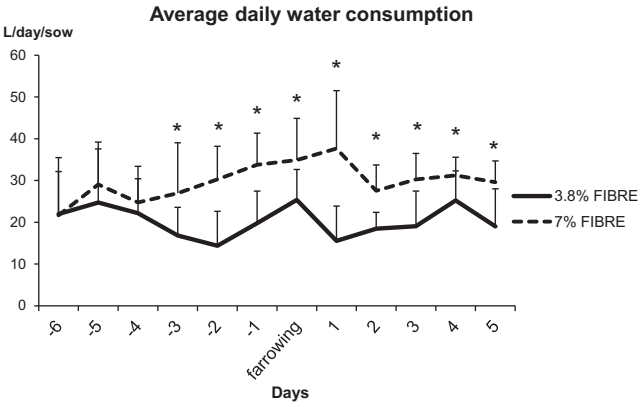

FIGURE 10 Average daily water consumption of sows fed two different levels of crude fibre (Oliviero et al., 2009). Significant difference ($p < .05$) is marked with an asterisk

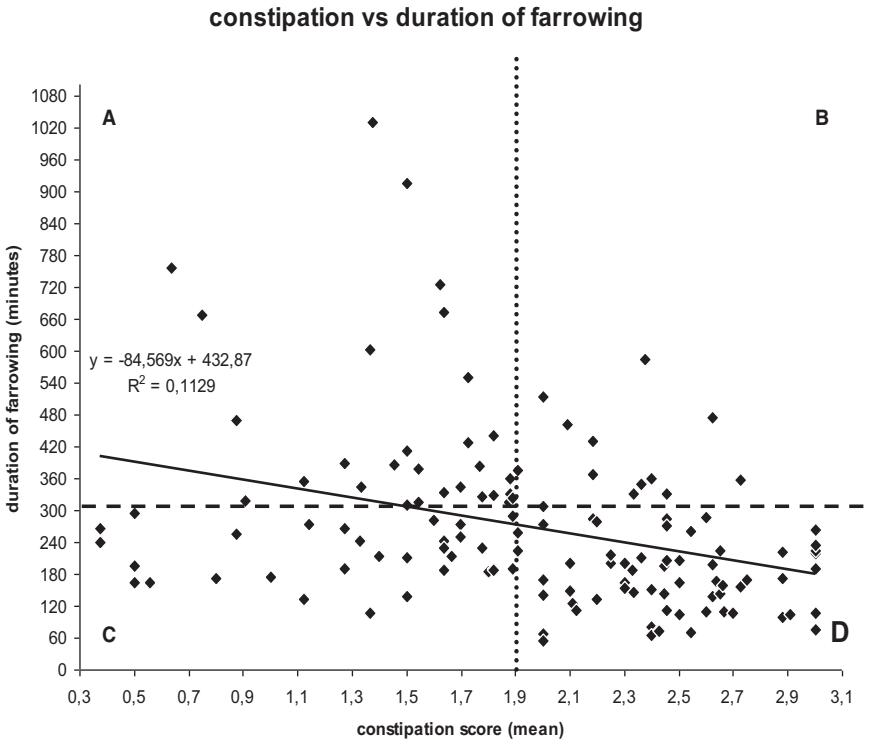

FIGURE 11 Individual sows plotted according to average constipation index score (CI) and duration of farrowing. Low CI values indicate constipated sows, whereas high CI values indicate unconstipated sows. The horizontal dashed line distinguishes prolonged farrowings (>300 min; areas a and b); the vertical dotted line distinguishes constipated sows (areas a and c). The solid regression line represents a negative relationship between the constipation index and the duration of farrowing

last phase of pregnancy (Oliviero et al., 2009; Tabeling, Schwier, & Kamphues, 2003). The provision of dietary fibre improves intestinal activity and reduces the degree of constipation. The use of high-fibre diets therefore appears to be a beneficial strategy to improve the health of the sow around farrowing. Giving roughage may not only be a way to increase fibre intake and alleviate constipation, but it can also serve as an appropriate material for nest building.

8 | CONCLUSIONS

There is increasing evidence to suggest that colostrum yield and subsequent fertility are affected by prolonged farrowing, retained placenta, development of PDS and impaired involution of the uterus. These causal factors appear to be correlated to each other in a way not yet fully understood. A prerequisite for successful farrowing is that the sow has the materials and the ability to move freely to express properly her nest-building behaviour. It is important that sows receive a diet that reduces the risk for obesity and incidence of constipation around farrowing. Furthermore, farrowing can be considered successful if the total duration of parturition is a few hours to avoid intrapartum hypoxia of the foetuses. In the contemporary sow, time required for expulsion of placenta appears highly variable and may take more than generally assumed (4 hr). In addition, placenta expulsion should start after the birth of the last piglet. If not, it can indicate that duration of parturition has been too long. Those sows will need attention towards the end of parturition and early lactation. Adequate production of colostrum by the sow and intake by piglets are of fundamental importance to successfully conclude the process of farrowing. Contamination of the birth canal during farrowing and inadequate management increasing the risk of subsequent inflammation of the mammary gland (PDS) should be avoided. The mechanisms involved in linking farrowing success, uterine health and fertility largely remain to be studied.

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WILEY Reproduction in Domestic Animals

WORKSHOP 1
PRODUCTION OF PORCINE EMBRYOS *IN VITRO* AND *IN VIVO*: APPLICATIONS AND POTENTIAL USE

WS 1.1 | Advances in embryo transfer with *in vivo* derived porcine embryos

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With the development and recent improvement of the non-surgical deep uterine (NsDU) embryo transfer (ET) technique, the main obstacle to the use of ET in pigs, i.e. the surgical transfer, has been overcome. With this technology, the commercial use of ET in pigs is now possible, which will result in important sanitary and economic benefits for the pig industry and will prevent welfare problems associated with transporting live pigs. Many factors that influence NsDU-ET effectiveness and the implementation of the ET technology have been recently evaluated, including superovulation treatments, the synchrony between donors and recipients, the embryonic stage at transfer, the use of defined medium in each step of ET, the recipients' parity, the future reproductive potential of donors and recipients, the potential for embryo re-vitrification and the safety of short-term vapor storage (dry shipper) of vitrified embryos during transport. Moreover, important advances have been achieved during the last decade in the development of short-term (48 h) and long-term (vitrification) storage procedures for *in vivo* derived morulae and blastocysts and excellent reproductive performance has been reported (70–90% farrowing rates and 9.0–10.5 piglets born) when these stored embryos were non-surgically transferred to the recipients. Since all of these studies were performed in controlled experiments, the challenge now is to translate the ET procedures into reliable on-farm practices. (Supported by Séneca (19892/GERM/15) and MINECO-FEDER (AGL2012-38621 and AGL2015-69735-R))

WS 1.2 | Current and potential commercial use of embryo transfer in pigs

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Genetic companies require a continuous exchange of genetic resources among nuclei selection and from these into commercial farms to achieve an optimal genetic progress. Currently, there are two ways to transfer genetic progress from selection nuclei, each with its drawbacks. The introduction of live animals involves health risks and can cause a pathological destabilization of destination farms. In addition, transport of live animals compromises their welfare and carries great economic costs. Alternatively, the use of semen from selected boars allows the introduction of genetic improvement, but only through the male, slowing genetic progress. In addition, semen can carry pathogens. Embryo transfer (ET) responds to this demand for an alternative, safe and economical way to mobilize genetic resources, with minimal risk of disease transmission, and without costs and disruption of animal welfare associated to livestock transport. Recent advances in short-term conservation and transport (up to 24 h) of *in vivo* derived embryos have allowed the first steps toward the commercial application of ET and, although results are promising, great disparity has been observed which shows the importance of factors such as genotype and management of donors (different response to superovulation treatments, variations in ideal moment for embryo collection) and recipients (farrowing rates ranging from 50 to 83.3% between different farms using same embryo donors). For these reasons, further research is required to evaluate additional difficulties when ET is applied under commercial conditions.

WS 1.3 | *In vitro* production of porcine embryos: present applications in reproductive biotechnology

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There is an increased interest in producing large quantities of porcine oocytes and embryos, through *in vitro* maturation (IVM), fertilization (IVF) and culture (IVC), for their use as biological material in a variety of reproductive technologies (RT). With the booming of CRISPR/Cas9-mediated genome engineering, it has been demonstrated that knockouts can be efficiently generated by co-injection of Cas9 mRNA and sgRNAs into pig zygotes. This approach offers new opportunities to improve porcine traits for agriculture, including the generation

of pigs resistant to specific disease. Because the pig resembles the human closely in size, anatomy and physiology, CRISPR/Cas9 technology has also a high potential to develop porcine models for biomedicine. Moreover, the generation of patient-derived human induced pluripotent stem cells makes possible the potential translation of stem-cell related studies into the clinic. As a strategy, the combined use of CRISPR/Cas9 and interspecies blastocyst complementation technologies could provide a relieve tool for the development of functional human cells in pig host. Since these technologies require high-quality zygotes and blastocysts it is needed to re-invent the *in vitro* embryo production conditions to decrease the major problem of the current IVF systems, the polyspermy, and to find appropriate supplementary factors for culture media. This presentation discusses advances on porcine IVM-IVF-IVC to be applied to newly proposed RT. (Supported by Seneca (19892/GERM/15) and MINECO-FEDER (AGL2015-69735-R))

WORKSHOP 2 ENDOMETRIAL FUNCTION AND PATHOLOGY

WS 2.1 | Immuno-endocrine regulation of uterine function: modulatory effect of endogenous and exogenous steroids

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The endometrium is a complex tissue, which is mainly regulated via cyclic changing patterns of ovarian steroid hormones 17β-estradiol (E2) and progesterone (P4). These hormones are known as crucial regulators of cell differentiation, angiogenesis, morphogenesis, and endometrial physiology. Several other factors, such as prostaglandins (PGs) and cytokines have also been shown to participate in endometrium function regulation throughout the estrous cycle and pregnancy. Phytoestrogens are similar to E2, due to the presence of phenolic rings in their structure, which enable their binding to estrogen receptors. Since phytoestrogens may compete with the endogenous estrogens, they may act as endocrine disruptors and subsequently influence several endocrine mechanisms. Phytoestrogens absorption, biotransformation, metabolism, and bioavailability depend on various factors. A number of studies have reported the ubiquitous effect of phytoestrogens on developmental abnormalities in female reproductive tract and fertility disorders, mostly in ruminants. Diverse effects of phytoestrogens may not only depend on different cell signaling pathways, but also on enzymes involved in PGs production. Our last studies have shown that phytoestrogens may act as endocrine disruptors in mares, by altering endometrial function, and hormone profiles in the estrous cycle. Phytoestrogens differentially modulate PGs synthesis in a cell-specific and species-dependent manner, by modifying $PGF_{2\alpha}/PGE_2$ ratio in bovine and equine endometrial cells. New data concerning intracellular, receptors and molecular mechanisms that are involved in phytoestrogens actions will be discussed from a scientific, as well as a practical perspective. (Studies supported by NSC Grant 2011/02/A/NZ5/00338.)

WS 2.2 | Function of conceptus-derived factors in endometrial gene expression during the implantation period in pigs

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For successful establishment and maintenance of pregnancy well-coordinated interactions between the implanting conceptus and the maternal uterus are required. In pigs, the implantation process begins on around day 12 of pregnancy. During the implantation period the conceptus undergoes dramatic morphological and functional changes, and produces various secretory factors, including estrogens and cytokines, interleukin-1β (IL1B) and interferons (IFNs). These conceptus-derived factors induce maternal recognition of pregnancy by changing endometrial function to become receptive to the implanting conceptus. Estrogens are known to act as the maternal recognition of pregnancy signal, which redirect the secretory pattern of prostaglandin $F_{2\alpha}$ from the uterine vasculature to the uterine lumen in the endometrium, and increase expression of many endometrial genes at the time of implantation. IL1B is a pleiotropic cytokine that functions in various biological processes. The role of IL1B of conceptus origin is not fully understood, but it has been shown that IL1B also induces many endometrial gene expression related to prostaglandin production and transport. Subsequently to the secretion of estrogens and IL1B, conceptuses produce IFND and IFNG into the uterine lumen, and they do not have any antiluteolytic activity as shown for IFNT in ruminant. Although the role of IFNs is not much understood, some data indicated that IFNs affect endometrial immune responses during early pregnancy. In this presentation some recent findings on the role of conceptus-derived factors for the establishment of pregnancy in pigs is discussed. (Supported by the Next Generation Biogreen 21 Program (#PJ01119103), Rural Development Administration, Republic of Korea)

WORKSHOP 3 CONTROLLING REPRODUCTIVE PERFORMANCE IN BEEF CATTLE HERDS IN EXTENSIVE PRODUCTION SYSTEMS

WS 3.1 | Maximizing the reproductive efficiency of beef cow herds

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Attaining a high level of reproductive efficiency in beef cows is underpinned by producers being aware of key targets throughout the production cycle and demands high technical competency. The lifetime productivity of the beef female commences before puberty and is dictated subsequently by the duration of the post-partum intervals, pregnancy rate and inter-calving intervals. Puberty in heifers is a consequence of genotype and both pre and post-weaning nutrition. Early puberty is essential to achieving first calving at two years of age. In calved heifers and mature cows, the onset of ovarian activity post-partum is a key event dictating the calving interval. This is the product of pre-partum nutrition with a recommended target BCS at calving of 2.5–3.0 (scale 0–5.0). There is evidence of modest genetic influences on this trait. Following the initiation of cycles post-partum, conception and subsequent pregnancy rate are largely a function of bull fertility in natural service herds and heat detection and timing of AI in herds bred by AI. Cows and heifers should be maintained on a steady plane of nutrition during the breeding season, but the contribution of significant excesses or deficiencies of nutrients including protein and trace elements is likely to be minor where adequate pasture is available. Progestgen-based ovulation control programs, combined with equine chorionic gonadotropin produce pregnancy rates of 50–60% following a fixed-time AI. The genetic improvement of cow fertility is a long-term strategy and will not replace the need for a high level of technical efficiency and management.

WS 3.2 | Beef cow herd reproductive performance in the extensive Mediterranean scenario

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The environmentally sustainable and economically efficient Mediterranean beef cow extensive production system relies on the adjustment of the cow reproductive cycle to forage availability. In the south of Portugal, climatic conditions restrict growth and availability of good-quality grass to the period from January to May. Food supplementation is often needed during the other months. Body weight (BW) and body condition score (BCS) may experience significant fluctuations throughout the reproductive cycle (up to 25% BW loss). Resistance to BW loss keeping acceptable fertility is genetically driven. Adequate BCS at calving is essential for normal postpartum uterine involution, early onset of ovarian activity, restoration of fertility and nursing capacity. A ≥ 3.5 BCS at calving may insure coping with BW loss. Cows calving from late winter to early summer achieve this goal with the lowest food supplementation, starting the breeding season with a positive/plateau BCS dynamic. Cows calving from the autumn to early winter need supplementation, otherwise the calving interval is increased. Control of reproductive efficiency in the extensive production system must minimize animal handling. Control of BCS in key points of the reproductive cycle enables adjusting feeding levels to target optimal BCS. Pregnancy diagnosis allows timely culling of open cows and projection of calving distribution within the calving season. Control of bull fertility and of venereal and other infectious diseases causing reproductive failure is a major step of veterinary beef cow herd practice. (Funding: UID/CVT/00276/2013)

WORKSHOP 4 ARTIFICIAL INSEMINATION IN THE BITCH WITH FRESH, CHILLED AND FROZEN SEMEN

WS 4.1 | Artificial insemination in the bitch with fresh, chilled and frozen semen

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Preservation of canine semen aims at maintaining motile, viable and healthy spermatozoa able to produce sustainable embryos resulting in the birth of healthy pups, preferably in a number expected for the breed. Semen treatment depends on the source of sperm, how is collected, selection procedure, and on the site of semen deposition by artificial insemination (AI). Processing, i.e. dilution, centrifugation, cell sorting or cryopreservation, damages sperm plasma membrane integrity and sperm chromatin. Semen handling from collection to use must be hygienic during benchtop and cool storage, and at AI. The procedures and extenders used depend on the time perspective of the preservation, i.e. AI directly after collection, hours later, days later or years later. Sperm microencapsulation offers new perspectives for timing of AI. For AI with freshly collected semen dilution is not necessary, and vaginal deposition is feasible. However, if transported or chilled, dilution is essential. Canine ejaculates with sperm concentrations within normal range are diluted to 1:3 to 1:6 to a concentration of 50–100 million sperm per ml. Dilution rate depends on the number of doses required and quality of the semen. For fresh semen 50 mill/ml may be sufficient but for frozen semen higher concentration is needed, typically 100–200 mill/ml. The volume inserted may be important depending on intrauterine (IU) or vaginal deposition. Too large IU volumes may cause reflux especially in small breeds. IU AI is mandatory to get consistently high fertility results with frozen semen.

WS 4.2 | SpermVital immobilization technology – approaches applicable for canine semen?

E Kommisrud

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Timing of artificial insemination (AI) relative to ovulation is of utmost importance to obtain high pregnancy rates. The SpermVital®

immobilization technique was developed to prolong shelf life of spermatozoa and hence make timing of AI less critical. The sperm cells are embedded in a homogenous gel network made of calcium alginate gel, representing a fundamentally different approach than previous reported encapsulation techniques leaving the cells in a liquid core surrounded by a membrane. Upon AI or application in artificial media, the gel will gradually dissolve, releasing sperm cells over a prolonged period. The technology is well documented in cattle, *in vitro* and *in vivo*, and has successfully been combined with both liquid preservation and cryopreservation. Immobilization combined with cryopreservation prolongs sperm shelf life in the reproductive tract after AI, while the combination with liquid preservation additionally prolongs sperm shelf life prior to AI. *In vitro* models mimicking *in vivo* conditions have demonstrated that gels with immobilized sperm may dissolve both fast and slow, depending on the alginate gel and the media composition. Several studies have been conducted to determine dissolving time in relation to prolonged survival of both immobilized and released sperm cells. Quality control *in vitro* includes e.g. viability, acrosome integrity and DNA integrity. *In vivo* AI field trials, conducted on several thousand heifers and cows have demonstrated that immobilized spermatozoa can be used at normal as well as early timing of AI relative to ovulation. The technology has also been tested and demonstrated to be applicable in the ovine, equine and porcine species.

WORKSHOP 5
HEALTH CARE OF NEONATES

WS 5.1 | Investigation of bovine fetopathy/ abortion – can we improve?

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Diagnostic rates for bovine fetal mortality (abortion) have not improved significantly in recent years. Diagnosis of bovine fetopathy involves an investigative triad involving the farmer, veterinary practitioner and the veterinary diagnostic laboratory; each can contribute to better investigative outcomes. From the farmer's perspective under and late reporting and placental non-submission are significant issues which can be improved. Veterinary practitioners can be more pro-active in awareness raising and client education regarding index case reporting, norm- or criteria-referenced investigative thresholds, proforma anamnesis recording and advising clients of possible causes of fetal loss. In addition, with closures of many government veterinary diagnostic laboratories, veterinary practitioners may need to upskill in fetal pathology and consider providing this new service for their clients. Veterinary diagnosticians can review the investigative protocols they currently adopt to see where they can improve their investigative / necropsy / sampling / testing SOPs. Issues of under- and over-triage and intra-institutional harmonisation can also be reassessed. Additionally, the educational role of veterinary diagnosticians to both veterinary practitioners and farmers can contribute substantially to improved field investigations. At an international level, a recent Delphi survey highlighted the current lack of homogeneity in the criteria used to define cause of death for bovine fetal mortality and the need for such standardisation (Mee et al. 2013, Reprod Dom Anim 48:651–659). Workshops such as this one on healthcare of the neonate can contribute to addressing these issues and act as a catalyst for future collaborations amongst participants.

WORKSHOP 6
ASSISTED REPRODUCTION IN CATS

WS 6.1 | Assisted reproduction in cats

E Axnér

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Assisted reproductive technologies (ART) in cats have mostly been developed with the domestic cat as a model for wild felids. However, especially the more basal techniques such as semen preservation and artificial insemination would be useful also for breeding pedigree cats if efficient methods for routine application could be developed. Kittens have already been born after insemination with fresh as well as frozen-thawed semen. Difficulties include a relatively low success rates, the low sperm numbers and the high proportion of morphologically abnormal spermatozoa produced by many cats, as well as the technical challenge to perform non-surgical intrauterine AI. Therefore, routine application of ART is more challenging in cats than in many other domestic species. Promising progresses in increased knowledge and development of improved methods are, however, continuously being published. For rare wild felids, post-mortem sperm collection is a valuable alternative to rescue genetic material. Usually, spermatozoa are collected from the cauda epididymides. To increase the amount of preserved genetic material also more immature spermatozoa may be of interest. It has recently been shown that spermatozoa from the corpus epididymis may have similar properties and fertilizing abilities as spermatozoa from the epididymal cauda and might, therefore, be of interest to preserve for future IVF or AI. The sperm numbers can be increased by including spermatozoa from the corpus. For more immature spermatozoa, from caput epididymis or even the testes, ICSI is likely to be required for production of offspring.

WS 6.2 | Endoscopic transcervical insemination in the queen

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The first successful Artificial Insemination (AI) in the cat was reported by Sojka in 1970. In the following years, different authors have

improved the technique providing substantial advances in feline assisted reproduction. Techniques for Transcervical Catheterization (TC) proposed by different authors have been certainly the most important step to improve the results in AI permitting insemination with a low number of sperm. Considering the particular characteristics of queen's vagina, for each technique a different catheter has been proposed, often associated with a speculum to perform a blind TC (Zambelli et al. 2015, Reprod Dom Anim 50:13–16). In 2015 the first successful endoscopic transcervical insemination in the queen was reported. The cervix was catheterized under endoscopic visualization with a human semi-rigid sialendoscope (length 120 and diameter 1.1 mm) using a 100 mm stainless steel rounded tip needle connected to a tom cat urinary catheter. For estrus induction, 4.7 mg deslorelin subcutaneous implants (Suprelorin; Virbac, Milano, Italy) were used and queens were monitored daily until estrus identification. Once the peak of estrus was identified, ovulation was induced with hCG, 100 IU total dose (Corulon; Intervet Italia Srl, Milano, Italia) and, 5 to 6 days after, serum progesterone was assayed to confirm ovulation. Artificial insemination was performed twice, 24 h and 48 h after hCG administration. Tomcat semen was collected with UrCaPI (Urethral Catheterization after Pharmacological Induction) technique (Zambelli et al. 2015, Theriogenology 84:773–778).

WORKSHOP 7
BOVINE HERD HEALTH MANAGEMENT: ANALYSIS OF REPRODUCTIVE DATA

WS 7.1 | Bovi-Analytics: a platform to educate veterinary students Big Data in dairy cows An initiative to create the veterinary stethoscope version 3.0?

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As in other sectors, Veterinary Medicine is evolving quickly in the 21st century due to new Precision Livestock Farming (PLF) technologies. At the Department of Reproduction, Obstetrics and Herd Health (ROHH) at the Faculty of Veterinary Medicine of the University of Ghent, Belgium, dairy researchers and veterinarians are trying to adapt to the wide range of new technologies. New mobile wearables during animal and herd checks offer valuable tools to increase accuracy of the diagnosis. Furthermore, the amount of data created on a dairy farm exponentially grew over the last decade to 100 MegaBytes each day (milk meters, accelerometers and vaginal or ruminal temperature loggers). However, trying to integrate the widespread data streams from PLF technologies is tempting. Researchers at ROHH are developing an online platform allowing students to have direct access to a wide range of data from dairy farms. Students are able to consult the herd and animals on- and offline, on and off site. The current initiative is focusing on a new tool called Bovi-Analytics to allow student access to herd data using Big Data technology. The proposed framework mainly focuses on efficient co-creation, co-coding and collaboration. The goal of the platform is to increase the student's ability to convert physiological knowledge into applied Veterinary Medicine using Big Data. The workshop will focus on the current experiences of implementing such framework in a large European project called GplusE (www.gpluse.eu).

WORKSHOP 8
ULTRASONOGRAPHIC EXAMINATION OF THE STALLION

WS 8.1 | Advances in Doppler ultrasonographic examination of the stallion

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Doppler ultrasonography has signified an improvement in diagnosis of testicular disorders. Testicular function is particularly susceptible to vascular insult, resulting in a negative impact on sperm production and quality of the ejaculate. Pulse Doppler ultrasound provides several parameters that can be used as indicators of testicular efficiency since significant correlations between them and parameters of sperm production have been determined. Moreover, preliminary studies have shown important correlations among Doppler parameters and sperm quality parameters such as integrity and viability of sperm, mitochondrial activity and DNA fragmentation index assessed by flow cytometry. Early diagnosis of subfertility problems triggered by vascular disturbance would enable implementation of appropriate treatment, hence improving fertility forecasts for stallions. In addition, Doppler ultrasound is an excellent tool to monitor therapeutic outcome after medical treatment such as pentoxyfilline therapy or for follow-up after surgical interventions such as hernioplasty. Furthermore, this imaging modality could be an alternative to invasive procedures traditionally used for diagnosis of sub-fertility disorders such as fine needle aspiration or assays to determine plasma concentrations of hormones. The main problem with this technique is that it suffers from a lack of standardization and reference values. Generally, healthy stallions tend to present high values for EDV, TAMV, TABF and TABF rates, whereas subfertile stallions tend to show high Doppler index values. Doppler ultrasound is being introduced ever more frequently into breeding soundness evaluations of the stallion and should be performed in all stallions with pathologies and abnormalities of sperm analysis. (Funding: IJCI-2014–2167)

WORKSHOP 9 AQUACULTURE

WS 9.1 | Bottlenecks for aquaculture growth

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Aquatic production from fisheries has been stabilizing over the last 10 years. Aquatic production from aquaculture has been growing worldwide for a long time, sometimes close to 10% per year, currently reaching close to 50% of the production through fisheries. It is generally accepted that aquaculture will need to keep growing to meet the growing worldwide demand. There are a couple of features that argues in favor for the stimulation of aquaculture. Most aquatic species have very low feed conversion rate (depending on the species and the way of calculation). Basically FCR is close to that of chicken and lower than for instance for red meat. Also a lot of aquatic production is based on extractive aquaculture systems (mollusks) which extract nutrients and is hence environmentally neutral. Yet aquaculture is also facing a lot of bottlenecks. For aquaculture species higher up in the food chain, marine proteins and lipid are used. Since these sources are limited alternative source will need to be developed to become fully independent from fisheries. In addition the following issues are considered as important research areas: 1) Complete independence from natural stocks through domestication, 2) Improved / more cost-effective seed production, 3) better targeted species selection, 4) Development of more efficient stocks through selective breeding 5) More microbial management for more sustainable production, 6) Better understanding of immune systems in vertebrates and invertebrates, 7) More integrated production systems for plant and animal farming, 8) coastal and off-shore farms of food and energy and finally more attention for integration of restocking activities with fisheries management.

WS 9.2 | Cryopreservation of germ cells for the production of marine species

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Germ cell cryopreservation is a safe method to store and preserve genetic material. Cryobanks in aquatic species were developed with different aims, benefiting fish farming, from management of reproduction to genetic selection of sperm from males with high reproductive value. Research has been conducted on the development of protocols for new/problematic species, for commercial species to improve gamete quality during storage or for conservation purposes. Protocols for sperm cryopreservation were successfully developed in fish and bivalve species, although some cell damage has been identified with relevance to oxidative stress. Nutritional supplementation of breeders can play an important role in this regard. The incorporation of antioxidants was proven to be favorable for sperm quality in gilthead seabream and seabass, especially when quality needs to be reinforced to sustain cryopreservation. Another source of cryobanking material is spermatogonial stem cells. Cryopreservation of testicular cells plays an important role in fish reproductive biotechnology. Cryopreserved spermatogonia can be transplanted between close-related species, differentiating into male or female gametes in the host gonads, allowing the preservation of the all genome. Due to this capacity, spermatogonia xenotransplantation is becoming a useful tool to produce surrogated broodstocks, particularly in species difficult to maintain in captivity, with high age at maturation or with reproductive problems as the Senegalese sole. (This work was supported by COST AQUAGAMETE (FA1205), CRIOBIV and REPLING projects (PROMAR).)

WORKSHOP 10 INTERACTIONS NUTRITION-REPRODUCTION IN SMALL RUMINANT SYSTEMS

WS 10.1 | Interactions between nutrition and reproduction in small ruminants

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Nutrition affects all aspects of reproductive process from onset of puberty to gamete development and successful fertilization, embryo survival and the establishment of pregnancy. The well recognized link between energy balance and reproduction is mediated by a plethora of metabolic hormones, metabolites, neuropeptides and growth factors, acting either through modulation of the hypothalamic GnRH neuronal network or through direct effects at the ovarian level. In seasonal breeding animals, like small ruminants nutrition interacts with photoperiodic signals to affect the seasonality of reproduction. There is also strong evidence that nutrition in utero may impact offspring's later reproductive performance and productivity. Specific outcomes depend on the severity, duration and stage of development when nutritional perturbations are imposed, while sex specific effects are also manifested. Mechanisms underlying reproductive programming are yet unclear, but may include epigenetic modulation of critical genes involved in the control of reproductive function. The goal of the workshop is to bring together recent findings on the effects and mechanisms governing the nutrition-reproduction interactions in small ruminants and to interpret them in the context of animal systems sustainability and efficient productivity.

WS 10.2 | Feeding strategies to improve reproductive efficiency in small ruminants

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Feeding strategies improve reproductive efficiency by enhancing reproductive performance and product yield. Nutritional requirements vary according to different productive and reproductive stages. In small ruminants the type of management (intensive vs. extensive or semi extensive) system is a crucial factor that determines nutritional requirements and feed strategies adopted. Especially in the Mediterranean area, where the majority of small ruminants are maintained in extensive or semi extensive systems, seasonal variations in food availability is another key factor determining reproductive and productive outcomes. In this workshop feeding strategies and techniques that could be successfully and cost effectively applied across a range of different small ruminant production systems, using also alternative local feed sources, will be discussed. With increasing concern focused on climate change, nutritional strategies possess a critical role to improve the reproductive outcome, while at the same time considering the environmental footprint.

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WILEY Reproduction in Domestic Animals

ORAL COMMUNICATIONS

OC 1.1 | Morphological, hormonal and histological modifications induced by immunisation against GnRH in stallions

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This study was conducted to evaluate in 10 adult non breeding male horses the effects of immunisation against GnRH, using the porcine "vaccine" Improvac®, on scrotum, on biomarkers in urine and blood and on tissues in testis. Ten stallions were injected intramuscularly with 1 mL anti-GnRH Improvac® twice at 28 days apart. Blood and urine samples, measurement of total scrotal width (TSW) were done during 8 months. Plasmatic GnRH antibodies titres were measured by ELISA, 8 steroids profiling were performed on urine by gas chromatography coupled to tandem mass spectrometry. After surgical castration, histomorphometric analysis was done from testicles using Johnsen's score. TSW decreased significantly after 2nd injection in 9/10 horses. For those 9 horses, concentrations of the 8 steroids in urine began to decrease from 1st injection to reach very low values during all the study. Anti-GnRH antibodies were detected 2 weeks after 1st or 2nd injection with individual variation, their levels reached maximum value 2 months after 1st injection and became indictable at different times among the 9 horses, 2/9 had yet antibodies 8 months after 1st injection. In comparison with testicles from control horses, histological analyses of testicles of treated horses showed a significant atrophy with a variable germinal hypoplasia without sign of non-reversible lesions. In this study, immunisation against GnRH induces in 9/10 horses dramatic modifications of scrotal morphology and steroidal secretion correlated with antibodies levels. However the intensity and the duration of effects show a large individual variability. Histological analyses suggest a reversible effect of this treatment. Further studies will evaluate which effects are induced by booster injections.

OC 1.2 | Identification and quantification of differentially expressed seminal plasma proteins between boar ejaculate fractions

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Boar sperm from sperm rich ejaculate fraction (SRF) show different reproductive performance than those from entire ejaculate, even for overcoming sperm technologies such as cryopreservation or sex sorting. These different performances could be related to seminal plasma (SP) composition, particularly in proteins. This study evaluated differences in the SP-proteome of the two main boar ejaculate fractions: SRF and post-SRF. Five SP-pools from 5 boars (4 ejaculates/pool) of each ejaculate fraction were analyzed. The SP-samples were subjected to a combination of SEC, 1-D SDS PAGE and NanoLC-ESI-MS/MS and identified SP-proteins were quantified by sequential window acquisition of all theoretical spectra (SWATH; Gillet et al. 2012, Mol Cell Proteomics, 11:O111.016717) using a spectral library (Deposited in ProteomeXchange <PXD003579>). A total of 447 SP-proteins (FDR≤1%, Confidence≥95%) were identified and quantified in the two-ejaculate fractions. No qualitative differences in the identified SP-proteins were found between both ejaculate fractions, but 34 SP-proteins were differentially expressed ($p < 0.05$) between SRF and post-SRF. Sixteen SP-proteins were over-expressed and 18 down-expressed in SRF regarding to post-SRF. In conclusion, the boar SP-proteome show quantitative differences between the two main ejaculate fractions: SRF and post-SRF. (Supported by Seneca Foundation (19892/GERM/15) Murcia, Spain; and FORMAS, Stockholm, Sweden)

OC 1.3 | Changes in flow Doppler indexes of the testicular artery in peri-pubertal donkeys

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Testicular growth and onset of spermatogenesis are dependent on the hormonal changes occurring during the peri-pubertal period. Puberty is achieved when a threshold number of motile spermatozoa in the ejaculate is present. Doppler ultrasonography allows to study testicular perfusion, which might be related to testicular function: in humans, resistivity index is lower in adults compared to pre-pubertal children. The aim of this study was to evaluate in peri-pubertal donkeys some blood flow indexes of the testis. Four Amiata donkeys were subjected to flow Doppler examination of the testicular artery at the sopratesticular (STS) position every two months between 8 and 24 months of age. Pulsatility and resistivity indexes (PI and RI, respectively) were evaluated. Semen collection was attempted every 20–30 days from 12 months of age, and puberty was considered attained when an ejaculate with >50 × 106 spermatozoa with >10% motility was collected for the first time. Both indexes were affected by age ($p < 0.01$): PI was significantly higher at 14 months compared to 24 months (median[IQR]: 4.76[1.77] and 1.69[0.21], respectively, $p < 0.01$), and the same occurred for RI at 12

and 14 months compared to 24 months (1.07[0.03] and 1.08[0.16] versus 0.77[0.02], p < 0.01). Puberty was reached by one donkey at 19 months and by the other three at 20 months. Mean RI of right and left STS evaluations was <1 in none, two, three and four donkeys at 14, 16, 18 and 20 months, respectively. These results, although on a small number of animals, showed that puberty was associated with a decrease of PI and RI, as described in humans.

OC 1.4 | Testicular hemodynamic changes in ram according to the ejaculation and the season of the year

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Color Doppler ultrasonography is used routinely to examine blood flow in the testicular artery, in the diagnosis of testicular pathologies to predict spermatogenesis and as a technique for guiding testicular sperm extraction in various species but not in the ram. The object of this study was to evaluate the testicular hemodynamic changes using pulsed wave Doppler sonography and to establish mean values for Doppler measures of blood flow in the testicular artery of the ram during and outside breeding season including the impact of ejaculation. Both testes from each of 10 mature, testicular pathology-free rams were examined using color and pulsed wave Doppler ultrasound with a linear array 3–13 MHz probe. Peak systolic velocity (PSV), end diastolic velocity (EDV), resistive index (RI), and pulsatility index (PI) of the testicular artery were measured in during and outside breeding season, both before and after ejaculation. There were no differences (p > 0.05) in testicular echotexture between images taken from left and right testes. All blood flow measures did nodiffer between left and right testes (p > 0.10). The different parameter values (mean ± SEM) were: PSV (cm/s)28.45 ± 0.53, 33.78 ± 1.24; EDV (cm/s) 7.96 ± 0.26, 8.71 ± 0.42; RI 0.71 ± 0.01, 0.71 ± 0.01; PI 11.38 ± 0.37, 1.51 ± 0.49 during and outside the breeding season, respectively. PSV (p < 0.0001) as well as PI were higher (p < 0.05) during the nonbreeding season. There were no significant differences (p > 0.05) for EDV and RI measurements between seasons as there was no differences for any of the parameters studied in respect to ejaculation. The studied measurements may provide useful reference values for clinical evaluation of rams.

OC 2.1 | Urine pregnancy-associated glycoproteins (PAG) in embryonic loss diagnosis in dairy cattle

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The aim of this study was to determine the urine pregnancy-associated glycoprotein (PAG) concentration in pregnant and open cows and their relationship with the ability to maintain pregnancy. For this purpose, combined measurements of PAG concentrations in urine and plasma and transrectal ultrasonographic examination of the conceptus were performed. The collected data were derived from 46 multiparous dairy cows. Animals were divided into 4 groups: nonpregnant cows (NP; n = 11), cows with demonstrated late embryonic mortality (LEM; n = 10), pregnant cows that aborted after 105 days of gestation (PA; n = 6), and pregnant cows that gave birth to healthy calves (PB; n = 19). Transrectal ultrasonographic examination, blood and urine samples were obtained on days 0 (AI day), 14, 21, 28, 35, 49, 63, 77, 91 and 105 of gestation. Radioimmunoassay (RIA −706) was used to determine cPAG (caprine PAG) concentration in blood and urine. Confirmed pregnancies were monitored to 105th day of gestation. At parturition the data on labor were collected and pregnant cows were divided into two groups PA and PB. During pregnancy (groups PA and PB), PAG concentrations in urine showed only a weak correlation (Pearson's correlation coefficients: r = 0.1) with pregnancy length. However, we found a statistically significant difference (p < 0.05) in the urine PAG concentration in group PA versus PB. In cows in which an abortion occurred after 105 days of gestation, the urine PAG concentration was higher than in cows which gave birth to healthy calves. Furthermore, there was a statistically significant difference (p < 0.05) in the urine PAG concentrations between groups PB and LEM from 7th week after insemination, as was confirmed by ultrasound examination.

OC 2.2 | Expression of VEGFC and its receptors in the porcine uterus during early pregnancy

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Early pregnancy is associated with morphological and functional changes within the uterus, accompanied by angiogenesis, increased vascular permeability and activation of immune tolerance. Lymphatic system is a key regulator of fluids homeostasis and is involved in adaptive immunity in uterus during implantation and further embryo development. VEGFC is proangiogenic/prolymphatic factor which activity is mediated by two tyrosine kinase receptors: Flk1 and Flt4. The aim of this study was to determine the expression of VEGFC system in porcine endometrium. Endometrium was collected from gilts on days 8, 10, 12, 14 of estrous cycle and early pregnancy (n = 5/group/day). The expression of mRNA was determined by qPCR, followed by statistical analysis using Kruskal-Wallis ANOVA and protein by immunofluorescence (IF). There were differences of VEGFC mRNA expression between d.8 and d.12 of estrous cycle (p < 0.05). During early pregnancy the increase in quantity of VEGFC transcripts was observed on d.14 (p < 0.05) vs. d.10 as well as to corresponding day of the estrous cycle. Differences between d.8 and d.12 of estrous cycle (p < 0.05) in Flk1 mRNA expression was also noticed. No changes in Flt4 transcripts

were observed. VEGFC IF was mainly localized in the luminal and glandular epithelium, endothelium of spiral arteries, in the trophoblast and fetal blood vessels. Flk1 slight IF was observed only in the apical pool of trophoectoderm and in the cytoplasm of luminal epithelium. Strong IF for Flt4 was found in the trophoectoderm and weak in the luminal and glandular epithelium. This study for the first time showed the presence of VEGFC in the porcine endometrium. Its differential expression suggests that VEGFC system may play a role during early pregnancy.

OC 2.3 | Immunization against GnRH in adult cattle: a prospective field study

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Suppression of cyclic activity in cattle is often desired in alpine farming and for feedlot cattle not intended for breeding. These cows - when untreated - cause a disturbance in the herd and are often pregnant when slaughtered. The aim of the present study was to evaluate the duration and reversibility of an active immunization against GnRH in Eringer cattle spending the summer on the Alps. Healthy Eringer heifers (n = 13) and cows (n = 64) which had been unsuccessfully inseminated until 5 weeks before the Alping season, were vaccinated 2 times 31.5 days apart (med; 25%/75% quartiles: 28/35 days) with an anti-GnRH vaccine (Bopriva®, Pfizer Animal Health, Parkville, Australia). In all animals, blood was sampled at 1st examination, at 1st and 2nd vaccination and in 1st heat after cycle blockade. Median GnRH antibody titer was 17'974 at the 2nd vaccination and decreased to 10'648 when the cows were back from the Alps in autumn. Median duration of cycle blockade was 93 days (25%/75% quartiles: 91/191 days). Cox Regression including the variables corpus luteum and progesterone value at 1st vaccination, and interval between the 2 vaccinations revealed a significant shortening of cycle blockade ($p < 0.0001$) if the 2nd vaccination was done earlier than 28 days after the 1st vaccination. A total of 35 cows got pregnant within 212 days (med; 25%/75% quartiles: 198/251 days) after the 1st vaccination, and 34 cows were slaughtered within 39 days (med) after the Alping season. In addition, 8 cows' owners indicated "infertility" as the reason for slaughter. We can recommend double vaccination with Bopriva® for a reversible cycle blockade of 3 months. Further studies using triple vaccination will be needed if a longer cycle blockade is demanded.

OC 2.4 | Serum equine alpha-fetoprotein (AFP) levels as an indicator of twin pregnancy in the mare

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Managing twin pregnancy is challenging in equine practice and is based upon the gestational age of recognition. Until now, no reliable blood test is available for twin diagnosis. In 1991, Sorensen et al. have reported that maternal serum alpha-fetoprotein (AFP) levels in the Thoroughbred are a sensitive tool for predicting pregnancy abnormalities, such as twins. In a recent study, we evaluated Lipizzaner mares with normal pregnancies and with pregnancy loss and AFP was proven to be a good indicator of these conditions. Reference values have only been established for AFP concentration in equine serum in Lipizzaners, with a reference value of 23.68–122.18 pg/ml in the normal group and 115.52–188.48 pg/ml in the pregnancy loss group with 95% Confidence Interval, respectively (Vincze et al. 2015). In the present study, two mares (Hungarian Sport Horse breed) with twin pregnancies were evaluated. After twin pregnancies have been confirmed by ultrasonography, four blood samples (during pregnancy day 246, day 256 and 7 and 14 days post partum) were taken, and sera were frozen for AFP measurement. Mare 1 had live twin foals but both foals died before 6 months of age, Mare 2 aborted. AFP concentrations have been measured with a commercially available ELISA test (MybioSource Ltd., San Diego, CA, USA). All of the measured "twin" AFP concentrations (117.7–133.5 pg/ml) were in the range for Lipizzaners of the complicated group published previously. Although the number of blood samples was limited, an elevation in AFP levels could be detected based upon previous data; and the role of AFP-testing as a possible diagnostic tool in twin- and complicated pregnancies has been confirmed in this study. Actual concentrations of AFP in equine twin pregnancies were measured for the first time.

OC 3.1 | Influence of an extended lactation on the fertility of high-yielding dairy cows

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Aim of this study was to compare the reproductive performance of high-yielding dairy cows (11 488 kg milk/305d) assigned to extended lactation periods. On day 40 post-partum, cows without clinical endometritis were randomly allocated to groups with a voluntary waiting period (VWP) of either 40 days (G40, N = 135), 120 days (G120, N = 135) or 180 days (G180, N = 131). For 40 days after the VWP cows were bred during natural estrus. Cows not showing estrus within 40 days were bred after synchronization with an Ovsynch protocol. Comparisons were performed with chi-square or Kruskal-Wallis test. A higher percent of cows exhibited estrus and conceived within 40 days in the G120 and G180 compared to G40 (88.9% and 56.4% for G120, 90.8% and 56.5% for G180, 70.4% and 34.1% for G40, respectively, $p = 0.001$). First artificial insemination (AI) success was greater in G120 and G180 compared to G40 (48.9% and 49.6% vs. 36.6%, respectively, $p = 0.05$). Cows in the G40 needed more AIs (1.8 ± 0.1) and more days from the end of the VWP (52 days) to conceive than cows of the G120 (1.6 ± 0.1, 28 days) and of the G180 (1.5 ± 0.1, 24 days) ($p < 0.05$). There was no difference ($p > 0.05$) between the 3 groups regarding percent of cows that did not conceive after 3 AIs, percent of cows that lost the conceptus between

d40 and d90 post AI and percent of cows culled within 305 days. Conclusively, the extension of the lactation period in high-yielding dairy cows can improve reproductive performance and reduce the use of hormonal interventions.

OC 3.2 | Fertility of lactating Holstein cows after synchronization of ovulation and timed artificial insemination versus artificial insemination after detection of estrus at a similar DIM range

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Our objective was to compare pregnancies per artificial insemination (P/AI) after synchronization of ovulation and timed artificial insemination (TAI) with artificial insemination (AI) after detection of estrus at a similar DIM range. Lactating Holstein cows (n = 542) were randomly assigned to receive their 1st TAI after a Double-Ovsynch protocol (TAI) or to receive 1st AI after estrus induced using $PGF_{2\alpha}$ (Estrus). All cows received their 1st insemination from 74 to 81 DIM. Estrus cows not inseminated within 7 days after the last $PGF_{2\alpha}$ treatment (n = 58) were excluded from the analysis of P/AI but were included in the calculation of insemination rate. Pregnancy status was assessed 33 ± 3 days after AI, and reconfirmed 63 ± 3 days after AI. Data were analyzed by ANOVA and logistic regression using MIXED and GLIMMIX procedures of SAS. DIM at AI did not differ between treatments (77.0 ± 0.2 vs. 76.9 ± 0.3 for TAI vs. Estrus). More ($p < 0.01$) TAI cows received AI within 7 days after the VWP than Estrus cows (100% vs. 78%). At 33 days after AI, TAI cows had more ($p < 0.01$) P/AI than Estrus cows (51% vs. 37%). At 63 days after AI, TAI cows had more ($p = 0.02$) P/AI than Estrus cows (46% vs. 34%), and pregnancy loss from 33 to 61 days after AI did not differ between treatments (11% vs. 4% for TAI vs. Estrus). In conclusion, synchronization of ovulation and TAI for first service increased the percentage of cows inseminated within 7 days after the VWP, and TAI cows had greater fertility at first service than Estrus cows at a similar DIM range. (Supported by USDA Hatch project 231440 and CEVA Sante Animale)

OC 3.3 | *Trueperella pyogenes* isolated from a cow with clinical endometritis showed a higher growth rate and increased mRNA expression of virulence factors compared with a strain isolated from a healthy cow

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The ability of cows to defend against *Trueperella pyogenes* in the uterus depends on host innate immunity and virulence of invading bacteria. This study aimed to compare growth rate and mRNA expression of virulence factors of two *T. pyogenes* strains. One strain was isolated from the uterus of a postpartum dairy cow with clinical endometritis (TP1) and one from a healthy uterus (TP2). Both strains were grown in BHI broth at starting OD600 of 0.05. The OD600 were measured every 2 h for 24 h. At 6, 12 and 24 h, bacteria were harvested, total RNA was extracted and subjected to RT-qPCR. Bacterial growth curve showed a higher growth rate of TP1 than of TP2 after 6 h. Both strains reached their plateau phase after 18 h. mRNA expression of cbpA and fimE were only found in TP1, whereas nanP was only expressed in TP2. TP1 showed higher expression of nanH and fimG than TP2 after 6 h. Virulence factors were expressed time-dependently in TP1 but not in TP2. Both strains expressed PLO and caused death of >95% of endometrial epithelial cells in vitro after 16 h. In conclusion, *T. pyogenes* recovered from inflamed uterus grew faster and expressed virulence factors at higher level than a strain isolated from a healthy uterus. Expression of these virulence factors in a time-dependent manner may reflect their importance in pathogenicity. (Supported by DFG (GA 1077/5-1) and Erasmus Mundus)

OC 3.4 | Effect of diet on energy balance and reproductive performance in primiparous Holstein and SRB cows

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The objective of this study was to investigate the effect of diet on energy balance (EB) and reproductive performance in primiparous Holstein and Swedish Red cows (SRB). A total of 44 cows (22 Holstein, 22 SRB) kept in a loose housing system were included in the study. The control group (HE, n = 23) was fed a diet for high producing cows (target 35 kg/day Energy-Corrected Milk, ECM). The lower feeding intensity (LE, n = 21) was achieved by giving −50% concentrate to target 25 kg/day ECM. Diets were implemented one month before calving and cows were followed from calving to 17 weeks postpartum (pp). The weekly EB was calculated using NorFor. Mixed linear models were used to analyze data (SAS 9.3; proc mixed). There was a large individual variation in EB, however Holstein cows tended to have lower mean EB than SRB cows (−4.67 ± 15.7 MJ vs 0.69 ± 14.2 MJ; $p = 0.078$). In early pp (within days 5 and 12) Holstein cows were in a deeper negative EB than SRB cows ($p < 0.001$), tended to be lower between Days 13 and 20 postpartum ($p = 0.08$), subsequently the curves met at Day 98. There was a tendency for a lower EB in the LE diet group between Days 45 and 52 pp ($p = 0.07$). No significant effects of diet or breed were found on % Pregnant at 1st AI. To conclude, despite trends were found for differences in EB between HE and LE diet groups, the stronger Negative

EB status in the LE diet group was not associated with lower reproductive performance. (Financed by "PROLIFIC" EU grant N°311776)

OC 4.1 | Progesterone addition to oocyte maturation medium enhances homologous sperm-zona pellucida binding in the equine species

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Equine in vitro fertilization (IVF) is not a commercial procedure due to its low repeatability. In the horse, oocytes are generally retrieved as germinal vesicles and matured in vitro prior IVF. Equine follicular fluid composition largely varies in vivo during the follicular wave prior to ovulation, being estradiol and progesterone the main changing steroids. The aim of the present work was to assess if progesterone and/or estradiol supplementation during equine oocyte in vitro maturation (IVM) influence homologous sperm-zona pellucida (ZP) binding. Germinal vesicles were retrieved and placed in culture in 500 µl of TCM-199 with 25 mM bicarbonate, 10% FBS and 5 mU/ml of FSH (base medium or BM; n = 62), in BM supplemented with 100 ng/ml progesterone (BMP; n = 52), in BM added with 1 µg/ml of estradiol (BME; n = 53) or in BM supplemented with both hormones (BMEP; n = 55) in a humidified atmosphere of 5% CO_2 in air at 38.5°C for 30 h. After IVM, oocytes were denuded and co-incubated for 2 h with equine sperm incubated in a capacitating medium for 4 h. An ANOVA on ranks followed by a Dunn's post hoc test was used to compare groups. The Mean ± S.E.M. number of sperm bound per oocyte and treatment were as follows: BM = 82.4 ± 7.7; BME = 95.8 ± 7.4; BMP = 125.1 ± 12 and BMEP = 108.7 ± 25. Statistically significant differences were found only between BM and BMP treatments ($p < 0.05$). Thus, progesterone addition during IVM seems to affect equine ZP structure increasing sperm binding ability.

OC 4.2 | Notch and Wnt interplay in the regulation of the pace of embryo developmental kinetics

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Mammalian embryo development involves timely coordinated cell proliferation and differentiation events. Notch and Wnt are signaling pathways that synergistically coordinate cell fate decisions in adult and embryonic scenarios. The aim of this work was to evaluate the interplay of these pathways in early embryo development. Female CD1 mice were superovulated and, from embryos collected at 2.5 days post-coitum (dpc), 8-16-cell embryos were randomly allocated to one of 4 groups: 1) Control; 2) DAPT (canonical Notch inhibitor); 3) DKK1 (canonical Wnt inhibitor); and 4) DAPT+DKK1. Embryos were in vitro cultured in KSOM until 4.5 dpc and their development evaluated at 3.5, 4.0 and 4.5 dpc. 3.5 dpc embryos were processed for gene transcription analysis by qRT-PCR. Developmental kinetics was slowed by DAPT at 3.5 and 4.0 dpc and fastened by DKK1 at 4.0 dpc. DKK1 fastened development at 4.5 dpc, compared to DAPT. Double blockade slowed developmental kinetics at all time-points and decreased hatching rates at 4.0 dpc. Transcription of Notch genes Jagged1 (ligand) and Hes1 (effector) was altered in DAPT, DAPT+DKK1 and also in DKK1 treated embryos. Transcription of Wnt3a (Wnt ligand) increased in DAPT+DKK1 but not in DKK1 blastocysts. Transcription of SOX2 increased in compact morulae of all treated groups and decreased in DAPT blastocysts. In conclusion, Notch and Wnt pathways interplay in pacing embryo developmental kinetics and coordinate proliferation and differentiation in preimplantation embryos. (Funding: EXPL/CVT-REP/1485/2012; UID/CVT/00276/2013)

OC 4.3 | Regulation of focal adhesion pathway genes in bovine preimplantation embryos

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Focal adhesion (FA) refers to extracellular matrix mechanical linkage with intercellular actin bundles and is involved in several biological processes. The regulation of FA is vital to maintain normal embryonic growth and development. We hypothesized that supplementation of in vitro culture media with different growth factors affects embryo development via regulation of FA pathway. For that, embryos after IVF were cultured in fatty acid free SOF media (G1), SOF + epidermal growth factor (EGF) (10 ng/ml) (G2), SOF + hyaluronic acid (HA) (1 mg/ml) (G3) and SOF + EGF + HA (G4). Following IVC until day7&8 blastocysts were used for mRNA, DNA methylation and protein analysis of FA related genes. Results indicated that EGF and/or HA affected mRNA and protein expression of COL1A2, COL4A1, VCL, FAK, PTEN, RAC1, PAK4, ACTG1, EGFR, HMMR and CD44 genes, which were highly abundant in G4 blastocysts. Moreover, the addition of HA enhanced embryo development rate, cryotolerance, and total cell count with lower apoptotic cells. To assess the effect of EGF and HA on FA pathway pre and post embryo genome activation, embryos were transferred from G1 to G4 culture media at 4-cell and 16-cell stage and reciprocal. The mRNA level of FA genes was higher with the supplementation of EGF + HA at 4-cell until 16-cell stage. Furthermore, CpG islands of COL1A2, COL4A1 and RAC1 promoter regions were differentially methylated due to supplementation of EGF and HA. In conclusion, the phenotypic changes observed in embryos from various supplements were associated with changes in methylation and subsequent alteration on transcript abundance of FA genes.

OC 4.4 | Immunolocalization of prolactin receptors in the equine oviduct in various stages of the estrous cycle and anestrus

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Prolactin (PRL) has been demonstrated as a critical factor for early embryo development and implantation in mammals. Its action on target cells is conditioned by the presence of specific prolactin receptor (PRLR). The aim of the study was to identify PRLR in mare's oviduct and compare immunolocalization of PRLR in the ampulla and isthmus during follicular and luteal phase of estrous cycle, as well as anestrus. Tissue samples were collected post mortem from 14 adult mares during follicular phase (n = 5), luteal phase (n = 5) and anestrus (n = 4). After that, oviducts were dissected free of mesentery, and cross sections of the ampulla and isthmus were obtained. Immunohistochemical (IHC) staining was performed with commercially available mouse anti-prolactin receptor antibody. EnVision System was used for visualization. The most intensive immunoreactivity for PRLR was observed in epithelium of ampulla comparing with isthmus during follicular phase of the estrus cycle. Although signaling was also noted in muscular layer. In follicular phase IHC reaction was observed mainly in cell cytoplasm, less in nuclei. In luteal phase staining intensity decreased, but still dominated in oviduct ampulla comparing with isthmus. It was observed more in cell nuclei, than in cytoplasm. In anestrus decreasing immunoreactivity was observed in isthmus epithelium (cytoplasm). It remained intensive in epithelial cytoplasm of ampulla. The strongest expression of PRLR in oviduct ampulla during follicular phase suggests strategic role of prolactin in equine fertilization as it was reported in rodents. Moreover, it may have variety of functional and modulating effects in horse oviduct.

OC 5.1 | Sperm motility is regulated by Notch signaling in the epididymal epithelium

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Sperm sequential maturation changes are modulated by the epididymal epithelium and require a finely tuned gene expression. The Notch cell signaling pathway is a major regulator of cell fate decisions. This study was designed to evaluate the role of Notch signaling in the mouse epididymis. The transcription and expression of Notch components (NC; Notch1-3, Dll1, Dll4, Jagged1) and effectors (NE; Hes1-2, 5) showed a specific and dynamic pattern along the epididymis and vas deferens (n = 4). Nuclear detection of NE indicates that Notch signaling was active. NC (but not NE) were identified in the cytoplasmic droplet of sperm and within exocytic vesicles in a dynamic pattern along the epididymal lumen. A purified population of these vesicles (epididymosomes) from different epididymal segments was obtained from other 4 mice, and dot blot analysis confirmed the presence of NC within them. Epididymosomes may allow Notch signaling at distance from epididymal epithelial cells to sperm. In vivo blockade of Notch signaling following administration of a Notch inhibitor (DAPT) during 13 days (n = 6) and 43 days (n = 12) induced significant changes in NE transcription levels in the epididymis (13 days: Hes1 decrease, 0.63 ± 0.13, p = 0.038); 43 days: Hes5 increase, 2.64 ± 0.31, p = 0.035), disrupted the expression patterns of NC and NE in the epididymal epithelium and in sperm, and decreased sperm motility (13 days: 0.40 ± 0.04 and 43 days: 0.32 ± 0.07 vs. control: 0.59 ± 0.04; p = 0.005 and p = 0.008, respectively). These results prompt for a regulatory role of Notch signaling in epididymal epithelial function and sperm maturation. (Funding: PTDC/CVT/105022/2008; UID/CVT/00276/2013)

OC 5.2 | Effect of supplemental antioxidants in soybean lecithin-based extender on bovine sperm quality after cryopreservation

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The objective of the present study was to assess the effect of two antioxidants supplemented in soybean lecithin (SL)-based extender on post-thaw quality of bull spermatozoa. Semen samples were collected from six Holstein bulls by artificial vagina twice a week at Iranian Progeny Test Center. Twelve different extenders were prepared by the addition of five levels of Vitamin E (VE: 0.1, 0.2, 0.4, 0.6 and 1 mM), or four levels of Glutathione (Glu: 0.5, 1, 2 and 3 mM) to the extender. Another extenders were used as a positive control (Con+: ethanol in extender), a negative control (Con-) and commercial extender (Andromed®). Quality of the spermatozoa was analyzed with CASA for sperm motility, hipoosmotic test (HOST), apoptosis status, mitochondria activity and acrosome integrity. Data were analyzed by GLM procedure using SAS 9.1. The post-thaw sperm motion characteristics of soybean lecithin-based extender were similar to those of Andromed® extender. Progressive motility (PM) and total motility (TM) of soybean lecithin-based extender was not improved by vitamin E and glutathione supplementation. However, the highest percentages of HOST (51.17%), live spermatozoa (73.94%, p ≤ 0.05) and least amount of MDA (0.69 nMol/ml, p ≤ 0.05) were found in semen diluted with soybean lecithin-based extender containing 1 mM glutathione (Glu 1 mM). Also, percentage of spermatozoa with intact acrosome (PSA+) was higher (p ≤ 0.05) in the CON- (76.23%) compared to the Andromed® (67.84%). The differences in RH+ among the three diluents were not significant. We concluded that soybean lecithin based extender containing 1 mM glutathione could be suitable for cryopreservation of bovine sperm.

OC 5.3 | Biological characteristics of spermatozoa and seminal plasma proteins in dogs with benign prostatic hyperplasia

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Benign prostatic hyperplasia (BPH) is a common disorder in male dogs over 5 years of age. Specific seminal plasma proteins (SPPs) related to this condition and their functional role on sperm parameters are important factors for successful preservation of spermatozoa from valuable individuals. The aim of this study was to analyze the differences in SPPs between healthy dogs and dogs with BPH. Radiographic and ultrasonographic imaging was performed for BPH diagnostics on 25 asymptomatic dogs of various breeds. Animals were divided into two groups: healthy dogs (Group 1, n = 10, mean age 4.6 ± 0.97), and dogs with evidence of BPH (Group 2, n = 15, mean age 6.95 ± 0.64). Whole ejaculates were collected by digital manipulation and evaluated by Computer-Assisted Sperm Analysis (CASA). Semen pH, SPPs concentration and High Performance Liquid Chromatography (HPLC) profiles of SPPs were determined. Semen pH values were more alkaline for Group 2 (pH 7.11 ± 0.15), compared to Group 1 (pH 6.79 ± 0.14). CASA demonstrated no significant differences between progression of spermatozoa from healthy dogs and dogs with BPH. However, the number of spermatozoa with rapid velocity was significantly lower in Group 2, compared to Group 1 ($p < 0.05$). Spectrophotometric analyses indicated tendency for lower protein concentration in the ejaculates from Group 2. HPLC profiles of SPPs in Group 2 demonstrated the presence of proteins with low molecular weight (under 12 kDa), defined as a protein peak on 18th minute. This peak was almost absent in normal ejaculates. The presence of such SPPs could be used as an additional biomarker along with standard sperm analyses in assessment of semen quality in dogs.

OC 5.4 | Comparative analysis of carp seminal plasma proteome from different freezability semen

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Our recent studies revealed that cryopreservation has deleterious effect on the carp semen proteome and consequently sperm quality. Determination of predictive indicators of the potentially high and low ability of semen to cryopreservation is highly desirable to improve the results in the cryopreservation. The aim of the study was to compare seminal plasma proteome of high and low semen freezability and identify differentially expressed proteins using two-dimensional difference gel electrophoresis (2D-DIGE) coupled with mass spectrometry (MS). The carp semen was classified as high (71 ± 6% motile sperm) and low (47 ± 3% motile sperm) freezability upon their sperm motility parameters assessed after freezing-thawing using computer assisted sperm analysis system. 2D-DIGE-MS resulted in the identification of 20 protein spots differentially expressed in seminal plasma of high and low freezability semen. Two proteoforms of warm temperature acclimated protein, apolipoprotein A and fetuin long form were identified in higher abundance in seminal plasma collected from carp with high semen freezability while six proteoforms of apolipoprotein E, three proteoforms of intelectin, cofilin and matrix metalloproteinase were more abundant in seminal plasma with low semen freezability. This work is currently underway to identify proteins differentially expressed in carp spermatozoa of high and low freezability semen. Our results suggest that these differentially expressed proteins play important roles in the survival after cryopreservation and can be used as potential biomarkers of high and low carp semen freezability. (Funded by National Science Centre 2011/01/D/NZ9/00628)

OC 6.1 | Microvesicles secreted from equine amniotic-derived cells and their role in in vitro equine endometritis model

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Endometritis are pathologies that reduce conception rate and increase delivery-to-conception intervals. Many treatments have been proposed to treat or prevent endometritis but in view of the embryo-maternal interaction, a different approach to the treatment of endometritis could be represented by regenerative medicine. It is known that paracrine communication between mesenchymal stem cells and target cells exists and may involve microvesicles (MVs) as an integral component of cell-to-cell communication during tissue regeneration. Based on this hypothesis, this in vitro study aims to understand the efficacy of MVs in a model of endometrial inflammation in view of potential application in vivo. Presence and type of MVs secreted by amniotic derived cells (AMCs) was investigated and the response of endometrial cells to MVs was studied using a dose-response curve at different concentrations (10-20-40-50 × 10^6 MVs/ml) and times (24, 48 and 72 h). Moreover, the ability of MVs to counteract in vitro inflammation of endometrial cells induced by lipopolysaccharide (LPS) was studied through the rate of apoptosis and prolificacy, the expression of some pro-inflammatory genes such as metallopeptidase (MPP) 1 and 9, IL-1β, IL-6 and TNF-α and the release of IL-6, TGF-β and TNF-α. Results show that AMCs secrete MVs ranging in size from 100–200 nm. The uptake of MVs is gradual over time but peak at 72 h. MVs decrease apoptosis rate, increase prolificacy rate, down-regulate gene expression and reduce secretion of pro-inflammatory cytokines after treatment with LPS. MiRNA 335, 146 and 26, present in these MVs, might modulate expression of the genes involved in the study.

OC 6.2 | Mare endometrosis is dependent on estrous cycle cytokine environment

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Structure and function disruption of endometrial tissue with excessive deposition of type I and III collagen (COL1, COL3), occurs in endometroses. Dissimilar COL1 and COL3 gene transcription in endometrium explants treated with fibrogenic cytokines Transforming growth factor beta 1 (TGF-β1), Platelet derived growth factor (PDGF) and Connective tissue growth factor (CTGF) was observed in follicular (FP) and luteal phases (LP). The aim was to assess estrous cycle influence on TGF-β1, PDGF and CTGF mediated mare endometrium fibrogenesis and secretory function (prostaglandins-PG). Endometria (n = 5 FP or LP) were cultured (24 h, 48 h) with different doses of TGF-β1, PDGF or CTGF. qRT-PCR was used for mRNA transcription. Culture medium protein content was assessed by Elisa. In LP, TGF-β1 directly acted on its receptors ($p < 0.05$), and induced MMP-9 expression ($p < 0.05$). MMP-9 may cleave latent TGF-β and activate it. In FP, but not in LP, a positive cross-talk between PDGF and TGF-β1 seems to exist by stimulating TGF-β1 via. TGF-β1 protein expression increased ($p < 0.05$), TGFRI mRNA ($p < 0.05$) and TGFRII ($p < 0.01$). In FP and LP, CTGF might mediate endometrium fibrosis by stimulating TGF-β1 receptors ($p < 0.05$). CTGF lowered PGE_2 protein expression ($p < 0.05$) and PGE_2 receptor mRNA transcription ($p < 0.05$) and raised $PGF_{2\alpha}/PGE_2$ ratio ($p < 0.001$) and TIMP-1 protein in LP ($p < 0.05$). A high $PGF_{2\alpha}/PGE_2$ ratio may enable specific cytokines milieu for CTGF induced fibrosis. In conclusion, endometrium cytokine environment may be steroid-sensitive, shifting the importance of cytokines involved in fibrogenesis. (Grants: PTDC/CVT-REP/4202/2014; UID/CVT/00276/2013)

OC 6.3 | Heavy mares that retain fetal membranes and those that deliver fetal membranes physiologically differ in production of $PGF_{2\alpha}$ and PGE_2

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We researched if, at foal delivery, heavy mares that retain fetal membranes and those that do not differ in terms of mRNA expression of prostaglandin synthases (COX-2, PGFS and PGES), protein content of prostaglandins ($PGF_{2\alpha}$ and PGE_2), and the tissue location of COX-2, $PGF_{2\alpha}$ and PGE_2, and of $PGF_{2\alpha}$ and PGE_2 receptors in the allantochorion and endometrium. We sampled the allantochorion and endometrium of 33 Polish heavy draft mares immediately after foal delivery. Twenty mares delivered fetal membranes physiologically (control) and 13 mares retained them (FMR). We performed histology, qPCR, EIA and immunostaining. The allantochorion was more hyperemic and had more infiltration by immune cells in FMR mares than in control mares ($p < 0.05$). FMR mares expressed significantly less COX-2 mRNA in the endometrium and COX-2 and PGES mRNA in the allantochorion (endometrium—COX-2, 26 times less, $p = 0.046$; allantochorion—COX-2, 6.3 times less, $p < 0.001$; PGES, 2.2 times less, $p < 0.001$). All other differences in mRNA expression were not significant. FMR mares had a significantly higher concentration of $PGF_{2\alpha}$ in both placental compartments (endometrium—FMR 44 pg/mg, control 19 pg/mg, $p = 0.017$; allantochorion—FMR 247 pg/mg, control 180 pg/mg; $p = 0.046$). Although the differences were not significant, FMR mares had more than twice as much PGE_2 in the endometrium (FMR 61 pg/mg, control 26 pg/mg, $p = 0.057$), but somewhat less PGE_2 in the allantochorion (FMR 470 pg/mg, control 520 pg/mg, $p = 0.53$). COX-2, $PGF_{2\alpha}$ and PGE_2, and receptors for $PGF_{2\alpha}$ and PGE_2 were present in epithelial and endothelial cells of both placental compartments. These results indicate that production of $PGF_{2\alpha}$ and PGE_2 differs in FMR mares and mares that deliver fetal membranes physiologically. (Supported by NCN grant 2012/07/D/NZ5/0429)

OC 6.4 | Prognostic value of uterine biopsies for the evaluation of female donkey fertility: a preliminary study

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Most donkey breeds in Europe present an aged population and low fertility rates. In mares, but not in jennies, endometrial biopsy has been used to detect endometritis/endometrosis and predict fertility. Endometrial biopsies of 21 jennies were classified according to Kenney's method. Animals in category II were non-significantly younger ($p = 0.2037$; $\chi2 = 1.615$) than those on category III (10.5 ± 5.56 years vs. 15.1 ± 8.4 years, respectively). Jennies that were pregnant at least once in their lifetime had a higher possibility of having a better endometrium ($p = 0.0237$). Just 15 jennies were mated after sample collection, with no previous knowledge of the endometrial classification. Ten were diagnosed as pregnant, 9 from category II and just one from category III. Jennies in category II showed a higher probability to get pregnant than the ones in category III ($p = 0.017$; $\chi2 = 7.643$). As in mares, we could observe that jennies that have less inflammatory cell infiltrate and/or endometrium fibrosis showed better fertility. However, the absence of category I animals, despite

good fertility results for category II suggests specific characteristics of asinine endometrium, and thus there is a need for further studies to establish a fertility prognosis based on biopsy evaluation.

OC 7.1 | A comparative analysis of the protein composition of the oviductal fluid and blood serum by two dimensional gel electrophoresis

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During the periovulatory phase of the estrous cycle processes important for fertilization are going on in the oviduct. These processes include, e.g., the transport and maturation of gametes and the formation of the sperm reservoir. The oviductal lumen contains a complex fluid, which interacts with gametes and the embryo; however, the composition is not entirely known. The oviductal fluid (OF) consists of secreted components from epithelial cells and transudate from blood serum. The identification of the components of this fluid involved in fertilization can be very useful for the design and improvement of culture media used in the techniques of in vitro fertilization. The aim of this study was to identify through a comparative study, differences in the protein composition of OF and blood serum. Six Simmental heifers were synchronized using gonadotropin-releasing hormone (Receptal®) in conjunction with two injections of $PGF_{2\alpha}$ (Estrumate®) and slaughtered three days after the second PGF injection. The blood serum and OF samples were obtained immediately after slaughter. Samples were first separated using immobilized pH gradient strips (3–10 pH). The second dimension was performed in a polyacrylamide gel. After separation, images were obtained (Typhoon 9410) and analyzed with the Progenesis SameSpots software v4.0. The results of image analysis showed that 263 spots were different between the two samples. Thus, a number of 118 and 45 spots were more abundant in serum and OF, respectively. Future studies will identify these different protein spots and this information would provide more detailed information about their roles during the fertilization. (Supported by MINECO AGL2012-40180-C03-02 and FEDER. Acuña OS received aCOST action fellowship (FA1201-010513-028404).)

OC 7.2 | Well-developed corpora lutea are important for establishment of the pregnancy of pigs

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Corpus luteum (CL) function is important for establishment of pregnancy. During the first 11 days, the CL grows autonomous and gains its maximum size. On day 14, the CL regresses if no embryos are present. In this study, we investigated whether a premature CL regression has an effect on pregnancy rate. We performed in 46 crossbred sows (Finnish Yorkshire x Finnish Landrace) a transrectal ultrasound examination (10 MHz, linear array probe, Esaote SpA, Italy) of both ovaries and their CLs at day 10 (CL10) and 13 (CL13) after mating. The ultrasound images were saved, and later exported in DICOM format and analyzed on the computer using IMPAX 6.5.5 picture archiving and communication system (Agfa Healthcare, Belgium). We measured of each ovary the size (area in cm^2) of the five biggest CLs and averaged them. We calculated the difference of the CL area between day 10 and 13 (CL13-10). Pregnancy detection was performed two weeks later. We analyzed the correlations between CLs' sizes and pregnancy rate with an independent two sample t-test (PASW Statistics v. 18.0.0). The mean parity was 3.8 ± 1, mean CL10 0.63 ± 0.11 cm^2, mean CL13 0.65 ± 0.13 cm^2, and mean CL13-10 −0.02 ± 0.12 cm^2. Forty sows were detected pregnant (PREG) and 6 not pregnant (NONPREG). NONPREG sows had similar CL10 (PREG 0.62 ± 0.12 cm^2 vs. NONPREG 0.69 ± 0.07 cm^2), but showed luteal regression (CL13-10; PREG 0.05 ± 0.08 cm^2 vs. NONPREG -0.15 ± 0.16 cm^2; $p < 0.001$) and had therefore smaller CL13 (PREG 0.66 ± 0.11 cm^2 vs. NONPREG 0.55 ± 0.18 cm^2; $p = 0.046$) compared to PREG sows. The results show, that CLs of mated sows that fail to become pregnant show a regression before the expected day 14. It indicates that insufficient CL function might imperil establishment of pregnancy.

OC 7.3 | Investigation of liver X receptor pathway in luteal regression

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Cholesterol is the precursor of steroid hormones in mammalian tissues. Liver X receptors (LXR) are members of the nuclear hormone receptor family and have roles in cholesterol efflux from the cells. The present study aims to understand whether reverse transport of cholesterol is a part of luteolytic mechanism. For the induced luteoylsis model, ewes were injected $PGF_{2\alpha}$ on 12th day of the estrous cycle and luteal tissues were collected at 0 hour (no $PGF_{2\alpha}$ injection, n = 4), 1 h (PG1, n = 4), 4 h (PG4, n = 4), and 16 h (PG16, n = 4) after injection. Total RNA extraction and cDNA synthesis were performed and mRNA expression levels of LXR pathway genes were quantified using qPCR. Expression of LXRα (NR1H3) was significantly downregulated in both PG1 and PG4, however, expression level increased in PG16. While expression of LXRβ (NR1H2) was significantly upregulated in PG1, it was significantly downregulated in PG4. Expression of LXR target gene ATP binding cassette subfamily A1 (ABCA1) was significantly downregulated in PG1. Expression of ABCG1 increased in PG16. Expression of receptors (LDLR, SR-BI) mediating cholesterol uptake were significantly downregulated both in PG4 and PG16. It is suggested that genes controlling intracellular cholesterol levels in the CL are regulated

during the luteolysis and that cholesterol uptake mechanism is more significantly affected than the efflux mechanism. The CL appears to retain cholesterol acutely to maintain steroidogenesis after the $PGF_{2\alpha}$ injection, however in long term cholesterol uptake was significantly impaired, which could be the main reason for the cessation of progesterone production. (M. Hitit was supported by OYP 2013-090.)

OC 7.4 | miRNAs expression during the peri-attachment period of pregnancy in the ovine endometrium

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In ruminants, early pregnancy is an important period that involves maintenance of pregnancy through luteolytic inhibition, endometrial receptivity and implantation of the embryo. Therefore, ovine endometrium is tightly regulated under the effect of consistently changing environment of hormones and embryo-related factors. These regulations involve extensive modulation of gene expression and microRNAs (miRNAs) are one of the epigenetic mechanisms that regulate gene expression. Aim of the present study was to evaluate expression profiles of miRNAs in the ovine endometrium during the peri-attachment (implantation) period of pregnancy. Pregnant ewes were slaughtered on day 12 (n = 4), day 16 (n = 4, pre-attachment) and day 22 (n = 4, post-attachment). Intercaruncular region of endometrium was collected. Total RNA was isolated and microarray analysis was performed using Genechip miRNA 4.0 Array platform (Affymetrix, USA). The array contains all currently identified ovine miRNA sequences along with miRNAs from different species. All miRNAs with a true positive signal were included in the statistical analyses and those with more than 2 fold change with significance were reported. A total of 64 miRNA between P12 and P16, 1040 miRNAs between P12 and P22, and 1135 miRNAs between P16 and P22 were detected to be significantly expressed. The results suggest that miRNAs expression is regulated in the endometrium by factors related to pregnancy. Especially, embryonic attachment seems to be intensively affecting epigenetic mechanism to modify gene expression at endometrial levels in favor of pregnancy. (This study was funded by TUBITAK grant 214O643 to M. Köse.)

OC 8.1 | Comparison of the stress response at birth in preterm and term calves

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In cattle, final fetal maturation occurs only during the last week before birth; however, viable calves are already born after induced parturition about 20 days before term. To compare the stress response in calves born spontaneously (TERM, n = 7, gestation length 286.3 ± 2.1 days) or after induction of parturition with the PGF_2-analogue cloprostenol (PRETERM, n = 7, gestation length reduced by 7 days; 279.6 ± 0.2 days), pre- and postnatal heart rate (HR) and heart rate variability (HRV; standard deviation of the beat-to-beat interval) and postnatal salivary cortisol concentration were investigated. Fetuses were studied from one month before until one day after birth. Fetal HR decreased during the last month of gestation in both groups ($p < 0.001$) whereas HRV increased only during the last 6 days before birth in TERM calves. In both groups, HR started to increase hours before birth, reached maximal values shortly after birth ($p < 0.05$) but was at all time higher in group PRETERM than TERM ($p < 0.05$). The HRV neither changed before nor after birth but was lower in PRETERM than in TERM calves during birth ($p < 0.01$). Cortisol concentration increased postnatally ($p < 0.001$) and correlated with gestation length ($r = 0.68$, $p < 0.01$). In conclusion, calves born only one week before term showed a certain degree of immaturity and their ability to cope with the challenge of birth and postnatal adaptation appeared to be impaired in comparison to calves born at term. (Supported by Hochschuljubiläumsstiftung of the City of Vienna)

OC 8.2 | Accuracy of endovaginal thermal telemetry on calving prediction in Piedmontese cattle

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The efficacy of endovaginal temperature telemetry to predict parturition and complete calving process monitoring, including evaluation of newborn, have been performed. In this study 248 Piedmontese cows were included to evaluate the trend of the pre-partum endovaginal temperatures (EVT) and to verify accuracy of calving prediction in relation to the time from parturition. All data about pre-partum signals sent by the Medria Vel'phone® calving system were recorded and analyzed. EVT was measured for 6 times every 12 h beginning 72 h prepartum until parturition. Although a linear decreasing of temperature was found ($p < 0.001$; $R^2 = 0.27$) a significant differences were found among EVT groups from 72 h to 12 h ($p < 0.05$) but not between 12 vs. 24 h, 48 vs. 60 h and 60 vs.72 h ($p > 0.05$). An EVT difference of 0.5°C (from 38.66 ± 0.024 to 38.11 ± 0.022°C) detected 60 h before indicates the beginning of the calving process within 24 hours. With a cut off of EVT <38.4°C (Se = 80%, Sp = 69%, VPP = 80%, VPN = 70%, Acc = 73%) is obtained a good accuracy for prevision of delivering within 24 h. No seasonal differences among EVT ($p > 0.05$) were reported and no difference were recorded between parity or partum typology (Eutocic, Dystocic, Caesarian) among the different time groups within last 24 h (0–6 h, 6–12 h, 12–18 h, 18–24 h). Medria Vel'phone® calving system gives

accurate information about an expectation of deliver and thank to the EVT analysis of this study, this tool could be a good predictor within 24 h from delivering.

OC 8.3 | Correlations between the applied traction forces and different body measurements of Holstein Friesian calves extracted with the use of an in vitro model

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Aim of this study was to examine if the body measurements of Holstein Friesian calves are related with the applied forces recorded during the extraction of the body with the use of an in vitro model. Ten stillborn calves were measured objectively with a calliper, a measuring tape and by computed tomography. In total 57 body measurements were obtained. Thereafter, the calves were pulled through a dissected pelvis with computer-controlled electric motors and the emerging forces were measured with the use of load cells and with appropriate software. Statistical analysis was performed with Pearson's correlation coefficients. The forces recorded for the extraction of the front part of the body (up to the elbows) were highly correlated with the body weight ($r = 0.83–0.86$, $p < 0.01$) and with the circumference of the head at forehead ($r = 0.80–0.83$, $p < 0.01$). The forces recorded for the extraction of the thorax were highly correlated with the cross-sectional area of the thorax ($r = 0.73–0.76$, $p < 0.05$) and with the circumference of the thorax ($r = 0.73$, $p < 0.05$) in the region of cranial sternum. Conclusively, the perimeter of the head of the calf can be used for the estimation of the severity of extraction of the front part of the body. However, there is no accessible body structure that can help predict how much force is needed for the extraction of the thorax.

OC 8.4 | Neonatal and early life performance of asphyxiated piglets

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This study aimed to document growth and survival in asphyxiated piglets. Data from 47 sows included time of birth and condition of each live born piglet, mixed cord blood lactate, colostrum intake based on piglet weight at birth and 24 h later, and performance to weaning (n = 515) and 10 wks of life (n = 302). Piglets born with a ruptured umbilical cord (21%) had higher blood lactate (6.20 ± 0.41 vs 4.91 ± 0.33 mmol/l) than piglets with an intact cord. Meconium staining and being wrapped in membranes did not affect lactate. Blood lactate averaged 4.23 ± 0.16 mmol/l for the first three piglets born in a litter and increased progressively to an average of 6.34 ± 0.30 mmol/l in piglet 13 and over. Stillbirth increased from 2% in the first three piglets to 19% in piglet 13 and over. Birth order and blood lactate also affected pre-weaning survival. Piglets with <4.5 mmol/l lactate had a 5% risk of mortality as opposed to a 10% risk in piglets with >4.5 mmol/l. A higher degree of asphyxia, based on lactate (classed as <3.36, 3.36 to 4.45, 4.46 to 6.40, or >6.40 mmol/l), was related to a lower birth weight ($p < 0.01$), less colostrum intake ($p = 0.10$), longer birth to first milk intake ($p < 0.01$), weight gain to weaning ($p < 0.05$), and weight gain to 10 weeks of life ($p < 0.05$). However, all these traits were related to birth weight, and when including birth weight as a covariate, there was no significant relationship between lactate and these traits. In conclusion, asphyxia increases risk of pre-weaning mortality and reduces pre- and post-weaning gain; however, the effects of asphyxia are hard to separate from effects of birth weight.

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WILEY Reproduction in Domestic Animals

POSTER PRESENTATIONS

P 1 | Nod-like receptor protein 1 and 3 are ovarian specific mediators of inflammatory response in the course of diet-induced obesity in mice

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Obesity can negatively affect fertility and inflammation is one of the features described in the ovary of obese females. Thus, inflammasome activation, which mediates inflammatory response, might lead to ovarian failure. In the present work we compared the inflammasome activation between somatic organs, liver and gonadal fat (GF), and the ovary. Mice (C57Bl/6J, n = 8/group) were fed chow diet (C) vs. high-fat diet (HFD) during four (4w) or sixteen weeks (16w). Tissue samples (liver, GF and ovaries) were obtained for mRNA analysis of Nod-like receptor protein (NLRP) 1, NLRP3, tumor necrosis factor-α (TNF), interleukin (IL) 1β, IL1R, and IL18 by Real-Time PCR. The results showed an increase in NLRP1, NLRP3 and IL1β mRNA in the ovaries after 4w-HFD comparing to C ($p < 0.05$), while no changes were seen in other tissues. Conversely, transcription of IL1β mRNA was augmented in all tissues after 16w-HFD ($p < 0.05$). TNF mRNA was increased after 4w-HFD in liver ($p < 0.05$), and 16w-HFD in ovaries and GF ($p < 0.05$). However, IL1R mRNA expression was diminished after 16w-HFD in liver and GF, comparing to C ($p < 0.05$). Finally, the expression of IL18 was decreased after 16w-HFD in GF ($p < 0.05$), whereas in the ovaries the opposite trend was evidenced ($p = 0.06$). In conclusion, our study shows a tissue-dependent mechanistic activation of inflammatory response in the course of obesity. Indeed, the mediators of inflammasome activation NLRP1 and NLRP3 might stem ovarian pathogenesis and functional failure, once markers of steroidogenesis (StAR) are also impaired soon after the introduction of HFD (4w-HFD; data previously shown). Further work is needed to characterize the dichotomy between inflammasome and ovarian failure during obesity (Work supported by National Science Centre (2014/15/D/NZ4/0115)).

P 2 | Recto-vaginal fistula associated to atresia ani and double vagina in a 63,X0 karyotype filly: a case report

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A 14 month old crossbred filly was referred to the Teaching Hospital with a history of retarded growth, poor appetite and of voiding feces, with tenesmus, through the vulva. At the clinical examination, the filly had overall poor development, a side-curved tail, absence of anal sphincter (atresia ani type II), and a recto-vaginal fistula. A mid-line vertical septum dividing the whole length of the vagina was observed by vaginoscopy and endoscopy. At the transrectal ultrasonography, the uterus was underdeveloped and the ovaries were hypoplastic. A blood sample was collected to perform a chromosomal analysis, which revealed a 63,X0 karyotype (Equine Turner's Syndrome). Surgical correction of the atresia ani and of the recto-vaginal fistula was performed. The filly lived plus 1½ year without improvement in body size and weight, and died of unknown cause. This filly presented a unique association of chromosomal and congenital abnormalities. The underdevelopment and genital abnormalities (uterus and ovaries) are typical of the karyotype 63,X0. The recto-vaginal fistula and atresia ani may be associated in foals. The complete vaginal septum, probably a persistent Mullerian remnant, was not reported in association with any of the other abnormalities. (Funding: UID/CVT/00276/2013)

P 3 | Semen cryopreservation in Puro Sangue Lusitano horses

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Fertility results after cryopreservation are lower in equine sperm than in other mammalian species due to low cryotolerance and individual variability. In this experiment, the effect of utilization of two centrifugation protocols (CI-600×g for 10–15 min, CII- 2400×g for 5 min) and two freezing methods (P-programmable freezing machine; C-conventional method, i.e. liquid nitrogen vapour in a Styrofoam® box) were studied. The effects of seasonality were also studied by analyzing cryopreserved sperm quality during the breeding vs. non-breeding seasons. Twenty-nine stallions, totalizing 327 ejaculates were used. Commercial Gent® extender was used and the median for the final concentration was 220×10^6 spermatozoa/ml. Sperm loss was 41% and 42% in protocols CI, CII, respectively; no statistically significant differences were found

between CI and CII (p = 0.452). When P and C cryopreservation methods were compared, significant differences were found for total (p < 0.001) and progressive motility (p < 0.001) and no differences were found in the total number of sperm (p = 0.278). Finally, the total number of spermatozoa during and out of the breeding season showed significant differences (p = 0.006), while total motility showed no significant differences (p < 0.001); and progressive motility showed significant differences when fresh and centrifuged were compared (p = 0.007). Significant negative correlations were found between and sperm concentration and total motility (Rho = −0; 239; p = 0.02) and also with the progressive motility (Rho = −0; 216; p = 0.03).

P 4 | Effect of the trypsin digestion in the zona pellucida of different mammalian species

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The zona pellucida (ZP) is a coat surrounding the oocyte, egg and preimplantation embryos that plays key roles during fertilization and block to polyspermy. It has been previously reported that the ZP can be removed by chemical or enzymatic treatment and the time for the ZP dissolution can be modified by oocyte maturation, after ovulation and after fertilization. The mammalian ZP is formed by three or four different proteins depending of the species; however, the significance of this different composition is not yet well understood. In the present study, the resistance to trypsin digestion of the ZP of different species with three or four proteins was evaluated to verify if the different composition has an effect on the properties of the ZP that could be related with the sperm penetration or fertilization. Ovarian oocytes from species with 3 proteins (cow, dog, ewe, pig and mice) and 4 proteins (cat, hamster, human, rabbit, and rat) were obtained by puncturing ovarian follicles. After washing, oocytes were incubated with trypsin (5 mg/ml) and the time needed to be completely degraded was recorded. A higher resistance to trypsin digestion was observed in the ZP from pig, cow and ewe oocytes which are formed by the proteins ZP2, ZP3 and ZP4 compared with the ZP from hamster, human, rabbit and rat oocytes with four proteins. The use of chemical crosslinker produces an increase of the digestion time suggesting a major contribution of the protein-protein interaction or protein folding compared to the aminoacid sequence in this process. There is not a direct relation between the ZP thickness and the digestion time. The use of phylogenetically related species suggests a role of the protein glycosylation in this process (MINECO AGL2012-40180-C03-01-02/FEDERALG2012-40180-C03-01).

P 5 | Ultrasonographic assessment of ductus venosus closure in 24 Great Dane neonates

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Ductus venosus (DV) closure plays a key role in hepatic circulation adaptation to postnatal metabolic function. While in humans (Kondo et al. 2001, Arch Dis Child Fetal Neonatal 85:F57-59) DV closure is assessed by Echo Color Doppler exam (ECD), only few papers describe its non-invasive ultrasonographic evaluation in dogs (Lamb and Burton 2004, Vet Rec 155:699-701). The aim of this study was to determine, by ECD, DV closure time in healthy Great Dane neonates. Patency of DV, in serial ECD, and bodyweight (BW) were recorded on days 0-3-6-9 in 24 neonates that were classified as having patent (PDV) or closed ductus venosus (CDV) based on ECD signal presence/absence. From D3, DV diameter was recorded. All dogs were healthy 1 year later. Data were analyzed by χ^2 and ANCOVA ($p < 0.05$). The number of PDV and CDV puppies at birth was not different compared to D3 (D0- 24 and 0 vs. D3- 22 and 2, PDV and CDV respectively), whereas it was different compared to D6 (D0- 24 and 0 vs. D6- 14 and 10) and D9 (D0- 24 and 0 vs. D9- 0 and 24); differences were also observed on D3 compared to D6 and D9; and on day 6 compared to D9. Reduction of DV diameter (0.17 cm and 0.09 cm on day 3 and 6) was positively influenced by neonatal BW growth. Our data confirmed that ECD is a valuable method for early DV functional closure monitoring in dogs (Lamb and Burton 2004); showed that the number of PDV puppies significantly reduced towards D9; suggest that ECD DV patency evaluation should not be done earlier than D6 after deliver. In humans, DV closes later in pre-term and low BW neonates (Kondo et al. 2001). Results of ANCOVA confirm DV functional closure is earlier in higher BW puppies and prompts further investigation to evaluate the role of delayed closure in pre-term puppies.

P 6 | The motility pattern of brown bear (*Ursus arctos*) sperm is modified by using gelatine as extender additive in prefreezing long-term storage (48 h)

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Solid media may be a tool to prevent sedimentation of sperm cells at the bottom of the tube when long-term storage (LTS) is required. Our aim was to assess the effect of the gelatine as an additive during long-term pre-freezing storage of bear sperm. Ejaculates from 5 males were obtained by electroejaculation during the breeding season. Two models of sperm storage were tested: 1) 1:1 dilution in liquid extender (TTF-ULE-Bear extender) at room temperature (RT), cooling up to 5°C

in a tube and then diluting down to a final concentration of 100×10^6 sperm/ml (Control-C-); 2) a treatment group using liquid extender supplemented with 1.5% gelatine (Gel) and cooled in 0.25 ml straws. The samples (C and Gel) were stored for 48 h before freezing. After thawing, a thermal stress test (ThS test) at 37°C was carried out for 2 h. The motility of the samples was assessed by a CASA system (%TM; %PM; μ/s VCL (curvilinear velocity) and μ/s VSL (straight line velocity)) in pre-freezing (PF), post-thawing (PT) and post-ThS test (PTT). Gel samples yielded lower VCL than C (PF: 134.7 ± 3.4 vs. 106.5 ± 1.1; PT: 129.9 ± 4.1 vs. 95.1 ± 3.6 for C and Gel, respectively, $p < 0.05$). After PTT, Gel showed higher VSL (19.6 ± 4.9) than C (12.3 ± 3.3). In conclusion, the Gel treatment decreases VCL and improves VSL in pre-freezing LTS (48 h). The solid state immobilizes sperm, reducing the metabolic demands of motion and distributing the cellular detritus homogeneously. This fact may be related to a VCL decrease in sperm, both before and after freezing. The increase of VSL could be related to a more sperm straightness. This change in the velocity pattern may improve the sperm transport in the female genital tract. (Supported by Cantur S.A and MINECO (CGL2013-48255-R))

P 7 | Cytokine TGFβ1-3 in seminal plasma: biomarkers for pig semen quality?

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The transforming-growth factor-β (TGF-β)-family is a regulatory cytokine group involved in testis development, spermatogenesis and fertility. Deviating TGFβ-levels relate to dysfunction as disruptor of the blood-testis-barrier and the epididymal epithelium. Whether its relative levels in seminal plasma (SP) are reflected in the spermiogram is not known. Specific ejaculate fractions (P1: 1st 10 ml of the sperm-rich fraction, SRF; SRF-P1: rest of the SRF; Post-SRF: rest of the ejaculate) were manually collected twice weekly from 3 Swedish Landrace breeding boars (A-C, 9–12 months) for 3 consecutive weeks. Ejaculate volume (VOL), sperm concentration (CON), total sperm production (PROD), total and progressive sperm motility (TM-PM) and velocity (VEL) were recorded. Relative concentrations of TGFβ1-3 in SP were measured using a Luminex xMAP multiplexed microsphere-based flow cytometer assay (3-plex kit, Cat#TGFB-64K-03 reactive for pig, Merck Millipore, USA). As expected, values of VOL, CON and PROD differed among ejaculate fractions, but not for variables TM, PM and VEL or TGF-β1-3. Boar A had lower TM, PM and VEL than boars B-C, depicting the highest P1-TGF-β1 levels were highest, whereas Boar B had the lowest TGF-β2. No differences were found in boar C among fractions. There was a negative correlation ($p < 0.05$) between both VEL and CON relative to TGF-β3 and TGF-β2, respectively. In contrast, positive correlations ($p < 0.001$) were found between TM and PM relative to TGF-β2. These results, albeit interesting for diagnostics, demand inclusion of a larger animal sample. (Supported by Vetenskapsrådet (VR), FORMAS and FORSS, Sweden)

P 8 | Effect of insulin-like growth factor-I on embryo quality of bovine oocytes subjected to heat shock

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The aim of this study was to evaluate the effect of different concentrations of IGF-I added to the medium IVM of bovine oocytes subjected to heat shock (HS) on embryo quality. Three concentrations of IGF-I (0, 25 and 100 ng/ml-Sigma) added to the IVM medium and two incubation conditions (conventional (CON): 24 h at 38.5°C and 5% CO_2; or HS: 12 h at 41°C followed by 12 h at 38.5°C and 5% CO_2) were tested. Oocytes were in vitro fertilized (IVF) and cultured. Ninety blastocysts with 8 days post-IVF were fixed and evaluated by TUNEL. Data were analyzed by generalized linear mixed models with Poisson distribution using the Proc Glimmix (SAS). The interaction between IGF-I concentration and incubation condition was not significant ($p > 0.05$). The total cell number of blastocysts (91.4 ± 3.8; 98.3 ± 3.6 and 100.2 ± 4.1 with 0, 25 and 100 ng/ml IGF-I, respectively) and the inner cell mass (ICM) index (30.5 ± 2.3%; 33.8 ± 2.4% and 36.9 ± 2.8% with 0, 25 and 100 ng/ml IGF-I, respectively) were not affected ($p > 0.05$) by IGF-I. However, IGF-I reduced ($p < 0.05$) the apoptosis index of blastocysts ($5.5 \pm 0.8\%^a$; $2.2 \pm 0.4\%^b$ and $2.7 \pm 0.5\%^b$ with 0, 25 and 100 ng/ml IGF-I, respectively) and apoptosis index of the ICM ($13.6 \pm 3.2\%^a$; $5.1 \pm 2.2\%^b$ and $8.5 \pm 2.5\%^b$ with 0, 25 and 100 ng/ml IGF-I, respectively). HS reduced ($p < 0.05$) the total cell number of blastocysts (CON = 104.3 ± 3.5^a; HS = 88.9 ± 2.3^b) and ICM index (CON = $40.1 \pm 2.1\%^a$; HS = $27.2 \pm 1.8\%^b$), increased ($p < 0.05$) the apoptosis index of the ICM (CON = $6.2 \pm 1.4\%^a$; HS = $12.0 \pm 2.7\%^b$) and had no effect on the apoptosis index of blastocysts (CON = 3.5 ± 0.6%; HS = 3.4 ± 0.5%). In conclusion, the addition of IGF-I, even at low doses, results in lower apoptosis of embryonic cells and improved embryo quality. **Acknowledgment**: Fapemig, CNPq and Embrapa.

P 9 | Prostatic volume and progression of Benign prostatic hyperplasia in dogs

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Benign prostatic hyperplasia (BPH) is a very common, age-related condition in adult intact dogs. Animals with BPH can present symptoms or not (in more early stages, subclinical form) being, therefore, difficult to diagnose. Clinical signs relate to the enlarged prostate exhibit including locomotor, gastro-intestinal and urinary tract disorders (Ponglowhapan and Mankong, 2015). The objective of this work was to measure the

size of the prostate using ultrasonography, to obtain more accurate values, to compare it to the expected size calculated through the weight, and finally to relate the increased size with the presence or absence of clinical signs. Prostatic volume (PV, cm^3) median values were 45.60 cm^3 (interquartile range [IQR]: 19.85–63.95) in dogs with BPH and 20.68 cm^3 (IQR: 16.18–27.46) in dogs without BPH ($p < 0.001$). Expected volume (EV, cm^3) median values were 15.15 cm^3 (IQR: 13.89–16.98) in dogs with BPH and 13.72 cm^3 (IQR: 12.00–15.86) in dogs without BPH ($p = 0.044$). Following these results, a correlation analysis was carried out using data of the two groups of animals. The correlation between de PV and the EV in dogs without BPH was found to be 0.7848 ($p < 0.000$) and 0.4492 ($p < 0.004$) in dogs with BPH. Therefore, it can be concluded: (i) the estimate of the EV using the reported formula by Sannamwong et al. (2012) must be used with caution, since correlations, although significant, are low, being necessary further studies. (ii) The formula is more reliable in dogs without BPH than in dogs with BPH, which reduces its usefulness. (Ponglowhapan and Mankong, Proc. 14th Chulalongkorn University Veterinary Conference; April 20–22, 2015; Bangkok, Thailand)

P 10 | Inhibition of NETs components on collagen 1 and 3 transcription in mare endometrium

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Endometrosis (endometrium fibrosis) is a major cause of subfertility/infertility in mares, which justifies the importance of fighting this pathology. Bacteria induce neutrophil extracellular traps (NETs) formation that besides killing pathogens in the uterus, may also contribute for endometrosis establishment. The aim was to evaluate NETs components and their influence on fibrosis formation and how this can be inhibited. Equine endometrium explants from mid luteal phase (n = 4) were cultured, for 48 h, with NETs components (elastase - ELA; cathepsin G - CAT), either with or without their specific inhibitors (Silvelestat - elastase inhibitor - ELA-IN; cathepsin G Inhibitor I - CAT-IN) or with inhibitors alone. In an inhibitors dose assessment trial, the best doses were between 1 and 10 μg/ml. Thus, 1 μg/ml was used. Collagen (COL)-1 and -3 gene transcription in cultured tissue was done by qRT-PCR. COL-1 transcription increased with ELA-IN alone and with ELA 0.5 μg/ml treatment, compared to control ($p < 0.01$), but was reduced with ELA-IN + ELA 0.5 μg/ml ($p < 0.01$). Stimulatory effect of CAT 0.1 μg/ml on COL-1 and 3 expression was also inhibited by CAT-IN pretreatment ($p < 0.05$). Since COL-1 mRNA increased with ELA-IN alone, higher doses should be tested. These findings suggest that by inhibiting NETs components action it may be possible to reduce fibrosis in equine endometrium (Grants: PTDC/CVT-REP/4202/2014; UID/CVT/00276/2013).

P 11 | Molecular variability in 64,XY SRY negative intersex horses: preliminary results

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Sex reversal syndrome is a genetic disorder in the horse associated with morphological abnormalities in the reproductive tract and infertility. The most common presentation is the 64,XY-SRY-negative DSD, i.e., animals present a male chromosomal complement (64,XY) but they are morphologically characterized as females. These individuals always show the absence of a functional SRY (sex-determining region) gene located on the Y chromosome (ECAY). The only previous comprehensive study described two possibilities: a massive ECAY deletion (two cases), or a 21-kb deletion surrounding the SRY region (11 cases) (Raudsepp et al. 2010). Here we present preliminary results of two cases evidencing a new larger deletion which has not been described earlier. Two phenotypic mares showing classic symptoms of sex reversal syndrome (sterility, small or hypoplastic ovaries and uterus and absence of sexual behavior or cyclicity in the breeding season) were studied. The absence of the SRY gene was determined by PCR. A further characterization of the SRY-surrounding region was performed by genome walking, using eleven newly designed primer pairs, covering a region of approximately 35 extra kb. Based on the combination of fragments amplified, it was determined that this deletion fell into a size ranging between 38.7 and 41.9 kb and was at least 15 kb larger than previously reported. Despite the fact that molecular differences with previous studies are remarkable, the reproductive morphology and behavior of the studied animals were similar. These results strongly suggest that the sole inactivation of the SRY gene is enough to produce the disorder, but also confirm certain variability in the deletion associated to this reproductive disease in horses.

P 12 | Effect of human chorionic gonadotropin administration on pregnancy rate in recipients after embryo transfer in dairy cattle

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The aim of the study was to evaluate the effect of human chorionic gonadotropin (hCG) administration after embryo transfer on the pregnancy rate in recipient heifers. Embryo collections were performed in dairy cows or heifers after superovulation by 8 FSH (Stimufol®, Reprobiol SPRL, Belgium) treatments. Recipients were randomly divided into three groups. Heifers in the first group (n = 49) were treated by 2000 IU of hCG (Pregnyl®, N.V. Organon, The Netherlands) intravenously and heifers in the second group (n = 46) were treated by 2000 IU of hCG intramuscularly immediately after transfer. Heifers in the third group (n = 125) were not treated. Pregnancy rates in

groups 1, 2 and 3 were 49/28 (57.14%), 46/25 (54.35%) and 125/63 (50.40%), respectively. In conclusion, the pregnancy rate tended to be higher in recipient heifers treated by hCG after transfer of embryos in comparison with untreated recipient heifers, however differences were not significant.

P 13 | Could chemokines participate in embryo-endometrial cross-talk at the time of implantation?

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Implantation is the major process of success pregnancy and requires an appropriate immune response to the embryo. One of the crucial factors determining this process are chemokines, the small cytokines, mostly produced by immune cells and involved not only in inflammation but also in chemotaxis, proliferation and angiogenesis. We suggest that during maternal recognition of pregnancy trophoblast cells produce chemokines which could influence the endometrium during preparation to embryo implantation. Endometria and/or trophoblasts were collected from mature crossbred gilts on days 10, 12 and 14 of the estrous cycle and pregnancy. Examination of gene expression of CC- and CXC-chemokines and its receptors was done using Real Time PCR. Data were analyzed by two-way Anova followed by Tukey's post hoc test. We found that during implantation (days 12–14 of early pregnancy) trophoblasts expressed mRNA for chemokines like: CCL2, CCL4, CCL8, CCL11, CXCL8 and CXCL12 and chemokine receptors: CXCR4 and silent chemokine receptors: DARC and D6. Endometrial CCR2, CCR5 as well as CXCR3 and CXCR4 mRNA expression strongly correspond to its ligands ($p < 0.001$) produced by trophoblasts. The embryo presence decreases D6 mRNA expression in the endometrium on day 12. During the estrous cycle DARC mRNA expression slightly increases with highest expression on day 14. There were no significant changes in gene expression of most studied chemokines and receptors during the estrous cycle. In conclusion we found that chemokines produced by trophoblast cells could affect endometrium via specific receptors like CCR2, CCR5 and CXCR4 and thus participate in tissue remodeling necessary for embryo implantation.

P 14 | Immunohistochemical localization of cocaine and amphetamine regulated transcript (CART) in murine eutopic and ectopic endometrium

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The present study was aimed at investigation whether CART (cocaine- and amphetamine-regulated transcript) may be expressed in uterine tissues and whether this expression may be present in heterotopic endometrial tissues in the murine model of endometriosis. The study was performed on 8-week-old inbred C57BL/6 female mice. Uterine fragments were transplanted into abdominal wall. The identification and visualization of CART was performed based on the EnVision method according to Herman and Elfont, 1991. Following 2, 4 and 8 weeks of observation the ectopic endometrial foci were excised and processed for immunohistochemical examinations. As control served normal eutopic uterine tissues. Tissues were fixed by immersion in 4% buffered formaldehyde, and, following dehydration, were embedded in paraffin wax. The sections were cut 4 μm thick and attached to FLEX IHC microscope slides (K8020, Dako, Denmark). All of the sections were stained with Vector QS hematoxylin (H-3404, CA, USA), mounted and evaluated under a light microscope. The specificity test performed for the CART antibody included: negative control, where the antibodies were replaced by normal rabbit serum (Vector Laboratories; Burlingame, CA, USA) at the respective dilution. Positive control was carried out for the specific tissue, as recommended by the producer (for our research we used mice stomach). Specific antibodies showed that this peptide is constitutively expressed in endometrial epithelial cells in normal eutopic uterus and heterotopic endometrioid cysts. CART is constitutively expressed in eutopic and ectopic endometrium mostly in glandular and covering epithelium. The possible role of this neuropeptide in the reproduction remains to be elucidated.

P 15 | Influence of copulation and artificial insemination on the dynamics of vaginal cell populations, pH and electrical resistance in the bitch

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The aim of the study was to investigate the effect of copulation and artificial insemination on the dynamics of vaginal cell populations, pH and electrical resistance in the bitch. Sixty-seven female and 27 male dogs of different breeds and ages were included in the experiment. The bitches were divided into three groups: Group I, naturally mated (females, n = 15 and males, n = 9), Group II, vaginally inseminated with fresh semen (females, n = 30 and males, n = 18) and Group III control, no insemination (females, n = 22). The insemination procedures were done twice on the 1st and the 3rd day after the ovulation, which was determined by progesterone assays. Cytological examination, pH and electrical resistance of vaginal mucus were performed daily from the first day of vulvar bleeding until the onset of cytological diestrus. The results were

expressed as mean ± SD and analyzed using ANOVA for repeated measures. $p \leq 0.05$ was considered significant. The percentage of keratinized epithelial cells in vaginal smears in bitches from group I and II was similar and increased in days after mating or insemination. The obtained values at the same days were significantly ($p < 0.001$) higher than those in group III. The pH and electrical resistance of vaginal mucus in inseminated bitches were lower ($p < 0.001$ and $p < 0.05$, respectively) in days after mating or insemination compared with the controls. The lowest values were determined in group I, followed by group II and III ($p < 0.05$). In conclusion, the mating and the artificial insemination caused an increase of the percentage of keratinized epithelial cells and a decrease of pH and electrical resistance of vaginal mucus in inseminated bitches.

P 16 | Assessment of sperm populations separated by single or double density gradient centrifugation in normospemic ram ejaculates

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The present study compares the sperm quality differences and the percentage of recovered spermatozoa in different phases (high, medium and low) obtained after single (SLG; 80%) or double (DLG; 45% and 90%) density gradient centrifugation in nosmospermic ovine semen, prepared at different concentrations (800 and 3000 × 10^6 spz/ml). Top (T), medium (M) and bottom (B) phases were isolated and motility, concentration, spermatozoa recovery rate, vitality, morphology and membrane functionality were evaluated. When sperm samples contained 800 × 10^6 spz/ml, the sperm recovery rate in T phase was significantly lower ($p < 0.05$) than in M and B phases, both in SLG (23.7%, 45.4% and 30.7%) and in DLG (10.4%, 47.5% and 41.9%). Different sperm centrifugation protocols and concentrations affected the sperm recovery rate at different phases. In this sense, it was observed that T phase in SLG with 3000 × 10^6 spz/ml contained a significantly higher percentage of spermatozoa (53.2%) than others (SLG800 = 23.7%, DLG800 = 10.4%, SLG3000 = 27.4%; $p < 0.05$), while the sperm recovery in M phase was lower (25.2%) than the others (SLG800 = 45.4%, DLG800 = 47.5%, SLG3000 = 31.5%; $p < 0.05$). This observation suggests that SLG does not allow the adequate separation of sperm populations when samples are highly concentrated; in this case, DLG is recommended. As expected, total motility was higher in lower layers, although significant differences were only detected when DLG was realized in sperm samples containing less sperm concentration (800 × 10^6 spz/ml). No significant differences were found for other studied parameters. Results support that the use of density gradient centrifugation does not offer advantages when is used in normospermic samples of ram sperm.

P 17 | Cholesterol imaging in living bovine oocytes treated with cholesterol-loaded ciclodextrin and its effect on development competence after vitrification

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The present study aimed to evaluate whether cholesterol-loaded methyl-β-cyclodextrin (CLC) could be incorporated into the bovine oocyte plasma membrane in order to increase its tolerance to a vitrification procedure. In a first experiment, an in vivo time-lapse imaging (0–60 min) by confocal microscopy was conducted to follow the internalization of 2 mg/ml of CLC into the oocytes. Then, the effect of a pre-treatment with 2 mg/ml of CLC for 30 min before vitrification of immature (GV) and in vitro matured (MII) oocytes on their developmental competence was evaluated. Statistical analysis was performed through an ANOVA ($p < 0.05$). Oocytes exposed to 2 mg/ml of CLC showed a clear immunofluorescence in the plasma membrane after 30 min. Vitrified/warmed oocytes displayed lower cleavage and blastocyst rates than non-vitrified oocytes. No significant differences in terms of survival, cleavage or blastocyst rates were observed between vitrified groups, regardless of CLC treatment or the meiotic stage (VG vs. MII). In conclusion, in vivo time-lapse analyses using fluorescent cholesterol allowed identifying the exact time at which the probe is mainly located at the plasma membrane. However, this treatment prior to a vitrification-warming procedure of immature or in vitro matured bovine oocytes didn't improve their developmental competence. (This study was supported by the Spanish Ministry of Science and Innovation (Project AGL2013–46769).)

P 18 | Comparisons of spermatologic-andrologic parameters and testosterone-phospholipase A2 levels during breeding and non-breeding seasons in Abasian goats

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The aim of current study was to investigate and compare effect of breeding (BS) and non-breeding (NBS) season on native spermatologic parameters and post-thaw sperm quality in Abasian goats. Also, andrologic parameters (AP), testosterone (T, ng/ml) and phospholipase A2 (PA2, mmol/min/ml) were determined in BS and NBS. Ejaculates (n = 105) from six Abasian bucks were collected weekly with artificial vagina during October-January (BS) and April-July (NBS), and AP was determined monthly. Serum (for T) and seminal plasma (for PA2) were collected monthly and stored. Results are mean ± SEM of BS vs. NBS values, respectively. Ejaculate volume (1.22 ± 0.05 vs. 0.89 ± 0.07 ml),

native sperm motility (80.6 ± 1.0 vs. 71.5 ± 2.0%) and post-thaw sperm motility (25.9 ± 2.6 vs. 14.7 ± 3.7%) were significantly higher in BS ($p < 0.001$). Sperm count per ml (2.7 ± 0.1 vs. 3.7 ± 0.2 × 10^9 cells/ml) was significantly lower during BS ($p < 0.01$). T did not show any significant change between BS and NBS (14.81 ± 1.26 vs. 13.52 ± 4.51 ng/ml). AP (testicular volume, 333 vs. 299 ml; scrotal circumference, 26.1 vs. 23.3 cm; mean height, 10.8 vs. 9.5 cm, and width, 6.3 vs. 5.6 cm, of testis) was higher in BS ($p < 0.01$). Individual PA2 levels were lower for goat with good post-thawing results during BS (38.01 vs. 97.64 mmol/min/ml, $p < 0.01$). In conclusion, Abasian goat semen must be collected during BS if cryopreserved. PA2 activity may be important factor affecting success of cryopreservation during BS. Moreover, native semen from Abasian bucks may be used for artificial insemination during NBS if used without semen cryopreservation. (Supported by TUBITAK (112O193))

P 19 | Effect of melatonin on developmental competence of bovine oocytes subjected to heat shock

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The aim of this study was to evaluate the effects of melatonin added to IVM medium on developmental competence of bovine oocytes subjected to heat shock (HS). Immature oocytes aspirated from ovaries obtained from slaughterhouse were selected and randomly allocated in factorial experiment design 3 × 2. Three concentrations of melatonin (0 M, 10–6 M and 10–4 M) added to the IVM medium and two incubation conditions (conventional (CON): 24 h at 38.5°C and 5% CO_2; or HS: 12 h at 41°C followed by 12 h at 38.5°C and 5% CO_2) were tested. After IVF, the presumptive zygotes were cultured in CR2aa medium for 8 days. Six replicates were performed with a total of 1157 oocytes. Data were analyzed by generalized linear mixed models considering the Binomial distribution using the Proc Genmod (SAS). We considered the effects of melatonin concentration, incubation condition, replicate and interaction between melatonin and incubation condition. Values are shown as lsmean ± SEM. There was no interaction ($p > 0.05$) between melatonin concentration and incubation conditions. Addition of melatonin (68.7 ± 4.3%; 73.2 ± 5.3% and 68.8 ± 3.5% with 0 M, 10–6 M and 10–4 M, respectively) and the HS (CON = 71.9 ± 3.5%; HS = 68.5 ± 3.6%) did not affect ($p > 0.05$) the cleavage rate. The blastocysts rate on day 7 (23.7 ± 3.1%; 26.8 ± 4.4% and 25.4 ± 2.4%) and day 8 (24.6 ± 3.3%; 27.0 ± 4.1% and 24.5 ± 2.6%) was not affected ($p > 0.05$) by melatonin with 0 M, 10–6 M and 10–4 M, respectively; however, was reduced ($p < 0.01$) by HS on day 7 (CON = 28.2 ± 2.9%; HS = 22.4 ± 2.4%) and on day 8 (CON = 29.7 ± 2.9%; HS = 20.9 ± 2.1%). It was concluded that melatonin supplementation did not improve the developmental competence of bovine oocytes subjected to heat shock.

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P 20 | Oxidative stress levels during the early period of pregnancy in dairy cattle

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This study aimed at assessing the levels of markers of oxidative stress during the early period of pregnancy in dairy cattle. Blood samples were collected from coccygeal vessels containing EDTA in pregnant females between 25 and 50 days after artificial insemination (AI) (n = 54). For the control group, samples were randomly collected during a stabling period and in the absence of males (n = 45). Plasma was obtained by centrifugation (1,500×g for 15 min) immediately after collection and was stored at −20°C until assayed. Measurement of superoxide dismutase (SOD) was carried out in pregnant and non-pregnant females using spectrophotometric method with a commercially available kit. NADPH-dependent membrane lipid peroxidation (LPO) was measured as thiobarbituric acid reactive substance using malonedialdehyde as standard. Measurement of reduced glutathione (GSH) concentrations was carried out according to the method described by a commercially available kit. The activity of the antioxidant SOD was decreased in pregnant females from days 25 to 50 after AI (6.79 ± 0.2 U/ml) compared with non-pregnant ones (7.51 ± 0.33 U/ml). However, this decrease failed to reach a statistical significant level ($p = 0.06$). Regarding the levels of LPO and GSH, the values obtained in pregnant (114.81 ± 5.97 μM and 6.41 ± 0.71 μmol/min/ml, respectively) and in non-pregnant females (125.48 ± 11.92 μM and 6.98 ± 0.88 μmol/min/ml, respectively) did not show statistical differences ($p = 0.40$). In conclusion, our preliminary results show that the markers of oxidative stress assessed did not show major differences between pregnant and non-pregnant dairy cattle in the early period of pregnancy. Additional studies are required to evaluate the levels of oxidative stress at later period of gestation.

P 21 | Establishment of semen bank of wild Ovis species and their hybrids with domestic sheep

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The aim was to develop a protocol and freezing media for cryoconservation of epididymal sperm from wild representatives of Ovis genus: mouflon (MF), argali (AR) and snow seep (SN), and ejaculated semen collected from their hybrids with domestic sheep. The semen from wild sheep was derived postmortem, while electoejaculation was used with their F1 hybrids. A modified Tris-based egg-yolk medium (15% egg

yolk, 4% glycerol) was used for cryoconservation. Sperm quality was evaluated before and after freezing. Sperm DNA integrity was checked by acridine orange test (AOT), with DNA fragmentation index (DFI) calculated as the percent of spermatozoa containing damaged DNA. Five measurements were performed for each semen sample. The motility in fresh-derived diluted epididymal semen of MF, AR and SN males was 89.4 ± 3.8, 90.2 ± 4.2 and 88.7 ± 8.3%, respectively, and decreased in frozen-thawed semen to 51.4 ± 6.2, 52.6 ± 7.2 and 41.5 ± 8.3%. SN showed the highest DFI: 20.4 ± 5.8% comparing to 16.3 ± 3.5 (MF) and 12.4 ± 3.2% (AR). The viability of cryopreserved semen of all studied wild Ovis species was confirmed by production of live hybrid offspring after intra-oviduct insemination of domestic (Romanov) sheep with low doses of frozen-thawed semen. All produced F1 hybrids had a normal growing capacity and were fertile. The sperm motility in fresh ejaculated semen was 91.4 ± 4.0, 92.7 ± 4.0 and 90.5 ± 3.0% in MF, AR and SN hybrid males, respectively, and in frozen-thawed semen was 47.9 ± 8.1, 52.3 ± 8.4, and 43.2 ± 5.4%. The values of DFI were 14.6 ± 3.2, 9.4 ± 3.6 and 12.7 ± 4.2%, respectively. The viability of collected semen was proved by production of F2 hybrid animals. (Supported by the RSF within project No. 14-36-00039)

P 22 | Uterine contractility after twin calvings in dairy cows

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Possible negative effects of twinning on uterine involution due to different contractility patterns in dairy cattle are hypothesized. Therefore, intrauterine pressure (IUP) changes were quantified in early postpartum (pp) dairy cows after twinning. Puerperal IUP of 16 twinning cows were measured using an open tip catheter technique, which had been applied 14–17 h pp. Initial 4-h recordings were followed by three 1-h recordings in 12-h intervals. Six from these animals had RFM in both horns, 5 only in 1 horn, whereas 5 delivered both placentas. As control, 22 cows after singleton calvings were used, 7 of which had RFM. Contraction frequency (FREQ), amplitude (AMP), duration (DUR), mean and total area under the curve (AUC, TAUC) were compared using T tests. Contractility did not differ after twin vs. singleton parturitions. Uteri with RFM were more contractile. After twinning this difference was more evident for FREQ, after singleton calving AMP and AUC were increased, and in both groups TAUC was increased. No differences were found between the two uterine horns in the twinning cows, independent of RFM. All IUP parameters had a time-related significant decline ($p < 0.001$ to $p < 0.05$). As a conclusion, IUP of puerperal cows were not significantly different after twin vs. single pregnancies. However, RFM had a positive influence on uterine contractility both after twinning and singleton parturitions.

P 23 | Aberrant imprinted gene expression in postnatal mice exposed to 5AzaC but not TSA during post implantation stages

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A number of studies have shown that exposure to drugs that induce DNA demethylation (5AzaC) and histone hyperacetylation (TSA) alter the expression of imprinted genes at later stages, little is known about the ability of treated fetuses to carryover imprinting reprograming to postnatal stage. The objective of this study was to evaluate lasting effects of treating post-implantation fetuses with either TSA or 5AzaC on the development outcome and imprinting patterns in postnatal mouse pups. Pregnant females were injected en utero with TSA or 5AzaC. Pups (D1) were weighed, dissected into liver for histological examination and external tissue was used for qRT-PCR. Our results show that while the pups from the TSA treated fetuses were overweight by 21%, pups from the 5AzaC treated fetuses were 16% underweight. Notably, external morphological abnormalities were not visible at birth in all groups. Internally however, histopathological examination of liver tissues revealed abundance of inflammatory cells and a slight decrease in glycogen levels in pups from the TSA treated fetuses. Whereas pups from the 5AzaC treated fetuses displayed more pronounced abnormalities such as hepatocytes degeneration, pyknotic nuclei and a substantial decrease in glycogen levels. Moreover, while pups from the TSA treated fetuses displayed upregulation of imprinted genes Igf2 and p57 (1.6, 1.5 fold respectively), pups from the 5AzaC treated fetuses exhibited an aberrant over/under expression of imprinted p57, H19, Igf2r, Igf2, Mash2, Ipl genes (1.2, 2.3, 0.2, 0.4, 1.6, 0.2 fold, respectively). Together, these results indicate that, in contrast to DNA methylation, epigenetic alterations created by histone hyperacetylation during post-implantation are somehow reset at postnatal developmental stages.

P 24 | Expression of glutathione peroxidase-5 in the boar genital tract

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Glutathione peroxidase-5 (GPX5) is an H_2O_2-scavenging enzyme identified so far exclusively in the epididymis of mammals. This study evaluates whether GPX5 is also expressed in other organs of the male reproductive tract, using boar as animal model. Tissue samples from the testis, epididymis and accessory sex glands of 6 healthy and fertile boars were fixed in buffered formaldehyde, embedded in paraffin blocks, sliced and mounted on slides. GPX5 was immunohistochemically localized using a rabbit primary polyclonal antibody (ab190733,

Abcam, Cambridge, UK). GPX5 was expressed throughout of boar genital tract. In the testis, it was expressed in the interstitium (blood vessels and Leydig cells) and the seminiferous tubules (Sertoli cell and elongated spermatids). In the epididymis, it was expressed in the lining epithelium and luminal spermatozoa. GPX5 was also expressed in the prostate (intracytoplasmic and nuclear localization in glandular and duct epithelia), seminal vesicles (intracytoplasmic localization) and bulbourethral glands (epithelial membranes). There were no differences in staining neither distribution nor subjective intensity among boars. In conclusion, GPX5 is widely expressed in the boar reproductive tract; including testis, epididymis and accessory sex glands. (Supported by MINECO & FEDER founds (AGL2012-39903), Madrid, and Seneca foundation (19892/GERM/15), Murcia, Spain; FORSS and The Swedish Research Councils VR and FORMAS, Stockholm, Sweden.)

P 25 | Comparative study of the testicular capsule of the Rothschild's giraffe (*Giraffa camelopardalis* ssp. *rothschildi*) and the Philippine mouse-deer (*Tragulus nigricans*)

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The available literature provides does not provide an entire description of the testicular capsule of the Rothschild's giraffe and the Philippine mouse-deer. The research material came from Warsaw Zoological Garden (one adult Rothschild's giraffe) and from Wroclaw Zoological Garden (two adult Philippine mouse-deers). The samples were collected after the natural death of the animals. Histological, histometrical and histochemical examinations were carried out. The testis of Rothschild's giraffe and Philippine mouse-deer were covered by a dense fiber capsule (tunica albuginea). Testicular capsule was formed by collagen fibers and numerous blood vessels. In the giraffe it forms a layer immediately adjacent to the marginal (outer) layer of the seminiferous tubules, while in mouse-deer the vascular layer was located in the medial part of tunica albuginea. Numerous intracapsular sebaceous glands were observed in the mouse-deer, while in the giraffe numerous clusters of adipocytes were present. Thickness of the testicular capsule in the giraffe was 1355.19–2407.08 μm, while in the mouse-deer was 1067.47–1492.5 μm. The connective tissue from capsule penetrated deep into testicular stroma, and formed septa, which divided testis into lobes. Lobes were evident in the giraffe testis (thickness approximately 1307.28–1554.07 μm), while in the mouse-deer were poorly marked (thickness approximately 81.52–93.14 μm). The results of this preliminary study showed significant differences in the microstructure of the testicular capsule between these two examined species of wild ruminants. These initial observations are completing material to the information in the reproductive male system and can be applied to other species, including endangered ones.

P 26 | Micro-CT imaging of chick lungs one day before hatching

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Using the lungs to breathe starts in chicks for many hours before they hatch while still in the egg. It uses air collected in the air cell. An increasing amount of carbon dioxide in the air cell induces the chick to start the shell destruction from the inside and to go out. Disorders in lung development may be a reason for abnormalities in the hatching process. Chicks from excessively large or small eggs are characterized by poor hatchability. Data have been collected using micro-CT 1 day before planned hatching, and subsequently, 3D reconstruction was done. Areas with a density corresponding to the air density have been visualized in the thoracic cavity, and subsequently analyzed using an adequate algorithm, allowing its accurate reconstruction and use for further measurements. Micro-CT is an excellent tool for imaging the internal structures of the egg without compromising its integrity. It enables diagnostics of the respiratory system, among others, including the assessment of the degree of lung aeration in relation to the egg size. This information may be helpful in efforts to improve hatchability of the flock and for evaluation of the chicks' growth rate after hatching, its general condition and the assessment of pathological processes in the lungs or the whole egg.

P 27 | Study of two local cattle breeds for occurrence of DUMPS mutation

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The aim of the work is to study the role of a point mutation of the gene DUMPS in the etiology of fetal mortality in cows. Deficiency of the enzyme uridinmonophosphate synthase is detected in the red blood cells of animals. DUMPS defect manifests itself in a recessive form. When heterozygous DUMPS cows are inseminated with sperm of a heterozygous bull, 25% of the embryos die in the early stages of development. So first of all control of siring bulls should be carried out. For the study, two groups of 300 animals each were selected. These were representatives of the Kazakh White-Headed and Auliekol breeds from farms "Zholdas", "Zhanabek" located in the Kostanai region. DNA was isolated from blood spots using a kit for automatic isolation of nucleic acids «iPrep ™ ChargeSwitch® Forensic and Buccal Cell Kits» manufactured by the «Life Technologies», USA. The reaction mix consisted of the following components: DNA Polymerase, 1.5 mM $MgCl_2$, mixture of dNTP, 10x Taq Buffer, primers, the DNA sample and deionized water. The PCR product was subjected to digestion with the enzyme Ava I, which allowed the detection of CT nucleotide substitution in codon 405

of exon 5 of the gene UMP. The resulting PCR product was 108 bp in length, which had two restriction sites. In healthy animals, 3 fragments were observed having a length of 19, 36 and 53 bp. According to the data obtained, no positive results were found, which allows us to suggest that Kazakhstan's domestic breeds have a high chance to be free of the mentioned mutation. It remains however necessary to test all sires imported for improving the quality of local cattle in order to prevent occurrence and spread of harmful recessive mutations in the population.

P 28 | The effect of natural or induced calving in full term beef heifers on maternal immune function, reproductive health and colostrum IgG concentrations

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The aim was to determine the effect of natural or induced calving of full term beef heifers on immune status and subsequent reproductive health and colostrum IgG concentration. At day 285 of gestation, 55 crossbred recipient beef heifers were assigned to one of three groups: (i) control (CON, n = 19); (ii) induction with corticosteroid (CORT, n = 20) and (iii) induction with corticosteroid plus prostaglandin (CORT+PG, n = 16). Vaginal mucus was collected on day 21 (D21) and 42 (D42) post calving (D0) and an uterine cytology sample was obtained on D21. Blood samples were collected 2 weeks before parturition (D-14), 1 day after parturition (D1) and 2 weeks after parturition (D14) and analyzed for cortisol and calcium (Ca) concentrations. Calf weight on D0 was recorded and a colostrum sample was taken for subsequent IgG concentration analysis. Data were analyzed using Spearman correlation and stepwise backwards linear regression using SPSS for Windows. There was a strong relationship (R2 = 0.86) correlation between plasma Ca concentration on D14 and mucus score and cytology score on D21. On D1 the control group had higher cortisol concentrations ($p < 0.04$) compared with both treatment groups. Colostrum IgG concentration at calving was lower ($p < 0.05$) for CON compared with either of the induced groups. Treatment group and Ca concentrations on D-14 were strong predictors of IgG concentration. Calf weight at birth was not different between groups correlated to mucus score, uterine involution and cytology on D21. In conclusion: Induction of parturition had a positive effect on colostral IgG concentrations. Calcium concentrations on both D1 and D14 post calving are associated with subsequent reproductive health.

P 29 | Detection of VEGF in the testicular interstitium of *Mesocricetus auratus* during testicular regression after to a short photoperiod

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The Syrian hamster is a good model for studying reproductive control. When subjected to a short photoperiod, hamster testes regresses and shows pronounced morphological, histological and physiological changes. VEGF (Vascular Endothelial Growth Factor) is a protein involved in the maintenance of spermatogenesis, the regulation of endothelial growth, the induction of angiogenesis. The aim of this work was to perform a morphological and semiquantitative study of VEGF expression in the testicular interstitium during testicular regression. For this, a total of 16 Syrian hamsters were divided into four groups: Control (C), Middle Regression (MR), Strong Regression (SR) and Total Regression (TR). The presence of VEGF was detected by immunohistochemistry in Leydig cells and the endothelium of vessels in all groups. The proportion of Leydig cells and capillaries positives for VEGF was determined. The rate of VEGF positive Leydig cells showed no significant difference during regression. The proportion of VEGF positive capillaries was significantly higher in SR and TR groups than in C and MR. In conclusion: (a) the decrease in Leydig cell function during regression before complete involution does not seem to be accompanied by a decrease in VEGF expression; (b) the increased expression of VEGF in the testicular microvasculature of regressed animals may reflect the beginning of a process of angiogenesis. The formation of new vessels would continue during recrudescence, until the full restoration of the microvasculature and its function. (Funded by GERM 19892/15 from Fundación Seneca CARM)

P 30 | The benefits of natural and lyophilized egg yolk plasma for freezing canine sperm

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Low density lipoproteins (LDL) constitute the sperm cryoprotective fraction of egg yolk. Six percent LDL-based extender was shown to be effective in freezing dog semen. Egg Yolk Plasma (EYP) is the soluble fraction easily obtained by simple dilution and centrifugation of the whole EY. It is composed of LDL (15%) and globular glycoproteins (85%) and has proved to be efficient in sperm cryopreservation. Unlike LDL, EYP can be industrially produced, easily sterilized and incorporated into ready to use extenders. Lyophilizaton of EYP has never been attempted yet. Therefore, the present work aims to determine the possible benefits of three concentrations of both natural and lyophilized egg yolk plasma in substitution to LDL for freezing canine sperm. Twenty ejaculates collected from 6 Beagle dogs were frozen with extenders composed of 6% LDL, 20%, 40%, 60% natural plasma (NEYP) and 20%, 40%, 60% lyophilized plasma (LEYP). Motility parameters were assessed before and after freezing by Hamilton Thorne analyzer (HTM-IVOS, 14.0). A linear model with mixed effects was applied using R® software. Forty percent of EYP appeared to be the optimal concentration for both natural and lyophilized egg yolk plasma with no significant difference ($p > 0.05$) when compare to the reference extender (6% LDL). In conclusion, motility characteristics of dog semen could be successfully cryopreserved in

both natural and lyophilized 40% EYP-based extender as effectively as in the reference extender (6% LDL). Lyophilized EYP seems to be a viable alternative to LDL in freezing extenders for dog semen.

P 31 | Reduced glutathione prevents the observed increases in intracellular levels of both free cysteine radicals and overall reactive oxygen species in boar sperm subjected to "in vitro" capacitation

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A study was carried to test the effects of reduced glutathione (GSH) on the changes in free cysteine radicals (FCR) and reactive oxygen species (ROS) levels in boar sperm during the "in vitro" capacitation (IVC). Four samples for each experiment (n = 6) were analyzed. Incubation for 4 h at 38.5°C of sperm in an IVC activation medium (CM) induced a significant ($p < 0.05$) increase in the intracellular levels of FCR in both head (from 3.0 nmol/μg protein ± 0.3 nmol/μg protein at 0 h to 17.4 nmol/μg protein ± 1.7 after 4 h of incubation) and tail sperm extracts (from 3.4 nmol/μg protein ± 0.4 nmol/μg protein at 0 h to 8.4 nmol/μg protein ± 1.0 nmol/μg protein after 4 h of incubation). Similarly, a significant ($p < 0.05$) increase was seen in the rate of sperm with high intracellular levels of both peroxides (from 2.4% ± 0.7% at 0 h to 18.6% ± 2.0% after 4 h of incubation) and superoxides (from 11.6% ± 1.7% at 0 h to 17.5% ± 1.9% after 4 h of incubation). These increases were concomitant with capacitation-like changes of parameters like sperm motility, membrane fluidity, mitochondrial membrane potential and P32 protein tyrosine phosphorylation levels. The addition of GSH at the CM prevented the majority of these changes in a dose dependent manner, reaching a maximal effect at concentrations of 5 mM. Our results seem to indicate that boar sperm IVC is related with a significant increase of both overall disrupted disulfide bonds and intracellular ROS levels which can be prevented by the addition of GSH to the medium. These phenomena could play a role in the achievement of the capacitation status.

P 32 | Messenger RNA expression and DNA binding activity of peroxisome proliferator-activated receptors β/δ and γ in periimplantation porcine conceptuses

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The present studies aimed to analyze (1) mRNA expression and DNA binding activity of peroxisome proliferator-activated receptor (PPAR) β/δ and γ isoforms in periimplantation porcine conceptuses and (2) the correlation between the expression of PPARs and genes important for conceptus development: aromatase, prostaglandin endoperoxide synthase (PTGS2), glucose transporter 1 (SLCA1) and interleukin 1β. Conceptuses were collected from 35 pregnant gilts and classified based on the size and morphology to the following days/groups: 10–11 (spherical), 11–12 (filamentous), 13–14 and 15–16 (elongated). Total RNA was isolated and used for Real-time PCR analysis. DNA binding activity was determined in tissue nuclear extracts using ELISA. One-way ANOVA followed by Bonferroni's post-hoc test was conducted for data analyses. Correlation was assessed using Pearson test. A considerable increase in PPARβ/δ and γ mRNA expression was detected between days 10–11 spherical and days 11–12 filamentous conceptuses ($p < 0.01$). On days 13–16, mRNA levels of both isoforms decreased ($p < 0.05$) but for PPARγ were still greater than on days 10–11 ($p < 0.001$). DNA binding activity of PPARβ/δ and γ was higher in conceptuses from days 11–12 than from days 13–16 ($p < 0.05$). PPARβ/δ expression showed strong positive correlation with SLCA1 and PTGS2 mRNA expression ($p < 0.001$). In summary, porcine conceptuses from periimplantation period express PPARβ/δ and γ isoforms, which may be involved in conceptus transformation before implantation. (Supported by NSC grant 2013/11/B/NZ9/00806)

P 33 | Prevalence and effect of subclinical endometritis on the pregnancy outcome of nulliparous dairy heifers

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Objectives of this study were to assess the prevalence of subclinical endometritis (SCE) and its effect on the pregnancy outcome in nulliparous dairy heifers. A total of 496 endometrial cytology samples were taken during artificial insemination (AI) from 339 Holstein-Friesian heifers by means of cytotape. Cytotape consisted of a 1.5 cm piece of paper tape rolled on the top of an AI catheter covered with a double guard sheet. After sampling, the top of the AI catheter was gently rolled onto a glass slide, air dried and stained using Diff-Quick®. Since PMNs were rarely found in the cytology slides (mean 0.32 ± 1.5, range 0–18%), an arbitrary dichotomization of the PMN% was performed: SCE positive ≥1% PMN vs. SCE negative = 0% PMN. An insemination was considered successful when pregnancy was confirmed by rectal palpation. Heifers were defined not pregnant when they received a subsequent insemination or were diagnosed empty by rectal palpation. Multilevel generalized mixed effect models were built to test factors affecting the pregnancy outcome and the occurrence of SCE at the AI. At the sample level (n = 496), prevalence of SCE at AI was of 7.86% (n = 39), and at the heifer level (n = 339) SCE prevalence at AI was 5.9% (n = 20). Conception rate in SCE negative samples (n = 457) was 62.8% (n = 287) while it was 38.46% (n = 15) in SCE positive samples. Risk factors for non-pregnancy were: performance of a previous

AI (Odds ratio (OR): 2.966) and the interaction between SCE × previous AI. The only risk factor identified as being associated with the occurrence of SCE was the performance of a previous AI (OR: 4.717). In conclusion, the performance of unsuccessful inseminations significantly affects the outcome of a subsequent AI and may cause SCE in nulliparous dairy heifers.

P 34 | Prediction of calving time in dairy cows

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The objectives of this study were to describe changes in rumination and locomotion behavior of dairy cows to predict the time of calving. N = 32 primiparous and multiparous highly pregnant (mean = 258 day) Holstein Friesian cows were included. The rumination and locomotion behavior was recorded from 10 d ante partum (a.p.) until calving using a noseband-sensor and a pedometer. The 72 h before calving were divided into 3-h periods. Every 3-h period from −15 h a.p. to calving was compared to its 3-h reference period 24 h earlier. In addition, the final 24 h a.p. was compared to the period 48–24 h before calving. In the last two 3-h periods before calving, cows had a significant increase in lying down events compared to the reference periods. The number of lying down events increased in the 24 h before calving, while the lying time decreased compared to the period 48–24 h before calving. Walking time and walking bouts increased in the last 3-h period before calving compared to the reference period. Cows made almost twice as many other leg movements and the time the cows spent ruminating decreased significantly in the last 3-h period compared to the reference period. In a second step, a ROC-analysis of all significant variables showed that a combination of the lying down events, walking time and other leg movements is the most useful combination for successfully predicting (sensitivity = 90.32%; specificity = 90.32%) calving within the next 3–6 h with the applied device. The non-invasive tools assessed in this study could support the farm personnel to predict the time of calving, reduce the number of unattended parturitions and calf losses and thus help improve animal welfare.

P 35 | Endometritis in pregnant Degu (*Octodon degus*)

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The degu is a small rodent endemic in central Chile, about 25 cm long and weighs about 300 g. The length of gestation is about 87 days. Endometritis in pregnant degu is very rare. Our patient, aged about 1 year, underwent a routine evaluation of pregnancy by ultrasound examination (10 MHz probe), and we measured the levels of glucose and progesterone from day 23 every week until 11 weeks. Last examination was performed 4 days before the miscarriage. No changes were observed in appearance of the amniotic fluid, placentas or fetuses state. Only a slight thickening of the uterine wall was noticed. Overall activity and patient's appetite before abortion was normal. The levels of glucose and progesterone were normal. Six stillborn fetuses appeared with signs of being bitten by their mother. The abdominal ultrasound examination post abortion revealed thickening of the uterine wall and fluid in the uterus. Clinical condition of the dam was good. During few days a slight purulent vaginal discharge was observed. The microbiological smear of vaginal secretions revealed the presence of *Streptococcus pyogenes* (SP), being sensitive to enrofloxacin. During 2 weeks the degu was treated with enrofloxacin (10 mg/kg, subcutaneous). Treatment was completed when the ultrasound examination of the uterus was normal and degu's behavior was normal. Eight weeks after finishing the treatment the patient was placed with a male where she stayed until the detection of a next pregnancy. In the next gestation she gave birth to five live, well-developed young. Ultrasound in degus allows monitoring the pregnancy, determining gestational age, detecting infections and controlling the treatment process. SP infection in pregnancy in the degus may lead to decay fetuses.

P 36 | Determine the effect of twin and single pregnancies on the results of rearing of Angus calves

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The course of cows' parturition is a complex trait conditioned by a variety of factors. Suitability for easy labors is associated with body weight of cows as well as the structure of the genital tract. Also important are hormonal mechanisms responsible for the ability to make an effort during labors. The aim of the study was to determine the effect of twin and single pregnancies on body weight and daily gain of Angus calves. The study evaluated 179 calves born in 2013. Data on the rearing of calves were collected from breeding documentation according to the guidelines of the PZHiPBM. The study analyzed the results of rearing calves depending on birth as a twin (16) vs. a singleton (163 pregnancies). Analyzed features included birth weight (kg), weaning weight (kg) and daily weight gain (kg). Analyzing the results revealed that calves born after a singleton pregnancy had a significantly ($p \leq 0.01$) higher birth weight (29.41 kg) compared to calves born as twins (25.69 kg). Singleton calves also had a higher weaning weight (267.33 kg vs. 253.38 kg). Analyzing the daily weight gain of the calves from birth to 210 days of age (g) revealed a greater advantage for calves born from twin (1084.13 vs. 1074.90 g) pregnancies.

P 37 | The development of puppies of various breeds

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The domestic dog (*Canis familiaris*) is the most morphologically diverse species (near 400 breeds) of the animals kept by people. Due to the changes that occur in populations of breeds of dogs should be monitored at an early stage of development of individual life dog breed is similar? Using breeders' records, we analyzed the development of puppies of various breeds (Chihuahua-Ch, Yorkshire Terrier-YT, Rabbit Dachshund-RD, Cavalier King Charles Spaniel-CKCS, Shetland Sheepdog-SS, Welsh Corgi-WC, Polish Hunting Dog-PHD, Labrador Retriever-LR, Alaskan Malamute-AM, Bouvier des Flandres-BF, Newfoundland-N, Great Dane-GD). The most numerous litters were found in breeds GD (8.7) and N (8.2), while the least number in CH (2.3) and YT (3.7). The highest mortality rate in puppies was found in YT litters (29%). Analysis compilation of the pups' gender revealed that YT born more females (66%) and N more puppies male (58%). Analyzed races are significantly different in terms of the average number of pups per litter (the biggest differences were found between breeds miniature and giant). Significant differences were also found in the body weight of pups after birth. Giant breed puppies had the lowest birth weight compared to the weight of an adult (N-1%; GD-1.1%) while YT puppies (miniature breeds) had a birth weight in the range of 3.6% by weight of an adult. Incremental analysis showed the highest growth rates occurred within the first 28 days of life (significantly higher in the case of medium and large dog breeds).

P 38 | Investigating the expression of genes encoding epigenetic enzymes in the bovine endometrium

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All cows experience an influx of bacteria after calving, but many fail to clear the resulting infection, causing uterine disease and subfertility. Epigenetic modifications, which affect gene regulation without altering the underlying DNA sequence, may contribute to individual differences in immune response and disease susceptibility. Epigenetic enzymes such as DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) affect transcription factor access to DNA and chromatin structure, respectively. The aim of this study was to investigate expression of these enzymes in postpartum cattle, with and without subclinical endometritis (SCE). Uterine biopsies were collected from healthy (n = 9) and SCE (n = 6) cows at 7 and 21 days postpartum (DPP). RNA was extracted using Trizol, and cDNA synthesis and RT-qPCR were performed. Results showed HDAC1 was increased 2-fold in SCE cows at 7 DPP compared to 21 DPP. In contrast, HDAC9 was significantly decreased in all cows at 7 DPP vs. 21 DPP. DNMTs were not differentially expressed. On-going analysis is investigating expression of these genes in primary epithelial and stromal cells and endometrial explants stimulated with E. coli lipopolysaccharide. Our preliminary results suggest that HDAC enzymes may be important in regulating the postpartum immune response, and further investigation could aid development of future intervention strategies for endometritis.

P 39 | Factors affecting the efficacy of progesterone supplementation post insemination on the fertility of dairy cows

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It was the aim of this study to evaluate the efficiency of progesterone supplementation post insemination (p.i.) in the pregnancy per artificial insemination (P/AI) of dairy cows under commercial farm conditions. Four hundred and five cows from 3 farms were bred after natural estrus or after synchronization and were randomly allocated to receive a progesterone-releasing intra-vaginal device (PRID®) between day 5 and day 17 p.i. (P4+, n = 213), or to remain untreated (P4-, n = 192). Differences in binary variables were evaluated with the use of chi-squared analysis and with a linear logistic model. Number of insemination, days open, parity, season, daily milk yield and P4 supplementation were included in the model. Progesterone supplementation had no overall effect on P/AI (42.3% vs. 41.2%). However, the treatment improved P/AI in cows enrolled in the study after the second post-partum insemination (46.8% vs. 25.5% for P4+ vs. P4-, respectively, p = 0.02) and as tendency in the farm that showed the lower overall fertility and the lower days to insemination (48.2% vs. 23.5%, for P4+ vs. P4-, respectively, p = 0.08). In the farm with the greater overall fertility the untreated group had better P/AI compared to the treated group (57.4% vs. 37.7%, respectively, p = 0.03). The interaction between the number of insemination and PRID satisfied the cut off level for entry in the final model (p = 0.15). Conclusively, P4 supplementation p.i. can be beneficial in farms with lower fertility and early in the lactation.

P 40 | Effect of insulin-like growth factor I (IGF-I) on oocyte in vitro maturation, apoptosis and steroidogenesis of cumulus-oocyte complexes in the guinea pig model

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The aim of this work was to evaluate the effect of different concentrations of IGF-I on in vitro maturation (IVM) of guinea pig oocytes. A total of 568 selected cumulus-oocyte-complexes (COCs) were matured with TCM199 and IGF-I at 0, 50, 100, 200 ng/ml or 10% FCS, respectively. Meiotic and cytoplasmic oocyte maturation rate in terms of cortical granule (CG) migration and mitochondrial distribution, and the apoptotic rate in COCs were assessed by confocal-scanning microscopy. Steroidogenesis was evaluated in the spent media by ELISA. Chi-square and one way ANOVA test were performed. Supplementation with 100 ng/ml of IGF-I significantly stimulated nuclear oocyte maturation (metaphase II; $p < 0.05$); in addition, 100 ng/ml of IGF-I induced a significantly higher rate of oocytes showing peripheral migration pattern of CG (compatible with cytoplasmic maturation) compared with non-supplemented group and with 50 ng/ml of IGF-I group ($p < 0.05$). However, the mitochondrial distribution patterns were similar for all groups. Apoptotic index of the COCs matured with 50 or 100 ng/ml of IGF-I was significantly lower than in group without IGF-I ($p < 0.05$). The highest oestradiol and progesterone secretion by COCs was found in 100 ng/ml group ($p < 0.05$). In conclusion, the more suitable concentration of IGF-I in the present IVM system was 100 ng/ml. (We acknowledge UTPL and UCM for funding.)

P 41 | Bone marrow derived mesenchymal stem cells therapy on the expression of pro-fibrotic cytokine TGF-β1 in mare's endometrosis

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Equine endometrosis is a chronic degenerative disease responsible for mare infertility, with no effective treatment. When tissue homeostasis is disturbed, cytokine Transforming Growth Factor β1 (TGF-β1) may lead to fibrosis. Mesenchymal stem cells (MSCs) may be an innovative therapy for fibrosis. The aim was to evaluate the expression of TGF-β1 and its receptors in endometrium after uterine infusion of bone marrow derived autologous mesenchymal stem cells (BM-MSCs) in mares suffering of endometrosis. Endometrium biopsies were collected before treatment (D0), and on days 30 (D30) and 60 (D60) after the endometrial infusion of $8x10^6$ BM-MSCs/mare (n = 7). In all mares, TGF-β1 and TGFβRI showed a similar immunohistochemistry pattern in the cytoplasm of glandular epithelium and endothelium. TGFβRII was found mainly in stromal cells and cilia of glands. A stronger TGF-β1 and TGFβRI cytoplasm intensity was observed in glandular epithelium of dilated glands or glands located in fibrotic foci. There was a decrease in the intensity of TGF-β1 and TGFβRI at D30 and D60, but not in all mares' endometria. TGFβRII expression did not change after BM-MSCs treatment. No clear effect of autologous BM-MSCs therapy for endometrosis was observed on the expression of pro-fibrotic cytokine TGF-β1 and its receptors TGFβRI and TGFβRII. (Grant: UID/CVT/00276/2013)

P 42 | Heparin concentration changes development of bovine blastocysts in vitro according to their sex

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Capacitation changes heparin binding to sperm, and sorting destabilizes sperm membranes in a capacitation-like effect. It is ignored whether X and Y-bearing sperm react differently to heparin. The objective was to investigate whether heparin concentration affects differentially blastocyst (B) sex in vitro. In vitro matured oocytes were fertilized with male (M)- and female (F)-sorted Holstein semen, with 10 or 20 μg/ml heparin (H10 or H20). Zygotes were cultured in SOF+BSA, and B, expanded B [XB], and hatched B [HB]) rates were monitored. Single bulls were then used as unsorted, and Bs were sexed by amelogenin gene amplification. Data were analyzed by GLM and REGWQ test. Sorted bulls differed in % Day-8 Bs with heparin dosage. Bull A: 20H-M = 17.4 ± 2.3 vs. 20H-F = 6.5 ± 3.2; 10H-M = 8.0 ± 8.0 vs. 10H-F = 25.2 ± 5.5 ($p < 0.05$). Bull B: 20H-M = 15.6 ± 1.4 vs. 20H-F = 10.4 ± 1.4 ($p < 0.05$); 10H-M = 5.0 ± 2.5 vs. 10H-F = 5.6 ± 2.5). Bulls C and D did not produce Bs in vitro as sex-sorted. Bull E tended ($p < 0.08$) to differ in Day-7% Bs (20H-M = 2.8 ± 1.5 vs. 20H-F = 6.9 ± 1.1). With unsorted sperm (N = 320 Bs sexed; bulls A,B,C,D) overall sex ratio with HB-H10 (38.4 ± 7.6) was lower than HB-H20 (70.5 ± 11.9) and XB-H10 (69.6 ± 6.4) ($p < 0.04$). Only sex ratio of bulls A (H10 = 45.8 ± 7.7 vs. H20 = 61.0 ± 0.8) and B (H10 = 50.0 ± 8.3 vs. H20 = 67.4 ± 9.6) was close to differ ($p = 0.11$), consistent with sex-sorted development. Heparin concentration might alter B sex independent on sorting in single bulls. (MINECO (AGL2012-37772) and FEDER; COST Action FA1201 (Epiconcept); Principado de Asturias, PCTI-2013-2017 (GRUPIN 14-114); Sexing Technologies)

P 43 | Synchronization with a 5-day PRID sync protocol for first service decreased age at first calving and rearing costs in Holstein dairy heifers

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Our objective was to compare reproductive performance and rearing costs in Holstein dairy heifers after synchronization of ovulation and timed artificial insemination (TAI) for 1st service and natural service

for subsequent breedings or natural service (NS) for all breedings. Nulliparous Holstein dairy heifers (n = 366) were managed to receive TAI after a 5-day PRID sync protocol (Day 0, GnRH+PRID; Day 5, PGF+PRID removal; Day 6, PGF; Day 8, TAI) for 1st service and NS for subsequent services or to receive only NS for all breedings. Heifers were ~14 mo of age when received TAI or were placed with the bulls. A partial budget was developed to calculate the economic differences between the reproductive programs using specific inputs for each heifer. The structure of the economic analysis included expenses with hormones for synchronization of ovulation, labor associated with hormone administration and AI, semen and AI supplies, costs of pregnancy diagnosis, costs of bull, and feed costs, as well as, genetic gain associated with the use of AI sires of greater genetic potential. Data were analyzed by ANOVA and logistic regression using MIXED and GLIMMIX procedures of SAS. Age at first calving was lower ($p < 0.01$) for TAI than for NS heifers (23.7 vs. 24.9 months). In addition, a higher ($p < 0.01$) proportion of TAI heifers calved with ≤24 months in comparison to NS ones [77% (141/183) vs. 44% (81/183)]. Considering all the economic inputs, rearing costs were 67.5 € less ($p < 0.01$) for TAI than NS heifers (1430.9 € vs. 1498.4 €). In conclusion, performing TAI for 1st service decreased age at first calving and reduced rearing costs of Holstein dairy heifers.

P 44 | Seminal plasma protein composition in vasectomized rams

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Seminal plasma (SP) is composed of secretions from the testis, epididymis and the accessory glands. In order to determine the protein contribution of the testis and epididymis to the SP, ejaculates from two vasectomized and three intact rams were collected by artificial vagina, and the SP was obtained by centrifugation at 12000xg. SP protein concentration was calculated by Bradford, and protein composition was analyzed by SDS-PAGE and difference gel electrophoresis (DIGE). Protein concentration was higher in SP from vasectomized vs. intact rams (64.6 ± 6.3 vs. 34.2 ± 5.4 mg/ml, $p < 0.001$). SDS-PAGE showed a decrease in high and medium molecular weight bands and an increase in the low molecular bands in the SP from vasectomized rams. DIGE revealed that the abundance of 40 spots increased and of 144 spots decreased in vasectomized animals ($p < 0.01$), along with the absence of six proteins identified as angiotensin-converting enzyme, lactotransferrin, phosphoglycerate kinase, sorbitol dehydrogenase, epididymal secretory glutathione peroxidase and epididymal secretory protein E1. In conclusion, vasectomy seems to change the protein profile of ram SP. (Grants: CICYT-AGL2013-43328P, CICYT-AGL2013-41200P, DGA-A26, Ministry of Higher Education and Scientific Research of Tunisia)

P 45 | Seminal plasma proteome in fertile and infertile rabbit male

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The present study was designed to compare seminal plasma proteins of two New Zealand White rabbit males (*Oryctolagus cuniculus*). Both males were of the same age, one was of proven fertility and the other of proven infertility. Ten seminal samples, five from each male, were collected during 5 weeks and semen quality was evaluated. Afterwards, seminal plasma of the two males was obtained and subjected to polyacrylamide gel electrophoresis (SDS-PAGE). The electrophoretic profile of both males resulted in multiple protein bands of different intensity ranging from 9.5 to 270 kDa. Results showed that eleven protein bands were significantly different ($p < 0.05$) between the fertile and the infertile male. The differentially expressed proteins were identified by MALDI-TOF/TOF or LC-MS/MS. Among the identified proteins, the relative quantity of the following ones was higher in the fertile male: Aldehyde oxidase 1 and 3 (165 kDa), FAM115E-like (98.8, 60.4 and 53.9 kDa), Annexin A5 (34.3 kDa), Carbonic anhydrase 2 (30.9 kDa), Glutathione S-transferase Mu 1 (26.5 kDa), Lipocalin allergen Ory c 4 precursor (19.6 kDa), Odorant-binding protein-like (17.2 kDa) and Epididymal secretory protein E1 (17.2 kDa). On the contrary, FAM115E-like (269 kDa) and Desmoplakin isoform X1 (9.5 kDa) proteins were more abundant in the infertile male. In conclusion, there were differences in the seminal plasma protein profile of the fertile and the infertile rabbit male. Furthermore, the distinct presence of some of these proteins could potentially be used to identify novel biomarkers of rabbit fertility/infertility.

P 46 | Anti-Müllerian hormone expression in testes of neonatal and prepubertal unilateral cryptorchid swine (*Sus scrofa domesticus*)

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The anti-Müllerian hormone (AMH) is synthesized by the gonads of all vertebrates. In mammals, AMH expressed by the testicular Sertoli cells causes regression of the Müllerian ducts during early fetal life, regulates gonadal function during postnatal life up to puberty, and has extra-gonadal effects on the hypothalamic-pituitary-gonadal axis. In mammals, circulating AMH likely derived from the gonads and its abnormal concentrations indicate the presence of several testicular pathologies, including cryptorchidism. These data confirm that the AMH production by Sertoli cells is correlated to testicular pathology. In this context, the potential role of AMH as an endocrine marker of cryptorchidism in boars remains unexplored. Thus, the goal of this

study was to evaluate by immunohistochemistry (IHC) and western blotting (WB), the expression of AMH in testes of neonatal (8 days, n = 4) and prepubertal (2, 3, and 5 months, n = 12) unilateral cryptorchid swine. Both IHC and WB findings revealed that all the neonatal and prepubertal cryptorchid testes show a high positivity for Sertoli cells, whereas in contro-lateral normally descended testes this positivity decreased (densitometric analysis, one-way ANOVA followed by Student-Newman-Keuls post hoc comparisons: $p < 0.05$) from neonatal to late prepubertal phase. These results suggest that the unilateral cryptorchid testis may influence the AMH plasma concentrations in prepubertal swine, with its persistent production by Sertoli cells.

P 47 | Sperm chemoattractant and capacitation modulator role of melatonin during the non-breeding season

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Chemotaxis is an essential mechanism for the sperm-oocyte binding. The aim of this study was to analyze the sperm chemoattractant ability of melatonin and whether the melatonin-sperm interaction is affected by the season when samples are collected. We evaluated viability (membrane integrity, CFD/PI), phosphatidyl-serine translocation (Annexin V) and capacitation state (chlortetracycline staining, CTC) comparing breeding (BS, October–February) and non-breeding (NBS, April–June) season. A dextran/swim-up technique was carried out in the presence of two doses of the hormone (high, 1 μM and low, 100 pM). No significant differences were found during BS in the studied parameters (viability, phosphatidyl-serine translocation and capacitation state). However, during NBS, the sperm recovery rate in the swim-up process was higher in the presence of melatonin (64.8 ± 7.3 and 59.3 ± 6.5% for 1 μM and 100 pM, respectively) than in the control (52.04 ± 10.46%, $p < 0.05$). Likewise, a higher percentage of capacitated sperm (CTC staining) was found in the samples with the lower melatonin concentration (23.0 ± 4.2 and 17.2 ± 3.7 for 100 pM and control group, respectively, $p < 0.05$). These results indicate a certain chemotactic effect of melatonin in spermatozoa, and a modulation of capacitation ability. (Grants: CICYT-AGL 2013-43328-P, AGL 2014-57863-R and DGA-A26)

P 48 | Factors influencing conception rate in dairy cows

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The aim of this study was to evaluate conception rate in relation to milk yield, season, lactation number and method of estrus detection or synchronization in dairy cows. Overall conception rate in observed cows (n = 1218) was 41.9%. Conception rate in cows yielding <30 kg/day was significantly higher than in cows yielding >40 kg/day (50.3% vs. 35.8%, $p < 0.01$). Conception rate in summer was significantly lower compared to other seasons (34.4% vs. 43.8%, $p < 0.01$). Cows in the 1st lactation showed higher conception rate in comparison with cows in the 3rd or 5th lactation (55.6% vs. 34.3% or 31.7%, $p < 0.001$). Conception rates after insemination in natural estrus, natural estrus with induction of ovulation by GnRH, after simple administration of cloprostenol in presence of CL, after ovsynch 56 or CIDR-ovsynch were similar (39.7%, 38.8%, 42.5%, 42.3%, 44.8%, respectively). Ovsynch 56 was started in various conditions (in a known stage of estrous cycle, a diagnosed stage of estrous cycle by rectal palpation, after treatment of acyclicity or ovarian cysts, and after pregnancy diagnosis) with similar conception rates (54.2%, 43.5%, 36.8%, 48.6%, 34.4% and 42.6%, respectively). A Pearson's chi square independence test was used for statistical evaluation. The results showed that conception rate in dairy cows is influenced by season, milk yield and number of lactation but the method of estrus detection or synchronization did not significantly influence conception rate.

P 49 | Responsiveness of rabbits to superovulation treatment by a single injection of a long-acting recombinant follicle stimulation hormone

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Efforts have already been made to simplify the hormonal superstimulation method. We investigated the applicability of a long-acting recombinant FSH as a simplified method of inducing superovulation in rabbits. New Zealand White rabbits were superovulated with one subcutaneous injection of corifollitropin alfa (3 μg, Elonva®). Sixty hour after, does were inseminated (AI) and the embryos were collected 72 h after AI. After recovery, morphologically embryos were classified as according to IETS classification. Normal embryos were transferred using laparoscopic technique. Embryo survival rates were assessed by laparoscopy at day 14 and at birthday noting implantation rates and birth rates, respectively. Whilst 100% of control does ovulated (26/26), only the 76.5% of the treated does for the corifollitropin alfa group ovulated (13/17). The ovulation rates and transferable embryos were significantly higher in superovulated does (50.2 ± 5.7 and 40.0 ± 5.4 vs. 12.4 ± 0.4 and 9.8 ± 0.4, respectively). The recovered oocytes and abnormal embryos were not significantly different (1.9 ± 1.3 and 2.1 ± 1.3 vs. 0.04 ± 0.04 and 0.2 ± 0.02, respectively). The rate of implantation and development to term were significantly lesser for the corifollitropin alfa group (64 ± 5.0% and 53 ± 6.0%) than that control (82 ± 5.0% and 73 ± 6.0%). Although hormonal superstimulation with corifollitropin alfa appears to affect the embryos

ability, the total number of offspring (about 3 times higher) indicates that corifollitropin alfa treatment by single injection, which is less time- and labor-consuming, is an alternative method that allows the collection of rabbit embryos. **Acknowledgement**: This work was supported by the Spanish Research Project (AGL2014-53405-C2-1-P).)

P 50 | Sensitivity and specificity of endometrial cytopathological diagnosis in the slaughtered cows

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This study was conducted to evaluate sensitivity and specificity of endometrial cytopathological diagnosis, considering histopathological diagnosis the golden standard in the uterus. Reproductive stages of the 66 slaughtered cows were determined by examinations of the ovaries, endometrial swabs were collected using cytobrush for cytological examination (Giemsa) and endometrial tissues were harvested for histopathological examination (H&E). Cells were enumerated at 10 areas randomly selected (x40) in both methods. Data were analyzed by two-way ANOVA to determine main effect of diagnostic methodology and reproductive stage as well as their interaction. ROC curve was also developed to measure sensitivity and specificity of cytopathological examination. There were no effects of reproductive stage and diagnostic methodology by reproductive stage interaction on response variable. For Giemsa and H&E, lymphocytes (5.31 vs. 10.72, $p = 0.002$), macrophages (1.40 vs. 3.00, $p = 0.011$) and plasmocitic cells (0.30 vs. 0.87; $p = 0.046$) differed, but not neutrophiles (0.25 vs. 0.38; $p = 0.85$) and eosinophiles (0.12 vs. 0.19, $p = 0.85$). There was no connective tissue proliferation difference in both methods ($\chi^2 = 0.08$, $p = 0.78$). The ROC curve analysis revealed that sensitivity and specificity of cytopathological diagnosis were 75.00 (53.3–90.2 at 95% CI) and 100 (85.8–100 at 95% CI), with positive and negative likelihood ratios of 1.00. In conclusion, acute changes in the tissue can clearly be visualized in endometrial cytology. However, chronic changes in stromal tissue could not be evaluated in superficial endometrial layer completely. In case of chronic endometritis, more severe chronic inflammations should be suspected in the profound endometrium than presented in endometrial layer.

P 51 | Non-esterified fatty acids affect the survival and increase lipid accumulation of bovine endometrial epithelial cells

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Elevated non-esterified fatty acid (NEFA) concentrations in blood are associated with impairments of reproductive function during the postpartum period. Adverse effects of NEFA have previously been shown on bovine theca, granulosa and oviduct cells. However, NEFA effects on bovine endometrial epithelial cells (bEECs) remain unknown. This study aimed to investigate the possible responses of bEECs following NEFA exposure in vitro. Post-primary bEECs from three cows (two Holsteins and one Swedish red) were cultured with 150, 300 or 500 μM of individual palmitic acid (PA), stearic acid (SA), oleic acid (OA), and their combination (150, 300 or 500 μM of each FA) and 0.5% final concentration of ethanol or ethanol alone (vehicle for NEFA) as a control. Viability was determined by trypan blue staining at 24 h and 48 h and evaluated by calculating the survival rate compared to untreated control cells at each time point. Lipid accumulation in cell plasma was also evaluated by Oil-Red-O staining. Addition of 300 and 500 μM of all NEFAs significantly decreased cell survival when compared to controls after 24 h and 48 h exposure ($p < 0.05$ to $p < 0.0001$), whereas no difference was observed with 150 μM of all NEFA treatments. Also, 4 h after exposure, higher amounts of intracellular lipid vacuoles were observed in 300 and 500 μM concentration of OA and combined NEFA-treated cells than in controls. These results show that combined NEFA, OA, PA and SA strongly impaired bEEC viability in a dose- and exposure time-dependent way and were associated with lipid accumulation in cell plasma. Both changes may be associated with alterations of endometrial function in cows submitted to negative energy balance during the post-partum period.

P 52 | Effect of the age at first calving on milk performance in Simental cows

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Research about reproductive efficiency in HF cows showed a progressive decline in fertility. Significantly longer calving intervals and an increased number of inseminations per pregnancy have been observed. An important factor influencing fertility and milk performance is the age at first calving (AFC). The aim of the study was to determine the effect of the AFC on milk yield in Simmental cows in the first 305-day lactation. The study included 54 individuals from a dairy farm. Data on milk yield, and fat and protein content and the AFC were obtained on the basis of the farm breeding documentation. The cows were divided into three groups on the basis of the AFC (I – under 26.0, II – 26.0–27.0, III – above 27.0 months of age). The significance of differences between the group means was determined using Tukey's test for unequal frequencies. The material analyzed statistically using the program Statistica®10 PL. The highest milk yield was characteristic for cows calving between 26 and 27 months of age (6158 kg/305-days). The highest fat and protein amount per 305-days was characteristic for cows calving under 26 months of age (247 and 212 kg, respectively). In terms of the fat and protein content, the highest values were measured in late calving cows after 27 months of

age (4.16% and 3.59%, respectively). In this study, no clear influence of AFC on milk yield and content was found. Therefore, in the herd examined, it's not possible to give an advice to optimize AFC in regard to milk yield and contents.

P 53 | Immuno-distribution of VEGF and its receptors in the canine endometrium in early pregnancy

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VEGF system is an important player of endometrial vascular permeability and angiogenesis in implantation. VEGF pathways include two receptors: Flk-1, a major VEGF transducer, stimulates the endothelial cells proliferation; Flt-1 inhibits endothelial cell mitosis and impairs endothelial cell assembly. In this work, we describe the endometrial location of VEGF, Flk-1 and Flt-1 in early canine pregnancy. Ten pregnant uterus samples were used in the study, grouped in 2 phases of canine implantation (apposition - days 12–15 and invasion - days 17–19); females were inseminated twice, starting on day 0 (= ovulation, blood P4 ≥ 5 ng/ml). Ten early diestrus endometrial samples were used as controls. An indirect immunohistochemistry method with polyclonal primary antibodies (VEGF: RB-222-P1; flt-1: RB-1527-P1; and flk-1: RB-1526-P1, NeoMarkers, USA) diluted at 1:100 was used. Immunostaining intensity was scored in 5 non-overlapping fields. VEGF showed higher immuno-scores in the trophoblast and the lacuna cells in the invading phase compared to controls and in apposition, which displayed a prevalence of moderate immunoreaction. For Flt-1, the scores were higher in controls and apposing phase, for the epithelium and stroma, but were lower during the invasion. Similarly, weak to moderate Flk-1 labelling was found in the stroma in controls and apposition phase, but the highest scores existed in the endometrial and embryo epithelia in all pregnancy stages compared to controls. These results suggest a complex interplay in the VEGF pathway in canine early pregnant endometrium; Flk-1 showed higher immunostaining scores in pregnancy than in controls, suggesting its contribution to endometrial angiogenesis in early pregnancy. (The Project UID/CVT/00772/2013 (FCT) sponsored this work.)

P 54 | Effect of season on sperm DNA fragmentation of cooled-stored stallion sperm

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Sperm DNA fragmentation analysis has been widely used to assess the sperm quality of equine semen. However, seasonal variations on sperm DNA fragmentation have not been determined yet. The aim of this study was to assess the influence of season on stallion sperm DNA fragmentation after 24 h of cool-storage. Ejaculates were collected from 23 stallions of different breeds by artificial vagina during 2015. Animals were located in Avila, Spain (40,66:N 4,70:W). According to the date of collection, ejaculates were classified as follow: winter (January to March); spring (April to June); summer (July to September) and autumn (October to December). After collection, sperm samples were slowly cooled to 5°C using a standard protocol with INRA96 extender and stored for up to 24 h. After that, an aliquot of each sample was incubated at 37°C for 10 min and sperm DNA fragmentation was assessed using the Halomax Equ kit (Halotech DNA SL, Madrid, Spain) following the manufacturer instructions. The percentage of sperm with fragmented DNA (sDF, %) was recorded and compared between seasons by ANOVA followed by Duncan test. Results (mean ± standard error) showed no significant differences of sDF between spring, autumn and winter (14.98 ± 0.86 vs. 18.46 ± 1.85 vs. 16.62 ± 1.12) respectively; however, sDF was significantly higher ($p < 0.001$) in summer (32.85 ± 2.24) in comparison to the other seasons. In conclusion, seasonal variations were found in stallion sperm DNA fragmentation after 24 h of cool-storage, obtaining the highest values in summer

P 55 | Experiences of a 2 years neutering program with shelter dogs organized for final year veterinary students

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Animals play a crucial role in the education of veterinarians whether in their initial professional training or subsequent advanced study for a clinical specialty. However, veterinary teaching hospitals are experiencing caseload trends that negatively affect efforts to prepare students for entry-level veterinary practice, particularly in the training of technical skills. The Faculty of Veterinary Science in Budapest, Hungary, has concluded an agreement protocol with the Ministry of Agriculture and ten shelters. In the contract, the Faculty undertook the neutering of 1000 dogs free of charge for shelters. Shelters agreed that students could perform the operations, with their work being monitored by experienced clinicians. The Ministry and the Faculty would pay for the cost of the ovariohysterectomy/castration. The aims of the spay program were (1) to increase the possibility to be actively involved students in surgery procedures and (2) to support the shelters. During the 2 years period, 1000 dogs (49% female, 51% male) have been spayed. During the fall and spring semester, Monday through Thursday, five animals were operated on each day. The neutering program allowed each final-year

student to take part in a surgical intervention a minimum of three-four times.

P 56 | Pro- and anti-inflammatory mediators change prostaglandins secretion from the inflamed porcine endometrium

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The goal of the study was to examine the influence of lipopolysaccharide (LPS) and pro-(tumor necrosis factor/TNF/-α and interleukin/IL/-1β) or anti (IL-4 and IL-10)-inflammatory cytokines on the release of prostaglandin (PG)$F_{2\alpha}$, PGE_2 and PGI_2 from the inflamed porcine endometrium. On Day 3 of the estrous cycle (Day 0 of the study), either 50 ml of saline or 50 ml of *Escherichia coli* suspension (containing 109 colony-forming units per ml) were infused into the uterine horn of the gilts (n = 12 per group). Endometrial explants, obtained eight (D8) and sixteen (D16) days later, were incubated for 24 h with LPS (100 ng/ml of medium), TNF-α, IL-1β, IL-4 or IL-10 (10 ng/ml of medium). PGs contents in the medium were determined by EIA method. Acute endometritis developed in all bacteria-inoculated gilts, however on day 8 (five gilts) of the study a severe form of acute endometritis was noted more often than on day 16 (two gilts). $PGF_{2\alpha}$ secretion was decreased ($p < 0.05$) after incubation of the D8 and D16 inflamed endometrium with LPS compared with the saline-treated uteri. PGE_2 release from the D8 inflamed endometrium was lower in response to LPS ($p < 0.01$), IL-1β ($p < 0.001$) and IL-10 ($p < 0.001$), while on D16 it was reduced by LPS ($p < 0.01$), TNF-α ($p < 0.05$) and IL-1β ($p < 0.001$). PGI_2 secretion from the D8 inflamed endometrium was decreased after incubation with LPS ($p < 0.01$), TNF-α ($p < 0.001$) and IL-1β ($p < 0.001$), whereas on D16, such effect occurred in response to LPS ($p < 0.001$) and TNF-α ($p < 0.05$). Data obtained show that pro- and anti-inflammatory mediators participate in the PGs secretion from an inflamed porcine endometrium.

P 57 | The impact of calving season on birth weight and daily gain of Angus calve

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Rearing during a year as many calves as possible is an important factor determining the profitability of beef cattle breeding. A number of factors affect the normal growth and rearing of calves, such as genotype and age of the mother, the course of cows' parturition and calf sex. The aim of the study was to determine the effect of calving season on body weight and daily gain of Angus calves. Material for the study consisted of 140 calves born in 2013. Data on the rearing of calves were collected from breeding documentation according to the guidelines of the PZHiPBM. The birth weight, weaning weight (kg) and daily gains (g) for the rearing period to 210 days of age of heifers and bulls were analyzed. The effect of calving season (summer and winter season) on the birth weight and daily gains of calves were estimated. The material was analyzed statistically using Statistica®10 PL software. The birth weight was significantly higher ($p \leq 0.01$) for calves that were born in winter (29.26 kg) in comparison to summer season (25.56 kg). Calves that were born in summer season reached a higher weaning weight at 210 days of age (267.33 kg) compared to calves born in winter season (254.32 kg). It was also found an advantage in daily gain of calves born during the summer season (1151.11 g). The course of cows' parturition in the analyzed herd was characterized by a much ease without the human help.

P 58 | The life and fate of mesenchymal stem cells after transplantation into the porcine uterine cervix

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Bone marrow-derived mesenchymal stem cells (MSC) are a promising source for cell-based treatment in reparative medicine, but existing strategies are restricted by low cell survival and engraftment therefore the in vivo studies of MSC survival in different species and tissues are needed. Six Polish Landrace female pigs, weighing 90–120 kg, have undergone general inhalational anesthesia (GIA) during which 40 ml of red bone marrow were gathered from the head of the humerus. Material was collected in sterile conditions, using special 13G needle, 20 ml syringe. The MSC from collected bone marrow were isolated and cultivated in vitro during 3 weeks as well as labeled with PKH 26 and DID. Their differentiation potential was also tested and cyto- and immunocytochemical stainings were performed. Afterward laparotomy (under GIA) through the midline was performed to expose the cervix of the uterus. The MSC suspension was transplanted into the muscle layer of the cervix on the dorsal part of the cervix, five on the left and five on the right side, with 1 cm gaps. Two weeks later pigs were neutered using similar procedures (GIA, laparotomy), the cervix of the uterus was collected and put into buffered formaldehyde. The organ was then cut crosswise and longitudinally into small slices. The localization of MSC were defined in cross-sections using cyto- and immunocytochemical stainings. Living MSC were found in all translantation places. The result of the study is very promising for the future research on the reproductive system, especially concerning cervical abnormalities (wounds, scars, cervical tumors, cervical incompetence, dysplasia). MSC could be useful in the future as a supportive therapy for some diseases of uterine cervix. However, further studies are required.

P 59 | Urolithiasis of the urethra in dog as an indication for penis amputation

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Total amputation of penis in dogs is rarely performed in veterinary practice. The most common indications for this treatment are malignant tumors and traumatic fractures of the os penis. Partial amputation of the distal part of the penis is indicated in cases of recurrent prolapse of the urethra with trauma. The occurrence of urolithiasis in males is quite high. Obstruction of the urethra usually occurs at the height of the penis bone; an unsuccessful attempt to unblock the urethra is an indication for urethrostomy. The aim of the study is to describe an unusual case of urethra obstruction in which, besides urethrostomy, amputation of the penis was necessary. An 8-year-old male Yorkshire Terrier came to the clinic with severe congestion and swelling of the penis and anuria. The X-ray study revealed urolithiasis of the bladder and urethra along with an incorrect position of the mineralized deposits suggestive of an extra-urethral location. The ultrasound indicated signs of congestion, oedema, and inflammation of surrounding tissues. Due to local necrosis, perforation of the urethra, and the presence of urolithiasis outside its canal, amputation of the penis with urethrostomy was required. After surgery and treatment, recovery was achieved and preventive dietary treatment was continued. Histopathological examination of affected urethra and penis tissues excluded neoplastic process and local necrosis. The probable causes of the described changes were: damage to the urethra by urolithiasis, bedsores as a result of local pressure, incorrect catheterization, or all of these causes together. Our case provides a new indication for surgery to remove the penis together with urethrostomy.

P 60 | Reproductive effects of postnatal androgenization in female domestic cats

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To test the hypothesis that in domestic cats, the same than in other mammals, postnatal androgens defeminize the hypothalamus inducing sterility, the aim of this study was to describe the reproductive effects of a postnatal administration of a long term release androgen in this species. Secondly, the clinical safety of the treatment was also assessed. Thirteen newborn littermate female kittens were randomly assigned to one of the following treatment groups within the first 24 h of birth: testosterone enanthate 12.5 mg total dose sc (TE; n = 8) or Placebo (PL; n = 5). The animals were followed up (physical and behavior examination, vaginal cytology, fecal testosterone, T) until puberty when they were exposed to fertile tomcats for matings. After 21 days, serum progesterone was measured, gestation was diagnosed by ultrasound and, then, all queens were ovariohysterectomized. In the TE-treated animals, fecal T was elevated during the first 2 postnatal weeks ($p < 0.05$), then T remained low. While all the females showed estrous behavior and were receptive during the pubertal estrus ($p > 0.1$), two TE (2/8) vs. all PL (5/5) cats ovulated ($p < 0.05$). Only one (1/2) vs. 3 (3/5) of the TE and PL queens, respectively became pregnant. All TE kittens presented vulva and clitoris enlargement during postnatal weeks 3–13, when they normalized. Anovulated TE-treated cats had no corpus luteum, and a significant diminution of the endometrial glands as well as of the height of the uterine epithelium. It is concluded that in domestic cats a single postnatal supraphysiological dose of testosterone enanthate caused a high proportion of anovulation (75%) and histological endometrial abnormalities.

P 61 | Effect of sucrose as cryoprotectant for donkey sperm cryopreservation

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The aim of this study was to evaluate the effect of different concentrations of sucrose, a non-permeable cryoprotectant, on post-thaw sperm motility, comparing the results with a control extender containing glycerol. Semen samples were collected from three Andalusian donkeys using an artificial vagina. Thereafter, semen was divided in 6 aliquots, centrifuged (400 g/10 min) and resuspended with commercial Gent extender for sperm freezing (Minitüb, Tiefenbach, Germany) containing glycerol or adding instead 1% BSA and different concentrations (Molar, M) of sucrose: 0.05, 0.1, 0.25, 0.35 and 0.45. After that, sperm aliquots were slowly cooled (2 h/5°C), filled in 0.5 ml straws and frozen in nitrogen vapors. After thawing (37°C/30 s), total (TM, %) and progressive (PM, %) sperm motility were assessed by CASA and compared between treatments by ANOVA followed by Duncan test. Results were expressed as mean ± standard error. Sucrose 0.25 M showed similar TM (28.22 ± 4.42 vs. 29.56 ± 4.48) and PM (17.11 ± 2.91 vs. 20.00 ± 2.89) after thawing when compared to control treatment with glycerol. However, the remaining sucrose concentrations resulted in lower sperm motility values. In conclusion, sucrose 0.25 M can be used as an alternative to glycerol for freezing donkey semen. Further studies are needed including fertility trials, using a larger number of animals and assessed sperm parameters.

P 62 | Notch expression in bovine spermatozoa

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Physiological sperm capacitation results from cell interactions (uterine/oviductal epithelia and spermatozoa - SPZ). Notch is a major cell signaling pathway involved in the regulation of cell-fate decisions. The objective of this study was to evaluate the presence of Notch components (receptors: Notch1-4; ligands: Dll1, Dll4, Jag1) in bovine SPZ with different capacitation status (CS). Viable frozen-thawed SPZ were obtained following swim-up and incubated in TALP-Sperm without or with heparin. SPZ were then processed for immunofluorescence using PNA and each of the anti-Notch antibodies. PNA allowed the differentiation of the following CS: non-reacted (NR), reacting (R) and acrosome reacted (AR). Notch1, 2 and 4, Dll1 and 4, and Jag1 were expressed in SPZ, whereas Notch3 was not detected. Notch1 had a post-equatorial (PE) expression in 91% of SPZ, irrespective of the CS. Notch2 had apical (A), PE or A+PE expression patterns, which were related to the CS (NR: 79% A, 21% A+PE; R: 26% A, 42% PE, 9% A+PE; AR: 77% PE). Notch4 had a calix (SPZ head baseline) or equatorial (E) expression, unrelated to the CS. Dll1, Dll4 and Jag1 had single expression pattern related to the CS. Dll1 had a tail expression (NR: 96%; R: 60%; AR: 40%). Dll4 had an acrosome expression (NR: 98%; R: 94%; AR: 0%). Jag1 had an A expression (NR: 73%; R: 32%; AR: 3%). These dynamic expression patterns prompt for a Notch involvement in SPZ capacitation. (Funding: EXPL/CVT-REP/1485/2012; UID/CVT/00276/2013)

P 63 | Analysis of sperm quality in a type I diabetes zebrafish model

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Diabetes is a fast growing disease in human population and the study of its impact on mammalian reproductive traits has been controversial. Some authors showed a negative effect on sperm motility and DNA fragmentation in some species, while others failed to detect any effects. In the present study zebrafish was used as a model to study the effect of diabetes in sperm traits such as motility, viability and DNA fragmentation. Additionally sperm from these males was cryopreserved with 10% DMF in Hank's solution. Ten transgenic zebrafish (InsNTR-mCherry) were injected with either citrate solution (n = 5, control) or metronidazole to ablate pancreatic β cells and induce a transient state of type I diabetes. Sperm was extracted through glass capillary tubes connected to a mouth piece and immediately diluted in 10 µl of Hank's solution. Sperm quality was assessed using sperm motility with CASA system (Proisier, Spain), viability was determined in a fluorescent microscope using PI/Syber green and DNA fragmentation was assessed by comet assay. Although there was a higher average motility in the sperm of diabetic fish, there were no significant differences on sperm motility and viability due to the dispersion in sperm quality within groups. Cryopreservation affected sperm quality independently of the treatment; therefore no association could be established with diabetes affecting sperm motility of zebrafish males. (This work was supported by COST AQUAGAMETE (FA1205), PEst-CCMAR/Multi/04326/2013 project and by FCT doctoral fellowship-SFRH/BD/97466/2013 (P. Diogo).)

P 64 | The *Dirofilaria repens* cases in the reproductive system of dogs in Poland

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Filariosis is considered as an important parasitic problem in dogs. Invasions are caused by several nematode species (Bowman 2000: Parasitology for Veterinarians, ISBN 978-0721670973). These are vector-borne diseases transmitted by blood-sucking ectoparasites. For several years an increasing number of nematode *D. repens* invasions have been noticed in Poland. The research conducted on the group of 529 dogs in 2013–2016 revealed the 191 positive cases – microfilariae in blood smears. There were also ELISA and PCR tests performed. There were 191 (36.1%) positive samples with microfilariae in blood smears, and 22 specimens (4.15%) isolated in dogs found. There were noticed 7 co-infections of *Babesia canis* and *D. repens*. Parasites were most frequently found in spermatic cord and scrotum in male, mammary glands and linea alba in female dogs (72.72%). Single parasites were isolated from fascia of stifle joint, medial side of cheek, superciliary arches – 6 cases (27.28%). There were mainly skin dirofilariosis described in literature (Tarello 2011, J Parasit Res 2011:578385). In currently conducted research adult parasites have been found in different parts of the body – mostly found in reproductive system of infected animals. It should be considered that higher ratio was due to large number of surgical interventions of the reproductive systems. Despite microfilariae in blood circulation and adults present in different part of the body, the course of infection is mostly non-pathognomonic and asymptomatic. The mechanisms of organism self-defense against currently ongoing infection and changes of the blood profiles have not been recognized yet. This is being currently intensively investigated during our studies.

P 65 | Preliminary semen collection and analysis in the Kea parrot

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Semen analysis and characterization is a prerequisite to correct semen handling and AI. This work describes the seminal characteristics of Kea parrot (*Nestor notabilis*), an endangered species endemic to the South Island of New Zealand. The current population is

estimated to be approximately 1,000–5,000 birds. Semen was collected during breeding season (from January to April) from three adult males of five (2/3) and six (1/3) years old, in the collection of an amateur aviculturist. The manual massage technique was used. A total of 18 ejaculates were collected and evaluated for volume, degree of contamination, spermatozoa concentration; motility and kinetic parameters were assessed on diluted samples (modified TALP, pH 8.2, temperature 37.5°C) with a computer-aided sperm analyzer (CEROS, Hamilton Thorne Research Inc.). Six ejaculates were not analyzable because of an excessively high degree of contamination. Semen color ranged from transparent or turbid yellowish to whitish. The geometric mean of spermatozoa number/ejaculate result was $105.15 \pm 85.43 \times 10^6$. Total and progressive motility were 78.92 ± 14.77% and 69.17 ± 9.38%, respectively. Great variability was observed both among birds and among different ejaculates of the same subject. The seminal characteristics of *Nestor notabilis* are worth further investigation, with the aim of relating semen quality to fertility and defining a minimum inseminating dose for breeding purposes. The kea can also represent an interesting study model for similar threatened species, such as the kaka (*Nestor meridionalis*) and the kakapo (*Strigops habroptila*).

P 66 | Efficiency of GnRH administration on days 5–7 after insemination in relation to follicular development in dairy cows

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Days 5–7 after insemination are considered to be suitable time for induction of the accessory corpus luteum by GnRH in cows because the 1st cyclic dominant follicle should be present on ovaries. Occurrence of 2 corpora lutea (CL) and pregnancy rate in cows treated by GnRH on day 5, 6 or 7 in relation to diameter of the largest follicle on ovaries in time of the treatment was object of this trial. Cows bearing 1 CL and follicle ≤9 mm (n = 34), 10–14 mm (n = 59) or 15–20 mm (n = 42) on day 5 (n = 40), 6 (n = 48) or 7 (n = 47) after insemination were treated by gonadorelin (day 0). Ultrasonographical examination was performed on days 0, 14, 28 and about day 90. Occurrence of 2 CL as well as pregnancy rate in all experimental cows were 42.2% (57/135) on day 28 after treatment. Pregnancy rate were higher in cows bearing 2 vs. 1 CL on day 28 as well as about day 90 (82.5 vs. 18.5% and 79.0 vs. 18.5%, $p < 0.001$). Occurrence of 2 CL on day 28 was significantly higher in cows treated on day 5 vs. day 6 or 7 after insemination (60 vs. 33.3 or 36.2%, $p < 0.05$). Pregnancy rate was not influenced by term of the treatment. Surprisingly none significant differences were found in number of CL and pregnancy rate in relation to follicle size in time of the treatment. Our results show higher efficiency of accessory corpus luteum induction by administration of gonadorelin in cows treated on day 5 vs. day 6 or 7 after insemination regardless size of the largest follicle on ovaries in time of treatment.

P 67 | Relationship between color flow Doppler sonographic assessment of corpus luteum in the pregnant and not pregnant mares

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Color-flow Doppler sonography has been described as a means of assessing corpus luteum (CL) function rapidly, because area of luteal blood vessels correlates well with circulating progesterone (P4) concentrations in estrous cycling mares. The aim of this study was to assess the relationships between CL size and vascularity evaluated by color Doppler ultrasonography, and to determine the differences in the corpus luteum blood flow (CLBF) between the pregnant and not pregnant mares. The ultrasound examinations were carried out on 20 Warmblood mares at 5, 10, 15 days after artificial insemination (AI). CL size was determined by measurement of the maximal cross-sectional area of the luteinized tissue (MCSL) and CL blood supply by the maximum colored area of the luteinized tissue (MCAL) from Doppler ultrasound images. There were no significant differences in MCSL ($p > 0.05$) between days 5, 10 and 15 in pregnant and days 5 and 10 in cyclic mares as well as in MCAL between days 5 and 10 in both pregnant and cyclic mares. Whereas at day 15 MCSL significant decreased in cyclic mares ($p = 0.016$) and MCAL significant decreased ($p = 0.0004$) and increased ($p = 0.003$) in pregnant and cyclic mares respectively. The increase of the blood supply of the CL (MCAL) corresponded with luteolysis manifested by MCSL decreasing at 15 day after AI. The lack of luteolysis at 15 day (pregnant mares) was related to constant MCAL and MCSL decreases. Color Doppler ultrasonography may be used to estimate the corpus luteum growing, maturation and luteolysis better than standard ultrasound, because more accurately corpus luteum aging. Therefore Color Doppler USG provides more quantitative information than the standard USG in phases of the estrous cycle and earl pregnancy examination.

P 68 | Effect of N-methyl-2-pyrrolidone and seminal plasma on cooled rabbit semen

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N-methyl-2-pyrrolidone (NMP) and N, N-Dimethylformamide (DMF) are amide solvents used in chemical reactions. Unlike DMF, the carcinogenic nature of NMP has not been demonstrated yet. The aim of this study was to assess whether NMP can be used as cryoprotectant for rabbit semen cooling and if the seminal plasma (SP) has beneficial effects on rabbit sperm preservation at 4°C over time. Ejaculated semen from 8 rabbits was diluted with INRA or INRA plus NMP. An aliquot of INRA and the entire INRA plus NMP were stored at 4°C. The other aliquot of INRA was centrifuged at 700 g for 10 min to remove SP, then

the sperm was resuspended in INRA and INRA plus NMP and stored at 4°C. Samples were analyzed at 4, 24, 48 and 72 h after collection by Integrated Sperm Analysis System (ISAS), eosin-nigrosin stain (vitality), hipo-osmotic-swelling (HOS) test and acrosome integrity test. The motility was affected ($p < 0.05$) by the SP and the extender. The sperm motility was higher ($p < 0.05$) in presence of SP (59.7 ± 3.0%) or INRA (57.6 ± 3.0%) than those without SP (45.9 ± 3.0%) or INRA plus NMP (47.9 ± 3.0%). The addition of NMP showed significant differences ($p < 0.05$) in other values related with motility such as progressive motility, LIN, STR and BCF, whereas INRA showed higher VCL, VSL and VAP. Significant differences ($p < 0.05$) over time were observed in motility, VCL, VSL, VAP, LIN, STR, WOB, ALH, BCF and HOS test. In contrast, there were no significant differences in vitality and acrosome integrity. These results suggest that extender, SP and time have influence on the sperm motility, whereas the addition of NMP did not offer advantages.

P 69 | Assessment of DNA integrity after freeze-drying of rabbit semen

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The aim of this study was to assess DNA integrity of rabbit semen after freeze-drying with different extenders. Semen was collected from seven rabbits with motility of spermatozoa higher than 85% and mixed to obtain a heterospermic sample. Heterospermic sample was diluted with INRA and divided into four aliquots and washed by centrifugation at 700 g for 10 min. Seminal plasma was removed and the sperm pellet was resuspended in basic medium (10 mM Tris-HCl buffer and 50 mM NaCl) supplemented with 50 mM EGTA (1), 50 mM EGTA plus 105 µM Rosmarinic acid (2), 50 mM EDTA (3) or 50 mM EDTA plus 105 µM Rosmarinic acid (4). Sample of 150 µl were poured into cryovials, plunged into liquid nitrogen and immediately lyophilized by Telstar Lyobeta 25©. Semen samples were kept at room temperature for at least 2 months. After rehydration with 300 µl bidistilled water, the analysis of DNA integrity was performed by Sperm DNA Fragmentation Test (Halomax®). DNA fragmentation was higher when semen samples were freeze-dried with EGTA (11% fragmented DNA) than with EDTA (4.4% fragmented DNA) ($p < 0,001$). Furthermore, the presence of Rosmarinic acid showed statistically significant influence ($p = 0.023$), causing 1,361 times more DNA fragmentation in samples without Rosmarinic acid. It was concluded that the use of EDTA and moreover supplementation with Rosmarinic acid improves sperm DNA integrity of freeze-dried rabbit spermatozoa.

P 70 | Control of myoelectric activity patterns in the porcine uterus by pacemaker cells

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The myoelectrical activity is a result of voltage- and time-dependent changes in membrane ionic permeability and may be detected directly in myometrium by electromyography (EMG). The ICLC (Interstitial Cajal-Like Cells) contained in myometrium are suspected to display characteristic patterns of rhythmical activity and to have dual functions as pacemakers and conduction pathways for the active propagation of electrical slow waves. The aim of the study was to describe relations between uterine EMG activity and ICLC occurrence in pig using an in vivo model. The spontaneous uterine myoelectrical activity in the 10 non-pregnant, mature sows during diestrus was recorded by the combination of three electrodes connected to transmitter used in large animals. The typical pattern of rhythmic electrical activity was recorded during 3 weeks of registration. Mean amplitude (A), mean root mean square (RMS), duration of electrical activity (D), duration of pauses between activities (P), number of excitations (N) were analyzed regarding to different topographic regions. In parallel immunofluorescent studies, the distribution of specific c-kit/CD 117 receptors (display on ICLC surface) in the same topographic regions of porcine reproductive tract was determined. The significant strong correlation (Pearson's r) between ICLC density and EMG parameters such as duration of electrical activity (D) ($r = -0.85$, $p = 0.03$) and duration of pauses (P) ($r = -0.81$, $p = 0.05$) in uterine horn tip were demonstrated. No significant correlations in any of the parameters studied were observed for the uterine body and horns. The electrophysiological studies indicate that ICLC in the horn tip myometrium may participate in the regulation of slow waves durations and frequency similar to ICC in gastrointestinal tract

P 71 | The effects of ram introduction or estrus synchronisation by MAP, FGA and CIDR-G on reproductive parameters in Awassi ewes during transition period

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Recently, overgrazed pasture areas have enforced inevitable attempts (using hormones/ram effect) for increasing the individual yield in sheep. For this, 100 Awassi ewes (aged 2–4 year old) were divided randomly into 5 equal groups (n = 20, each): Group I; ewes given 30 mg FGA vaginal sponge (Chrono-Gest®) for 12 days and 500 IU eCG (Gonaser PMSG®) i.m. upon withdrawal. Group II; ewes received 60 mg MAP sponge (Esponjavet®) for 12 days and 500 IU eCG, as above. Group III; 0.3 gr P4 (CIDR-G®) inserted for 12 days and 500 IU eCG injected, as above. Group IV; ewes received no hormone, but 10 rams (aged 4–5 year old; known fertile) were kept 12 km away for 2 months and re-introduced rotationally with them after grouping. Group V; as control, no hormone given. Following sponge withdrawals, estrus signs were monitored (teaser rams) twice

a day 24 h onwards. Estrous ewes were allowed to hand-mate. Data from routine reproductive parameters were recorded. Estrus rates were 100% (20/20) in treatment groups (I–IV) while it was 55% (11/20) in controls (Group V), with significant ($p < 0.05$) differences between them. For interval to the onset of estrus, there was significant difference between Group III (24 h to 48 h) and Group IV (96 h to 120 h). Pregnancy rates (2.5-month) in treatment groups (I-IV) were 70% (14/20), 75% (15/20), 95% (19/20) and 10% (2/20), respectively while it was 27.27% (3/11) in Group V. The differences between Groups "I-III" and "IV-V" were significant. Twining rates in Group I (7.14%; 1/14) and in Group III 27.27%; 3/11) were significantly higher than those (zero %, all) in other groups (Groups II, IV and V). We conclude that both ram effect and "P4 and eCG" administrations should be combined for superior fertility in Awassi ewes during transition period.

P 72 | Embryo transfer in Andalusian donkeys: preliminary results

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This preliminary study was conducted to evaluate the effect of several factors (individual donor, donor age, day of flushing, number of cycles in the same donor, and number of ovulations per cycle) on the outcome of the non-surgical embryo collection technique, based on positive flushing (flushings where at least one embryo was recovered) and embryo recovery (embryos recovered per cycle) rates as well as on the quality of the embryos. Secondly, it aimed to serve as a starting point for the use of vitrification and embryo transfer in the conservation of the Andalusian donkey. Six to nine days after ovulation, donors were flushed three times for embryo recovery and each recovered embryo was evaluated for morphology and measured. Data were evaluated by the chi-square test and the Kruskal-Wallis ANOVA. A total of 15 embryo collections were performed, of which 12 were positive flushings (80%). Thirteen embryos were recovered out of 15 estrous cycles (86.7%) and 16 ovulations (81.3%) of eight Andalusian jennies, 5–10 years old, naturally mated. All recovered embryos but one were classified as Grade 1 (excellent or very good). Mean diameter of donkey embryos was 158.33 ± 8.33 μm (range: 150–175 μm) for 6-days-old embryos; 366.67 ± 124.44 μm (range: 175–600 μm) for 7-days-old embryos; 685.00 ± 139.33 μm (range: 375–1100 μm) for 8-days-old embryos; 3000 μm for 9-days-old embryos. None of the factors studied affected ($p > 0.05$) the outcome of the non-surgical embryo collection technique. In conclusion, this preliminary study resulted in a good embryo recovery rate but failed to identify factors that affect the rate of positive flushings, the embryo recovery rate, and the quality of the embryos. Finally, embryos collected on days 6 and 7 had a diameter lesser than 300 μm.

P 73 | Implementation of "on-farm" method for early detection of non-pregnant cows by a single ultrasonographic examination of the ovaries on day 21 after artificial insemination

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When an ultrasound is used for early pregnancy diagnosis, the emphasis must be given to identify non-pregnant rather than pregnant cows which allow their immediate re-insemination, and thus to increase the rate of successful fertilization. The aim of the present study was to implement an on-farm method for early detection of non-pregnant cows by single ultrasonic examination of the ovaries on day 21 after artificial insemination. A total of 98 Holstein cows with regular estrous cycle, from three dairy farms were included in this study. The cows were scanned by B-mode "real time" ultrasound scanner equipped with 7.5 MHz trans-rectal linear probe. The cows were declared "non-pregnant" if either a "poor echogenic" Corpus Luteum (CL) with a diameter <21 mm and concomitant follicle with a diameter >15 mm were observed or absence of visible functional CL. If a CL with "granular, grayish echogenic structure", at least 25 mm in diameter and present follicle/s, with a diameter no larger than 13 mm was observed, the cows were considered "pregnant". Confirmation of the test results was carried out on day 33 after AI, also by ultrasonography. General accuracy (pregnant vs. non-pregnant) was 78.57%; however, the greatest accuracy was obtained for the "negative predictive value" with 100% of sensitivity and 57.14% of specificity. Based on the results it can be concluded that this method is suitable and applicable as "on-farm" examination for detection of non-pregnant cows which can be used without any additional blood or milk sampling and laboratory analysis for pregnancy diagnoses. Furthermore, all cows declared as "non-pregnant" when this method is used, could be inseminated on the same day, if a follicle larger than 17 mm is observed.

P 74 | The influence of elevated temperature on the morphology of bovine oviduct epithelial cells (BOECs) cocultured with cattle embryos

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The objective of this study was to evaluate the morphology of BOECs cocultured with cattle embryos at the elevated temperature of 41°C. Bovine oviducts and ovaries were collected post mortem from slaughtered cattle. Oviduct epithelial cells were isolated from oviducts, which were obtained from cattle between days 0 and 4 of estrous

cycle. Cattle embryos were obtained based on in vitro fertilization of oocytes matured in vitro. BOECs were cocultured with cattle embryos at control (38.5°C) and elevated (41°C) temperatures for 168 h. Statistical analyses were performed by Statgraphics 5.0 Centurion using T-test. After 168 h, the percentage of blastocysts was significantly higher at 38.5°C than at 41°C (30.03 ± 2.07 vs. 0), ($p < 0.001$). The percentage of viable cells was similar in the BOECs from control (84 ± 3.02) and elevated (82 ± 2.16) temperatures. Analysis of the cilia movement of ciliated cells indicated no difference between control and elevated temperatures. The SEM micrographs' analysis indicated that the length of cilia on BOECs surface at 38.5°C was similar to 41°C (5.5 µm ± 0.3 vs. 5.3 µm ± 0.2). The number of microvilli on secretory cells was also similar at control (44.5 ± 1.3) and elevated (43.7 ± 1.2) temperatures. The TEM micrographs' analysis showed that the number of secretory cells of BOECs cultured at control temperature (12 ± 3.6) was similar to elevated temperature (11.9 ± 4.1). In conclusion, the elevated temperature has no effect on the morphology of BOECs but negatively influences cattle embryo development. (GRANT 453/N-COST/2009/0)

P 75 | Effect of ethanol addition to extender on quality and fertility of chilled boar semen

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The aim of this study was to evaluate the effect of sub-lethal exposure of liquid stored boar semen to ethanol as antioxidant additive on the spermatozoa quality and fertility. In Exp. 1 the semen samples (n = 6) from 4 Large White boars were collected and pooled. Pooled samples were divided into 5 equal parts and each part was extended with Glucose-tris-bicarbonat extender containing 0 (control) or different concentrations of absolute ethanol (0.05; 0.1; 0.2 and 0.3%). The extended semen was stored at 17°C for up to 10 days and evaluated for progressive sperm motility (CASA), acrosome morphology (Watson technique), viability (eosin-nigrosin), longevity and malondialdehyde concentration. In Exp. 2 the effect of ethanol addition to diluted semen (0.1%) on the fertility results of inseminated sows was evaluated. Sows in Exp. group (n = 38) and control group (n = 40) were inseminated twice in spontaneous estrus using 24 h stored semen (3×10^9 motile spermatozoa/dose). Statistical analysis was made using a Students t-test and a chi-square test. Progressive sperm motility and longevity were higher in the group with 0.1% ethanol than control ($p < 0.05$) at 6 and 8 d of storage. Malondialdehyde concentration was lower in ethanol treated groups (0.1–0.3%) at 3 and 5 d of storage compared to the control group ($p < 0.05$). There was no statistically significant difference in farrowing rates between sows inseminated with sperm in which 0.1% ethanol (84.2%) was added vs. control (82.6%). Our results show that although addition of ethanol at a 0.1% concentration affects the in vitro motility and longevity of chilled boar sperm, their fertility is not improved.

P 76 | Testosterone and 11-ketotestosterone level according to gonadal and gametic development in male trout (*Oncorhynchus mykiss*)

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The aim of this study was determined the testosterone (T) and 11-ketotestosterone (11KT) plasma levels in male trout with different testicular development. 35 male trout 1 year old were clustered according gonadal growth: 11 trout showed gametogenesis and sperm production (batch 1), 9 trout showed testicular development and azoospermia (batch 2), and 15 trout showed underdeveloped testes (batch 3). Testosterone level increased since September and reached a peak in January in all batches (52.61 vs. 58.41 vs. 44.95). The maximum values corresponded to batch 1 and 2. The low levels were registered during the period between April and September (<10 ng/ml). The 11KT levels increased from October to January (62.76 vs. 66.15 vs. 53.17) and decreased from March to October (<13 ng/ml). The highest values were reached by male trout with developed testes with or without sperm production. The hormonal profile showed similar modification in every batch during experimental period. In three batches, over the year, T concentrations were significantly related to 11-KT levels ($p < 0.001$). In all batches these low T and 11-KT, concentrations in spring and end-summer were followed by increased levels in winter (for May to November, Dunn's test, $p < 0.01$) and a sharp rise in January (for November to January, Dunn's test, $p < 0.05$), with levels steadily decreasing thereafter. Maximum values were obtained by male trout with gonadal development, although male trout with underdeveloped testes showed high values during breeding season of Rainbow trout (January to March).

P 77 | Stallion sperm cryopreservation: effect of α-tocopherol on the quality and heterologous in vitro fertilization capacity under different freezing rate

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The effects of supplementation of α-tocopherol and different freezing rates (FR) on the ability of stallion sperm to heterologous in vitro fertilization (IVF) of bovine oocytes with zona pellucida intact (ZP-I) were investigated, in an attempt to develop a model for assessing cryopreserved sperm function. Semen (four ejaculates per stallion) was obtained from four purebred Lusitano stallions. Each ejaculate was subjected to cryopreservation with a commercial extender (Ghent, Minitube Iberia, Spain), without any supplementation (control) or supplemented with 2 mM α-tocopherol. The semen was exposed to two different FRs between 5 and −15°C: slow (5°C/min)

and moderate (10°C/min). After thawing, the viability (Syber-14 and PI), mitochondrial membrane potential (MMP; JC-1) and membrane lipid peroxidation (LPO; C11-BODIPY581/591) of each sample were determined by flow cytometry. For both extenders, the viability was higher for spermatozoa cooled at slow FR ($p < .05$). The α-tocopherol extender improved ($p < .05$) post-thaw LPO; however, it did not improved viability and MMP. Regarding the heterologous IVF rate, in the moderate FR, α-tocopherol supplementation showed a higher percentage of IVF ($p < .05$), comparing with the control. Regarding the slow FR, no significance differences were observed for percentage of IVF between the two extenders and the FRs. However, it seems that the α-tocopherol supplementation improve the IVF rate. In conclusion, this research demonstrated that bovine oocytes ZP-I can be used to evaluate the quality of frozen-thawed stallion semen, as well as, α-tocopherol supplementation in the stallion freezing extender might exert a protective effect against oxidative damage during heterologous IVF.

P 78 | Reproductive performance in aging and young roosters from the endangered breed 'Gallina Valenciana de Chulilla'

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Reproductive failure is a common process in aging animals, declining fertility after 45 week in broiler roosters. The aim of this study was to compare some in vitro quality parameters and in vivo fertility of young (Y: 24–30 week of age) and aging (A: >5 years) roosters from the endangered autochthonous breed 'Gallina Valenciana de Chulilla'. 13 Y and 17 A roosters were used for the evaluation of valid ejaculates (VE), volume (V; ml), concentration (C; × 10^9 sperm/ml), plasma membrane integrity (PMI; %) and abnormal sperm (AS; %) in 53 ejaculates (20 from Y and 33 from A). Twenty-six A hens (48 eggs) were inseminated with 9 of the A roosters and 36 Y hens (215 eggs) were inseminated with 8 of the Y roosters. Fertility at d7 (FR) of incubation and hatching (from fertilized eggs; HR) rates (%) were recorded. All the protocols are described in Blanch et al. (Theriogenology 2014, 81: 1174–80). VE and V were higher in Y (95% and 0.43 ± 0.03) than in A roosters (46% and 0.3 ± 0.03) while C, PMI and AS were similar between Y (1.2 ± 0.3 C, 90 ± 5 PMI and 33 ± 7 AS) and A (1.3 ± 0.4 C, 99 ± 6 PMI and 36 ± 9 AS). FR and HR were also similar between Y (66 ± 12 and 82 ± 3) and A (80 ± 11 and 76 ± 7) groups. In conclusion, the number of VE and V declined in A roosters, but the other quality parameters were similar in both groups. Albeit not statistically different, it appears that higher fertility rates can be obtained in the eggs layed by A hens inseminated with the sperm from A males. (Funded by INIA (RZP2014-00002-00-00) and FEDER funds)

P 79 | Weight of the reproductive tract in relation to the age in wild sows (*Sus scrofa*)

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Wild boar population management is necessary in countries like Spain due to its increase in number over the last years. In order to achieve an adequate management, it would be important to have a good understanding of the reproduction parameters in the current population. From 2011 to 2015, we have studied the genital tract of 125 non-pregnant wild sows caught during the hunting season in the northeast region of Spain. Wild sows were classified according to their weight or age: Young (<6 months) Young1 (<30 kg) Young-adult (30–35 kg) and adult (>35 kg). The weight of the ovaries, uterine horns and entire uterus were statistically compared among ages. The weight of ovaries (left vs. right) was: Young (0.39 ± 015 vs. 0.42 ± 009 g), Young 1 (0.67 ± 0.72 vs. 0.56 ± 05 g), Young-Adult (1.12 ± 0.38 vs. 0.86 ± 0.16 g) and Adult (1.12 ± 0.09 vs. 0.94 ± 0.07 g). The weight of ovaries increased with the age, as illustrated by the significant difference between the Young and Adult groups ($p < 0.05$). However, there was no significant difference in weight between the right and left horn in any age group. The weight of the entire uterus increased according the age (13.16 ± 3.14, 23.89 ± 4.40, 53.99 ± 7.60, and 59.35 ± 24.28 to Young, Young 1, Young-Adult, and Adult, respectively). Further studies should be carried out to evaluate the female puberty moment so as to come up with an adequate management system to foster the reproductive control of the wild pig populations.

P 80 | Relationship between semen analysis, chromatin integrity (SCD), motile sperm organelle morphology examination (MSOME) and testicular hemodynamic changes in ram according to the breeding season

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The aim of this study was to establish the correlation between sperm motility, SCD (sperm chromatin dispersion test), MSOME (motile sperm organelle morphology examination) and testicular hemodynamic changes in the ram according to the breeding season. In the study, 18 rams free of testicular pathologies were examined using color and pulsed-wave Doppler ultrasound with a linear array 3–13 MHz probe MyLab 30 Vet Gold (Esaote, Italy). Peak systolic velocity (PSV), end diastolic velocity (EDV), resistive index (RI), and pulsatility index (PI) of the testicular artery were measured. For sperm motility assessment, the Sperm Class Analyser

CASA system (Microptic S.L, Spain) was used. Furthermore, samples were taken to determine sperm DNA fragmentation by SCD test (Halomax, Halotech DNA, SL Spain) and MSOME (Leica DMi8 DIC inverted microscope). During the breeding season, several significant correlations were detected: straight-line velocity (VSL) and PI (r = 0.60; p = 0.017) and PSV (r = 0.66; p = 0.007); linearity (LIN) and PI (r = 0.78; p = 0.001), RI (r = 0.67; p = 0.007) and PSV (r = 0.62; p = 0.013); straightness (STR) and PI (r = 0.83; p < 0.001), RI (r = 0.73; p = 0.002) and PSV (r = 0.74; p = 0.002). Furthermore, it was observed that the USG parameters were significantly lower than these detected in the non-breeding season. In conclusion the positive correlation between the USG parameters and sperm motility during breeding season would reflect that lower value in the peak systolic velocity and pulsatility index associated with a decrease of blood flow in the testicular artery can favorably influence the spermatogenesis process. The study showed that the observed correlation between sperm motility and USG parameters may provide a useful reference for male clinical evaluation.

P 81 | High milk production does not diminish the sexual response of anestrous goats primed with progesterone and stimulated with male effect

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We determined if high milk production provokes a diminution of the sexual response of anestrous goats primed with progesterone and submitted to male effect. During the sexual rest period (March), two group of goats (n = 14 each) with different milk yield were exposed during 5 d to two sexually active males previously treated with 2.5 mo of long days (16 h/light) from November 1st. In goats from Low Production (LP) group the daily milk production was 0.729 ± 0.65 kg, whereas in the High Production (HP) group was 1.386 ± 0.85 kg (p < 0.001). Parturition of all goats was on January 21 ± 5 d and they were milked manually once daily. Forty-eight hours prior to introduction of males, all goats were treated IM with 25 mg of progesterone. Estrous behavior was determined each 3 h during 5 d by direct visual observation. Ultrasonography of the ovaries was performed three times daily until the ovulation was inferred by disappearance or collapse of a potential preovulatory follicle previously identified. Latency to estrus was not different between groups (45.5 ± 1.0 and 37.5 ± 4 h, for LP and HP, respectively, p > 0.05). Latency to ovulation was similar between LP (72.8 ± 3.9 h) and HP (66.9 ± 3.9 h, p > 0.05). Proportion of females that showed estrus was of 92.9% in two groups (p > 0.05). Proportion of goats that ovulated did not differ between those from LP (85.7%) and HP (92.9%; p > 0.05). We conclude that high milk production level does not affect the response of anovulatory female goats exposed to male effect.

P 82 | Disruption of the splicing factor ZRSR2 impairs folliculogenesis in mice

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Mammalian oocytes are arrested at prophase I. During ovulation, germinal vesicle breakdown marks the resumption of meiosis I and progression to meiosis II. Events controlling the progression of folliculogenesis require a tight regulation of gene expression to produce functional gametes. Splicing of mRNA, a post-transcriptional modification, should modulate the expression of some essential genes involved in follicle maturation. ZRSR2 is a splicing factor necessary for the recognition of 3′ splice site in unprocessed mRNA. Interestingly, ZRSR2 is located on the X-chromosome in all mammals. Quantitative PCR analysis revealed that ZRSR2 is overexpressed in primary follicles compared to more mature stages of female gametes, suggesting an important function of ZRSR2 during oocyte maturation. To study the function of ZRSR2 in folliculogenesis, we used CRISPR-nCas9 technology to generate three mutant mice lines with ZRSR2 disrupted at its RNA-recognition motif. Homozygous mutant females exhibited severe defects in growing oocytes as they did not respond to superovulation, ultimately leading to female sterility. At 3 months of age, mutant ovaries were smaller than controls and histological sections showed evident defects during follicle maturation: the presence of less advanced-stage follicles, abnormal antral follicles and less fully-grown oocytes suggest that oocyte development is impaired in ZRSR2 mutants. Moreover, the number of antral stage follicles, when oocyte acquires meiotic competence, decreases dramatically in ZRSR2 mutants, likely impeding proper meiotic resumption. Our findings uncover a functional link between ZRSR2 and splicing governing meiotic progression during folliculogenesis.

P 83 | Distribution of serum PSPB concentration and pregnancy loss in pregnant dairy cows

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Since early 2012 a fully quantitative assay to measure serum pregnancy-specific protein B (PSPB) level (BioPRYN® ELISA) is available. The aims of the study were to analyze the distribution of PSPB levels in pregnant cows (>0.6 ng/ml) and to compare these values with pregnancy losses. Blood samples were routinely assayed in 23 Hungarian dairy herds from April 1st 2012 to October 30th 2015 in cows 29–35 days post AI. Rectal palpation was done 60 days after AI to confirm pregnancy. A total of 53915 blood samples were measured and 25325 were found pregnant. 4493 of these cows were not pregnant by rectal palpation, so pregnancy loss was 17.7%. In 4.8% of

the pregnant cows (1225) the serum PSPB levels were between 0.6 and 1.1 ng/ml, in 86.5% (21907) they were between 1.1 and 4 ng/ml, whereas in 8.7% of the cows the serum levels were above 4 ng/ml (2193). Pregnancy loss in cattle with higher PSPB levels (>1.1 ng/ml) was lower (15.2%), whereas cows with low levels (0.6–1.1 ng/ml) had the highest pregnancy losses (67.0%; $p < 0.0001$). Pregnancy loss did not differ between cows with serum PSPB levels of 3–4 ng/ml (9.8%), and cows with levels >4 ng/ml PSPB in serum (9.8%). Calving data of 2 farms were additionally analyzed for twin calving. Based on the analysis of 3933 deliveries, 4.8% twin calving was recorded in cows with serum PSPB levels between 0.6 and 4 ng/ml, whereas cows >4 ng/ml PSPB serum level had 30.2% twin deliveries ($p < 0.0001$). Based on these findings PSPB serum level showed negative correlation for pregnancy loss, but in cows with higher than 4 ng/ml serum PSPB level pregnancy loss did not decrease which may be caused by twin pregnancy.

P 84 | Epithelial-stromal crosstalk via hand2 in the canine endometrium

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Recent studies concerning the murine and human endometrium demonstrated the importance of progesterone (P) signaling in endometrial stromal cells to activate hand2 expression. Hand2 acts as a transcription factor that blocks fibroblast growth factors (FGFs) production by endometrial stromal cells, and thereby triggers a signaling cascade to block estrogen receptor activity and cell proliferation in endometrial epithelial cells. This mechanism regulated by hand2 is important for successful embryo implantation. FGFs act as paracrine mediators of mitogenic effects of estrogen (E) via FGF receptors (FGF-Rs) and murine uteri lacking stromal hand2 expression maintain epithelial proliferation, leading to impaired implantation and cystic endometrial hyperplasia (CEH). The aim of the study was the assessment of hand2, FGFs and FGF-Rs in samples of healthy and CEH affected canine endometria as well as in P and E stimulated stromal cells in vitro by means of qRT-PCR, Western blot and immunohistochemistry. In healthy endometria cyclic alterations were observed for stromal FGF and epithelial FGF-R expression patterns with highest levels of FGF-Rs in oestrous surface epithelium comparable to CEH affected epithelium. Higher levels of hand2 positive fibroblasts were observed in healthy metestrous than in CEH affected endometria indicating that reduced levels of hand2 expression in canine endometrial stromal cells are associated with epithelial proliferation and cystic alterations. Combination of E and P stimulation in vitro induced a significant increase of hand2 expression in stromal cells compared to single dose experiments of E or P suggesting an involvement of the combination of E and P in hand2 regulation rather than P alone.

P 85 | Congenital prostatic-urethral fistula in a dog

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Urogenital fistulas in dogs may be acquired, or more often, they are congenital defects. They could be rectovaginal, anogenital cleft or urethrorectal fistulas. Congenital prostatic-urethral fistula in dogs has not been described so far. German shepherd dog, 5 months-old, with a history of a cyst-like deformation in ischiadical region was referred for the evaluation of the urinary tract. Digital rectal examination did not reveal any abnormality. Transabdominal sonography, positive contrast uretrography and cystography were performed. Additionally, computed tomography with positive contrast was performed and revealed a fistula communicating the prostate and the cyst in the ischiadical region. Parapenile minilaparotomy, open cystotomy and prostatic uretrotomy revealed a fistula 6 × 4 mm-sized in the prostatic urethra just behind the seminal colliculus. The fistula continued to the caudal direction ending as a urinary cyst in retroperitoneal space under the muscles of the ischiadical region. The fissure was resected with partial prostatectomy. Additionally, the cyst was resected from the perianal surgical access. Numerous samples of lesion's wall with surrounding tissues were examined histopathologically. Slides were stained with hematoksylin and eosin method and inspected under light microscopy. Inner surface of lesion was lined by one row of epithelial cells, more peripherally connective tissue with thick bands of collagen fibers was present. On the basis of clinical outcome (appearing clinical signs in early age) the presumptive diagnosis was congenital prostatic-urethral fistula. After surgical resection in the postoperative time the cyst did not appear again. To the confirm congenital origin prostatic-urethral fistula is carried out a genetic test.

P 86 | Correlation between phenotype and ovarian failure in different mice strains subjected to diet-induced obesity

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Obesity leads to ovarian failure and infertility. However, the diversity of responses to an obesogenic environment might determine different consequences to fertility. Presently, we used three mouse strains with different susceptibility to diet-induced obesity, in order to correlate the phenotypic response with the level of ovarian failure. An in vivo study was conducted in C57Bl/6J (B6), AXB8 (B8) and 129S1 mice (n = 8/group) fed chow diet (CD) or high-fat diet (HFD) for 16 weeks. Changes in phenotype were followed by nuclear magnetic resonance and glucose and insulin tolerance tests (GTT and ITT), while ovarian function was assessed by mRNA quantification of folliculo-stimuline hormone

receptor (FSHR), luteinizing hormone receptor (LHR), the steroidogenesis marker Star-protein (StAR), and the inflammatory cytokines tumor necrosis factor α (TNF) and interleukin 1β (IL1). The B6 mice presented the highest body mass (BM), fat mass (FM) and adiposity index (AI) after 16 week HFD, while 129S1 mice showed the lowest levels ($p < 0.05$). The B8 mice presented an intermediate phenotype, with BM, FM and AI measurements between the aforementioned strains. Regarding GTT and ITT, B6 and B8 mice presented severe levels of glucose impairment, with higher blood glucose concentration after glucose and insulin challenge, comparing to 129S1 ($p < 0.05$). Concerning the ovarian function, B6 and B8 mice showed lower mRNA transcription of FSHR, LHR and StAR then 129S1, after 16 week HDF ($p < 0.05$). Conversely, TNF and IL1 showed the opposite trend between strains ($p < 0.05$). To conclude, phenotypes presenting higher levels of FM and AI gain are associated with higher degree of ovarian dysfunction. (Funded by NSC (2014/15/D/NZ4/01152) and Intramural Grant (GW17))

P 87 | Study of corpora lutea dynamic under effect of different FSH doses in ewes superovulated

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This study aimed to evaluate the dynamics of corpora lutea under the effect of exogenous FSH doses in a superovulatory protocol for embryo donor ewes. Twenty-nine Santa Ines ewes were used, which received an intravaginal progesterone device (CIDR®) on Day 0, remaining until Day 8. At the beginning of the protocol and at CIDR removal 0.5 ml of an analogue of $PGF_{2\alpha}$ (Sincrocio®) were administered. The gonadotrophin treatment began 48 h prior to CIDR removal (Day 6) when the ewes were randomly divided into groups: G200 (n = 9), G133 (n = 10) and G100 (n = 10) with 200, 133 and 100 mg of pFSH (Folltropin®), respectively. On D6, females also received 300 IU eCG (Novormon®). The B-mode ultrasonographic evaluations were daily performed during the luteogenisis luteogenesis period, from Day 11 to 15 (corresponding embryo recovery day), for the purpose of following the dynamics of luteal structures (diameter). Statistical analysis was performed using ANOVA, with Tukey test (mean ± SD, $p < 0.05$). The G100 and G133 showed a gradual increase in the diameter of the corpora lutea over the post-ovulation days (G100: 5.21 ± 1.10^{c}, 6.10 ± 1.40^{b}, 6.78 ± 1.80^{a}, 6.80 ± 1.80^{a} and 6.99 ± 2.02^{a}; and G133: 5.72 ± 1.03^{c}, 6.41 ± 1.17^{b}, 6.69 ± 1.51^{ab}, 6.95 ± 2.02^{ab} and 7.23 ± 2.06^{a} mm of diameter from Day 11 to 15, respectively ($a \neq b \neq c$, $p < 0.001$). In contrast, the G200 showed increased diameter from Day 11 to 13 (5.23 ± 0.86^{b}, 5.67 ± 1.04^{b} and 6.43 ± 1.32^{a}, respectively) and decreased from Day 14 to 15 (5.75 ± 1.57^{b} and 5.86 ± 1.79^{b}, respectively) ($a \neq b$, $p < 0.001$). In summary, the lower exogenous FSH doses (ie. 100 and 133 mg) were sufficient promote appropriate formation of corpora lutea in embryos donors ewes. (Financial support: EMBRAPA no 02.13.06.026.00.00 and PROPE no TC1288/te)

P 88 | Localization of melatonin synthesis and degradation enzymes on testicular parenchyma of bull and red deer

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Melatonin is a crucial hormone for reproduction. It accumulates in seminal plasma, not being subjected to circadian variations (Casao 2010, Reprod Biol Endocrinol 8:59). Thus, reproductive tissues should metabolize melatonin independently from the pineal secretion (González-Arto 2016, Andrology 4:163–71). We tested the localization of synthesis (AANAT: aralkylamine N-acetyltransferase; ASMT: N-acetylserotonin O-methyltransferase) and degradation (IDO: indolamine-2,3-dioxygenase; MPO: mieloperoxidase; CYP1A2: cytochrome P450 1A2) enzymes in the testicular parenchima of the non-seasonal domestic bull (*Bos taurus*) and the highly seasonal red deer (*C. elaphus hispanicus*). Samples (3 males/species) were collected in a slaughterhouse (bull) and after regulated hunting (deer, breeding season) and fixed in Bouin. Tissues were included in paraffin. Slices were successively hydrated, submitted to peroxidase inhibition, blocked, incubated with the primary and secondary antibody (Vectastain HRP) and developed (DAB). Microphotographs were taken at different magnifications, comparing with the different negative slides. Localization varied between species and enzymes. AANAT: Sertoli and Leydig cells cytoplasm in bull; espermatocytes and Leydig cytoplasm in deer. ASMT: spermatogonia to early spermatids (not in late spermatids or spermatozoa) in bull; low labelling in spermatocytes and strong in Leydig in deer. IDO: Leydig in bull and no labelling in deer. MPO: low general labelling, stronger in Leydig in deer; CYP1A2: not detected. Results must be confirmed by Western and PCR, but support the participation of testicular cells in melatonin metabolism, with a contribution of testicular parenchyma in the melatonin detected in the seminal plasma. (Supported by MINECO (AGL2013-43328P))

P 89 | Expression of prolactin receptors in normal feline mammary tissue and different types of feline mammary tumors

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Prolactin (PRL), synthesized in the anterior pituitary and to a lesser extent in numerous extra-pituitary tissues, acts by binding to a

specific membrane receptor (PRLR). PRL affects several physiological processes, such as mammary development, and is suspected to influence mammary cancer initiation and progression. We studied the role of PRLR expression in the various types of feline mammary tumors. The indication was defined by immunofluorescence on routinely processed normal (N), dysplastic (D) and neoplastic (A-C, adenocarcinoma; A, adenoma) mammary tissue. The samples were taken at surgery from 48 mature queens during radical mastectomy. The sample sections were stained with HE, labeled with primary (anti-PRLR) and fluorescent secondary antibodies linked with 7-AAD, then imaged using light and confocal microscopy and scanning cytometry. The epithelial cell cytoplasm was immuno-positive while the stroma cells were mostly negative. No differences in PRLR expression (mean% ± SEM) were observed between A-C (22.13 ± 4.10), A (24.01 ± 7.30), D (20.59 ± 7.67) and N (19.00 ± 3.69) groups, although a low PRLR expression (5.52 ± 0.86) was observed in 10% normal (N), 10% dysplastic (D) and 25% neoplastic (19% A-C; 6% A) samples. A moderate PRLR expression (25.37 ± 1.46) was observed in 13% normal (N), 2% dysplastic (D) and 21% neoplastic (15% A-C; 6% A) samples whereas an overexpression of PRLR (50.42 ± 2.61) was demonstrated in 2% normal (N), 2% dysplastic (D) and 14 neoplastic (10% A-C; 4% A) samples. We suggested that a specific factor may affect PRLR expression in different mammary tumors, but this needs further investigation. Results also point that PRL may prove a more important factor in mammary gland carcinogenesis that it was supposed so far.

P 90 | Evolution of the corpus luteum volume and its relation to the plasmatic progesterone concentration after artificial insemination in pregnant and non-pregnant dairy cows

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Assessing if the corpus luteum (CL) volume during the early days of pregnancy could be used to predict pregnancy status in dairy cows. Thus, we evaluated the relationship between the evolution of the CL volume and progesterone concentration in blood plasma of non-pregnant vs. pregnant cows during the first 22 days of pregnancy. This study was carried out with 76 lactating cows. Every cow was artificially inseminated and examined (ultrasound scanner and plasmatic progesterone analysis) every 48 h, until the pregnancy was confirmed by an ultrasound exam on day 30 post-insemination. After pregnancy diagnosis, cows were retrospectively allocated as pregnant (n = 33) or non-pregnant (n = 43). CL volume and plasmatic progesterone concentration correlated significantly in both groups (Pearson). In the non-pregnant cows, the correlation was r = 0.50, p < 0.001 (day 0–11: r = 0.50, p < 0.001; day 12–23: r = 0.38, p < 0.001). In the pregnant group, the correlation was r = 0.37, p < 0.001 (day 0–11: r = 0.63, p < 0.001; day 12–23: r = 0.12, p < 0.001). Since the results showed a moderate correlation between the CL size and plasmatic progesterone concentration, the ultrasonography estimation of the CL volume could be considered useful for assessing the presence of a functional CL. These findings suggest that the ultrasonography scans of the CL volume is found to be useful for an early pregnancy diagnosis in dairy cows.

P 91 | Variation in theriogenology teaching methodology in Europe

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European students are encouraged to broaden their knowledge by international exchange programs. However in a clinical setting, a load of both theoretical knowledge and practical skills is necessary before joining a host university. Later on, the graduate might exercise his profession everywhere in Europe. Though, veterinary education is organized in various ways making this exchange not always obvious. This study describes the similarities and differences in methods and hours granted to theriogenology teaching in Europe. A survey containing 38 questions was sent to 97 universities spread over Europe. Questions were related to the selection criteria of students (fixed vs. open), methodology of teaching (modular vs. linear), degree of differentiation (species specific vs. general), available caseload, hours granted to teach theriogenology, available material, animal and human resources and student evaluation methods used. The survey is currently still ongoing, but some notable trends become obvious. Although a minimal supervision and caseload per student is essential for a good educational outcome, a huge variation in all questioned items was observed. Some veterinary faculties are well staffed with personnel but caseload is lacking whilst others are in serious lack of personnel. The variation in importance awarded to theriogenology is clearly illustrated by a large variation in hours (and ICTS credits) granted to teach theriogenology. The amount of theoretical courses and time to acquire skills in undergraduate studies are surely not coordinated among the different European faculties. This warrants some concern and the results of this survey might be base for discussion in institutions that score below average compared to fellow institutions.

P 92 | Multicystic degeneration of the Cowper's gland in a boar

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Reduced fertility in boars can be attributed to many different factors, such as environmental impacts, loss of libido and anatomical problems of the reproductive tract. Therefore, a thorough clinical history and a detailed clinical examination are necessary to identify the cause of

any reduced fertility. The present report describes a case of multicystic degeneration of the Cowper's gland in a 1.3 year old purebred intact Large White boar with reduced fertility. General physical examination, followed by an andrological investigation and ultrasound was performed. The physical examination revealed no abnormalities apart from the findings in the genitals, where several pathological conditions were found. The tissue of the testis was softer than normal. The size of the paired bulbourethral gland was slightly increased, the texture was rough, and the manual palpation was painful for the pig. A transrectal ultrasonography was conducted and multiple cysts in the bulbourethral gland filled with fluid were seen. For further diagnostics, a pathological examination including histopathology of the gland was performed. On gross examination there were multiple cysts filled with mucous within the glandular tissue. In the histopathological examination the epithelium of the cysts of the endpieces and excretory ducts of the glands were flattened and the cysts were filled with a large amount of basophilic mucous compressing the surrounding tissue. To the authors' knowledge, multicystic degeneration of the bulbourethral gland in pigs has not been described before. Although this degeneration seems to be rare in pigs and the cause is unknown, it should be contemplated in the differential diagnoses of andrological disorders.

P 93 | Reduced litter size in the domestic dog after the mating of C295G T-box gene mutation carriers

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T gene, belonging to the large family of T-box genes, encodes transcription factors that affect the development of the rear body structures. Mutations within this gene can lead not only to tail shortening but also to other serious malformations. The aim of the study was to analyze litter size originating from three types of mating (short tail × short tail; long tail × long tail; long tail × short tail) in the Polish Lowland Sheepdog breed. A total of 208 puppies of 44 litters (18 - short tail × short tail; 10 - long tail × long tail; 16 - long tail × short tail) were analyzed. To identify the C295G mutation within the T-box gene, a restriction enzyme BstEII was used, which digested the PCR product of 702 bp length. Statistical analysis for the size of derived litters depending on the type of mating was performed using Chi square test of the SAS® package (proc freq). The decrease of average litter size was found to come from mated C295G mutation carriers (4.28 ± 2.05), when compared with the average litter size derived from the other two types of matings (long tail × long tail 5.0 ± 0.89; long tail × short tail 5.32 ± 1.92) (not significant). One of the reasons of lower litter size could be embryonic mortality of dominant homozygotes during embryonic development. The factor confirming the lethality of mutation in dominant homozygotes during embryonic development was the fact that no such homozygote puppy/dog was found in molecular studies.

P 94 | Morphological assessment of sexual dimorphism abnormality cases in Japanese quail (*Coturnix japonica*)

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The identification of sex based on cloaca in Japanese quail chicks is much more difficult than in domestic chicken. However, quails have many advantages as a research model, namely due to its clear sexual dimorphism during the first month of life. These dimorphisms manifests itself through faster body weight gain in females and feathers color changes in males at the age of about 3 weeks. This enables unambiguous identification of the sex of sexually immature individuals. Recently, cases of sexual dimorphism development disorders have been seen in the flock, which could have negative effects both on reproduction and experimental work, as well as in the production of meat and eggs. In this experiment 15 Japanese quails (6 M, 6 F and 3 ND-not determined) at the age of 8 months were dissected and measurements of the reproductive system taken. All 3 birds from ND group had secondary sexual characteristics disorders. In each case single oviduct and ovary was present, showing evident hypoplasia compared to mature females. Both organs were immature, typical in size of 2–3 weeks old quails. The reproductive system weight was on average of 0.23 g (SD = 0.14 g), and the total length of the oviduct 6.50 cm (SD = 1.00 cm). The measurement results in 6 dissected birds with correctly developed female organs (F) were respectively 6.92 g (SD = 4.58) and 32.75 cm (SD = 3.17).

P 95 | The effect of various blood chemistry parameters on the pregnancy rates in embryo transferred cows and heifers

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The objective of this study was to determine the association of some biochemical blood parameters with the pregnancy rate at embryo transfer (ET) in cows and heifers. The research was performed on 18 cows and 19 heifers from East Mediterranean Agriculture Research Institute farms in Adana. The body condition score (BCS) was determined and then the blood samples was collected at ET date in recipient cows and heifers. Biochemical parameters such

as Glucose, Ca, P, Mg, Na, GGt, Ldh, AST, ALT; TP, ALP, Fe measured by spectrophotometer (Roche cobas 8000 Modular Analyzer) methods. The pregnancy rate for cows was 5/18 (27.7%) and for heifers was 5/19 (26.3). There was statistically no significant difference between the groups ($p > 0.05$). The mean BCS of the animals were 3.75 ± 0.52 and differences between the BCS values were not significant ($p > 0.05$). There was not statistical difference at the blood biochemical parameters between pregnant and non-pregnant embryo transferred cows and heifers. Embryonic loss was observed in 5 of 10 pregnant cows. Some biochemical values were (Ca: 9.20, 8.40; AST: 48.8, 53.4) in the embryonic loss observed and pregnant cow respectively. Although the differences were not statistically significant, Ca and AST values were quantitatively different in the embryonic loss cases.

P 96 | Use of embryo biopsy primary culture for bovine embryo epigenotyping

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The principal limitation of embryo epigenotyping is the reduced sample amount from an embryo biopsy, insufficient for immunoprecipitation analysis. We developed a system to in vitro expand trophoblastic cells (EX) from a biopsy that solves this limitation. The aim of this work was to analyze if EX is representative of the embryo or if, as a consequence of adaptation to culture, the EX has an altered epigenome that no longer represents that of the embryo. Bovine Day 8 blastocysts were produced in vitro, the ICM was separated from the trophectoderm (TE), which were biopsied (20–30 cells) and cultured for 5 days to produce 10e4 cells (EX). Then, differences in methylation were examined between TE and EX using whole-genome bisulfite sequencing (WGBS). Sequencing read counts and levels of methylation were calculated using Seqmonk. As a consequence of adaptation to culture, the EX showed an increase in passive demethylation along the entire genome. However, from 386394 probes selected after quantification of three replicates, 94% of probes did not show differences in differentially methylated regions (DMR) between TE and EX. After replicate set statistical analysis, it was found that only 5.3% of the DMRs had differences between TE and EX ($p < 0.05$). Analysis of individual embryos indicated that the regions with similar methylation between TE and EX were enriched in CpG islands, promoters and transcription units (TU), while transposons were not frequent among these regions. These findings suggest that methylation mostly remains conserved between TE and EX. In conclusion, the major part of the methylation marks of the TE in CpG islands, promoters, and TU were maintained in the EX, indicating that this system of biopsy primary culture is a good proxy for embryo epigenotyping.

P 97 | Freezing protocol influences post-thaw effect of seminal plasma in stallion epididymal sperm

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Seminal plasma (SP) activates epididymal sperm and post-thaw addition of SP increases motility. We investigated the effect of 50% SP on motility patterns and the effect of the SP donor stallions in relation to the freezing protocol. Epididymal sperm of 10 stallions were frozen after routine castration using a programmable freezer. Cooling rates were −1.0 (P1) or −0.1°C/min (P2) from 20 to 4°C; freezing rate for all samples was −60°C/min between 4 and −140°C. Then samples were plunged into liquid nitrogen. After thawing (38°C, 30 s), a skim milk-based extender containing 0 or 50% SP from 6 donor stallions was added to dilute samples to 25×10^6 sperm/ml and samples were stored at 37°C for 10 min before motility analysis. In accordance to previous studies from our laboratory, 50% SP increased total motility in all samples after thawing [P1: 4 (1–18) and 21 (1–59), P2: 3 (1–11) and 23 (1–45)% for 0 and 50% SP, respectively; $p < 0.05$]. While there was no difference in total (TM, %) and progressive motility (PM, %) in P1 samples among SP from donor stallions 1 to 6 [TM: 27 (2–50), 19 (1–47), 17 (1–44), 23 (2–47), 31 (1–47), 15 (1–32); PM: 19 (1–42), 12 (0–36), 11 (0–35), 17 (0–39), 25 (0–40), 10 (0–25); $p < 0.05$], there was a distinct effect using P2 protocol [TM: 18 (1–41), 16 (1–36), 18 (2–34), 27 (2–42), 29 (2–44), 20 (1–38); PM: 12 (0–32), 10 (0–27), 10 (0–25), 17 (0–34), 19 (0–35), 13 (0–31); $p > 0.05$]. In a previous study, SP from individual stallions had no effect on post-thaw motility of epididymal sperm cooled at −1.0°C/min. However, different changes of plasma membranes induced by different cooling and freezing protocols might be responsible for the diverse effects of SP.

P 98 | The effect of ischemia on expression and localization of inducible NO synthase (iNOS) in porcine myometrium

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Nitric oxide synthase (iNOS) is responsible for cytotoxicity or cytoprotection through the production of large amounts of NO in ischemic tissue. In this study we investigated the effects of ischemia on expression and localization of iNOS conducted in two different stages of physiological blood supply of uterus during estrous cycle. Four groups of gilts were used in this experiment: on 9–10 or on 1–2 day of estrous cycle with physiological blood supply (control groups, CG) and followed experimentally induced 60-min of ischemia (occlusion of uterine artery; ischemic groups, IG). Uterine samples were collected: close to the ovary (A), from the middle part of the uterine horn (B) and close to the uterine body (C). Cryostat sections were stained using immunohistochemistry method. The sections were viewed and optical density was measured using an

automated Zeiss Axio Imager.Z1. iNOS mRNA expression levels were determined by RT PCR and the concentration of protein was estimated by Western blot analysis. Immunoreactivity (IR) of iNOS was higher in IG compared to CG on day 9–10 in parts A, B ($p < 0.01$) and on day 1–2 in B, C. The level of mRNA expression was lower in IG on day 1–2 in all analyzed regions of uterus ($p < 0.05$) and no significant differences on day 9–10 were observed. On the contrary, iNOS protein level was lower only in B ($p < 0.01$) on day 1–2 but higher ($p < 0.01$) in A, B, C on 9–10 day. Higher immunoreactivity and protein level of iNOS on day 9–10, when physiological uterine blood flow is down to 30–40% of the level characteristic for the beginning of cycle, suggest that iNOS may be involved in adaptation tissue to changing blood supply. (Supported by statutory research founds of MSHE (GW-15) and NCS Grant NN311527040.)

P 99 | High progesterone levels at farrowing impair colostrum IgG concentration and colostrum yield in sow

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Lactogenesis is induced hormonally by a dramatic drop of progesterone (P4) concentrations which leads to a pre-partum peak of prolactin (PRL). In sows, impaired production of colostrum has been found to be related to a delay in the decrease of P4 concentrations during the pre-partum period. We assumed that this condition might affect also the quality of colostrum (IgG concentration). Our aim was to investigate the relationship between abnormally high P4 levels at parturition with colostrum yield (CY) and its IgG content in sows. Blood samples (n = 38) were collected from vena saphena medialis to assess P4 concentration at the beginning of parturition. Colostrum samples (n = 38) were obtained between 0 and 3 h after the birth of first piglet. CY was calculated according a formula accounting birth weight difference at 0–24 h. P4 samples were analyzed using a radioimmunoassay (RIA) kit and the colostrum IgG were analyzed using a pig IgG ELISA. The plasma P4 concentrations at the beginning of farrowing were 3.37 ± 2.06 ng/ml (mean ± SD; range 0.93–10.71 ng/ml). Sows having values of P4 > 4.00 ng/ml had lower IgG in colostrum (52.75 mg/ml vs. 66.56 mg/ml; $p = 0.01$). Similarly, sows having values of P4 > 4.00 ng/ml had an average CY of 2125 ± 1779 g while those with P4 values <4.00 ng/ml had CY of 4114 ± 1155 g ($p = 0.01$). Our results indicate that high P4 level at farrowing have negative effects on piglets' colostrum and IgG uptake.

P 100 | Effect of repeated ablation of dominant follicles on superovulation treatment in heifers

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Ultrasound-guided follicle ablation is used to eliminate the suppressive effect of the dominant follicle and to synchronize the emergence of a follicular wave before superstimulation. The aim of the study was to induce new waves and to recruit additional follicles before the initiation of superstimulation by means of repeated puncture of dominant follicles. In total 9 Simmental heifers were used in a crossover design. The animals were randomly assigned to experimental (DP-group) or control group (C-group). The animals were synchronized and all follicles over 6 mm were ablated by ultrasound at Day 4, 6, and 8 (DP-group) or only at Day 8 of oestrous cycle (C-group). Thirty-six hours later superstimulation was started using 8 decreasing doses of FSH during 4 days. Day 7 embryos were recovered by combined endoscopic flushing of oviducts and uterine horns. Numbers of CL's and recovered embryos did not differ between DP- and C-group (16.8, 17.7 and 10.7, 9.4, respectively). The number of recovered morulae and blastocysts at Day 7 was marginally significantly lower in the DP- compared to the C-group (4.8 and 6.1, respectively, $p = 0.051$). The proportion of degenerate embryos did not differ between both groups, but the proportion of unfertilized oocytes was higher in the DP- compared to C-group (31.2% and 11.8%, respectively). In conclusion it was shown that the repeated ablation of dominant follicles did not have an effect on follicle accumulation for superovulation. However, DP-treatment caused a higher number of oocytes incapable for fertilization.

P 101 | The weaning of the goat kids at day 30 post-partum is not necessary to stimulates ovulation by natural sexually active males in goats kidding during breeding season

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The aim of this study was to determine if the weaning of the goat kids at day 30 postpartum is necessary to stimulate ovulation by natural sexually active males in anovulatory goats kidding during breeding season (September 1st ±0.1 days) A first group of goats nursed their kids during the whole study and was isolated from males (IG, n = 15; prolificacy = 1.9 ± 0.2). In a second group goats also nursed their goat kids, but at day 30 postpartum was exposed to two natural sexually active males (MEG, n = 15; prolificacy 1.9 ± 0.2). In a third group, goats also nursed their goat kids, but at day 30 postpartum were weaned and mothers were exposed to two natural sexually active males (W+MEG, n = 15; prolificacy 1.9 ± 0.1). The post-partum ovulation was assessed by transrectal ultrasonography. The proportion

of goats that ovulated at day 15 after male introduction did not differ (p > 0.05) between the W+MEG (15/15, 100%) and MEG (11/15, 73.3%), while none ovulated in the IG (p < 0.0001). We concluded that in subtropical goats giving birth during the breeding season, the weaning of the goat kids at day 30 postpartum is not necessary to stimulate ovulation by natural sexually active males.

P 102 | Addition of 4-hydroxyestradiol (4-OEH$_2$) improves implantation rates of murine embryos

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It has been shown that epidermal growth factor (EGF) is essential for successful embryo implantation and administration of exogenous 4-OHE$_2$ induces overexpression of EGF and its receptors. Objective: to evaluate if supplementation of the embryo culture media with 4-OHE$_2$ improves the implantation rate of murine embryos obtained by in vitro fertilization (IVF). Methods: B6D2 female mice were hormonally stimulated to trigger ovulation. Mature cumulus-oocyte complexes were obtained, IVF was performed and 3 experimental groups were evaluated. (A) a control group of *in vivo* produced blastocysts obtained from pregnant females (IVP; n = 498); (B) a control group of *in vitro* produced blastocysts using IVF in which the embryos were cultured in KSOM to the blastocyst stage (IVFc; n = 223); (C) a last group of IVF produced zygotes were cultured to the morula stage (day 3) and subsequently moved to KSOM medium supplemented with 0.1 µg/ml of 4-OHE$_2$ and cultured to the blastocyst stage (IVF 0.1; n = 200). All blastocysts were transferred to pseudopregnant female mice that were euthanized 15 days after the embryo transfers and implantation rate was evaluated. Results: An ANOVA was used to compare groups, implantation rate was 25.7 ± 4.1% for IVP, 7.9 ± 4.3% for IVFc and 13.3 ± 3.8% for IVF0.1 (mean ± SEM; p > 0.05). Conclusion: supplementation of the embryo culture medium with 4-OHE$_2$ could potentially improve the implantation rate of murine embryos obtained by IVF, although more experiments are required to further confirm our observations.

P 103 | Comparison of extenders containing egg yolk 6% and lecithin soybean on post-thaw sperm quality in native Colombian deers (*Odocoileus virginianus*)

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Soybean lecithin extenders have been used for semen cryopreservation in domestic animals. However, their suitability for semen cryopreservation in native Colombian deer (*Odocoileus virginianus*) has not been tested. This study aimed to compare the effect of two extenders, egg yolk 6% (EY) and soybean lecithin (SL) with a concentration 60 million sperm on post-thaw µm/s curvilinear velocity (VCL), straight line velocity (VSL) motility (M) and viability (V) of Colombian deer sperm. Semen samples (n = 21) were collected by electro ejaculation from seven Colombian deers. After collection, each semen sample was divided in two aliquots and diluted into two extenders; EY and SL. Later, semen was slowly cooled for 2 h at 5°C, filled in 0.25 ml straws and frozen in nitrogen vapours. After thawing (37°C/30s), the Sperm Class Analyser (Microptic SL, Spain) software was used to assess VCL, VSL, M and V. Parameters between extenders were compared by ANOVA (R studio). Obtained explain LSM ± EE and coefficient of variation (CV) showed no differences (p > 0.05) among semen samples frozen with EY and SL when compared for VCL (65.55 ± 0.38 vs. 65.75 ± 0.54 µm/s; CV = 19.3%; respectively), VSL (33.48 ± 0.3 vs. 32.81 ± 0.42 µm/s CV = 20.4%; respectively) and V (55.83 ± 3.86% vs. 55.44 ± 3.06%; CV = 8.22%; respectively). In contrast, M differed (p < 0.05) (34.99 ± 4.24% vs. 35.37 ± 3.82%; CV = 12.3%) among semen samples frozen with EY and SL, respectively. According to these findings, extenders containing SL could be considered as good as EY for cryopreservation of deer semen. (Financial support: SENNOVA, 2015).

P 104 | Testosterone levels and testimetry evaluation in tomcats

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The aim of the present study was to evaluate the relationship between the body size and the testis morphometric parameters (weight, length, width, height) in tomcats during the year and to analyze peripheral testosterone levels before and after GnRH stimulation test. Testosterone levels were measured by Chemiluminescent Microparticle Immunoassay from samples collected from the v. cephalica antebrachii. Fifty-one tomcats of different breeds were divided by weight into 3 categories (≤3 kg; 3.01–3.99 kg; ≥4 kg). All tomcats in the study were admitted to our clinic for the purpose of elective castration. The measured values were expressed as mean ± SD and analyzed by ANOVA using GraphPad Prism 6 software. Positive correlation before and after the GnRH stimulation was observed for all groups (p < 0.001). The elevation of peripheral blood testosterone levels after GnRH administration varied by group, with an increase between 28 and 46%. There was no significant difference in morphometric parameters between left and right testis in any of the groups (p > 0.05), except height values in ≥4 kg group. The height of the right testis in this group was significantly lower than in the left testis (p < 0.001). In addition, there was a positive correlation between the tomcat body weight and the testimetry in all groups (p < 0.0001). (This study was supported by VEGA 1/0090/14.)

P 105 | Inhibition of FGF signaling pathway promotes the expression of pluripotency marker genes in porcine embryo

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During mammalian pre-implantation embryo development, two distinct lineages at the blastocyst stage have been formed, trophoblast and inner cell mass (ICMs). The latter segregated into two lineages, hypoblast and epiblast. In mouse, segregation of hypoblast lineage depends on FGF/MAPK signaling, which promotes the formation of cell lineage. Moreover, mouse embryos stimulated with FGFR inhibitor form ICMs composed entirely of epiblast cells and no hypoblast cells. However, it is unclear how FGF signaling pathway modulates segregation of porcine embryos, which has to be studied further. Here we investigated the effect of BGJ398 on swine parthenogenetic embryo development and related genes expression. Porcine parthenogenetic embryos were cultured in medium with the addition of DMSO (control) or BGJ398 (10 μM) post-activaction. Compared to control group, the results showed that BGJ398 conditions increased the total cell numbers of blastocyst at day 7 determined by cell counting (54 vs. 44; $p < 0.05$), but not significantly affected the rate of blastocyst development (47% vs. 44%). Meantime, it promoted the expression of epiblast and trophoblast marker genes (Sox2, Oct4, Klf4 and Cdx2), but not significantly affected that of hypoblast marker gene (Gata4). In summary, inhibition of FGF signaling could improve the quality of the porcine parthenogenetic embryo and may promote segregation of trophoblast and epiblast lineages, but not hypoblast lineages.

P 106 | Stress response of mares during gynaecological examination in veterinary medicine

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Stressful interventions in animals are seen increasingly critical. In this study, the stress response of horse mares (n = 21) to transrectal palpation and ultrasonographic examination of the genital tract was quantified based on salivary cortisol concentration, heart rate (HR) and heart rate variability (HRV). Mares differed in experience with regard to the examination and were either pluriparous broodmares (n = 13) or maiden mares (n = 8). The mares were either examined every 6 h (n = 10; insemination with cryopreserved semen) or at 24–48 h intervals (n = 11, insemination with cooled semen). All mares were followed for 3 examinations and 13 mares for 4 examinations. We hypothesized that gynaecological examination causes a stress response which decreases with repeated examinations. HR increased ($p < 0.01$) during the gynaecological examination but neither changed from examination 1 to 4 nor differed between experienced and inexperienced mares. The increase in HR was higher in mares examined every 6 h compared to every 24–48 h. During gynaecological examinations, HRV did not change while cortisol concentration increased. The cortisol response was more pronounced in mares examined at 6-h intervals (from 1.5 ± 0.7 to 2.4 ± 1.3 ng/ml) than in mares examined every 24–48 h (from 1.5 ± 1.1 to 1.9 ± 1.2 ng/ml; time × group $p < 0.01$). No differences existed between experienced and less experienced mares and between examinations 1 to 4. In conclusion, transrectal examination was not perceived as a major stressor by mares but the stress response was influenced by examination frequency.

P 107 | Changes in chromatin state derived of sperm capacitation in red deer spermatozoa

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In this study it was evaluated the state of chromatin after a capacitation time in red deer spermatozoa. Thawed semen from three red deer was used after selection in 45% Percoll. Spermatozoa were capacitated with a synthetic oviductal fluid (SOF) with 20% estrus sheep serum (ESS), and the high DNA stability (HDS) was evaluated at different incubation times (1, 5, 15, 30, 45, 60, 120 min and 24 h) in the capacitation medium. In addition, samples of non-capacitated spermatozoa (incubated with SOF without ESS) were evaluated at 0, 120 min and 24 h. A heterologous vitro fertilization trial, oocyte from sheep and spermatozoa from deer, was performed with capacitated sperm samples for 5, 15, 30 and 60 min and non-capacitated sperm samples at 0 min. Results showed that HDS increased with the incubation time with the highest values at 15 (6.48), 30 (6.14) and 45 (6.06) min. of incubation in relation to non-capacitated sperm samples initially evaluated. However, the highest percentage of cleaved embryos was register for capacitated spermatozoa for 5 (19.5%), 15 (19.88%) and 30 (18.19%) min., with lower values for non-capacitated samples (5.6%) or 60 min capacited (8.36%). More studies are needed to know the relation of the changes in chromatin state and the fertilization ability after capacitation time.

P 108 | Does equine fetus express Major Histocompatibility Complex Class I (MHC I) only of maternal origin?

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During pregnancy the semi allogenic fetus controls Major Histocompatibility Complex Class I (MHC I) expression in order to protect itself from maternal immune system. We hypothesized that the fetal part of the placenta (allantochorion) expresses only maternal

MHC I genes. Samples of endometrium and allantochorion were taken from 13 mares 3 – 8 months pregnant. Eleven MHC I genes were amplified in 2-steps experiment: first with genomic DNA as a template to determine the MHC I gene presence, and then with cDNA as a template to determine the MHC I gene expression. In the first step presence of the products of the same base pair length in both parts of the placenta suggested that both the mare and the fetus carried the same MHC I gene allele; whereas the absence of the product in one of the placenta parts suggested that the mare and the fetus carry different MHC I gene allele (the fetus inherited the allele from a father). In the second step presence of the product suggested expression of the gene. Out of 106 combinations from the first experiment where the fetus and the mother shared the amplified MHC I gene allele in 83 combinations the MHC I gene was expressed by the fetus and the mother. MHC I gene of paternal origin was present in 1 combination and it was not expressed. Above results suggest that expressed MHC I genes in the allantochorion are mainly of maternal origin and that there is tight epigenetic control of MHC I genes expression of paternal origin. (Approved by the Local Ethics Committee for Experiments on Animals in Olsztyn. Supported by NCN grant (2012/07/D/NZ5/04290).)

P 109 | Effect of fibroblast growth factor 2 (FGF2) on the bovine corpus luteum (CL) depends on the stage of estrous cycle: interactions with prostaglandin $F_{2\alpha}$ (PGF)

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The aim of the study were (1) to examine the effects of FGF2 on bovine CL functions in early (PGF-resistant) and mid (PGF-responsive) luteal stage of the estrous cycle, and (2) to investigate PGF and FGF2 interactions during the luteal phase in cows. The animals at early (day 3, n = 30) or at mid luteal phase of the cycle (day 10, n = 30) were divided into six groups; (1) intraluteal Saline injection (control), (2) intraluteal FGF2 injection, (3) intraluteal FGF antagonist injection (aFGF), (4) i.m. PGF injection, (5) intraluteal FGF2 application followed by i.m. PGF injection and (6) intraluteal aFGF application followed by i.m. PGF injection. Blood samples were collected frequently from the jugular vein before and after treatment till the end of the cycle. Concentrations of P4, PGE2, PGFM were determined by EIA. At early luteal phase, PGF, FGF2 or aFGF did not change P4 profile and cycle length ($p < 0.05$). However, PGF inhibited CL formation in the cows pretreated either with FGF2 or aFGF, which was shown by low P4 secretion during the cycle ($p < 0.05$). At mid luteal phase, FGF2 inhibited luteolysis (P4 level remained at 3.6 ng/ml till the end of cycle). Luteolytic effect of PGF injection was observed 2 days later in cows pretreated either with FGF2 or aFGF compared to PGF-treated group ($p < 0.05$). FGF2 plays a modulatory role in CL development and luteolysis. Moreover, it may sensitize early CL to the luteolytic action of PGF. (Supported by NSC 2011/03/B/NZ9/01634)

P 110 | A case of pregnancy in FeLV positive cat with advanced lymphoma

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A normal pregnancy coexisting with an advanced stage of T-Cell lymphoma in a female positive for feline leukemia virus (FeLV) is a very rare event. FeLV is suspected to be a cause of infertility and can be vertically transmitted, causing abortion. Lymphomas, on the other hand, are responsible for multiple symptoms depending on the localization. A 2-year-old mix-breed cat was presented with signs of vomiting, dyspnea and mild cachexia. Clinical examination revealed no further changes. X-ray examination evidenced advanced hydrothorax. The cat was found FeLV positive by a rapid immuno-enzymatic test (Vetexpert, Poland). Results from basic hematological and blood biochemical tests were within the reference range. Ultrasound examination revealed an apparently normal 25–28-day pregnancy of 3 fetuses, and a large mass (35 × 18 × 15 mm), with few anechoic caverns, located cranial to the left adrenal gland. Due to very poor prognosis, the owners decided to euthanize the patient. The necropsy revealed two tumor-like white colored masses – one as described in the ultrasound and another grossly similar but much bigger (100 × 80 × 50 mm) located in the thoracic cavity, cranial to the heart, co-existing with hydrothorax and focal lung congestion. Histopathological examination described the masses as blastic lymphomas with necrosis. The lungs showed changes typical for pulmonary hypertension, namely mild bronchial, bronchioleal and vascular inflammation, and arterial wall hyperplasia. The gross uterine appearance was typical for mid-pregnancy. The case presented here suggests that pregnancy in cat may be resilient to advanced changes in the maternal organism. However, this was just the first half of pregnancy and we do not know if it would reach term and whether the kittens would be healthy.

P 111 | Mammary gland calcification on CT scanning of goats (*Capra hircus*) infected with caprine arthritis-encephalitis virus

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Mammary gland is one of target organs for caprine arthritis-encephalitis virus (CAEV), which can cause indurative mastitis of goats ("hard udder"). It is characterized by migration of infected monocytes into the udder, their differentiation to macrophages and subsequent attraction of lymphocytes and induction of chronic immune-mediated inflammation and fibrosis. No data about potential subsequent calcification could be found. The aim of the study was to evaluate calcification

of the udder in CAEV infected goats on computed tomography. We used 10 dry dairy goats of Polish white ennobled breed, 2–5 year old. CT scan was performed on sedated animals using 16-detector row CT scanner Phillips & Neusoft Medical Systems NeuViz. On the obtained tomograms we evaluated the extent and location of calcification and its symmetry between both udder halves. Nine out of 10 goats had different pattern of calcification (diffuse, local or mixed) in the udder and only 1 goat had no calcification at all. The extent of calcification varied from single small (5 mm in diameter) calcifications spread randomly in the udder to diffuse calcification involving almost every part of the mammary gland. In 3 goats calcification was asymmetric with one of the halves being clearly more calcified. Goats infected with CAEV can have their mammary glands calcified and the degree of calcification is various. Heterotopic calcification of chronically inflamed mammary gland might be the potential reason of this finding. Different degree of calcification may come from different age, clinical condition or individual characteristics of the animals.

P 112 | Identifying serum levels of natural antibodies and beta-hydroxybutyric acid in Tuj and Hemşin Sheep in the peripartum period

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The aim of this study is to identify the level of serum beta-hydroxybutyric (BHBA) and titer of natural antibodies (NAb) in ewes during the peripartum period. The study was conducted on 25 Tuj (T) and 25 Hemşin (H) ewes. The ewes' breeds by rams were recorded, and pregnancy was confirmed with an ultrasound (Titan®, Sonosite, USA) 30 days after mating. Blood samples were taken from the ewes on days 30 and 15 prepartum, on the day of birth (day 0) and on days 15 and 30 postpartum. NAb titer was determined in the manner reported by van Knegsel et al. (2012). The titers were measured at a wavelength of 450 nm with ELISA Reader (Epoch®, Biotek, USA). BHBA levels were determined using the commercial kit (Randox Laboratories Ltd., UK). There was no difference in the NAb titer in any of the ewes on day 15 prepartum and day 30 postpartum ($p > 0.05$), but there was a significant difference on days 0 and 15 post partum ($p < 0.001$). In these ewes, the NAb titer fell from day 30 prepartum (T: 8.74 ± 0.06; H: 7.04 ± 0.08) to day 0 (T: 5.22 ± 0.11; H: 4.16 ± 0.14) and rose on days 15 (T: 6.61 ± 0.07; H: 5.07 ± 0.19) and 30 (T: 7.51 ± 0.09; H: 6.30 ± 0.01) postpartum. However, BHBA levels rose significantly from day 30 (T: 0.43 ± 0.01 mmol/l and H: 0.43 ± 0.01 mmol/l) to day 0 (T: 0.71 ± 0.01 mmol/l; H: 0.72 ± 0.01 mmol/l), peaking on day 0 ($p < 0.001$). BHBA levels on days 15 and 30 postpartum were significantly lower than on day 0 ($p < 0.001$). No significant difference was found between Tuj and Hemşin breeds of sheep on any of the days on which blood was drawn in terms of either NAb titers or BHBA levels. In conclusion, NAb titer fell in the last 30 days of pregnancy with the rapid growth of the fetus, but BHBA levels rose. Unlike the prepartum period, NAb titer rose in the postpartum period while the BHBA level fell.

P 113 | A case of pyometra in pregnant queen

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Cases of pyometra during pregnancy in cats are very rare. There is very few information about these conditions occurring simultaneously. A 7-year-old Norwegian Forest queen was presented with a history of infertility. Ultrasound examination, during anestrous, revealed no changes in reproductive tract. In the queen's next cycle, ovulation was stimulated with a use of hCG (Chorulon, Intervet, Poland). On day 26 after copulation, ultrasound examination (UE) showed 3 healthy fetuses. On day 42 the queen was presented with signs of vaginal discharge. UE revealed normal pregnancy with blood progesterone of 4.2 ng/ml. A diagnosis of hypoluteoidism was made. Progesterone injections (Agolutin, Biotika, Slovakia) at a dose of 2 mg/kg i.m. and amoxicillin (Betamox L.A., Scanvet, Poland) at a dose of 15 mg/kg s.c. were administered every third day starting from day 43. Described discharge was observed until day 58. On day 50, UE showed advanced decomposition of one fetus. On day 58, UE revealed 3 dead fetuses and hyperechoic amniotic fluids. Exploratory laparotomy revealed one normally developed and one partially macerated fetus, high amount of intrauterine pus and hyperplastic endometrial glands. Histopathological examination of uterus showed chronic inflammation, local necrosis, local proliferation of glandular epithelium and cystic endometrial hyperplasia. Bacteriological examination of dead fetus and of amniotic fluid reveled gram-negative anaerobic *Bacteroides* spp. A specific diagnosis in this case is complicated. Histopathological findings and bacterial infection separately or together could be responsible for the fetal death. Cases of pyometra during pregnancy are extremely rare, with no available treatment protocols for this condition.

P 114 | Relationships between follicular fluid steroid concentrations and uterine infections in ovarian cystic cows

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A total of 21 bovine genital tracts with ovarian cysts were collected from the abattoir to determine the relationships among uterine bacterial infection, ovarian cysts and subclinical endometritis. The uteri samples did not have any purulent discharges and all were

grossly involuted (more than 40 days postpartum). Ovarian cysts were defined as follicular structure ≥17 mm on the ovary with no concurrent presence of corpus luteum. Cystic fluids were aspirated from ovarian cysts (follicular cysts, n = 10; luteal cysts, n = 11) and 8 large follicles (<15 mm) with clear appearance as control for hormone assay. Samples from the internal surface of the uterine body, and the right and left uterine horns were collected and cultured for bacteriological examinations. In addition, samples from the cystic walls, uterine horns and bodies were subjected to histopathological examinations. Endometritis was defined as the presence of congestion and edema of the endometrium associated with infiltration of the inflammatory cells including neutrophils, macrophages, plasma cells, and lymphocytes. E2/P4 ratio was higher in follicular fluids in cows without ovarian cysts but with endometritis than that in cows with both ovarian cysts and endometritis (3.47 ± 1.37; 0.25 ± 0.11, p = 0.04). Furthermore, cows that had endometritis and no ovarian cyst had a higher E2/P4 ratio than those of endometritic cows with either follicular cyst (3.47 ± 1.37; 0.51 ± 0.17, p = 0.03) or luteal cyst (3.47 ± 1.37; 0.003 ± 0.002, p = 0.01). As the E2/P4 ratio increased, the numbers of isolated bacterial species decreased from 9 to 1 species of bacterial isolates. In conclusion, as the E2/P4 ratio increased in the follicular fluid, the numbers and diversity of bacterial species significantly decreased irrespective of pathogenicity.

P 115 | Behavioral effects of deslorelin implantation in livestock guardian male Turkish Kangal Dogs

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Chemical castration using GnRH agonists has proven to be an effective method of reversible suppression of fertility in male dogs. However, little is known about the behavioral effects of chemical castration. In this study, the effects of chemical castration on aggression, sexual behavior, livestock guarding behavior and obedience in male livestock guardian Turkish Kangal Dogs were assessed by means of a behavior test and a questionnaire that was filled out by the dog-owners. Eleven dogs were subcutaneously inserted either 4.7 mg (n = 7) or 9.4 mg (n = 4) deslorelin implants, and 5 dogs were served as controls. Their behavior was tested on the day of, but prior to treatment and 2 months after treatment and a questionnaire was filled out by the dog-owners 2 and 6 months after implant insertion. None of the dogs could concentrate on the simple learning test, in which a clicker was used, when a female dog in heat was around before the treatments. At the test 2 months after implant insertion 5/7 of dogs in group I and 3/4 of dogs in group II dogs responded to the learning test while all dogs in control group were unresponders ($p < 0.05$). In the first behavior test, all dogs were assessed as aggressive to unfamiliar male dogs. The most reported behavior problems were hunting (10/11), leaving the herd (10/11) and attacking the other livestock (6/11). Two months after the treatment, the owners have reported that motivation of livestock protection increased as sexual behavior ($p < 0.01$), leaving the herd ($p < 0.01$) and obedience ($p < 0.01$) were positively changed. Aggression and hunting behavior were not affected by the treatments ($p > 0.05$). Overall, results show that chemical castration in male Turkish Kangal dogs has positive effects on livestock guarding behavior.

P 116 | Determination of the gestational age of the fetus in Bulgarian local goats by ultrasonographic measurement of some uterine and fetal parameters

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The aim of the current study was to determine the gestational age of the fetus in Bulgarian local goats by ultrasonographic measurement of some uterine and fetal parameters. Twenty-four pregnant Bulgarian local goats, aged 2–4 years, body weight 42–55 kg and housed in the same management were used. Transrectal and transabdominal ultrasonographic examinations were performed weekly between days 21 and 49 of pregnancy and 2 weeks apart until day 133 of pregnancy. The uterine lumen diameter (ULD), outer and inner placentomas diameter (IPD and OPD), crown-rump length (CRL), biparietal, orbital, trunk and fetal aortic diameters (BPD, OD, TD and FAD) were measured in fixed images by an ultrasound electronic caliper. The results were processed by statistical computer software. The relationships between gestational age and each parameter were expressed with linear ($y = a + bx$), quadratic ($y = ax2 + bx + c$) and power ($y = axn$) equations and the coefficients of determination (R^2) were calculated. Highly positive correlations ($R^2 \geq 0.90$) with low standard error estimation (SEE ≤ 8) were calculated for parameters ULD, CRL, BPD, TD and FAD whereas lower correlations ($R^2 \leq 0.90$) with higher SEE (≥8) were registered for IPD, OPD and OD. In conclusion, the gestational age of the fetus in Bulgarian local goats could be determined correctly by measurement of uterine lumen diameter and crown-rump length between days 21–49 day, biparietal and trunk diameters after day 49 and fetal aortic diameter after 77 day of pregnancy.

P 117 | SIPS, uterine rupture, peritonitis and ovarian cyst in 4-year old bitch - a case report

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Subinvolution of placental sites (SIPS) is a rare complication of postpartum in bitch but its cause is still unclear. Throphoblasts do not degenerate and continue to invade the endometrium or myometrium. Persistent hemorrhagic uterine discharge is a typical clinical sign. Spontaneous remission occurs and medical or surgical therapy

is not always needed. A 4-year old Yorkshire Terrier was referred to our clinic due to persistent hemorrhagic vaginal discharge since 4 weeks after the parturition (two live and one dead puppies were born). She was treated before with amoxicilin together with metronidazole and meloxicam at proper doses. The ultrasound examination revealed a large structure (36 mm) inside left uterine horn. During laparotomy, peritonitis with peritoneum imbibition of green color was firstly noticed. Also, the left uterine horn was enlarged with 3 perforations and with cauliflower-like growths (placental sites) inside, and a cyst on left ovary and intraabdominal liquid were observed. Ovariohysterectomy was performed. Histopathological examination of uterus and ovaries revealed SIPS. The wall of uterus was thickened with foci of necrosis, hemorrhages, blood clots and clusters of bacteria. Syncytiotrophoblast-like cells were seen mainly around blood vessels. Endometrial glands were extended with mucous discharge inside. These changes were extensive, reaching serosa and could have caused peritonitis. Lack of sytemic signs makes SIPS difficult to diagnose. In rare cases if severe hemorrhage, ulceration of endometrium or uterine perforation is present ovariohysterectomy is required. Uterine rupture and peritonitis are rare consequences of SIPS.

P 118 | Determination levels of ceruloplasmin, haptoglobin and serum amyloid A in cows with endometritis

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The present study aimed to determine the serum ceruloplasmin (Cp) levels in cows with endometritis of varying degrees of severity, and to establish whether or not there is a correlation between haptoglobin (Hp) and/or serum amyloid A (SAA) levels. The study was conducted with 100 Brown Swiss cows 3–8 years of age on days 28–32 postpartum. Cows were grouped into endometritis (mild, moderate and severe endometritis) and healthy cows based on rectal examinations, ultrasonography (7.5 MHz, Titan®, Sonosite, USA), vaginoscopy and cytological examination. Blood samples immediately after examination were collected from all cows. Levels of Hp, SAA and Cp were analyzed. Serum Cp levels in cows with endometritis (mild = 18.57 ± 0.36 mg/dL, moderate = 22.14 ± 0.75 mg/dL, severe = 27.64 ± 0.87 mg/dL) were significantly higher than healthy cows (13.52 ± 0.32 mg/dL). Similarly, serum Hp (mild = 154 ± 5.16 μg/ml, moderate = 183 ± 6.2 μg/ml, severe = 234 ± 10.7 μg/ml) and SAA levels (mild = 20.25 ± 0.65 μg/ml, moderate = 28.17 ± 1.22 μg/ml, severe = 34.62 ± 1.28 μg/ml) were higher in cows with endometritis than in healthy cows (72 ± 2.76 μg/ml, 14.24 ± 0.52 μg/ml, respectively; $p = 0.001$), and the levels of these acute phase proteins increased in parallel with the severity of the endometritis ($p = 0.001$). A significant correlation was found between Cp levels and Hp and SAA levels (Cp-Hp: $r^2 = 0.783$, $p < 0.001$; Cp-SAA: $r^2 = 0.739$, $p < 0.001$; Hp-SAA: $r^2 = 0.723$, $p < 0.001$). In conclusion, it was determined that serum Cp levels increase significantly in parallel with the severity of endometritis. It was concluded that serum Cp levels can be used in the diagnosis of endometritis as an alternative to Hp and SAA levels.

P 119 | Early pregnancy regulates genes of hyaluronan system in ovine endometrium

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The glycosaminoglycan hyaluronic acid/hyaluronan (HA) is a key constituent of the extracellular matrix (ECM) and important in the tissue remodeling. Hyaluronan synthesis is modulated by three main hyaluronic acid-synthase (HAS) enzymes: HAS1, HAS2, and HAS3 and enzymatically catabolized by two hyaluronidases (HYALs) HYAL1 and HYAL2. The HA binds to cell surface receptor CD44 and facilitates its adhesion, proliferation and migration. The endometrial tissue undergoes extensive remodeling under cyclic conditions and pregnancy. The objective of this study was to elucidate the impact of early pregnancy on HA system in ovine endometrium. The endometrial tissue samples were collected from pregnant ewes on days 12 (n = 4), 16 (pre-attachment phase, n = 4), and 22 (post-attachment phase, n = 4) of pregnancy. Total RNA was extracted followed by cDNA synthesis and mRNA expression levels of HA system components were determined using qPCR. The expression of HAS1 and HAS2 mRNA did not differ among the days of pregnancy. Expression of HAS3, CD44, HYAL1 and HYAL2 were significantly upregulated on day 22 of the pregnancy ($p < 0.05$). The data suggest that genes related to HA system are significantly regulated in the endometrium after the post-attachment phase of the embryonic development. It may be concluded that regulation of the HA genes contributes to composition of ECM, and also enables the cell-cell contacts as pregnancy advances and implantation process starts. Moreover, the results provide insight into remodeling of the endometrium during early pregnancy. (This study was partially funded by TUBITAK grant 214O643 to MK.)

P 120 | Effect of the age at first calving on milk performance in PHF HO cows

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Currently in case of dairy cattle breeding more and more attention is paid to indexes related to reproductive efficiency. The most important index in cows is the age at first calving (AFC). The value of this trait affects on reproductive parameters and economic efficiency of milk

production. The aim of the study was to determine the effect of the AFC on milk performance of Polish Holstein Frisian Black-and-White (PHF HO) cows. The study included 959 heifers, which were kept in free stall barn and fed TMR system (total mixed ration). Data on milk, fat and protein yield, and fat and protein content and the AFC were obtained on the basis of the farm breeding documentation conducted by PFHBiPM. The cows were divided into three groups according to the AFC (under 750; 751–900; above 901 days of age). Differences between the groups were examined using Statistica®10 PL (StatSoft, Inc 2011). The highest ($p \leq 0.01$) milk yield (8994 kg) in the first lactation was characteristic for cows calving before 750 days, while the lowest milk yield (8572 kg) was found in the group of cows calving late (>901 days). The highest fat (340 kg) and protein (306 kg) yield and the highest fat ($p \leq 0.05$) 3.88% and protein ($p \leq 0.05$) 3.50% content in milk was obtained by cows calving between 751–900 days. AFC had a significant influence on milk yield (under 750 days of age) and fat and protein content in milk (751–900 days of age) ($p \leq 0.05$ and $p \leq 0.01$).

P 121 | Ex vivo developmental potential of caprine cloned embryos is affected by inducible epigenetic modification of both adult cutaneous fibroblast cells and activated nuclear-transferred oocytes

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The objective of our study was to explore the influence of sequential trichostatin A (TSA)-mediated epigenetic transformation of nuclear donor fibroblast cells and artificially activated oocytes that had been reconstituted with them on the developmental abilities of cloned goat embryos. Enucleated in vitro-matured oocytes were subzonally-injected with adult dermal fibroblast cells treated or not treated with TSA (Groups I and II, respectively). Successfully reconstructed and activated oocytes (clonal cybrids) were incubated for 24 h in SOF medium supplemented with TSA (Group I) or lacking TSA (Group II). Cleaved embryos were cultured up to morula/blastocyst stages for an additional 144 to 168 h in the TSA-free medium enriched with FBS. Among 247 cloned embryos assigned to Group I, 189 (76.5%) were able to divide ex vivo. The percentages of embryos that reached the morula and blastocyst stages were 143/247 (57.9%) and 85/247 (34.4%), respectively. In Group II, out of 212 cultured embryos, 136 (64.2%) underwent cleavage divisions, but 92 (43.4%) and 48 (22.6%) developed to morula and blastocyst stages, respectively. Collectively, two-step TSA-dependent epigenomic modulation of caprine cutaneous fibroblast cells and clonal cybrids brought about the considerable increase in the morula and blastocyst formation rates due to presumptive improvement of donor cell nuclear reprogrammability. (This study was conducted as a part of statutory activity No. 02-011.1, which is financed from 2015 to 2017 by the Polish Ministry of Science and Higher Education.)

P 122 | Influence of the length of intercalving period on the milk performance of cows

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The length of the intercalving period (ICP) affects udder health, conception rate after parturition, the course of the subsequent intercalving period, the condition of the cows and, therefore, the economical aspect. Good herd fertility is the basis of cattle breeding. The aim of the study was to determine the effect of the ICP on milk performance of PHF HO cows. The study included 962 individuals, which were kept in free stall barns and fed TMR system. Data on milk yield, fat and protein yield, and fat and protein content and the ICP were obtained on the basis of the farm breeding documentation provided by Polish Federation of Cattle Breeders and Dairy Farmers. The cows were divided into three groups depending on the length of the ICP (under 360; 361–520; above 521 days). Groups were compared using Statistica®10 PL (StatSoft, Inc 2011). The study revealed a positive correlation of ICP and milk yield. This relationship is evident in the case of ICP lasting more than 521 days (10,895 kg). Along with the extension of the ICP fat (426 kg) and protein (377 kg) yield also increased. The highest fat content (3.94%) was observed in the milk of cows with ICP between 361 and 520 days, while the highest protein content (3.50%) in the milk of cows with ICP under 360 days. Statistical analysis of the ICP length showed significant differences ($p \leq 0.01$, $p \leq 0.05$) among the groups.

P 123 | Expression of mRNA and protein for membrane progestin receptors in the bovine uterus during the estrous cycle and the first trimester of pregnancy

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Progesterone (P4) can affect bovine uterus function via nongenomic pathway, presumably involving the membrane progesterone receptor. The aim of the present study was to determine mRNA (Real Time PCR) and protein (western blot) expression and cellular localization (immunohistochemical analysis) of membrane progestin receptors (mPR) alpha (mPRα), beta (mPRβ) and gamma (mPRγ) in bovine endometrium and myometrium during the estrous cycle (day 2–5, 6–10, 11–16 and 17–20) and the first trimester (weeks 3–5, 6–8 and 9–12) of pregnancy ($n = 5$/each period). Expression of mRNA and protein for mPRα and mPRβ receptors increased ($p < 0.05$) on days 11–16 of the estrous cycle in endometrium, while in myometrium on days 2–5 and 17–20. There were no changes ($p > 0.05$) in mPRγ mRNA expression in uterus during the estrous cycle. mRNA and protein expression for all mPRs increased also during pregnancy. A strong immunostaining for all mPRs proteins was observed in the luminal and glandular epithelium; this reaction was less evident in the stromal cells and myocytes. All proteins were also

localized in the endothelial cells of blood vessels in uterus. This data suggest that mPRs receptors can participate in the nongenomic P4 action on blood flow and this way may regulate uterine function. Moreover, obtained data indicate that P4 may regulate uterine function via these receptors and this way participates in the regulation of the estrous cycle and maintenance of pregnancy. (Supported by National Science Center (2012/05/B/NZ4/01810) and by Polish Academy of Sciences.)

P 124 | Oestrogen receptors α and β characterized by immunohistochemistry in the mare oviduct

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The oviduct is an important organ that affects reproductive capacity, since it is the site of fertilization and passage of the early embryo. On the other hand, oestrogens are important regulators of female reproductive physiology. They mediate multiple changes in mares reproductive tract during the estrus cycle. Their role in the equine oviduct still remains unclear. The objective of the study was to characterize the expression of oestrogen receptors α and β (ERα and ERβ) in mare's oviduct using immunohistochemistry (IHC). Tissue samples were collected post mortem from 14 adult mares (n = 14) during various stages of estrus cycle. After that, oviducts were dissected free of mesentery, and full cross sections of the ampulla and isthmus were obtained. As primary antibodies for IHC staining, monoclonal mouse IgG1 Er-α (NCL-L6F11 clone, Novocastra) and monoclonal mouse IgG1 Er-β (NCL-ER-β clone EMR02, Novocastra) were used. Visualization was performed with AEC, EnVision System. Immunoreactivity for the presence of ERα and ERβ was observed in oviduct epithelium, both in the nucleus and cytoplasm. α-Receptors were also noted in the muscular layer, while β in both muscular and connective tissue. Moreover, a diversity of expression and intracellular localization between ERα and ERβ within various cycle phases was observed. There were also differences in ERα and ERβ expression between ampulla and isthmus of oviduct. Results of the current study suggest multiple effects of oestrogens on equine oviduct functions depending on estrus cycle stage.

P 125 | Development competence of porcine oocytes selected by brilliant cresyl blue before vitrification

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Brilliant cresyl blue (BCB) staining has been used for selection of the functional status of oocytes from several mammalian species, including pig (Ishizaki et al. 2009, Theriogenology 72:72–80). Native BCB+ oocytes had significantly higher development competence than BCB− oocytes. The aim of the present study was to evaluate the development competence of devitrified BCB+ and BCB− oocytes. Before vitrification cumulus oocyte complexes (COC) were incubated in BCB solution (13 μM) for 60 min. Oocytes were divided into BCB- (colorless cytoplasm) and BCB+ (colored) and then COC were incubated 40 min in follicle fluid (d ≤ 3 mm). Vitrification was performed by equilibration of oocytes in CPA (Cryoprotective Additive) - 1: 0.7 M dimethylsulphoxide (Me2SO) + 0.9 M ethylene glycol (EG) (30 sec); CPA-2: 1.4 M Me2SO + 1.8 M EG (30 sec); CPA-3: 2.8 M Me2SO + 3.6 M EG + 0.65 M trehalose (20 sec) and loading into straws. After thawing COC washed by step-wise dilution in 0.25 M,0.19 M and 0.125 M trehalose in TCM-199 and finally in TCM-199 alone. COC were cultured in NCSU - 23 with 10% (v:v) follicle fluid, 0.1 mg/ml cysteine, 10 IU/ml eCG and 10 IU/ml hCG. COC cultured with the pieces of follicular wall (600–900 μm in length). After IVM oocytes were fertilized in vitro and embryos were cultured by standard protocols (Stokes et al., 2005, Dev Biol, 284:62–71). Cleavage was significantly higher in BCB+ oocyte in compared to BCB− oocytes [32% (80/250) vs. 12% (22/185), respectively, $p < 0.001$, χ^2 test]. Blastocyst development rate was significantly higher in BCB+ vs. BCB− oocytes [12% (30/250) vs. 3% (6/185), respectively, $p < 0.05$, χ^2 test]. In conclusion, BCB test is an effective method for selection of more competent porcine GV− oocytes for vitrification.

P 126 | Genes associated with polycystic ovarian syndrome in women (PCOS) are changed in bovine blastocysts deriving from insulin treated oocytes

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PCOS is a common cause for infertility in women and associated with hyperandrogenism, obesity, increased risk for Type-2 diabetes mellitus and insulin resistance. Genetic studies revealed a dysregulation of genes involved in steroid biosynthesis and metabolism in PCOS ovaries. Our aim was to study gene expression associated to PCOS when oocytes were challenged with insulin during maturation. Bovine embryos (n = 120) were produced in vitro according to standardized methods with three different insulin levels (INS10 = 10 μg/ml; INS0.1 = 0.1 μg/ml; INS0 = control) during maturation. Gene expression data of day 8 blastocysts (D8BC) were received through microarray-studies at the EmbryoGENE platform. Differentially expressed transcripts between control and insulin groups were searched using an empirical Bayes moderated t-test (limma-package of R) defined as having a 1.5 fold change difference and $p < 0.05$. By using IPA (www.ingenuity.com), gene expression was further investigated and compared with results of similar studies on PCOS. Interestingly, genes with known association to PCOS (CYP11A and IGF2R) were changed in the D8BC deriving from insulin treated oocytes, while others as the INSR were not

influenced. Genes involved in related pathways, such as PRPF6 (androgene receptor binding), DHCR7 (Vitamin D metabolism) and NR3C1 (glucocorticoid receptor) showed different expression. These findings could help to understand mechanisms leading to decreased developmental competence and show that an insulin challenge during maturation changes the transcriptome of D8BC which could be one reason for impaired fertility of women with PCOS. (Funded by FORMAS)

P 127 | Modulating the quality of bovine oocytes aging in vitro by cumulus cells and prolactin

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Mammalian oocytes attained the metaphase-II stage are undergone time-dependent aging processes leading to a decline in the ovum quality. The aim of this work was to study effects of cumulus cells (CCs) and prolactin (PRL) on the quality of bovine oocytes aging in vitro. Bovine cumulus-enclosed oocytes (CEOs) were matured in vitro for 20 h. After IVM, CEOs and oocytes denuded of their CCs (DOs) were cultured for 24 or 48 h in the absence (Control) and in the presence of 50 ng/ml PRL and/or inhibitors of three pathways: PP2 (an inhibitor of Src-family tyrosine kinases), triciribine (an inhibitor of Akt kinase), and calphostin C (a protein kinase C inhibitor). Oocyte apoptosis was detected by the TUNEL method and the level of zona pellucida (ZP) hardening was assessed using the half-time (t50) for chymotrypsin-mediated dissolution of ZP. During aging in the control medium, the rate of apoptotic CEOs increased from 5.6 ± 2.4% (0 h) to 24.5 ± 3.3% (24 h, $p < 0.01$), whereas the rate of apoptotic DOs was unchanged. PRL decreased the frequency of oocyte apoptosis in the case of CEOs (up to 8.2 ± 3.3%), but did not affect this frequency in the case of DOs. Concurrently, the effect of PRL on oocytes was abolished by calphostin C (1 μM). ZP hardening became detectable only by 48 h of aging and was more marked in DOs than in CEOs. Meanwhile, t50 of ZP dissolution was unaffected by PRL or the inhibitors tested. Thus, CCs accelerate apoptosis of aging bovine oocytes, whereas PRL may eliminate the pro-apoptotic action of CCs by activating the protein kinase C-dependent pathway. At the same time ZP hardening does not contribute significantly to the time-dependent senescence of bovine oocytes and it is not enhanced by CCs. (The study was supported by FASO Russia and RFBR (15-08-99473).)

P 128 | Interacting seminal plasma proteins revert capacitation in ram sperm

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Cryopreservation induces premature capacitation in sperm decreasing its fertilizing capacity. Capacitation has been associated with protein tyrosine phosphorylation (pY) and it has been demonstrated that seminal plasma (SP) partially reverts this modification in cold-shocked sperm. We developed a methodology to obtain the fraction of SP proteins that bind to the sperm membrane. This fraction, interacting SP proteins (ISPP), may vary according to the season and collection. Our aim was to evaluate whether ISPP obtained by artificial vagina (AV) or electroejaculation (EE) during the breeding and non-breeding season can simulate the effect of complete SP on thawed ram sperm. Ejaculates from 8 Assaf rams obtained during the breeding or non-breeding season were pooled by collection method (AV or EE) and processed to obtain SP and ISPP. Cryopreserved semen from 10 Assaf rams was incubated either with 20% (v/v) SP or an equivalent amount of ISPP plus 5 mg/ml fructose. The presence of pY was detected by Western blot using anti phosphotyrosine followed by anti-tubuline (loading control). Two protein bands of 45 and 40 kDa were detected. We found that the pY signal induced by cryopreservation was reverted after incubation with SP and ISPP with differences among treatments. The relative intensity for the 45 kDa band was: 0.17 ± 0.03 AV SP, 0.13 ± 0.03 EE SP, 0.21 ± 0.04 ISPP AV, 0.36 ± 0.15 ISPP EE, $p = 0.04$; and for the 40 kDa band: 0.37 ± 0.14 AV SP, 0.38 ± 0.14 EE SP, 0.38 ± 0.14 ISPP AV, 0.53 ± 0.14 ISPP EE, $p = 0.010$. Season showed no effects. We concluded that ISPP obtained by AV and EE reverted premature capacitation of cryopreserved sperm as complete SP does. This can be a step to develop extenders to improve the quality of cryopreserved ram sperm. (Supported by BEC.AR)

P 129 | Effect of metabolic components on fertility of Holstein cows in early lactation

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The aim of this study was to examine the metabolic status of dairy cows in the first third of lactation and its relationship with reproductive capacity. Metabolic status was evaluated twice: at the end of the transition period (18–29 days) and at the second month of lactation (46–60 days). The concentration of total protein, albumin, glucose, triglycerides (TG), cholesterol, urea were determined in serum. Body condition evaluated in scores (BCS) was also determined. The obtained data were processed using the software SigmaPlot 12.5. Animals were divided into two groups according to dynamic changes in the concentration of TG: group 1 ($n = 8$) and group 2 ($n = 6$). In group 1 the concentration of TG decreased by 40% - from 0.063 ± 0.006 mmol/l (transition period) to 0.038 ± 0.005 mmol/l (46–60 days after calving), $p < 0.01$. In group 2 TG increased by 97% - from 0.033 ± 0.006 mmol/l to 0.065 ± 0.006 mmol/l, $p < 0.01$. The duration of open days for cows in group 1 was 135 ± 15 days, and in group 2 was 201 ± 21 days. Milk yield at 100 days of lactation was higher in group 1 than in group 2

(4342 ± 154 and 3808 ± 118 kg, respectively; $p < 0.05$). The concentration of total protein, albumin, glucose, cholesterol, urea, and BCS were not significantly different between the groups at studied periods. The study shows that the dynamics of change in the concentration of triglycerides in the first 2 months of lactation may determine subsequent reproductive ability.

P 130 | Protein profile of boar sperm from different ejaculate portions: a preliminary assay

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Boar sperm perform in different way, including for overcoming sperm technologies, according to the ejaculate portion where they came from. In addition to seminal plasma composition, differences in sperm structure, including protein composition, could explain this circumstance. This study evaluates putative differences in protein profile among sperm from the main ejaculate portions (EP). Boar ejaculates (n = 3) were collected into 3 portions: EP1: the first 10 ml of sperm rich fraction (SRF), EP2: the rest of SRF and EP3: post-SRF. Sperm proteins from each EP were extracted using a RIPA-buffer protocol and separated by 2D-PAGE. Then, gels were silver-stained, scanned, and analyzed. Qualitative analysis showed over 200 spots in EP1 and EP2 sperm while only 99 spots in EP3 sperm. The most differently expressed spots (n = 80) were studied. The results showed that out of those 80 spots, 15 (18.75%; 67% of them under 26.6 kDa) were only present in EP1 sperm, 11 (13.75%; 55% of them under 26.6 kDa) only in EP2 sperm and 6 (7.5%; 33% of them under 26.6 kDa) only in EP3 sperm, suggesting that boar spermatozoa from different EP differ in protein composition. This differing protein composition could explain differences in sperm tolerance to biotechnologies. However, further analysis including identification and quantification of these differing proteins should be performed to determine its physiological functionality, as well as its potential utility as biomarkers of boar sperm fertility or freezability. (Supported by Seneca Foundation, Murcia, Spain (19892/GERM/15) and CSC (China).)

P 131 | Differential protein expression in porcine corpus luteum during its formation from preovulatory follicles

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Luteinization of the follicular cells causes extensive changes in preovulatory follicles that lead to ovulation and ultimate formation of corpus luteum. Molecular determinants of these processes are not well understood. The objective of this study was to identify proteins expressed in porcine granulosa cells of preovulatory follicles and early corpus luteum, 1–3 days after LH surge. We used 2D-gel electrophoresis based proteomics followed by tandem mass spectrometry to address our aim. Protein lysates were prepared from granulosa cells (GC) isolated from preovulatory follicles before LH surge (4 biological repeats) and early corpus luteum (4 biological repeats). A comparison of granulosa cell proteome with that of early corpus luteal (CL) proteome revealed that out of a total of 173 identified proteins, only 43 were common between two structures. Functional analysis using Ingenuity Pathway showed that most of the follicular cell proteins were involved in cellular growth and proliferation followed by cell death and survival, protein degradation and post translational modifications. Most of the early luteal proteins were associated with cell death and survival followed by cellular movement, cell to cell signaling and free radical scavenging. An analysis of the proteins that were significantly altered with luteal formation, i.e. 1.8 times up- or down-regulated in CL as compared to GC and $p < 0.01$, were found to be associated with lipid metabolism, small molecule biochemistry, vitamin and mineral metabolism and cell death and survival. We have identified novel proteins that are either down- or upregulated after the LH surge in porcine preovulatory follicles. These data will aid in understanding physiology of luteinization process.

P 132 | Analysis of systemic changes in cows with subclinical endometritis

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One of the major causes of decreased fertility is endometritis and the diagnosis of sub-clinical disease is limited by the lack of early, reliable biomarkers. We hypothesize that early systemic immune changes associated with sub-clinical endometritis may provide predictive indicators of cows which are likely to develop uterine disease. Uterine cytology, vaginal mucus scores and peripheral blood evaluations from postpartum cows (n = 139) were performed at 7 and 21 days postpartum (DPP). Tempus tubes were used for the collection of blood and RNA extraction and RT-qPCR. An 18% polymorphonuclear (PMN) cell cut off was used to classify subclinical endometritis 21DPP. Two groups were identified; resolvers (n = 10) and non-resolvers (n = 10). Both had subclinical vaginal mucus scores and high PMN 7DPP. However, resolvers had 8.9% PMN compared to 57.3% PMN for non-resolvers 21 DPP. Pre-calving, non-resolvers had increased relative gene expression of immune receptors, cell surface markers (TLR2 [p = .012], CD68 [p = .047], CD80 [p = .025] and CD172 [p = .009]); as well as pro-inflammatory cytokines (TNF [p = .06]) at 21DPP. At 7 DPP, CD172 and TNF were significantly increased in non-resolving cows. Resolvers had significantly ($p < .001$) increased relative gene expression of TLR4 at 7DPP.

Conclusions: The current standards for diagnosing endometritis have limitations in how early subclinical disease can be reliably predicted. Our results have shown significant systemic immunological changes between non-resolvers and resolvers as early as 62 days pre-calving as well as at 7DPP. These early immunological profiles suggest that changes in the immune response may be useful in predicting subclinical endometritis before the manifestation of poor reproductive outcome

P 133 | The effect of an aromatase inhibitor on estrus, hormonal pattern and conception in the blue fox vixens (*Vulpes lagopus*)

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In this study, the contraceptive effect of a non-steroidal aromatase inhibitor (Finrozole) was evaluated at the time of estrus in female vixens, as a model for canine bitches. Blue fox vixens present hormonal pattern similar to those of female dogs. A total of 80 vixens were allocated into four treatment groups, according to the oral administration of either placebo or Finrozole at a dose of 0.5, 3.5 or 24.5 mg/kg, for 21 consecutive days beginning at proestrus and terminating at 1–2 weeks after artificial insemination (AI). The vaginal electrical resistance (VER) was monitored daily from proestrus to AI. The estradiol and progesterone concentrations in plasma were monitored twice a week for first 3 weeks and once a week for six following weeks starting at proestrus. After a 50 Ω decline from the maximum reading in daily VER values (peak VER values equal LH peak ±2 days), the vixens were artificially inseminated by the second week of treatment. Pregnancy was confirmed by ultrasound between D30 and D40. The administration of Finrozole significantly decreased plasma oestradiol concentrations in female vixens. Progesterone profile and VER values were similar in all treatment groups. In the three treatment groups, pregnancy rates were nearly linearly reduced as the dosage of Finrozole increased: 62.5%, 50.0% and 47.1%, respectively, when compared to a pregnancy rate of 73.7% for the placebo treatment group. Further, when the two highest dosages of Finrozole were administered at least 4 days before insemination, no pups were born. Estrus and pregnancy can be completely prevented by Finrozole as long as treatment is initiated at least 4 days before artificial insemination in vixens.

P 134 | Effect of sperm selection by colloid single-layer centrifugation on fertile and subfertile donkey ejaculates

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Single-layer centrifugation (SLC) is a procedure to improve sperm quality by selecting the best spermatozoa from semen samples of different species. The aim of this study was to determine the effect of SLC on the sperm motility parameters from fertile and subfertile donkeys. Ejaculates from one subfertile (pregnancy rates lower than 40%) and three fertile Andalusian donkeys were collected and divided into two aliquots. One of them was immediately analyzed (control) and the other one was subjected to SLC using Androcoll-E Large. Total (TM, %) and progressive sperm motility (PM, %) parameters were analyzed by CASA. The results were compared between subfertile and fertile ejaculates and treatments (SLC vs. control) by ANOVA and expressed as mean ± standard error. TM (30.1 ± 6.0 vs. 71.4 ± 4.7) and PM (19.7 ± 4.6 vs. 57.7 ± 4.3) were significantly lower ($p < 0.01$) for subfertile donkey ejaculates in comparison to fertile donkey semen, respectively. After applying SLC protocol to subfertile donkey semen, TM (65.7 ± 7.8 vs. 30.1 ± 6.0) and PM (45.4 ± 5.8 vs. 19.7 ± 4.6) of SLC samples were significantly higher ($p < 0.01$) in comparison to control samples, respectively; however no significant differences were found in fertile donkey semen between SLC and control samples for TM (78.8 ± 5.0 vs. 71.4 ± 4.7) and PM (66.8 ± 5.1 vs. 57.7 ± 4.3). In conclusion, SLC improved sperm motility of subfertile donkey ejaculates but no effect was found in ejaculates from fertile donkeys. Further studies are needed, including a large number of animals and sperm parameters assessed.

P 135 | Exposure to high hydrostatic pressures allows improving viability of rabbit sperm cells after cryopreservation

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Application of sublethal stress treatment to gametes and embryos may induce adaptation and increase in tolerance of various in vitro procedures such as cryopreservation. This could help to achieve a successful cryopreservation of sperm cells from species with usually non-optimal results. The aim of this study was to evaluate the viability of rabbit sperm cells after cryopreservation when they were previously exposed to high hydrostatic pressures (HHPs). Sperm samples from a total of 15 males were pooled and diluted with Galap diluent (IMV, France) in 5 replicates. Samples were packed into 0.25 ml straws and exposed to different HHPs (50, 100, 150 or 200 MPa) for 5, 10, 30 or 60 min (HHP samples). After HHP, samples were evaluated for sperm cell viability and acrosome status and motility parameters through eosin-nigrosin staining and computer-assisted sperm analysis (CASA), respectively. Control and HHP samples were centrifuged and resuspended with Gent B medium (Minitüb, Germany) for cryopreservation following a conventional procedure. Viability and acrosome status and motility parameters were again assessed after cryopreservation. Best results in viability after cryopreservation were obtained when the sperm samples were exposed to 50 MPa for

30 min (92.8% ± 5.5), showing values significantly higher with respect to the control cryopreserved samples ($p < 0.01$). However, motility was significantly reduced in HHP samples ($p < 0.05$). Acrosome status was not negatively affected by cryopreservation. In conclusion, the exposure of rabbit sperm cells to a sublethal stress in form of HHPs allowed obtaining promising results in sperm viability after cryopreservation but further experiments are needed to get optimal results in motility.

P 136 | A novel culture device suitable for culturing bovine oocytes using a cyclic olefin co-polymer-based chip

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Over the last decade, assisted reproductive technologies have greatly improved although its efficiency remains low. The use of microfluidic devices has allowed optimizing some procedures by automating the handling, minimizing the required manual operations and therefore reducing manipulation stress on the cells. The aim of this study was to test the suitability of cyclic olefin co-polymer (COC) as a microfluidic device substrate for in vitro maturation of bovine oocytes. Bovine oocytes were loaded and cultured into a microfluidic device and a four-well culture dish (conventional system). After 24 h of maturation at 38.5°C in a 5% CO_2 incubator, oocytes were collected and co-incubated with sperm cells for 20 h with fertilization medium. Finally, oocytes were fixed and stained with DAPI for pronucleus assessment. The analysis of differences among devices was carried out using contingency tables and Pearson's Chi-squared statistical test. Oocytes cultured in the four-well dish had significantly higher percentage of sperm penetration rates (61.4 vs. 35.6%; $p < 0.05$). However, in terms of normal fertilization with male pronuclear formation, no significant differences were observed between devices ($p > 0.05$). In conclusion, COC microfluidic device enables the functional maturation of oocytes and it is non-toxic for gametes.

P 137 | Multiparametric resonance imaging (mp-MRI) of the prostate, preliminary results in a canine model for the future MRIgFUS-preclinical evaluation

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Multiparametric resonance imaging (mp-MRI) is the gold-standard in imaging for the prostate cancer (PC) in humans. It consists of high-resolution planar T2WI, diffusion weighted imaging (DWI) and dynamic contrast enhanced MRI (DCE-MRI), optionally MR spectroscopic imaging (MRSI). PC shares the common features in dogs and humans: tumor growth outside the prostate, pulmonary and bone metastases and is diagnosed in older age. As dogs are used as a preclinical model for PC therapy in humans, we aimed to create feasible mp-MRI protocols for imaging PC in canines based on PI-RADS™ v2 human protocols and implement it on 3.0T MR scanner for the future ex-Ablation procedure (MRIgFUS). Four dogs (years 4, 9, 12, 13) with prostatic pathologies confirmed with ultrasound examination underwent the procedure. Under general anesthesia, dogs were positioned prone, feet first on the MR table. Protocols were validated with MR imaging on Discovery MR750w 3.0T, coils GEM Anterior Array and GEM Large Flex. Final protocol consist: T1WI Ax–FSE; TR/TEeff <1000/min ms; slice thickness 3 mm; slice spacing 1 mm; T2WI Sag-FRFSE; TR/TEeff >2500/~100 ms; sl. thick. 3 mm; sl. sp. 1 mm; T2WI Ax–FRFSE; TR/TEeff >2500/~100 ms; sl. thick. 3 mm; sl. sp. 1 mm; T2WI Cor-FRFSE; TR/TEeff >2500/~100 ms; sl. thick. 3 mm; sl. sp. 1 mm; DWI Ax-Focus TR/TE 3500/min ms; sl. thick. 3 mm; sl. sp. 1 mm; b-value 0, 50, 200, 800 s/mm^2. MRI examination in this canine model was challenging due to small FOV that increase scan time, distortion, and noise level. Canine patients differ more than tenfold in body mass, which preclude fully standardized protocol in a whole patient population. MRI sequence parameters need to be individually fitted in terms of number of slices and FOV.

P 138 | The sexual behavior of sexually inexperienced photo-stimulated male goats is more intense when they are in contact with multiparous than with nulliparous goats

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The aim of this study was to determine whether sexual behaviors such as ano-genital sniffing and nudging displayed by the sexually inexperienced photo-stimulated males can be different in the first contact with multiparous or with nulliparous anestrous goats (male effect). Six male goats were submitted to 2.5 months of artificial long days to stimulate their sexual activity during the sexual rest. Males were isolated from females from the weaning (40 day of age) until the male effect (15 months of age). In April, one group of multiparous goats (n = 30) was exposed to 3 males during 15 days. Simultaneously, another group (n = 30) of nulliparous goats was in contact with the other 3 males. During the first 3 days post male introduction, the ano-genital sniffing and nudging were registered (1 h/day). The frequencies of sexual behaviors were compared between males exposed to nulliparous and those multiparous females using a Chi-square test. Although the frequency of ano-genital sniffing was higher for males in contact with multiparous (423) than those with nulliparous females (365), the difference did not reach significance ($p > 0.05$). However,

the frequency of nudging was greater in males in contact with multiparous (1268) than those with nulliparous females (841; $p < 0.001$). These results show that the sexually inexperienced photo-stimulated male goats display sexual behavior more intense when are exposed to multiparous in comparison with those in contact with nulliparous anovulatory goats.

P 139 | Effect of fibroblast growth factor (FGF)2 on prostaglandins secretion from bovine corpus luteum in vitro

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FGF2 is a potent luteal pro-angiogenic factor highly expressed in both steroidogenic (LSC) as well as endothelial cell types derived from bovine corpora lutea (CL). A limited number of studies have shown that FGF2 may also affect luteal cell functions and revealed its pleiotropic nature. The aim of this study was to investigate the influence of FGF2 on prostaglandin (PG)s production by bovine LSC and CL explants. Both LSC and CL explants were obtained from early and mid-luteal phases (n = 5), and cultured for 24 h either with FGF2 (10 ng/ml) or FGF2 inhibitor (FGF2-I, 100 ng/ml) alone or with $PGF_{2\alpha}$ (10-7 M). Concentrations of PGE_2 and $PGF_{2\alpha}$ in culture media were measured using EIA. $PGF_{2\alpha}$ level was decreased after FGF2-I treatment in early CL explants and LSC, and in mid CL explants ($p < 0.05$). FGF2 increased $PGF_{2\alpha}$ output from early CL explants and LSC and also form mid LSC ($p < 0.05$). FGF2-I didn't change PGE_2 secretion in LSC from both stages but treatment with FGF2-I and $PGF_{2\alpha}$ increased PGE_2 level, which was with the same strength in case of treatment with $PGF_{2\alpha}$ alone ($p < 0.05$). Incubation of LSC (both stages) with FGF2 increase PGE_2 release ($p < 0.05$). Moreover, combinations of FGF2 and $PGF_{2\alpha}$ shown synergistic effect ($p < 0.01$). PGE_2 secretion from CL explants (both stages) was decreased after FGF2-I alone or FGF2-I plus $PGF_{2\alpha}$ ($p < 0.05$). Collectively, our findings suggest that FGF2 could regulate secretory function of LSC and its action may depend on the interactions between LSC and other accessory cells in bovine CL (Supported by NSC Grant (2011/03/B/NZ9/01634)).

P 140 | Optimization of electrical stimulation semen collection methods and semen quality analysis in forest musk deer (*Moschus berezovskii*)

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Forest musk deer (*Moschus berezovskii*) is a kind of ruminant, which was listed as Near Threatened (LR/nt) on the IUCN Red List of Threatened Species. Forest musk deer is pretty characteristic for secretion musk from male musk gland, which is famous as traditional Chinese medicines for several thousand years. Until now, it is unstable on its electrical stimulation semen collection methods or rarely reported on its semen parameters. In this study, three bucks were selected to optimize the semen collection methods. It showed that the forest musk deer were narcotized and lie on their back then electrical stimulated with a probe in the rectum. As Lu Mianning II anesthetic dosage 0.041 ± 0.006 ml/kg; average voltage 5.4 ± 1.4 V; depth 7.25 ± 0.65 cm and steadily electrical stimulated 5 s and interval 5 s, ejaculation could produce ideal semen quality. Data on evaluation semen quality of 6 semen samples showed the ejaculation volume, pH value, osmotic pressure, sperm density, sperm live rate and abnormality sperm rate were 0.240 ± 0.056 ml, 7.30 ± 0.24, 354.50 ± 27.62 Omsm/kg, $(1.79 \pm 0.38) \times 10^8$ cells/ml, (81 ± 4)% and (13.48 ± 4.90)%, respectively. The total length of forest musk deer sperm averaged 69.45 ± 3.38 μm with the length of head, the tail main section and middle to end section were 9.54 ± 0.42 μm, 16.02 ± 0.97 μm; 42.45 ± 2.25 μm, respectively.

P 141 | Vascular endothelial growth factor A (VEGFA) and its receptors expression in bovine uterus during adenomyosis

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Adenomyosis is a uterine dysfunction defined as the presence of endometrial glands within the myometrium. It is suggested that increased angiogenesis plays a role during adenomyosis. VEGFA is the main pro-angiogenic factor that regulates uterine vascularization via two receptors: VEGFR1 and VEGFR2. The aim of the study was to determine differences in VEGFA, VEGFR1 and VEGFR2 mRNA expression and localization in normal bovine uterus and during adenomyosis. Uterine tissues (days 8–10 of the estrous cycle) were divided into non adenomyotic (n = 8) and with adenomyosis (n = 11). mRNA expression for VEGFA, VEGFR1 and VEGFR2 was determined by real-time PCR. Results were statistically analyzed by Student's t-test. Immunohistostaining was used to localize VEGFA and its receptors in the tissues. VEGFA and VEGFR2 gene expression was decreased in tissues with adenomyosis when comparing to normal uteri ($p < 0.05$). VEGFR1 mRNA was upregulated during adenomyosis comparing with control group ($p < 0.01$). In all samples VEGFA and its receptors were mainly localized in blood vessel cells, however during advanced adenomyosis VEGFA and VEGFR2 immunoreactivity was increased in vessels adjacent to lesions. In conclusion, VEGFA and its receptors expression and localization were changed during adenomyosis when comparing to control tissues. Obtained results suggest that VEGFA may participate in adenomyosis pathogenesis by modulation of angiogenesis. (Study supported by National Science Centre 2014/15/N/NZ9/02414)

P 142 | Study of advanced DNA fragmentation parameters in fertile and infertile stallions

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Diminished male fertility has been related to DNA fragmentation, which can be assessed by flow cytometry by Sperm Chromatin Structure Assay (SCSA). SCSA yields a DNA fragmentation index (%DFI) and distinguishes between high DNA (%hDFI) and moderate DNA (%mDFI) fragmentation; also a High DNA Stainability (%HDS) population due to lack of full protamination can be detected. Although these parameters are readily used in human sperm to predict fertility, in the horse, these values are still under investigation. In the present work we compared the differences existing between 2 fertile stallions (IP and JC) with >65% pregnancies per cycle and an infertile sire (MB; 0% pregnancy per cycle). Ejaculates (2 per stallion) were collected, diluted in TNE buffer and frozen at −80°C until analyzed. %DFI showed the highest value in the infertile stallion among all the studied (3.77% and 3.23% for MB; 0.52% and 0.61% for IM and 0.86% and 2.25% for JC); %hDFI varied from 0.06% to 0.19% in the fertile stallions, while for MB the values were 0.95% and 2.51%. %mDFI ranged from 0.33 to 2.06% in the fertile stallions and reached 0.72% and 2.82% in the infertile male. %HDS varied from 1.03% to 2.95% in all the stallions and did not discriminate between fertile and infertile stallions. Our preliminary data show that %HDS did not vary between fertile and infertile stallions, while other variables related to DNA fragmentation could discriminate between fertile and infertile stallions.

P 143 | Calcium Sensing Receptor (CaSR) regulates in vitro bovine embryo development but not bovine oocyte maturation

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Calcium Sensing Receptor (CaSR) is a ubiquitous G-protein coupled receptor present in different somatic cell types that modulates calcium homeostasis. The aim of this work was to study the role of CaSR in bovine oocyte maturation and embryo development. First, the presence of CaSR was demonstrated by immunofluorescence; then, bovine oocytes were matured in vitro in the presence or absence of 15 μM NPS2143 (a specific CaSR inhibitor) and chromatin conformation was assessed by fluorescence microscopy. To study the role of CaSR on embryo development, bovine oocytes were fertilized by conventional in vitro fertilization and subsequently incubated in TCM-199 in presence or absence of 15 μM NPS2143. On days 2, 6 and 7 after insemination cleavage, morula and blastocyst rates were evaluated. Maturation rate (mean % ± SEM; 72.5 ± 1.8; vs. 78 ± 5.4; $p > 0.05$), and cleavage rate (79.1 ± 6.8 vs. 73.7 ± 5.3; $p > 0.05$) were not modified by 15 μM NPS2143 addition compared to the control group (control vs. inhibitor respectively). Conversely, development to the morula stage (46.6 ± 7.3 vs. 24.3 ± 4.3; $p < 0.05$) and blastocyst rate (29.9 ± 9.0 vs. 9.9 ± 3.6; $p < 0.05$) decreased significantly in presence of NPS2143 (control vs. inhibitor, respectively). These results demonstrate the presence of CaSR in bovine oocytes and a core role in embryo development. In addition, CaSR does not seem to play a significant role during oocyte in vitro maturation in the bovine species.

P 144 | Effect of the FSH dose on superovulatory response in Santa Inês ewes

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This study aimed to evaluate the superovulatory response under effect of different FSH doses in superovulatory protocols. Santa Inês ewes (n = 24) received an intravaginal progesterone device (CIDR®) on Day 0, which remains until the Day 8. On Day 0 and 8 were also administered 0.125 mg of a synthetic analogue of $PGF_{2\alpha}$ (Sincrocio®). Gonadotrophic treatment started on Day 6 when females were divided into three groups according to the total dose of exogenous pFSH (Folltropin®): G1 (n − 8) – 100 mg; G2 (n = 8) – 133 mg; G3 (n = 8) – 200 mg. FSH total doses were administered in eight injections given twice a day in descending order. On Day 6, all ewes also received 300 IU of eCG (Novormon®). Ultrasonography was performed to assess the follicular growth of the wave. Six days after the onset of estrus, a videolaparoscopy for counting the number of corpora lutea and anovulatory follicles was performed. Data were compared using Kruskal Wallis test and posttest Dunns ($p < 0.05$) using the software R®. Rates were compared between treatments by Chi-square test. There were no differences ($p > 0.05$) among groups for ovulations (12.46 ± 5.93) and anovulatory follicles (0.96 ± 1.23) numbers and score of superovulatory response (1.5 ± 0.59). However, G1 showed higher ovulation rate (96.85 ± 3.53) compared to the G2 (86.30 ± 21.75) ($p = 0.016$) but similar to G3 (93.74 ± 6.85). G1 showed the lowest anovulatory failures (3.13 ± 3.51) compared to the G2 (13.70 ± 21.75) ($p = 0.016$) but similar to G3 (6.26 ± 6.85). In conclusion, best results in superovulation treatment in ewes were obtained using FSH dose of 100 mg. Higher doses of FSH (133 mg and 200 mg) are not indicated. (Financial support: Fapesp no 2014/04614-6, EMBRAPA no 02.13.06.026.00.00, PROPE no TC1288/2015)

P 145 | Influence of the age of breeding bulls on parameters of frozen-thawed sperm

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The aim of the study was to examine influence of age of Holstein breeding bulls (n = 16) on viability and motility of frozen-thawed sperm. The sperm ejaculates collected from younger bulls (2.5–3.5 years; n = 8) and older bulls (3.6–6.0 years; n = 8) were diluted in AndroMed® extender, frozen in a programmable freezer and stored in liquid nitrogen for 6 months. After thawing, sperm motility was determined by CASA (SpermVision®) and acrosome-membrane integrity and live/dead spermatozoa were analyzed on unfixed sperm using fluorescent assays. Bull age affected neither the sperm acrosomal integrity, detected by PNA-AlexaFluor, nor the apoptotic ratio detected by Yo-Pro-1, whilst the dead sperm ratio (propidium iodide labelling) was slightly higher in older (20.5 ± 1.98) compared to younger (15.75 ± 1.86) bulls. Total (TM) and progressive sperm motility (PM) assessed immediately after thawing were not affected by the bull age; however after 2 h post-thawing, the sperm samples from older bulls showed higher TM (35.37 ± 2.40) and PM (32.38 ± 2.63) than younger bulls (28.5 ± 2.14 and 26.56 ± 2.27, respectively). Our data show that bull age does not have a negative effect on sperm parameters if bulls are younger than 6 year-old. (Grant support: APVV-0556-11)

P 146 | Lambing rates after laparoscopic insemination with frozen semen of ewes in natural heat drafted once daily in the morning vs. once daily in the evening

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The purpose of the study was to compare two drafting methods of ewes in natural heat for laparoscopic AI (LAI): daily in the morning (group M) vs. daily in the evening (group E). Two experiments were performed in the breeding seasons of 2014 and 2015. Workers exposed ewes to the direct contact of a teaser ram (belly covered with sackcloth) in a fenced area, either in the morning or in the evening, for 1 h. Considering the response of the ewes to the teaser male, they separated those showing behavioral symptoms of estrus, which were LAI-ed with frozen semen from two Suffolk rams with the aid of a Robertson gun on the same day in group M or on the next day in group E. The lambing rates in the first and second experiments in groups M and E were: 18.8% (9/48) and 46.7% (14/30) ($p < 0.05$); 32.4% (12/37) and 39.0% (23/59) ($p > 0.05$); total 24.7% (21/85) and 41.6% (37/89) ($p < 0.05$), delivering 1.19 and 1.35 lambs/lambing, respectively. The same frozen semen from two Suffolk rams in a previous study on LAI in ewes in natural heat drafted as in group M resulted in significantly ($p < 0.01$) lower lambing rates of 24.4% (10/41) and 23.5% (8/34) in comparison to Dorset (53.1%, 52/98) or South-African Meat Merino (54.9%, 28/51). It was concluded that daily evening drafting of ewes in natural heat followed by LAI with frozen semen on the next day provides higher lambing rates than daily morning drafting with LAI on the same day. Further research is needed to confirm these results.

P 147 | Interrelations of GH, IGF-1 y progesterone concentrations during luteal phase of estrous cycle in healthy Spanish Purebred mares

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In women (Taketani et al. 2008), bitch (Kooistra et al. 2000), cow (Liebemann et al. 1996) and sheep (Khan-Dawood et al. 1994) GH and IGF-1 plays an important role in corpus luteum (CL) function, stimulating pulsatile P4 secretion during diestrous. The aim of present study was analyze the relationship between GH, IGF-1 and P4 during luteal phase, which has not been previously reported in healthy mares. Daily blood samples from 24 cycling mares aged between 4 and 17 years were taken from the day of ovulation until day 5 of diestrus. Serum IGF-1 and P4 concentrations were analyzed by competitive immunoassay and GH by EIA sandwich. Mean values ± SD of IGF-1, GH and P4 increased progressively from the day of ovulation (228.25 ± 99.23 ng/ml; 2.48 ± 1.49 ng/ml; 0.178 ± 0.010 ng/dl) until day 5 of diestrous (287.73 ± 116.12 ng/ml; 3.01 ± 2.23 ng/ml; 2.523 ± 0.083 ng/ml; $p < 0.05$). Correlations between GH-IGF-1, GH-P4 and IGF-1-P4 were $r = -0.13$, $r = 0.26$ and $r = -0.57$, respectively. These results suggest that P4 production by CL increases by GH, but not by IGF-1 stimulation. Unlike in other species, in which the effects of GH on the synthesis of P4 is mediated directly through their own ovarian receptors rather than the IGF type 1 receptors, in the mare GH could stimulate partially the synthesis of P4 by a different route independent of IGF-1.

P 148 | Long-standing granulosa-theca cell tumor in a cow

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Granulosa-theca cell tumors (GTCTs) are the most common ovarian neoplasms in cattle. Presumptive diagnosis may be made based upon clinical presentation, ultrasound, and endocrine assay. Cows with GTCT are generally slaughtered or undergo ovariectomy to restore fertility. A 6-year Friesian cow was followed from 90 to 780 days post partum. During this period the cow had irregular heats. Cystic ovarian disease at the left ovary (10x5x3 cm) was firstly diagnosed. After unsuccessful treatments, a GTCT was strongly suspected. The ovary,

with a multicystic appearance at ultrasound, doubled in about 9 months reaching an impressive size (22 × 15 × 12 cm). The right ovary was inactive and almost impalpable at the end (4.0 × 1.5 × 0.4 cm). The uterus was constantly oedematous and fluid-filled. Vaginoscopy revealed an open cervix with a slight mucous discharge. Sex steroids maintained estrous values. Considering the lack of discomfort for the cow and the average of milk production (35 l), the farmer gave no consent for surgery. At the end, the cow was slaughtered and genital organs were processed for pathology. The typical follicular pattern of a benign GTGT associated to atrophy of the right ovary, mucometra and severe thickness of cervix and vagina were diagnosed. Moreover, immunohistochemistry for alpha-inhibin revealed a strong and diffuse expression in tumor cells. This case, contributing to the modest caseload of bovine GTCTs in literature, reports the effects of the long-standing (2 years) exposition to estrogens in uterus, cervix and vagina. The GTCT's inhibin production, poorly studied in this species, is suggested to be the main responsible of the marked atrophy of the contralateral ovary.

P 149 | Effect of different cocktails of cryoprotectants and calcium on bovine oocyte membrane permeability and cryosurvival

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Successful cryopreservation of the mammalian oocyte could expand the scope of human infertility treatment and the conservation of animal genetic resources. Mature bovine oocytes were completely vitrified (exp 1) or only exposed (exp 2) to different cocktails of cryoprotectants (CPA), including ethylene glycol (EG), dimethyl sulfoxide (DMSO) and sucrose (EGDMSO group) or 1,2-propanediol (PrOH) and sucrose (PrOH group), diluted in calcium (Ca^{2+}) free/-containing media. The effect of both protocols on oocyte viability and competence for development and cortical granules (CG) location was assessed. Membrane permeability of oocytes exposed to different CPA was also analyzed (exp 3). In exp 1, developmental rates were higher ($p = 0.0005$) for vitrified oocytes submitted to EGDMSO, independently of Ca^{2+} concentration. An enhanced CG exocytosis induced by CPA and chilling was denoted. Higher cleavage rates were obtained in EGDMSOCa^{2+} compared to Ca^{2+} free PrOH group ($p = 0.02$). In exp 2, this group also presented lower cleavage rates compared to control ($p = 0.04$). Exposure to PrOH enhanced the oocytes minimum volume and their permeability through the interaction of this CPA and Ca^{2+} ($p \leq 0.007$). In conclusion, the association of EG and DMSO is the most effective protocol for cryopreserving bovine oocytes, independently of Ca^{2+} presence. However, an interaction between the CPA and Ca^{2+} on some parameters was identified demanding further research. (Funded by FCT project PTDC/CVT/2863/2012)

P 150 | Effects of melatonin supplementation of IVM, IVF and culture media on fertilization and embryo development in pigs

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The presence of melatonin (MEL) during in vitro culture (IVC) improves embryo development (ED) in some species due in part to its antioxidant potential. However, its role during porcine in vitro embryo production (IVP) using in vitro fertilized oocytes has not been established. This study evaluated the impact of MEL on porcine IVP outcomes. In vitro matured oocytes (N = 3183) were inseminated with thawed sperm and cultured for 18 h or 7 days to assess fertilization and ED parameters, respectively. In experiment 1, IVM and IVF media were supplemented with or without 1 nM MEL in all possible combinations. When only IVM medium was supplemented, normal fertilization rate was higher than that of the control. This effect disappeared when MEL was added to both IVM and IVF media. No differences were found among groups in ED outcomes. In experiment 2, MEL (1 nM) was added or not to both IVM and IVC media or only to IVC medium. Supplementation of IVC medium with MEL increased ($p < 0.001$) cleavage rate (63.5 ± 1.5%) and blastocyst yield (42.1 ± 2.3%) compared to control (45.8 ± 2.4% and 28.7 ± 1.6%, respectively), regardless of whether or not the IVM medium contained MEL. Moreover, compared with the control, higher ($p < 0.05$) proportions of blastocysts were produced by Day 6 of culture in the presence of MEL. In conclusion, the addition of 1 nM MEL to IVM and/or IVF media failed to improve ED, whereas MEL supplementation of IVC medium not only increased porcine IVP outcomes, but also accelerated the kinetics of ED. (Supported by Séneca (19892/GERM/15))

P 151 | Apoptosis of Sertoli cells in the Syrian hamster (*Mesocricetus auratus*) during testicular recrudescence after exposure to a short photoperiod

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During testicular regression due to short photoperiod in the Syrian hamster, the loss Sertoli cells by apoptosis has been observed and after this period testicular recrudescence spontaneously occurs. This study aims to determine the apoptosis index (AI) of Sertoli cells during this process. A total of 27 hamsters were used (21 treated and 6 controls (CT)). The treated animals were subjected to an 8:16 light-dark photoperiod, while the control animals were subjected to a 12:12 light-dark photoperiod. Seven treated animals plus two from the control group were sacrificed at 16, 19 and 21 weeks. Testes

were fixed in methacarn and embedded in paraffin. Three recrudescence groups were established: Initial (IR), Advanced (AR) and Total (TR). Histological sections of testes were submitted to different histochemical protocols for light and fluorescence microscopy: TUNEL, and vimentin immunohistochemistry. For each group the apoptosis index of the Sertoli cells was studied. The results revealed the existence of TUNEL+ and vimentine + cells in all groups, which corresponded to Sertoli cells in apoptosis. In regard to AI (IR: 0.14%; AR: 0.19%; TR 0.23% and Control 0.28%) no significant differences were found between the groups ($p < 0.05$). The index was lower than observed for testicular regression in other studies. In conclusion: a) the apoptosis of Sertoli cells was observed under normal physiological conditions by both light and fluorescence microscopy; b) the AI of Sertoli cell remained stable throughout the recrudescence process and c) very probably, Sertoli cell proliferation restores their total number during the process of recrudescence. (Funded by GERM 19892/15 from Fundación Seneca CARM)

P 152 | Male and sampling time effects on sperm chromatin of Bufo calamita

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Amphibians are globally endangered, while its commercial culture is rising. Development of amphibian assisted reproductive techniques is a priority, in order to contribute to conservation and captive breeding. We assessed the male effect and semen collection technique on the chromatin of *Bufo calamita* sperm, given its importance for fertilizing ability (first report using SCSA, Sperm Chromatin Structure Assay). Six males were captured in their natural habitat and maintained in captivity during the study. Sampling was carried out during November, inducing spermiation with an intraperitoneal injection of 10 IU hCG/g (b.w.). Spermic urine was collected after 2, 3 and 4 h after the injection, and kept at 5°C. An aliquot was mixed with TNE buffer and analyzed by SCSA and flow cytometry. We obtained DFI for each cell (DNA fragmentation index), calculating fragmentation as SD-DFI (the SD of DFI) and %DFI (% of cells with elevated DFI), and %HDS (spermatozoa with high DNA stainability, a measurement of decreased chromatin compaction). Each experiment was replicated 2–5 times per male, and data was analyzed by linear mixed-effects models (time, male and their interaction). Male effect was significant ($p < 0.05$) for the 3 variables (mean ± SD: 36.5 ± 19.7, 8.0% ± 19.0 and 16.4% ± 18.8, respectively). Time of collection and the interaction were significant ($p < 0.001$) only for SD-DFI (mean ± SEM 2 h: 32.1 ± 2.6, 3 h: 30.3 ± 3.5, 4 h: 48.3 ± 4.6), with males showing different profiles (only 3 males showing a clear increase with time). Bufo sperm chromatin was successfully analyzed, showing that successive extractions had little effect, but with a high variability between males. At advanced times, extractions might force out lower quality sperm.

P 153 | Comparative evaluation of biomarker canine-prostate specific arginine esterase (CPSE) for the diagnosis of benign prostatic hyperplasia

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Benign prostatic hyperplasia (BPH) is the most common canine prostatic disorder. Although most dogs may develop BPH, many show no clinical signs. Taking into account the non-specific character of clinical and ultrasonographic findings, a new diagnostic approach has recently been proposed based on the increase of blood canine prostate-specific arginine esterase (CPSE) in dogs with BPH. The aim of the present study was to verify CPSE levels in dogs with (n = 29) and without (negative controls; n = 31) BPH, considering cytological findings as the reference method and taking into account the fact that controls were middle-aged dogs (median of 5.0 years). Significant differences of CPSE levels were found between controls and BPH dogs (29.1 vs. 160.7 ng/ml, respectively); and significant positive correlations were found between CPSE levels and age or prostatic volume ($r = 0.549$ and 0.448, respectively; $p < 0.001$). Sensitivity, specificity, positive and negative likelihood ratios put into evidence the good performance of the test. The agreement between methods was found to be very high, notably between CPSE levels and cytological results (Cohen's kappa coefficients above 0.8). Considering the results all together, measurement of CPSE is confirmed as a useful and accurate method and should be considered as an alternative or complementary tool to conventional methods for the diagnosis of BPH in middle-aged dogs.

P 154 | Comparison of closed vs. open vitrification systems on prolonged murine oocyte storage: impact on blastocyst rate

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Oocyte vitrification is used in sub-fertile mice colonies as an alternative to embryo cryopreservation. Metaphase II (MII) oocytes are damaged during vitrification and often require of long storage periods prior in vitro fertilization (IVF); the choice of open or closed vitrification devices is also controversial. Objective: to determine blastocyst rates of murine vitrified oocytes in 0.25 ml straws (closed system) and Cryoleaf™ (open system) during moderate (90–100 days) or prolonged (150–160 days) storage. Methods: B6D2 female mice were

stimulated to trigger ovulation. MII oocytes were equilibrated in M2 medium added with 7.5% of DMSO and 7.5% ethylene glycol (v/v) for 3 min., moved to a vitrification solution for 1 min. (15% ethylene glycol, 15% DMSO (v/v) and 0.5 M sucrose in M2), loaded and plunged into liquid nitrogen. Oocytes were stored for 90–100 days in a Cryoleaf™ (G1; n = 50), or in straws (G2; n = 50) and for 150–160 days in a Cryoleaf™ (G3; n = 50) or in straws (G4; n = 50). After warming (0.5 M sucrose in M2), viable oocytes were subject to IVF using fresh oocytes as controls (G5; n = 50). Results: A Kruskal-Wallis test was used to compare groups. There were no significant differences in blastocyst rate between G1 (91.25 ± 16.85%), G2 (79.86 ± 22.36%), G3 (84.40 ± 19.07%), G4 (74.11 ± 25.20%) and G5 (95.31 ± 10.07%) (mean ± SD; $p > 0.05$). Conclusion: Moderate and prolonged storage does not impair mice blastocyst rate independently of the vitrification system used (open vs. closed).

P 155 | Are we misdiagnosing many Neospora and Leptospira-positive bovine aborted and stillborn fetuses using fetal serology?

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As diagnosis of *Neospora caninum* and *Leptospira hardjo* in bovine abortions relies mainly on fetal serology, under-diagnosis is likely. Hence, the objective of this study was to compare the infection detection rate of serology and molecular diagnostics. Fetal fluid, kidney and brain samples were collected from 141 aborted fetuses and pre-colostral perinates in 36 dairy herds in Ireland. Neospora serology was conducted using an IgG ELISA (Bovine Serum Neospora Caninum, IDEXX, Bern, Switzerland). Leptospira serology was conducted using an ELISA (PrioCHECK *L. hardjo* Ab, Prionics, Zurich, Switzerland). Following gDNA extraction of kidney (for Leptospira) and brain tissue (for Neospora), purified samples was tested by real-time PCR with the target-specific TaqMan primers. In total 26 (18.8%) fetuses were either Neospora ELISA or qPCR test-positive; 8 (5.8%) and 18 (13%) Neospora ELISA and qPCR test-positive, respectively. Of 8 Neospora ELISA test-positives, 2 (25%) were Neospora qPCR test-positive. Of 18 Neospora qPCR test-positives, 2 (11.1%) were Neospora ELISA test-positive. In total, 2 (1.5%) fetuses were both Neospora ELISA and qPCR test-positive. In total 26 (18.4%) fetuses were either Leptospira ELISA or qPCR test-positive; 0 and 26 (18.4%) Leptospira ELISA and qPCR test-positive, respectively. Of 26 Leptospira qPCR test-positives, 0 were Leptospira ELISA test-positive. In total, 0 fetuses were both Leptospira ELISA and qPCR test-positive. In conclusion, there was a higher fetal infection detection rate with qPCR compared to fetal serology. There was poor agreement between fetal serology and qPCR results for both fetopathogens. The higher detection rate of fetopathogens by qPCR warrants wider usage.

P 156 | Factors influencing the postweaning reproductive performance of sows in a subtropical environment

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The aim of this study was to analyze the post-weaning reproductive performance of commercial hybrid sows in relation to temperature-humidity index (THI) at artificial insemination (AI), season, occurrence of estrus >8 days post-weaning, repeated estrus, insemination technique (cervical or post-cervical) and parity, under sub-tropical conditions (20°N). Data included 8851 reproductive records from a large pig farm. A decrease ($p < 0.05$) in pregnancy (87.7 vs. 93.6%) and farrowing (85.8 vs. 90.8%) rate following AI during high THI (>84), when compared to the farrowing rate following AI during mild average ambient temperatures (<72 THI), were observed. The summer season was associated with decreased ($p < 0.05$) pregnancy rate (89.3% vs. 92.2% for all other seasons), farrowing rate (87.8 vs. 90.3 for all other seasons) and repeat breedings (8.8% vs. 5.7% for all other seasons). When AI occurred at higher temperatures (THI > 84), the resultant litter size was decreased (9.5 ± 4.7; $p < 0.01$) compared with AI with cool temperatures (10.2 ± 4.2). The insemination technique did not have a significant effect on pregnancy and farrowing rate, but live pigs per litter were higher ($p < 0.05$) in sows inseminated in the cervix (10.2 ± 4.4) as compared to sows inseminated post-cervically (10.0 ± 4.3). This study reaffirms the negative effects of the hot season on reproductive performance of sows, although exposure of sows to thermal stress at AI did not cause fetal losses. It also indicates that, with high numbers of sperm cells per insemination, there is no advantage in using the post-cervical insemination technique as compared to the cervical AI in sows.

P 157 | Relation of the seminal plasma ions with sperm freezability in Iberian pig

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Ions can affect to hyperosmotic stress resistance or even protection during cryopreservation process. In addition the sperm preincubation in seminal plasma (SP) or the inclusion of SP in freezing extender can improve the post-thawing quality. The objective of this work was to relate the ions levels in the SP with sperm freezability. Two aliquots from 35 Iberian boar ejaculates were centrifuged to recover the SP (5000×g/30 min) or the sperm pellet (2400×g/3 min) which was frozen with fructose-egg yolk-glycerol (1×10^9 sperm/ml). Post-thaw sperm samples were assessed at 30 min for the percentage of total motile sperm (%TM; CASA system), and the percentage of live sperm (%LS; fluorescence microscope SYBR14/PI). After this, 2 groups were determined by their freezability resistance with

significance difference ($p < 0.05$) (High: H, n = 19; Low: L, n = 16; %TM: 50.1% vs. 29.9% ± 1.44; %LS: 55.6% vs. 42.3% ± 1.37; respectively) by cluster sorting. Ion composition (chlorine (Cl: mmol/l), calcium (Ca: mg/dl), iron (Fe: µg/dl), potassium (K: mmol/l), magnesium (Mg: mg/dl), sodium (Na: mmol/l), phosphorus (P: mg/dl)) of SP were determined with a commercial kit by spectrophotometry. The resulted showed significant differences between good and bad freezers in Cl, Ca, Na and P (L: 63.03, 7.37, 90.5, 4.01; H: 73.03, 6.83, 98.5, 3.63) and there was no difference in Fe, K and Mg. In conclusion the SP ion composition is different according to sperm freezability, this may serve as an indicator of future freezability of the boar ejaculates.

P 158 | A postovulatory decrease in the expression of somatotrophic axis genes in the sheep endometrium that apparently does not correlate with MUC1 mucin mRNA levels

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MUC1 mucin gene codes for a glycoprotein present on the apical surface of several cells. In the endometrium, MUC1 protein plays a key role in protection from microbial attack, but must be down-regulated in the receptive phase to allow embryo implantation. In sheep, it decreases in the luteal phase of the estrous cycle and in early pregnancy. Identification of factors that regulate MUC1 expression would be valuable to increase fertility. A role for growth hormone (GH) in blastocyst development, implantation, and fetal growth was proposed. Expression of somatotrophic axis genes was reported in the uterine endometrium of sheep. The objective of the present work was to preliminarily investigate if there is an association between the expression of the GH, GHR, STAT5 and IGF1 somatotrophic axis genes and the MUC1 gene in sheep endometrium. We tested, by real-time PCR, the cDNAs obtained from 8 endometrium samples, collected from the 2 uterine horns of 4 sheep - 2 in the follicular phase and 2 in the postovulatory stage of the estrous cycle. Our results showed expression of somatotrophic axis genes in sheep endometrium, in both stages of the estrous cycle, although GH gene was expressed at very low amounts. GHR and STAT5 mRNA levels were higher ($p < 0.05$) in the endometrium from the follicular phase compared to those from postovulatory stage. IGF1 expression levels showed the same trend, but without significance. A correlation between GHR and STAT5 (83.1%; $p < 0.05$) was identified. MUC1 mRNA level in sheep endometrium was not significantly different between follicular and postovulatory stages and apparently was not correlated to the expression of the somatotrophic axis genes. (Funded by FCT project PTDC/CVT/112054/2009 and FCT grant SFRH/BPD/100565/2014)

P 159 | Scriptaid-dependent epigenomic modulation of nuclear donor fibroblast cells impacts on the in vitro developmental outcome of cloned pig embryos

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The current study was conducted to assess the developmental capacity of nuclear-transferred (NT) pig embryos generated using fetal fibroblast cells that had been epigenomically modulated via exposure to new-generation non-selective inhibitor of histone deacetylases (HDACs), designated as scriptaid. Prior to use for somatic cell nuclear transfer, the permanent fibroblast cell lines (between passages 1 and 2) that had been established from the primary cultures originating from dermo-integumentary tissue explants of the conceptus at Day 35 of gestation were treated with 350 nM scriptaid during 24-h contact inhibition of mitotic divisions. Enucleated oocytes that had been reconstituted with epigenetically transformed fibroblast cells were simultaneously fused and electrically activated. The frequencies of cleaved embryos (242/278; 87.1%), morulae (191/278; 68.7%) and blastocysts (107/278; 38.5%) developing from NT oocytes descended from fetal fibroblast cells that had been subjected to scriptaid exposure were significantly higher than in a control group (167/236; 70.8%, 133/236; 56.4% and 62/236; 26.3%, respectively). In conclusion, the enhancements in cleavage activity and morula/blastocyst yields of porcine cloned embryos appear to result from increased functional capabilities for proper induction of epigenetic reprogramming of scriptaid-treated fetal fibroblast cell nuclei in a cytoplasm of reconstituted oocytes. (The work was supported by The National Centre for Research and Development (grant number INNOMED/I/17/NCBR/2014).)

P 160 | The embryo transuterine migration at early canine pregnancy

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An equal distribution of embryos within the uterine horns is of upmost importance in polytocous species; it may be achieved by transuterine migration of embryos prior to implantation. Still, this mechanism has not been clearly demonstrated, especially in dogs, a species that presents major physiological differences compared to other domestic mammals. This work aims to assess the existence of transuterine embryo migration in dogs, estimated from the difference in numbers between recovered embryos/unfertilized oocytes in each uterine horn and the number of ipsilateral ovarian corpora lutea (CL). Embryos

were collected from 19 bitches at elective OVH after artificial insemination procedure with fresh semen and allocated into two groups: the pre-adhesion phase, from pregnancy days 11 to 15 (G1, n = 7), and the apposition and attachment phases, from days 16 to 20 (G2, n = 12). Gestational age was aligned to the day of LH surge (P4 = 2 ng/ml). A total of 105 embryos and 22 unfertilized eggs were collected from 19 bitches, which presented a total of 134 CL (recovery rate: 84.0 ± 5.2%) with an average of 5.5 ± 0.7 embryos per bitch. The mean ovulation rate was 7.1 ± 0.5 (right ovary: 4.0 ± 1.9 vs. left ovary: 3.2 ± 1.5). The transuterine migration was presumed in 52.6% (10/19) pregnancies (G1: 40% vs. G2: 70%). Migration was more frequent between days 15 and 17 (n = 6/10). Before day 15 (n = 2/10), migration involved sets of 2–4 embryos, but after day 15 differences represented only one embryo. These results support the existence of transuterine migration during early canine pregnancy, which seems to operate both for embryos and unfertilized oocytes, beginning at the entrance of the embryos in the uterus and stabilizing only near implantation.

P 161 | Successful use of deslorelin implant in a Border Collie female after a prolonged anestrus

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A 3-year old female Border Collie came to our Reproduction Service due to the owner's will to breed. No estrous behavior has been detected during her life despite living with males. On clinical examination we observed a small and retroverted vulva, anestrous vaginal cytology (56% parabasal, 43% intermediate and 1% superficial cells), serum progesterone (<1 ng/ml) and sonographically normal uterus and inactive ovaries. After thyroid and adrenal pathology was eliminated, based on a possible failure of the hypothalamic-pituitary-ovarian axis, the female was treated with a deslorelin implant (Suprelorin® 4.7 mg, Virbac). After 4 days (D4), a bloody vaginal discharge was observed with proestrus cytology (RBCs and 15% of surface, 84% intermediate cells and absence of parabasal cells). From D7 to D14, cytology was consistent with the estrus (absence of RBCs and 100% of surface cells). On ultrasound ovarian follicular grew up to diameters of 0.88 and 0.96 cm, ovulation and subsequent presence of corpora lutea was observed. On D7, progesterone began to rise above 1 ng/ml. The bitch was inseminated with fresh semen on D12 and D14, with progesterone 11.97 and 47.34 ng/ml, respectively. On D15, cytology indicated the beginning of diestrus (9% superficial, intermediate 88% and 3% parabasal cells). Gestation was diagnosed by ultrasound 18 days after the first IA (52.3 ng/ml progesterone) and the implant was removed. Six healthy puppies were born 57 days after the vaginal cytology change from estrus to diestrus. Previous studies reported the use of deslorelin implant to induce ovulation in bitches with normal reproductive cycles. In this female, at least 18 months without heat, deslorelin has been able to induce a normal ovulation and a successful reproduction.

P 162 | Blood metabolite and thyroid levels in postpartum dairy cows with reproductive dysfunction

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Reproductive function of postpartum dairy cows is highly dependent on their metabolic status. The goal of the present research was to compare levels of blood metabolites and thyroid hormones between first-calf heifers with and without different reproductive disturbances. Blood samples were collected simultaneously from 32 Russian Black Pied cows (between the 7th and 12th week postpartum), which were either normally cycling (CY, n = 24) or having inactive ovaries (IO, n = 8). The diagnosis was confirmed by ultrasonography and the serum progesterone concentration. Thereafter, CY cows were divided into two groups: animals become pregnant within 1 year after calving (PREG, n = 18) and animals remained non-pregnant (NPREG, n = 6). Serum metabolite concentrations were determined by biochemical analyses and hormonal levels were measured by ELISA. In CY cows, the serum concentrations of total cholesterol, phospholipids, and magnesium were 1.2–1.4 times higher than those in IO cows ($p < 0.05$). By contrast, the serum content of triglycerides was 1.4 times higher in IO cows than in CY cows ($p < 0.05$). Among CY cows, lower levels of urea and aspartate aminotransferase were found in the NPREG group than in the PREG group ($p < 0.05$). Serum concentrations of thyroid hormones did not differ between the compared groups, although there was a positive correlation between concentrations of triglycerides and free triiodothyronine when combining CY and IO cows ($r = 0.395$, $p < 0.05$). Thus, in postpartum first-calf heifers, the ovarian inactivity was associated with a modified lipid and magnesium metabolism, whereas the inability of CY cows to conceive was attended by a low activity of the urea cycle. (The study was supported by the Federal Agency for Scientific Organizations and RFBR (16-34-00875).)

P 163 | Effect of the equilibration length on the quality of crypreserved goat buck sperm: preliminary data

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The long protocol for the cryopreservation of goat buck sperm is on occasion incompatible with a workday length. The sperm is equilibrated with the cryoprotectant during 1.5 h, being this step one possible pause for the continuation of the protocol the following day. Besides, during the equilibration, lipid remodeling also occurs in the plasma membrane, rendering spermatozoa fitter for the cryopreservation process. The aim of this study was to determine the effect of the equilibration length (1.5 h vs. overnight equilibration-OE) on the motility and viability of

goat buck sperm. A total of 8 ejaculates coming from 5 males from the Murciano-Granadina breed were cryopreserved to a concentration of 500 × 10^6 sperm/ml in skimmed milk with 11.1 mM glucose and 7% glycerol. Each ejaculate was split in two aliquots after the equilibration (1.5 h): one was frozen after equilibration while the other was frozen the following day (OE). After thawing at 37°C, total motile (TM), progressively motile (PM) and plasma membrane intact (PMI) sperm were evaluated. The data were analyzed with a mixed model including equilibration length (2 levels) as fixed and male as random effects. OE sperm exhibited lower values than 1.5 h for all the parameters evaluated, although differences were not significant ($p > 0.05$) because of the sample size: 45.7% ± 7.8 TM, 29.6% ± 6.1 PM and 41.8% ± 6.2 PMI for 1.5 h and 32.4% ± 7.8 TM, 17.1% ± 6.1 PM and 35.4% ± 6.2 PMI for OE. Apparently goat sperm does not benefit from longer equilibrations, although the effect of this increase in the equilibration length on the in vivo fertilizing ability of the sperm should also be determined.

P 164 | Use of a tissue sealing device for gonadectomy in cats and dogs

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Advances in technology, and development of new surgical techniques, in particular those of laparoscopic surgery, have led companies to develop more sophisticated systems for tissue dissection and coagulation. The system ENSEAL® combines the effect of compression and temperature, with the possibility of dissecting the tissue after coagulation. The peculiar electrodes allow a controlled temperature (100°C) in the tissue, and reduce to few millimeters the area were tissue temperature increase. In addition, design of the blade maximizes the pressure on the tissue during cutting. The aim of the study was to test this device for routinely open gonadectomy in cats and dogs. The instrument has been used for the gonadectomy of 100 cats (50 female and 50 male), and 50 dogs (25 female and 25 male) at the Veterinary Teaching Hospital of the University of Padua and in a private clinic. The instrument was applied on the spermatic cord and on the ovarian pedicle and at the utero-ovarian junction. After 10–20 s the instrument signaled the accomplished coagulation; after an inspection of the site of coagulation it was applied again and at the next signal of coagulation the dissection was carried out. In cats of both sexes and in male dogs bleeding was not observed after resection; in 4/25 of the bitches it was necessary to cut the pedicle in two distinct sections due to the diameter of the structure and in one case, due to the amount of adipose tissue, it was considered safe to add a ligature before cutting. In conclusion, the use of this instrument showed advantages, the procedure, fast and easy, permits to reduce the use of suture and does not show negative effects for the patient. The cost/benefit of the device should be pondered, despite having adopted an effective re-sterilization system.

P 165 | Sperm encapsulation in dogs: preliminary study

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Encapsulation is a technique of enclosing living cell with a semipermeable membrane, which may be composed of different biodegradable polymers such as sodium alginate. The aim of this study was to determine the best concentration of sodium alginate (SA) and the best media to encapsulate canine spermatozoa for artificial insemination. Four experiences were developed. Semen samples from 4 mature dogs were extended in CaniPro®. Media used for prepare SA solutions were: Phosphate Buffered Saline (PBS), Saline Solution (SS), CaniPro® (C) and Fraction 1 Fiser extender (F1) including 1 or 1.5% of SA. Semen extended was diluted in different SA, and the resulting suspension was forced through a needle attached to syringe into plastic dish containing 1.5% calcium chloride solution. The capsules were collected and transferred into 0.1% poly-L-lysine solution first and then into 55 mM sodium citrate solution. After 5 min, the capsules were resuspended and stored in the appropriate extender (PBS, SS, C or F1) at 4°C. At 24 h capsules conformation was evaluated based on their shape (1–5) and resistance based on their response to the pressure cover (1–5). Taking account all parameters evaluated, the optimal concentration of SA was 1% for all media. F1 1% SA showed the best conformation and resistance of sperm capsules (3.5). In addition, the sperm motility for F1 1% SA offers the best results, with 37% motility. C 1% SA was similar than SS 1% SA medium: 10 and 8%, respectively. The PBS 1.5% SA medium produced the worst results, less than 5%. Successful canine sperm encapsulation was achieved with Fiser extender including 1% of sodium alginate.

P 166 | Conceptus-induced changes in the porcine endometrial protein abundances

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Establishment of early pregnancy in pigs requires hormone estradiol (E2) secretion by the conceptus on days 11–12 of pregnancy. Conceptus secreted factors such as E2 induce molecular changes in endometrium that have effect on conceptus implantation. To better understand the molecular basis of embryo implantation, 2D-differential gel electrophoresis (DIGE)-based proteomic approach was employed to identify porcine endometrial proteins that have different abundances between cyclic and pregnant animals on day 13. Analysis of fluorescently labeled protein lysates on 2D gels showed about 39 significantly different protein spots ($p < 0.05$ and fold change >1.8) between cycling and pregnant animals. Principal component analysis of the differentially abundant spots was performed by the Extended Data Analysis module of the DeCyder software. The scatter plots of the principal components of the endometrial samples

on day 13 showed a clear separation between cyclic and pregnant animals. Identification of these proteins using tandem mass spectrometry followed by functional analyses using Ingenuity Pathway Analysis (IPA), revealed that free radical scavenging, cell death and survival, molecular transport and cellular assembly and organization are the top most functions associated with differentially abundant proteins that are altered due to presence of conceptuses. The overall processes influenced by E2, EGF, lipopolysaccharide and IL-1 were activated (*z* score > 2.0) on the basis of known interactions with the proteins in our dataset. In conclusion, data provide information on changes in molecular processes associated with porcine early pregnancy which may potentially lead to identification of proteins associated with successful embryo implantation.

P 167 | The efficacy of the homogenate of the antlerogenic stem cells (MIC-1) in the improvement of dairy cows' fertility

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Processes of reparation and regeneration are very rapid in the uterus of healthy cow. Disturbances of endometrium functions could be observed in infertile females. The aim of this study was to evaluate the usefulness of the homogenate of the antlerogenic stem cells (MIC-1) in improvement of dairy cows' fertility. The study was performed on 26 dairy Holstein-Friesian and 1 Polish Red-White cows divided into 2 groups: postpartum cows (PP, 15 animals, 7 treatment, 8 control) and low fertility cows (LF, more than 3 unsuccessful AI, 11 animals, 5 treatment, 6 control). All cows received 60 ml of fluid intrauterine i.e. MIC-1 in treatment groups and placebo in controls, respectively. Gynecological examination was performed at the beginning and the end of the study. Samples for cytological, histopathological, microbiological and PCR examination were collected in PP group. Cows in both groups did not show any signs of clinical disease of the reproductive tract during the experiment. We did not find any negative impact of the homogenate on uterus involution and ovarian function. In PP cows, 86% of cows showed estrous signs in the treatment group and 38% in the control group. Pregnancy was diagnosed in 42.9% of the cows in the treatment while in 12.5% of the control group. In the LF cows AI was successful in 33% and 50% of the cows in the treatment and control groups, respectively. Intrauterine infusion of MIC-1 can have a positive effect on the process of uterine involution in PP cows. However, further research on a larger group of animals is needed to obtain more reliable results. In the LF group, the positive effect seems to be connected with mechanical irritation of the endometrium by the fluid itself, but further studies are necessary to confirm this finding.

P 168 | An implemented dextran/swim-up technique with estradiol for the separation of a sperm subpopulation enriched in non-capacitated with no phosphatidylserine exposure cells

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Estradiol receptors are present on the ram sperm surface, but the information about specific effects of estradiol on ovine spermatozoa is very limited. In this study we used a ram sperm washing and selecting-procedure, a dextran/swim-up method, including two different concentrations of estradiol (E2) in the upper layer, one low (100 pM, LEsw) and one high (1 μM, HEsw). Both E2 concentrations resulted in a higher cell recovery rate than the standard swim-up (STsw, with no hormone) (59.2 ± 1.1, 53.7 ± 2.5 and 47.4 ± 1.1% for LEsw, HEsw and STsw, respectively; $p < 0.001$ for LEsw). The inclusion of E2 led to a significant increment in the percentage of non-capacitated (NC, CTC staining) and a decrease in the inverted-phosphatidylserine sperm rate (Total Ann+ [PI+/PI−]) compared with the ejaculate (68.6 ± 4.4, 74.2 ± 4.6 and 53.4 ± 5.0% of NC; 32.90 ± 1.5, 33.52 ± 3.1 and 48.27 ± 4.4% of Ann+ sperm for LEsw, HEsw and ejaculate, respectively; $p < 0.05$). However, non-significant changes were found by the addition of E2 compared to the STsw sample. None of the E2 concentrations assayed showed effect on motility (by CASA) or membrane integrity (CFDA+/PI−). With the aim to know whether the presence of estradiol influences the E2 receptor distribution, indirect immunofluorescence assays are now in progress comparing the STsw sample with the selected subpopulation in the presence of both E2 concentrations. (Grants: CICYT AGL 2014-57863R, DGA-A26)

P 169 | Post-thawed semen quality of Arab stallions: a comparative analysis of the sperm motility stored in two freezing media

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The objective of this study was to compare the sperm motility of Tunisian Arab stallions frozen according two protocols using two based-media: INRA96® and INRA Freeze®. Ejaculates (n = 56) were collected from 18 stallions (9–28 years). After filtration, each ejaculate was assigned to 2 fractions and frozen according 2 protocols: in the first protocol (P1, n = 32), semen was extended in the INRA96® based-media. For the second protocol (P2, n = 24), semen was extended in the INRA Freeze® based-media. After thawing, the sperm motility was analyzed for the percentage of progressive sperm (%PROG), average path velocity (VAP, μm/s), straight line velocity (VSL, μm/s) and curvilinear velocity (VCL, μm/s) using Hamilton Thorn Motility Analyzer (HTM,

version 12.1 M). ANOVA was performed to compare variables between the two protocols using a software SAS (SAS, Institute, Inc). Our results showed that the %PROG, VAP and VCL were higher in semen frozen in the INRA96® as compared to the semen frozen in the INRA Freeze® (%PROG = 26.5 ± 13.2 vs. 17 ± 11.8%, $p < 0.0001$; VAP = 91 ± 22 vs. 75 ± 25 μm/s, $p < 0.0001$; VCL = 165 ± 20 vs. 130 ± 34 μm/s, $p < 0.0001$). However, VSL doesn't varied in the 2 semen frozen in the two media (VSL = 74 ± 12 vs. 68 ± 21 μm/s, $p > 0.05$). Our results suggested that the use of the INRA96® extender in the freezing process gave better results on sperm motility after thawing.

P 170 | Mathematical modelling of liver glucose and blood insulin in post-partum dairy cows

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During the last four decades, the level of milk production has increased in dairy cows due to a combination of improved genetics, feeding and management. Now, there is evidence showing that high milk production is associated with a decrease in the reproductive performance of dairy cows, mainly in the Holstein breed. Negative energy balance, an unavoidable post-partum event, has one of the most important roles in the resumption of the reproductive functions in dairy cows. A mechanistic mathematical model of the role of liver glucose on the pancreatic and blood insulin alterations is designed in the present research. On the basis of the mechanisms of synthesis and secretion of metabolites and Insulin, ordinary differential equations are produced. Some of the most important parameters studied include milk production, diet propionate, adipose lactate, muscle glucogenic amino acids (mostly alanine), liver and blood glucose, pancreatic insulin and blood insulin. This model contains 3 ordinary differential equations and 14 parameters. Moreover, the differential equations are fitted with experimental data (generated by published data) by adjusting some of the parameters. The results of the model against the published data are in good agreement. The average absolute relative deviations (AARD%) obtained by the model are 0.388% and 0.638% for blood glucose and blood insulin respectively which shows convincing and closely fitted with the published data. The main efficiency of this model is to predict the effects of some metabolic factors involved in the occurrence of negative energy balance through glucose deceleration. This model is also able to mathematically predict the changes of blood glucose, pancreatic insulin and blood insulin in high producing dairy cows.

P 171 | Stallion semen quality after oral supplementation with beta-carotene

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Feeding beta-carotene is often suggested to increase fertility. The aim of this study was to investigate the influence of a commercially available supplement for breeding stallions containing 4000 mg/kg beta-carotene. Semen was collected from nine stallions three times a week and sperm parameters were compared in the week before (T1), in the last week during (T2) and 9 weeks after the end (T3) of treatment. The supplement was administered daily per os at a dose of 250 mg/kg bodyweight for 9 weeks. Differences among sperm parameters were evaluated by Wilcoxon test. Volume [25 (7–70), 36 (2–83), and 32 (8–60) ml], concentration [232.7 (22.7–766.4), 185.1 (21.9–629.1), and 179.2 (38.0–679.5) × 10^6/ml), and total sperm count [6.13 (0.34–16.36), 6.15 (0.31–13.25), and 6.51 (0.30–9.66) × 10^9] did not differ among time periods [median (range) for T1, T2, and T3, respectively; $p > 0.05$]. Further, progressive motility values were similar among time periods at the day of semen collection (H0) and 48 h (H48) after cooled storage [H0: 64 (9–80), 66 (9–77), and 71 (4–85); H48: 37 (0–66), 35 (0–66), and 30 (0–71)%; $p > 0.05$]. In contrast, curvilinear velocity differed significantly among time periods on either day (H0: 164.9 (136.3–193.0), 147.6 (105.5–188.6), and 163.8 (131.8–188.4); H48: 161.6 (0–195.7), 155.3 (0–207.7), and 167.8 (134.4–275.1) μm/s for T1, T2, and T3, respectively; $p < 0.05$]. Neither the amount of sperm production nor motility were influenced by supplemental feeding. Values of curvilinear velocity decreased during the time period of supplementation. In conclusion, administration of supplements containing beta-carotene using this concentration did not result in major effects of sperm parameters.

P 172 | Correlation between the level of respiratory stimulation by 2,4-DNP and fertilizing ability of frozen sperm

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Mitochondria are very sensitive to the negative effect of low temperatures. In this study we analyzed the effect of freezing-thawing on the mitochondrial respiratory chain of spermatozoa from boars, bulls, stallions and chicken. Respiratory activity was assessed by testing substance - 2,4-dinitrophenol (2,4-DNP). The more conjugation of respiration and phosphorylation is, the more 2,4-DNP increases the respiration of sperm. Study of respiration from boar, bull, stallion and chicken spermatozoa showed identity in responses to 2,4-dinitrophenol. Only the power of the response to test substance was different. We studied correlation between the level of respiratory stimulation by 2,4-DNP and fertilizing ability of sperm. Semen samples (4–5) from 10 chickens were frozen and used for evaluation and insemination. Respiratory stimulation by 2,4-DNP in these samples varied from 1.39 to 2.25 and fertility from 24 to 95%. The correlation between egg fertilization and respiratory stimulation by 2,4-DNP was high ($r = 0.76$, $p < 0.01$). A high positive correlation was observed between the stimulation of respiration by 2,4-DNP and fertilizing ability of ovine sperm ($r = 0.86$, $p < 0.01$). The positive correlation between conception rate in cows and stimulation of the respiration by 2,4-DNP of bovine sperm after cryopreservation

was found (r = 0.62, p < 0.05). These studies have shown that the stimulation of respiration by 2,4-DNP in spermatozoa after thawing can serve as criterion for predicting their fertilizing ability.

P 173 | Influence of an extended lactation on the milk production of high-yielding dairy cows

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Aim of this study was to compare the milk production of high-yielding dairy cows assigned to extended lactation periods. A prospective field study took place on a large dairy (1092 cows, 11488 kg milk/305 days) in Saxony/Germany. On day 40 post-partum cows were randomly allocated to groups with a voluntary waiting period of either 40 days (G40), 120 days (G120) or 180 days (G180). There were 120, 115 and 107 cows that completed the lactation in the G40, G120 and G180, respectively. Comparisons were performed with ANOVA. G40 cows had an energy-corrected milk yield (ECM) of 12025 kg in 346 days, G120 cows 13840 kg in 396 days and G180 cows 15454 kg in 442 days. Accordingly, ECM per day of lactation did not differ (p > 0.05) between groups (34.8 vs. 34.9 vs. 34.9 kg/days). Milk production within 305 days was greater in the G180 compared to G40 (12133 vs. 11417, p < 0.05), with G120 being intermediate (11849). However, cows in the G120 and G180 had greater 305 days ECM compared to G40 (11334 and 11707 vs. 10892, respectively, p < 0.05). There were no differences (p > 0.05) between the 3 groups regarding the somatic cell count or the percent of animals culled due to mastitis. Conclusively, the extension of the lactation period of high-yielding dairy cows up to 440 days can lead to improved productivity due to improved persistency as reflected by a significantly higher ECM yield within 305 days.

P 174 | Effects of the addition of ascorbic acid to the maturation, fertilization or culture media on porcine in vitro embryo production

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Ascorbic acid (AsA) has been used to promote in vitro embryo development in different species, but with controversial results. This study aimed at evaluating the effects of AsA on maturation, fertilization and developmental ability and quality of in vitro produced porcine embryos. In vitro maturation (IVM), fertilization (IVF) and embryo culture (IVC) were performed in the presence or absence of 50 μg/ml AsA in all possible combinations. A total of 2744 immature oocytes (6 replicates) were matured for 44 h and incubated with thawed sperm (1000 sperm per oocyte) for 5 h. The presumed zygotes were cultured for 18 h (N = 1142) to evaluate the fertilization parameters or for 7 days (N = 1602) to assess in vitro embryo development. The data are presented as means ± SEM, and differences among groups were analyzed by ANOVA. All groups displayed similar maturation (range: 89.5 ± 1.4% to 93.6 ± 1.9%) penetration (range: 63.3 ± 4.4% to 78.3 ± 6.0%) monospermy (range: 59.1 ± 6.9% to 73.7 ± 5.7%) and efficiency (number of monospermic oocytes/total inseminated; range: 37.6 ± 3.4% to 47.3 ± 5.6%) rates. Also, AsA did not affect the cleavage rate at Day 2 (range: 45.4 ± 3.0% to 54.9 ± 5.2%), the blastocyst rate at Day 7 (range: 29.5% ± 4.2 to 39.2 ± 7.1%) or the total number of cells in blastocysts (range: 39.8 ± 2.4 to 53.0 ± 3.4). In conclusion, the supplementation of IVM/IVF/IVC media with AsA at a concentration of 50 μg/ml failed to affect in vitro porcine embryo production outcomes. (Supported by Séneca (19892/GERM/15), Murcia, Spain)

P 175 | Fertility after OvSynch protocol with double $PGF_{2\alpha}$ injection during summer heat stress in dairy cows

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During summer when heat detection is difficult OvSynch protocol (GnRH-PG-GnRH) is suggested to improve fertility. The efficacy of this method might be decreased due to incomplete luteolysis after a single $PGF_{2\alpha}$ injection. The aim of this study was to compare OvSynch efficiency with a single (PG1 group) and double $PGF_{2\alpha}$ injection 24 h apart (PG2 group). Hormonal drugs were injected according to the order: PG1 group: 1st day – GnRH, 7th – PGF, 9th day – GnRH, 17–24 h later TAI; PG2 group: 1st day – GnRH, 7th and 8th day – PGF, 9th day – GnRH, 17–24 h later TAI. The trial was carried out on three dairy farms (A,B,C) with Holstein-Friesian cows during summer months (June-August). OvSynch protocol and its modification were applied if a follicle over 10 mm of size was found during gynecological examination of cows. A total of 64 cows were included and 60 of them were inseminated (PG1 n = 32, PG2 n = 28). Four cows were excluded from the study due to severe lameness or insemination just after the first GnRH injection. The pregnancy check was performed by ultrasonography 35–37 days after TAI. The following pregnancy rates have been obtained: PG1 group - (herd A- 0%, B- 33%, C- 40%) PG2 group - (herd A- 29%, B- 0%, C- 44%) In this preliminary study, no benefit of double $PGF_{2\alpha}$ injection in OvSynch protocol for fertility was observed. The trial will be continued on a larger number of cows and additionally progesterone levels will be measured on days 7 and 9 of the OvSynch protocol.

P 176 | Histological examination of uterus of European lowland bison *Bison bonasus*

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Bison is an endangered species on a global scale. There are many breeding programs and research, in situ and ex situ, regarding this species. Reproduction, which determines the survival and development of the population, takes a prominent place in this area. The aim of this study was to describe the microstructure of the uterus of the bison. Specimens of organs have been collected from 55 individuals. Two groups have been created on the basis of known information about the reproduction of this species: I - 36 sexually immature cows (2 months–1.5 years), II - 19 sexually mature cows (2–20 years). Specimens have been H&E stained. Microscopic measurements and analysis have been done using MultiScanBase v. 8.08™ software. Obtained results have been analyzed statistically. The endometrium, depending on the phase of the estrous cycle, is covered by simple columnar and sometimes stratified epithelium with average height 12.55 μm. Under the epithelium is the most developed stroma, which separates the functional layer from the basal layer. Vascular layer, between muscular layers, is well developed (1595.41 μm). In the analyzed bison females from group II thickness of all layers of the wall of the uterus is significantly higher than in group I. The study of uterine microstructures of lowland bison is important to increase the population of this species and allow its preservation for future generations.

P 177 | Influence of piglet's birth weight, sow's parity number, season and herd size on piglet pre-weaning mortality (PWM) in 46 swine commercial herds in Thailand

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The present study aims to determine the occurrence of PWM in swine commercial herds in Thailand. Data were collected from 46 commercial swine herds during 2007–2013. Data of each sow were obtained from a computerized data-base system of the herds. The data included 205,924 litters from 74,941 sows. Size of the herd was classified into three groups as small (300–999 sows, n = 27 herds), medium (1,000–1,999 sows, n = 11 herds), and large (>2,000 sows, n = 8 herds). Mortality data were analyzed by using general linear model procedure. On average, PWM was 12.8% (median 9.1%, 6.4–20.6%). Factors influencing PWM included piglet's birth weight (BW), parity, season, herd size, interactions between BW and herd size, season and herd size and parity and herd size ($p < 0.001$). Piglet pre-weaning mortality was 12.3%, 12.6% and 14.0% in sow parity number 1, 2–4 and 5–9, respectively. Piglet pre-weaning mortality in the litter with an average BW of <1.30 kg (15.5%) was higher than the litters with a BW of 1.3–1.8 kg and >1.8 kg (12.6% and 9.5%, respectively; $p < 0.001$). Cool season (12.9%) was higher PWM than rainy season (12.4%, $p < 0.001$). Small herds (13.6%) was higher PWM than large and medium herds (12.0% and 12.0%, respectively; $p < 0.001$). Low BW piglets in small herds (15.5%) had a higher PWM than those in the large herds (14.8%, $p < 0.05$). In cool season, small herds (13.6%) had a higher PWM than medium (12.9%, $p < 0.01$) and large herds (11.9%, $p < 0.001$). The highest PWM was found in sows with parity >6 in small herds (15.3%). In conclusion, the significant risk factors causing PWM included low BW, sow parity number above 6, small herds and cool season. Therefore, management in the farrowing house should be emphasized on small herds, cool season, low BW and in sow parity number >6.

P 178 | Identification of differentially expressed proteins between sperm of males (XY) and sex-reversed rainbow trout females (XX)

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The sex determination model of rainbow trout is of the XY type; to produce all-female stocks eggs must be fertilized by X chromosome spermatozoa. Such sperm can be produced from masculinized females (sex-reversed females, srf) containing only X spermatozoa. The aim of the study was to compare spermatozoa of males (X or Y) and srf (X) and the identification of more abundant proteins of males spermatozoa by two-dimensional difference gel electrophoresis coupled with mass spectrometry. The Ingenuity Pathway Analysis (IPA) was performed to predict the top canonical pathways of these proteins. Twenty eight differentially abundant proteins of males sperm were identified, mainly involved in citric acid cycle, aspartate and methionine degradation, activation of peroxisome proliferator-activated receptor with retinoid X receptor complex and AKT- phosphatidylinositol 3-kinase signaling (IPA). An analysis of „molecular function" indicated that the majority of proteins were classified as proteins with binding (ions 33%, proteins 23%, nucleic acids 15%, nucleotides, cofactors and lipids) and catalytic activities (oxidoreductases 35%, transferases 29%, hydrolases 24% and lyases 12%). In conclusion, our results highlighted a prevalent proteins and pathways in fish male sperm which are putatively related to energy production which is prerequisite for sperm motility. Identification of differentially expressed proteins in males sperm may provide theoretical guidance for further studies on the developmental processes of X and Y sperm and on molecular mechanisms of sex formation in fish. (Funded by National Science Centre 2011/01/D/NZ9/00619 and funds of Institute of Animal Reproduction and Food Research PAS, Olsztyn.)

P 179 | Use of rosmarinic acid as antioxidant on ram sperm freeze-drying

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Sperm DNA damage can be induced by mechanical or oxidative stress during freeze-drying but also during the holding period before

intracytoplasmic sperm injection (ICSI) after rehydration. Several studies have shown the beneficial effect of antioxidant therapy on oxidative stress in mammalian spermatozoa. The rosemary, and in particular the rosmarinic acid (RA) has a beneficial effect on post-thaw sperm parameters providing protection against oxidative stress during cryopreservation. The aim of this study was to evaluate the effect of RA added to rehydration solution on sperm DNA integrity and the in vitro development of IVM ovine oocytes after ICSI using freeze-dried (FD) ram sperm. Ejaculated sperm from three ram of Rasa Aragonesa were FD in a medium containing: NaCl 50 mM + TRIS-HCl 10 mM + EGTA 50 mM and stored for 1 year at 4°C. Samples were rehydrated by adding 150 µl of Milli-Q water (control) or 150 µl of RA solution (105 µM) and subjected to DNA damage detection using the Sperm Halomax kit. All data obtained from this study were compared by a chi-square test by using SSPS version 17.0 for Windows. It was found that the level of DNA integrity was significantly (p = 0.02) higher when samples were rehydrated with AR solution (99.7%) than those with water (97.4%). There were no significant differences between FD sperm and frozen-thawed sperm (control) on the blastocyst formation rates when ICSI was performed. These results suggest that the rehydration of FD ram sperm with RA solution improves sperm DNA integrity and embryos can be obtained by ICSI from these sperm samples. (Supported by DGA (Fondo Social Europeo))

P 180 | Corpus luteum of the European elk *Alces alces*

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In order to assess the state of the Polish population of elk, a limited hunting has been carried out in the program „Strategy of preservation and management of the elk population in Poland". Obtained animals have been examined and among others information regarding their reproduction abilities has been collected. Corpus luteum from 16 pregnant elk females (age 4–18 years) have been analyzed. Analysis included shape, size, vascularization and localization of large luteal cells (LLC) small luteal cells (SLC) and connective tissue. LLC cells were the majority of the cells of the corpus luteum. These cells are large, oval or polygonal with foamy cytoplasm. The nucleus is large and oval, is located in the cell marginally, nucleoli are usually several (2–3). SLC cells are localized mainly on the edges of corpus luteum, under the connective tissue capsule. SLC are also located near the connective tissue straps that penetrates deep into the corpus luteum. SLC cells are smaller than LLC cells and its shape is irregular. The nucleus is oval or cup-shaped, have a few nucleoli arranged on the edge of the nucleus. Clusters of connective tissue in the middle of the corpus luteum are not very clear. LLC cells are arranged randomly in relation to the clusters. Larger vessels, mainly veins are located subcapsular, on the edges of the corpus luteum. Such studies are pioneering and in the future may help in more accurate assessment of the reproductive capacity of wild animals threatened with extinction.

P 181 | Effect of ovarian status at the beginning of 17β-estradiol plus progesterone protocol on follicular dynamic in ewes

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This study was designed to evaluate the effect of ovarian status at the beginning of the 17β-estradiol plus progesterone (P4) protocol on follicular dynamic in ewes. In a random day of the estrous cycle (D0), twenty-four Santa Ines ewes received a P4 device (CIDR®) and an injection of 17β-estradiol (Sincrodiol®) in different doses (350, 500 or 1000 µg). The transrectal ultrasound examinations were performed daily during the CIDR permanence (10 days) using MyLab 30Vet equipment (Esaote, Italy). Data were analyzed by ANOVA with Tukey test (mean ± SEM; p < 0.05) using SAS software. Half of the animals (G1) had corpus luteum and large follicles (LF: 4.0–5.75 mm of diameter) in the ovaries while other half had no CL and had LF (G2). Initially, the largest follicles present in the ovaries showed a growing period (G1: 24.0 ± 0.0 and G2: 44.0 ± 7.4 h, p = 0.06) and then an atresia period (G1: 84.0 ± 10.4 and G2: 96.2 ± 6.5 h, p = 0.34). There was no difference (p > 0.05) for emergence day of the new follicular wave (G1: 4.0 ± 0.4 and G2: 4.1 ± 0.6 day), maximum diameter of the largest follicle of this wave (G1: 5.6 ± 0.2 and G2: 5.3 ± 0.2 mm), maximum diameter day (G1: 9.3 ± 0.4 and G2: 9.0 ± 0.4 day) and growth duration (G1:128.0 ± 8.0 and G2:116.6 ± 9.7 h). In conclusion, it was possible to confirm that regardless of the presence or absence of the CL at the beginning of the 17β-estradiol plus P4 protocol the ewes showed similar follicular dynamics. (Financial support: CNPq and FAPESP)

P 182 | Effect of a late pregnancy diet supplemented with hydrolyzed yeast on sow colostrum yield and its composition

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The aim of this study was to examine whether yeast derivative (YD) based on brewery yeast hydrolysate added to a late pregnancy diet affected colostrum composition and yield (CY) in sows. 37 sows were randomly allocated to two groups as follows: a negative control diet (n = 19) and the same diet supplemented with 3.5 g YD/kg (n = 18) during the last 3 weeks of pregnancy. The YD used was Progut®

(Hankkija Oy/Suomen Rehu, Hyvinkää, Finland). Within the first 2 h from the beginning of farrowing, a 10 ml colostrum sample was obtained to check for nutritional composition (protein, fat, lactose, dry matter, with Fourier transform infrared spectroscopy - FTIR), and immunoglobulin content (IgA, IgM and IgG with ELISA analysis). All piglets were individually weighted at birth and 24 h later in order to calculate CY. Colostrum content of protein, lactose and dry matter did not significantly differ between the two groups, while YD fed sows had higher level of fat in colostrum (5.1% vs. 4.2%; $p < 0.05$). IgA, IgM and IgG levels in colostrum did not significantly differ between the two groups. CY was 3701 g in the control group and 4580 g in the YD fed group ($p < 0.05$). In conclusion, adding YD to late pregnancy diet in sows did not affect immunoglobulin level, protein and lactose content in colostrum, but contributed to higher fat content and increased the CY. Therefore YD added to sow diet may increase colostrum availability and also its energy content through fat increment for neonate pig.

P 183 | Red deer sperm volume determinated by electronic estimation is related to sperm velocity

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Morphometry studies using image analysis reported a relationship between sperm volume and velocity (Ramón et al. 2013, Biol Reprod 89:110). This approach takes time and does not take into account sperm viability. Some flow cytometers are equipped with Coulter effect detectors, allowing to estimating the electronic cell volume (eV) while analyzing other physiological variables. We tested the relationship between eV and CASA parameters in deer sperm (*C. elaphus hispanicus*). Epididymal spermatozoa from 17 males were frozen. Thawed samples were assessed for viability (YO-PRO-1/PI), eV (Cell QUANTA, Beckman Coulter) and motility (CASA) before and after incubation (37°C, 2 h). Pearson correlations were used for the analysis, and results are given as mean ± SEM. Apoptotic (171.6 ± 13.8 fl), living (132.7 ± 13.2 fl) and necrotic sperm (160.8 ± 13.8 fl) showed different eV ($p < 0.001$). Incubation did not have an effect on total eV, but changed it in live cells (138.2 ± 12.3 vs. 127.1 ± 11.8 fl; $p < 0.001$) and in necrotic cells (156.7 ± 12.6 vs. 164.9 ± 13.8 fl; $p < 0.001$). eV correlated with MT (−0.20, $p = 0.018$), MP (−0.30, $p < 0.001$) and LIN (−0.23, $p = 0.007$). The eV of living sperm correlated with VAP (−0.25, $p = 0.018$), VSL (−0.25, $p = 0.022$), LIN (−0.35, $p = 0.003$) and WOB (−0.37, $p = 0.002$) at 0 h; at 2 h, eV correlated with VAP (0.312, $p = 0.01$) and VCL (0.342; $p = 0.005$). High eV in sperm might be related to sperm velocity. Coulter effect combined with flow cytometry could enhance the study of sperm subpopulations, and might help to better understand sperm physiology.

P 184 | Comparative study of three different pregnancy diagnosis techniques in rabbits

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Seven randomly selected pluriparous non-gravid does and two sexually matured bucks were utilized for the study. They were fed with commercial standard ration and grass and water was given ad libitum. The rabbits were stabilized for 30 days after which they were all mated. A successful mating was established by signs of recoil of the male, a fall backwards or sideways and emission of a cry. Count down of gestation began 10 h after successful mating. Pregnancy detection was performed by weight gain, abdominal palpation and ultrasonography techniques at days 6, 9, 13, 18, and 23. Abdominal palpation of does was carried out by gentle palpation of nodule-like tissues in the ventral abdomen. Ultrasonography was performed transcutaneously using portable ultrasound machine Kaixin KX2000®. Weight gain was monitored with the use of a sensitive weighing scale. Early pregnancy was diagnosed by ultrasonography and abdominal palpation on days 6.00 ± 0.00 and 7.5 ± 1.29 post-copulation, respectively. Pregnancy was diagnosed by abdominal palpation in 28.57% of the does on day 6 and increased to 100% on day 9 post-copulation; however diagnosis by ultrasonography was 100% on day 6. The average weight gain was 0.029 ± 0.029 kg by day 6; 0.057 ± 0.4 by day 13, then plateaued till parturition. It was concluded that ultrasonography could be used effectively for pregnancy diagnosis as early as day 6 of gestation in rabbit does while abdominal palpation could serve in absence of ultrasonography by day 9 of gestation. Weight gain is a positive adjunct to other pregnancy diagnostic techniques.

P 185 | The expression of CD10 relate to inflammatory response intensity (IRI) in normal feline mammary tissue and mammary neoplasms

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The deregulation of CD10 expression leads to the accumulation or loss of peptides, disturbing the regulation of cellular proliferation and differentiation, and altering intracellular signaling pathways therefore is associated with the development or progression of a variety of tumors. The CD10 expression has been reported to correlate with tumor progression in feline mammary gland tumors (fMGT). We examined the CD10 expression during fMGT development relates to inflammatory response intensity (IRI). Full thickness samples of normal mammary glands (N), adenoma (A) and adenocarcinoma (AC in general and grade I, II, III) were collected from 61 mature queens during mastectomy. Samples were routinely processed, stained with HE and anti-CD10 antibody, then evaluated by light and confocal

microscopy and scanning cytometry respectively. The IRI (degree of infiltrating immune cells was scored on a 3-point scale (0 to 2+)) (mean ± SEM) was significant higher ($p < 0.0001$) in A (0.89 ± 0.20) and AC (1.55 ± 0.20), (GI, GII, GIII) (1.50 ± 0.56; 0.66 ± 0.49; 1.84 ± 0.22) with respect to N group (0.50 ± 0.20). The CD10 was expressed in tumor cells (tCD10), stromal cells (sCD10) and infiltrating immune cells (iCD10). Inflammatory cells with CD10 expression (iCD10) were predominantly neutrophils. The positive rate of iCD10 expression was 78% in A, 77% in AC and 36% in N. iCD10 expression level (mean% ± SEM) was the highest in AC (AC(+) 27.00 ± 2.82; AC(++) 30.76 ± 3.01) compared to all other tissues (A(+) 17.77 ± 2.67; N(+) 6.79 ± 0.39) ($p < 0.05$). CD10 expression may be induced by the local inflammatory process in malignant tumors, because inflammatory and healing processes accompany tumor cell invasion of the surrounding tissue. CD10 expression in this tumor microenvironment is involved in fMGT development.

P 186 | The cell nuclei of bone marrow-derived mesenchymal stem cells are more competent to support the ex vivo development of cloned pig embryos than the cell nuclei of blood-derived fibroblast-like cells

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The present study was carried out to compare the effect of adult bone marrow-derived mesenchymal stem cells (ABM-MSCs) or adult peripheral blood-derived fibroblast-like cells (APB-FLCs) that were used as nuclear donor cells on the in vitro developmental potential of porcine cloned embryos. Oocytes that had reached the metaphase II stage following IVM provided a source of nuclear recipient cells for the somatic cell cloning. The complexes of ooplasts and either ABM-MSCs (Group I) or APB-FLCs (Group II) underwent simultaneous fusion and electrical activation. The clonal cybrids were exposed to 5 µg/ml cytochalasin B for 2 h, followed by culturing to morula/blastocyst stages for 6–7 days. In Groups I and II, 219/235 (93.2%) and 201/223 (90.1%) oocytes were successfully fused/activated and selected for in vitro culture, respectively. Out of 219 and 201 cultured cloned embryos allotted into Groups I and II, 198 (90.4%) and 127 (63.2%) were able to divide, respectively. The percentages of embryos that completed their development to the morula and blastocyst stages were 165/219 (75.3%) and 94/219 (42.9%) or 104/201 (51.7%) and 53/201 (26.4%) in Groups I or II, respectively. Summing up, cloned pig embryos reconstituted with ABM-MSC nuclei displayed remarkably higher frequencies of cleavage divisions and morula/blastocyst formation as compared to those reconstituted with APB-FLC nuclei. (This study was conducted as a part of statutory activity No. 02-011.1, which is financed from 2015 to 2017 by the Polish Ministry of Science and Higher Education.)

P 187 | Comparison of number of follicles, oocyte recovery rate and oocyte maturation stage between horses and donkeys: preliminary results

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Equid oocytes recovered post mortem and maturated in vitro can be used to speed up research in the field of assisted reproduction, which is not developed enough in these species and it could be applied to improve genetics and preserve endangered species. The aim of this study was to compare the number of follicles, oocyte recovery rate and in vitro maturation stages. For this purpose, fourteen ovaries obtained post mortem from twelve mares and two jennies aged between 1 and 15 years old were used. Oocytes were obtained after measuring and grasping follicles with a bone curette. Oocytes were in vitro matured for 42 h at 38.2°C and 5% CO_2, afterwards, denudation, fixation and staining were carried out in order to evaluate maturation stage. Recovery rates and number of follicles were compared by ANOVA. Chi-square was used to determine differences between maturation stages percentages. There were no significant differences ($p > 0.05$) between species (horse vs. donkey) in the number of follicles per ovary (15.33 ± 2.33 vs. 18.50 ± 2.5), oocyte recovery rate (46.97 ± 4.16 vs. 30.93 ± 4.67) or percentages of maturation stage: non-matured (NM, 26.1 vs. 25%), metaphase I (MI, 23.9 vs. 12.5%), metaphase II (MII, 19.6 vs. 37.5%), and degenerated (D, 30.4 vs. 25.0%). Although there were no significant differences between species, apparently donkey oocytes showed a higher tendency for MII. In conclusion, there are no significant differences between response to in vitro maturation between jennies and donkeys. However, further studies with a larger number of jenny ovaries are needed in order to elucidate differences between horse and donkey oocytes.

P 188 | Influence of photoperiod on viability and chromatin integrity of ram sperm collected by electro-ejaculation

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To investigate the impact of photoperiod on viability and chromatin integrity of ram sperm collected via electro-ejaculation (EE), six semen samples were collected once a week from 5 males (5 years old) during spring and autumn of the same year. Sperm viability was determined by eosin-nigrosin stain while chromatin integrity by the 2,2′-dithiodipyridine technique (Flores et al., 2011. Theriogenology 76, 1450–1464). Briefly, fresh semen was centrifuged at 5000g for 10′ at 5°C and the pellet submerged in liquid nitrogen before storage at −80°C. Stored samples were homogenized through sonication, centrifuged at 850g for 20′ at 4°C and the supernatant discarded. The pellet was resuspended in PBS, diluted (1:100) in a 2,2′-dithiodipyridine

solution (0.4 mM) and incubated at 37°C for 1 h. Levels of free cysteine radicals from the disruption of the overall disulfide bonds in sperm head nucleoproteins were determined using spectrophotometric analysis. The results were normalized against the total protein content of the samples using the Bradford method. Statistical analysis was performed on the six replications using the General Lineal Model (SPSS 19.0). Sperm parameters showed no significant differences within males irrespective of season. Also, results (mean ± SE) showed no statistical difference on sperm viability percentage (49.4 ± 4.6 and 50.2 ± 4.8) and free cysteine radicals levels (4.0 ± 0.8 and 5.3 ± 0.8 nmolCys/ug protein) between spring and autumn, respectively. In conclusion, photoperiod did not affect studied sperm parameters in semen collected via EE. (Supported by INIA (RZP2014-00001-00-00))

P 189 | Expression of interferon stimulated genes (ISGs) is extended to pituitary in early pregnant ewes

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In ruminants, the embryo must regulate endometrial $PGF_{2\alpha}$ secretion to sustain progesterone production by the corpus luteum (CL) during early pregnancy. This regulation is driven by interferon-tau (IFNτ) produced by embryonic trophoblast. The IFNτ induces uterus for interferon stimulated genes (ISGs) expression such as ISG15 and Myxovirus1 (Mx1) but its effect is also extended to the extra-uterine tissues such as the CL, peripheral blood leucocytes, and liver. The present study aimed to evaluate the extends of endocrine effects of embryonic IFNτ on the pituitary (PIT) in early pregnant ewes. For this purpose, pregnant (n = 5) and cyclic (n = 5) ewes were slaughtered on day 16 of estrous cycle or pregnancy. Tissue samples including pituitary, CL, and intercaruncular area of endometrium (EIC) were collected. Total RNA was isolated and qPCR was employed to detect expression levels of ISG15 and Mx1 mRNAs. As expected, expressions of ISG15 and Mx1 mRNA were upregulated in both endometrium and the CL. Expression levels of both ISG15 and Mx1 were significantly increased in the pituitary ($p < 0.05$). These results suggest that effect of IFNτ reaches pituitary in addition to the other extra-uterine tissues at early pregnancy. The role of IFNτ stimulation on such a distant tissue like pituitary, which is an important gland for reproduction, warrants further investigation. (This study was partially funded by TUBITAK grant 214O643 to M. Kose).

P 190 | Collection method of ram semen affects sperm viability and chromatin integrity

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To study the effect of semen collection method on sperm viability and chromatin integrity, 12 rams (5 years old) were divided into two equal groups. Semen samples in the first group were collected via electro-ejaculation (EE) and in the second by artificial vagina (AV) during autumn on weekly basis. Sperm viability was assessed by eosin-nigrosin stain while chromatin integrity by the 2,2′-dithiodipyridine technique (Flores et al., 2011. Theriogenology 76, 1450–1464). Briefly, fresh semen was centrifuged at 5000g for 10′ at 5°C and the pellet submerged in liquid nitrogen before storage at −80°C. Stored samples were homogenized through sonication, centrifuged at 850g for 20′ at 4°C and the supernatant discarded. The pellet was resuspended in PBS, diluted (1:100) in a 2,2′-dithiodipyridine solution (0.4 mM) and incubated at 37°C for 1 h. Levels of free cysteine radicals from the disruption of the overall disulfide bonds in sperm head nucleoproteins were determined using spectrophotometric analysis. The results were normalized against the total protein content of the samples using the Bradford method. Statistical analysis was performed on five replications using the General Lineal Model (SPSS 19.0). Sperm analyses showed no significant differences within males of the same group. However, the results (mean ± SE) showed significant differences on sperm viability (80.3 ± 3.2; 56.0 ± 3.3) and free cysteine radicals levels (1.9 ± 0.5; 5.1 ± 0.5) between AV and EE collected sperm samples, respectively. Therefore, semen collection by EE seems to negatively affect these sperm parameters. (Supported by INIA (RZP2014-00001-00-00))

P 191 | Distribution of the egg shell thickness on cross-section, 1 day before hatching - micro-CT

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Egg shells, including chicken egg, evolved to protect the developing embryo against the harmful influence of external factors. Only gases may pass through pores filled with cuticle, other substances are blocked. It can therefore be assumed that the effectiveness of this mechanism depends not only on the amount of cuticle but also on shell thickness, which is not the same at all places. In order to determine the shell thickness distribution in the cross-section, chicken eggs were examines with micro-CT the day before hatching. Analysis of the data showed significant local differences in the eggshell thickness, in a range between 300 and 350 μm. Therefore, measurements of the thickness in one point may be burdened with a large error. From a practical point of view, values considerably below the local minima may indicate areas with increased risk of bacteria penetration into the eggs, in that in these areas barrier is thinnest. Analysis of the distribution of the egg shell thickness may be in the future a useful method of screening for early diagnosis of hens diseases in the flock at the stage in which changes in the eggshell are not yet detectable visually.

P 192 | The role of iodine in cow fertility

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Iodine is an essential trace element to animals for the production of the thyroid hormones. The required amount of daily intake of iodine varies according to age and lactational stage. Iodine deficiency is known to affect fertility in different ways. In some areas there is a low concentration of iodine in grass. In this pilot study, T4 concentrations (μg/l) were measured in blood of lactating cows (n = 24), dry cows (n = 24) and young stock (n = 24) on farms with good (C; n = 4) and poor fertility (P; n = 4) using a Eurolyser T4 Solo testkit. In the included lactating cows (n = 24) iodide concentrations (μg/l) were also measured in milk samples using ICP-MS. Daily iodine intake at the farms included was calculated. Statistical analyses were performed (IBM SPSS 21). A significant positive correlation (r = 0.414, n = 24, p = 0.044) between iodine in milk (μg/l) and T4-values in plasma (μg/l) was shown in the lactating cows. At the P-farms a low estrus expression was reported. This was confirmed by detecting lower T4 concentrations in both pregnant and non-pregnant young stock in these farms compared to the C-farms. In addition, insemination number was significantly higher in the P-farms compared to the C-farms in cows (2.26 vs. 1.86) and heifers (1.95 vs. 1.34). Preliminary analysis of the data shows that calving interval was longer and age at first calving was higher in the group of animals with low estrus expression (P-farms). Occasionally high iodide concentrations in milk were found at P-farms most likely caused by the use of a teat dip containing iodine. The present results suggest a negative correlation between T4 in blood and iodine concentrations in milk on reproductive capacity in dairy cows.

P 193 | Comparative radial analysis of the skull of dog puppies with or without hydrocephalus

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Hydrocephalus is a condition in which the ventricular system of the brain is secondarily increased and leads to an abnormal flow of cerebrospinal fluid and its impaired absorption into the systemic circulation. Although it can happen under the influence of inflammation, carcinogenesis, or hemorrhage, it is usually a congenital condition affecting predisposed "toy" breeds. Hydrocephalus may result in severe neurological disorders and deterioration in the quality of life of the patient. Therefore, early diagnosis and appropriate treatment are important. Many dogs with hydrocephalus have an enlarged head and persistent fontanelle. In this situation, USG examination and visualization of the ventricular system are possible, but some patients have too small or closed fontanelle difficulting this exam. Ventricular system of the brain is also visible in the CT and MRI examination, but they require general anesthesia, which is problematic in young animals. This study describes the radial analysis of the skull of the dog with or without hydrocephalus. Images of cross-sections of the skull at the same height have been obtained using a 3D optical scanner. Enlargement and distortion of the skull contour, typical for hydrocephalus, was clearly visible. Radial analysis enables rapid and non-invasive evaluation of the shape and the outer surface of the skull, regardless of the fontanelle' diameter. It can be considered as an alternative method of early diagnosis of hydrocephalus in puppies.

P 194 | Sensitivity of lectin for identifying apoptotic cells in the seminiferous epithelium of Syrian hamster (*Mesocricetus auratus*) during testicular regression due to short photoperiod

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Many cell types suffer alterations in their glycoconjugate pattern in pathological situations. Changes in the photoperiod in seasonal breeding animals such as Syrian hamster lead testicular regression accompanied by depletion of the seminiferous epithelium, which involves cell apoptosis. This phenomenon is studied using the classical histochemical technique of TUNEL. In addition, it has previously been found that lectin histochemistry is useful for identifying cell apoptosis in the seminiferous epithelium during testicular regression. The aim of this study is to determine the sensitivity of lectin histochemistry as a marker of apoptosis compared with the standard technique of TUNEL. For this purpose, we used the TUNEL technique in testicular sections of strongly regressed animals and made a count of both TUNEL+ and TUNEL− spermatocytes (SC) and round spermatids (SD) per seminiferous tubule. The lectin histochemistry technique using PNA or Con-A peroxidase-labeled was performed in the same sections. Both positive and negative SC and SD were determined for each lectin and the number of positive cells for both techniques was counted by superimposing the images. The results pointed to a significantly higher number of lectin + cells than TUNEL + cells in the case of both spermatocytes and round spermatids ($p < 0.05$), with no significant differences between TUNEL+ and TUNEL+ / lectin+ cells. In conclusion, lectin histochemistry is more sensitive than the TUNEL technique for identifying spermatocytes and round spermatids undergoing apoptosis, probably because the technique covers more stages than the TUNEL technique. (Funded 19892/GERM/15, Fundación Seneca, CARM)

P 195 | The pattern of oviductal myoelectric activity relate to hormonal status (estradiol, progesterone and LH plasma concentration) during the estrous cycle in gilts

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The adequate level of uterine and oviduct (isthmus and ampulla) contractility at about the time of ovulation appears to be important for the rapid transport of gametes as well as fertilization efficiency. The aim of present research was to record oviductal myoelectric activity in sows during the estrus cycle. The studies were carried out on 6 polish landrace sows (90–110 kg body weight). The 3 silicone base silver bipolar electrodes were adapted to oviduct (isthmus and ampulla) and uterine horn and surgically sutured on them under general anesthesia. Electrodes were connected to TL10M3-D70-EEE telemetry implant surgically positioned between the abdominal muscles. The electromyography (EMG) signals were recorded using the DL10 analog output coupled to PowerLab and sampled 40 points/s with band pass filters at the level 10 and 50 Hz. P4, estradiol and LH were evaluated in plasma during the estrus cycle. Data were analyzed with Kruskal-Wallis followed by Dunn post hoc test. A high plasma estradiol concentration was found during proestrus. At the same time no significant differences ($p > 0.05$) in major EMG activity parameters such as: root mean square (RMS) and amplitude were found. Nevertheless the significant RMS as well as the amplitude increase ($p < 0.01$) was correlated with the LH peak. Moreover the significant increase of above mentioned parameters ($p < 0.01$) from day 0 (estrus) to the second day of the estrus cycle was recorded. The correlations between significant decrease of the amplitude and RMS ($p < 0.05$) and high plasma progesterone concentration were found. The preovulatory LH surge may play an important role in the oviduct relaxation in sows. The telemetry EMG registration is an accurate and useful technique to determinate the oviduct activity in sows.

P 196 | Guiding framework for case-based learning in reproductive medicine course

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The Bologna reforms put the focus on self-learning and skills acquisition. In veterinary education, among other strategies, the case-based learning has been adopted to stimulate students' clinical skills and vets' lifelong learning. This paper presents the main constraints found in the implementation of a case-based activity using collaborative groups, framed by adapted FRISCO Guidelines (Payan-Carreira et al., 2015, https://goo.gl/m7oK2u) in a Gynaecology and Obstetrics course (8th semester; 2015/16). Sixty-six students were randomly assigned to 11 groups and confronted with the assessment of a case of granulosa cell tumor in the mare (McCue, 2013, http://goo.gl/WAjct3). An instructional file covering the learning outcomes, the FRISCO framework and the description of the activity was offered 10 days in advance, in the platform Moodle; it included the demand for an individual concept map on primary ovarian tumors. The teacher presented in-class the case scenario and questionnaire; students had 2 classes of 1 h to perform the task and 1 additional week to upload it in Moodle. To facilitate the task, the teacher gave further clinical information upon request. Moodle records were used to assess the students' involvement. Results showed that (a) most students failed to prepare themselves for the activity; (b) 42.4% of the students never consulted the supporting material; (c) only 5 students, representing 2 groups, presented the concept map. Still all students considered having made major contributions to the submitted work. They also objected to working in randomly assigned groups and tended to distribute tasks instead of joining forces. This preliminary work suggests that the students need to be trained and motivated to change working habits.

P 197 | Effect of P2Y2 receptors modulation on bovine oocyte cryosurvival

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The improvement of the mammalian oocyte survival after cryopreservation is imperative for the conservation of animal genetic resources. Our objective was to investigate if the purinergic G-protein P2Y2 receptors (P2Y2R) in cumulus oocyte complexes (COC) could be important players for the cryopreservation success. Firstly, RNA was extracted from immature and mature bovine oocytes and cumulus cells and real-time PCR performed to quantify P2Y2R. Then the function of P2Y2R was investigated by measuring changes in intracellular calcium concentration $[Ca^{2+}]i$ of COC using 100 μM UTP (stimulator) and/or 100 μM suramin (inhibitor) and by analyzing whether this modulation of P2Y2R prior to oocyte cryopreservation leads to changes in oocyte cryosurvival (4 replicates). After maturation COC were randomly distributed into five groups (control n = 120, cryoprotectant only n = 82, suramin n = 81, UTP n = 89 and suramin+UTP n = 75) and vitrified/warmed. Viable oocytes were inseminated with frozen/thawed bovine semen and embryo production evaluated. Results showed that P2Y2R were expressed in both bovine oocytes and cumulus cells and that $[Ca^{2+}]i$ was affected by UTP and suramin stimulus. An improved cryosurvival rate was identified in suramin group compared to cryoprotectant alone (82.7 vs. 74.4%, respectively). The lowest cleavage rates were attained in the UTP group. These preliminary data pointed out to the improvement of bovine oocyte cryosurvival when the P2Y2R were inhibited during cryopreservation. (Funded by project PTDC/CVT/2863/2012 and grant SFRH/BPD/100565/2014)

P 198 | Reproductive performance of males from several falcon species at a raptor farm in Spain

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Artificial insemination is a routine technique in raptor farms, in order to improve fertility and allow insemination in animals unable to mate. Due to the scarcity of systematic studies on the application of assisted reproductive techniques in these species, it is necessary to revisit the topic in order to improve their reproductive management and conservation. Sperm production depends on male training and age, but there are other less studied factors. This survey was carried out in a raptor farm in the North of Spain (42.6° N, 5.5° W) during 2015, with a reproductive period from March to mid-May, in order to study the effect of collection date and species on semen production. Semen was obtained from 14 males (2–11 years old) by massage (2 peregrine falcon, *F. peregrinus*, 4 saker, *F. cherrug*, and 8 gyrfalcon, *F. rusticolus*). The cloaca was cleaned and the semen was collected in 75-µl capillary tubes. Data analysis was carried out by linear mixed-effect models, correcting data by male age. A total of 182 extractions were attempted, resulting in 157 collections (86%; most unsuccessful from mid-April to May). Discarding 5 males with <5 extractions, mean ± SD extractions per male were 19.1 ± 8.5, with 43.8 ± 26.5 µl per successful extraction. Semen volume was affected by date, being lower by the end of the season ($p < 0.001$; mean ± SEM March to April 20th: 24.6 ± 2.3; to May 15th: 10.5 ± 4.4 µl). Semen production was higher for peregrinus (mean ± SEM, 30.7 ± 4.2 µl) than for cherrug (9.7 ± 2.6 µl, $p < 0.001$) or rusticolus (24.6 ± 3.4 µl, $p = 0.016$). However, rusticolus yielded the highest volumes (not corrected by age, maximum of 131 µl vs. 90 µl of peregrinus and 75 µl of cherrug). This information can be used for better management of artificial insemination in raptor farms.

P 199 | Embryo cryopreservation by slow-freezing and vitrification in Spanish jennies

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The aim of this study was to describe the results obtained in an embryo recovery program carried out in endangered Spanish donkey breeds and to compare two methods for embryo cryopreservation. Embryo recovery was carried out between day 6.5–8.5, but only morulas or early blastocysts with grade 1 or 2 were used for cryopreservation, comparing two different methods: slow-freezing (SF) (n = 4) with 1.5 M ethylene glycol (EG) in 0.25 ml straw using Cryologic CL-3300 controlled-rate freezer (from −6 to −35°C at 0.5°C/min); and vitrification (VF) (n = 4) by exposure to 1.5 M EG for 5 min and 7M EG+0.6M galactose for 1 min, loaded into Fibreplug© and it was put in contact with CMV block surface (CryoLogic, Pty Ltd, Victoria, Australia) for some seconds. Then, embryos were stained for in vitro assessment, and images were obtained by confocal laser-scanning microscopy. Results are showed as mean ± SEM. The embryo average diameter was 187 ± 16 µm in SF group and 221 ± 37 µm in VF group, showing a total number of cells of 620 ± 195 and 1208 ± 310, respectively. It was observed that the percentage of dead cells was slightly higher after slow-freezing (4.6 ± 3.2%) than after vitrification (2.5 ± 1.1%) ($p = 0.06$). The assessment of apoptotic cells (SF = 0.52 ± 0.2%; VF = 0.83 ± 1.8; n.s.) and cytoskeleton quality (SF = 1.25 ± 0.5; VF = 1.20 ± 0.4; n.s.) did not show significant differences between methods. Results show a more reduced impact of vitrification on the cell vitality of donkey embryos, in contrast to slow-freezing, although the apoptosis and embryo skeleton were not affected by the cryopreservation method. Further studies should be carried out to evaluate the viability of these embryos in recipients. (Supported by INIA-FEDER RZ2008-00025)

P 200 | The inclusion of progesterone in the dextran/swim-up procedure increases the recovery of ram capacitated sperm rate

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The presence of receptors for progesterone (P4) on the mammal sperm surface, leads to speculate about a relevant role of this hormone on sperm functionality. However, the information about their specific effects on ovine spermatozoa is scarce. In this study we used a ram sperm washing and selecting-procedure, a dextran/swim-up method, including two different concentrations of progesterone in the upper layer, one low (100 pM, LPsw) and one high (1 µM, HPsw). We compared cell recovery and sperm quality with the standard swim-up obtained sample (STsw, with no hormone). Both P4 concentrations resulted in a significantly higher cell recovery rate (RR: 52.3 ± 4.8 and 53.4 ± 5.8% for LPsw and HPsw, respectively) and percentage of capacitated sperm (CTC: 35.0 ± 6.2 and 38.0 ± 5.8 for LPsw and HPsw, respectively) than STsw (42.5 ± 3.7 and 26.6 ± 6.7% for RR and CTC, respectively; $p < 0.05$). However, the inclusion of P4 showed no effect on motility (by CASA), membrane integrity (CFDA+/PI−) or phosphatidylserine exposure (Annexin−). Indirect immunofluorescence assays are currently in progress in order to determine whether there are changes in P4 receptor distribution in the sperm subpopulation selected by LPsw and HPsw, compared with STsw. (Grants: CICYT AGL 2014-57863-R and DGA 2015-A26 FSE)

P 201 | Diagnostic value of pregnancy-associated glycoproteins (PAG) measurement in blood and urine in late embryonic mortality diagnosis in dairy cattle

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The objective of this study was to evaluate whether urine pregnancy-associated glycoprotein concentration measurement in pregnancy cows is an appropriate predictor of late embryonic mortality (LEM). The data analyzed were derived from 40 multiparous dairy cows. Animals were divided into 3 groups: pregnant (n = 19), nonpregnant (n = 11) and late embryonic mortality (n = 10). Transrectal ultrasonographic examination, blood and urine samples were obtained on days 0 (AI day), 14, 21, 28, 35, 49, 63, 77, 91 and 105 of gestation. Radioimmunoassay (RIA-706) was used to determine cPAG (caprine PAG) concentration in blood and urine. The study of PAG concentrations in blood and urine showed the detectable concentration of these proteins (>0.2 ng/ml) in the group of pregnant (P), non-pregnant cows (NP) and cows with LEM. The PAG concentrations in urine demonstrated poor positive correlation with the time of pregnancy duration. The study of urine showed an elevated content of these proteins in group C and LEM after abortion (>1.5 ng/ml), and lower levels during pregnancy (<1 ng/ml). In the context of late embryonic mortality diagnosis, PAG urine test proved to be equally sensitive method as ultrasound.

P 202 | Local and systemic actions of prostaglandin $F_{2\alpha}$ (PGF) on genes expression related to steroidogenesis and angiogenesis in the bovine corpus luteum (CL): cycle dependent manner

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The aim of the study was to compare the systemic and local effects of PGF on genes expression involved in angiogenesis (FGF2, VEGF, FGFR1, FGFR2, FLK1 and FLT1) and steroidogenesis (StAR, 3β-HSD and P450scc) in CLs. Cows at day 4 (n = 24, 6/treatment) or day 10 of the cycle (n = 24, 6/treatment) were treated as follow; (1) intramuscular (i.m.) Saline injection, (2) i.m. PGF injection (25 mg; Dinoprost), (3) intraluteal Saline injection or (4) intraluteal PGF injection (2.5 mg). The cows were ovariectomized and the CL tissue was collected 4 h after injections. The mRNA relative abundance was evaluated by qRT-PCR. Intraluteal and i.m. PGF injections up-regulated FGF2 expression at early and mid CL ($p < 0.05$). Both ways of PGF treatment increased FGFR2 expression ($p < 0.05$), while FGFR1 expression was increased only after intraluteal PGF injection ($p < 0.01$). Intraluteal and i.m. PGF injections decreased VEGF expression at both luteal stages ($p < 0.001$). Although FLT-1 expression was increased by intraluteal PGF injection in early CL, FLK-1 was down-regulated by i.m. PGF injection ($p < 0.05$). Both treatments of PGF, increased STAR expression at early CL ($p < 0.05$). Intraluteal PGF injection up-regulated 3β-HSD expression at early ($p < 0.05$), while i.m. PGF application inhibited it's expression at both stages ($p < 0.001$). Gene expressions profiling reveal stage-specific response to PGF administration depending on local or systemic actions. (Supported NSC 2011/03/B/NZ9/01634)

P 203 | A method of evaluation and selection of cocks for cryotolerance of their sperm with aim of gene pool preservation

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Conservation of genetic resources acts as a tool of preservation of genetic diversity. However, even in chickens, sperm cryopreservation is not yet widely used in conservation programs because its success is highly depended on the fertility rates of frozen-thawed semen and there is a high individual within-breed variability. The development of new predictors of individual suitability of sperm for cryoconservation is required. Differences between species of domestic birds in semen freezability partly reflect the differences in cholesterol content of spermatozoa membrane. The goal of this investigation was to develop a method for cocks' sperm evaluation for cryotolerance and hence further use in gene pool preservation programs. The study was carried out using Rod Island cocks (n = 16, age 40 weeks) from gene pool farm, raised in individual cages under 14:10 D photoperiod on commercial diet. Semen was collected by dorso-abdominal massage. Spermatozoa concentration was evaluated by centrifugation; cholesterol concentration was measured by enzymatic colorimetry assay. Freezing was carried in pellets with DMA. The semen pellets were thawed at 60°C. Ninety-seven hens were inseminated and 617 eggs incubated. Results showed for cryopreservation one should select the cocks with sperm concentration ≥4.0 bln/ml; activity ≥80% and sperm cholesterol concentration ≤M in each experiment (in this trial ≤0.56 mmol/l). In case of use of this selection parameter egg fertility in selected cocks (n = 8) was 59.4% ± 6.0. The fertility after use of rejected cocks (n = 8) was 36.6% ± 5.0 ($p < 0.05$). Thus, additive criterion of sperm evaluation- cholesterol content- enables to increase significantly fertility of eggs after insemination by frozen-thawed semen.

P 204 | Failure to conceive in deslorelin-induced estrous bitches with regard to removal of hormone implants after ovulation

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Removal of a GnRH agonist deslorelin implant either before or during ovulation in deslorelin-induced estrous bitches is suggested because prolonged administration of the agonist may cause luteal failure secondary to pituitary down-regulation. This study aimed at investigating the effect of a 4.7 mg Deslorelin (Suprelorin®) on estrous induction and the conception rate after removing the implant 72–96 h post-ovulation to ensure that the entire ovulation process was completed. Ovulation began when serum progesterone reached 5–6 ng/ml. The implants were inserted subcutaneously in the umbilical area in 5 intact anestrous beagles (1.5–3 years). In all bitches, vaginal cytology was abruptly changed on day 3 and estrous signs were observed on day 5 post-implantation. Ovulation occurred on day 11.4 ± 0.9 post-implantation (11–13 days) (ovulation rate = 100%). Transcervical artificial inseminations with chilled semen (>75% sperm motility) using Scandinavian catheter were performed on the 2nd and 4th day post-ovulation. Pregnancy was confirmed on day 35 post-AI by transabdominal ultrasonography and serum relaxin test (WITNESS® Relaxin). No fetuses were detected and relaxin tests were negative (pregnancy rate = 0%). Progesterone levels remained higher than 1 ng/ml for approximately 57 days. Unsuccessful pregnancy outcome possibly related to delayed removal of the implants. Physiological changes in the oviduct and/or uterus associated with time of implant removal should be further investigated.

P 205 | Case report: ovarian fibroma in a mare – hormonal considerations

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History: A large ovary is incidentally palpated in a 13 year-old mare presented for colic with an alimentary obstruction. Clinical investigation: There was no uterine oedema. The left ovary was large (5.8 cm), hard and ultrasonography showed a large echogenic area (5 cm) with some small follicles. A corpus luteum and small follicles were present on the right ovary. Testosterone was below 25 pg/ml; estradiol below 5 pg/ml; progesterone 1.98 and 14.87 ng/ml 2 days later. Hormonology didn't confirm the clinical suspicion of an ovarian tumor. At control 6 weeks later, no changes on the left ovary were observed; the right ovary presented a corpus luteum and some small follicles. Steroids values were: progesterone = 8.7 ng/ml; testosterone below 25 pg/ml; Anti-Mullerian Hormone (AMH) = 2.21 ng/ml. Treatment: Unilateral ovariectomy (left ovary) was performed by laparoscopy on the standing sedated mare. The surgery and postoperative period were uneventful. Histopathology: A well-circumscribed neoplasm, partially encapsulated is identified. The ovarian stroma is collagenous. Small hemorrhages and cystic areas containing basophilic material are observed. The tumor cells have thin cytoplasm with elongated, regular, hyperchromatic nuclei and inconspicuous nucleoli. Mitotic figures are rare (<1/10 fields). Diagnosis: Benign Ovarian Fibroma Discussion: The aim was to describe equine ovarian fibroma. In contrast with Granulosa Theca Cells Tumors (TGCT) who are secreting steroids, inhibin and AMH in the mare and the woman, human ovarian fibroma don't produce AMH. This case also suggests that equine ovarian fibroma are endocrinally inactive, thus preserving cyclicity.

P 206 | Aquaporins 3 and 7 as cryotolerance markers in boar semen

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Although recent studies have reported that aquaporins 3 and 7 (AQP3 and AQP7) are present in boar sperm, their putative relationship with the sperm ability to withstand freeze-thawing procedures remains untested. In the present study, we aimed at determining whether the relative amounts of AQP3 and AQP7 assessed in refrigerated boar sperm are related to their cryotolerance. With this purpose, 17 ejaculates were used in the current study. The sperm motility and membrane integrity were evaluated through a computer assisted sperm analysis system (CASA) and a sperm viability kit (SYBR14/PI) for fluorescence microscopy. Proportions of total motile sperm and viable sperm in all fresh semen samples were higher than 85%. The ejaculates were split into two aliquots. One was used to determine the relative amounts of AQP3 and AQP7 in refrigerated semen through immublotting, whereas the other was cryopreserved following the Westendorf method (0.5 ml straws). At 30 and 240 post-thawing, sperm motility and viability were evaluated as aforementioned. After checking the normality and homogeneity of variances, Pearson and Spearman correlations were calculated between the relative AQP-levels and sperm motility and viability. While the relative amounts of AQP3 in refrigerated semen were found to be significantly correlated with sperm viability evaluated at 30 and 240 min post-thawing, those of AQP7 were only found to be significantly correlated with sperm viability determined at 240 min post-thawing. We can conclude that AQP3 and AQP7 appear to be related with the boar sperm resilience to withstand cryopreservation and that their relative amounts in refrigerated semen may predict the sperm cryotolerance before undertaking freeze-thawing procedures.

P 207 | Plasma cortisol concentrations in Holstein Friesian and Belgian Blue newborn calves born by different types of delivery

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Cortisol (C) is a major stimulus for fetal and neonatal lung maturation and for surfactant production. Neonatal calves born by caesarean section (CS), particularly the double-muscled Belgian Blue (BB), are more proned to develop the respiratory distress syndrome (Cambier et al. 2002 Vet Res 33, 283–290; Danlois et al. 2003 Vet J 165, 65–72). The aim of this study was to investigate C plasma levels in 15 Holstein Friesian (HF) newborn calves born by spontaneous vaginal delivery (VD) and in 25 BB newborn calves born by elective CS. Blood samples were taken at 10, 20, 30 min and at 6, 24 h after birth and at 7 and 14 days of age. Plasma C concentrations were analyzed by RIA. Statistical analysis evidenced an influence of both time ($p < 0.05$) and group ($p < 0.0001$) on C concentrations. In agreement with previous studies, high C levels at birth were followed by a reduction at 6 h and by a further decrease on day 7 after birth in both groups. Cortisol concentrations were different between BB and HF calves, with significantly higher levels in HF calves. These findings are in agreement with studies in newborn babies, while previous studies on calves mentioned no differences in C levels between calves born without assistance vs. calves born by CS. The present results suggest that both breed and type of delivery are associated with plasma C levels in the newborn calf.

P 208 | Udder firmness as a possible indicator for clinical mastitis

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Swelling and increased firmness of the udder is an important sign to detect clinical mastitis (CM) in dairy cows. However, data on objective methods to examine udder firmness are scarce. The overall objective of this study was to evaluate if udder firmness can be used as a cow-side indicator for mastitis. A dynamometer was used to objectively determine udder firmness in 45 cows with CM and 95 healthy cows. Udder firmness of both hind quarters was measured daily from the day of mastitis diagnosis till day 7 and again on day 14. Udder firmness before milking was similar in quarters without and with CM. After milking, quarters with CM were firmer than healthy quarters. An increase of firmness of a quarter with mastitis did not affect firmness of the healthy neighbor quarter nor did firmness of all healthy quarters differ. To reduce data clustering, we used one firmness value per cow i.e., Δ firmness (difference in udder firmness between both hind quarters after milking) for all further calculations. In all cases, CM affected Δ firmness. The threshold for detection of CM using Δ firmness was 0.282 kg (area under the curve: 0.722, sensitivity: 64.29%, specificity: 89.74%) and 0.425 kg (area under the curve: 0.817, sensitivity: 62.50%, specificity: 96.67%) in 1st parity and older cows, respectively. Cows with CM had a higher Δ firmness compared to cows without CM throughout the 14 days after the mastitis diagnoses. Depending on systemically signs of sickness, mastitic cows were divided into cows mild to moderate (n = 21) or severe mastitis (n = 24). Cows with severe mastitis suffered from a firmer udder on all measuring days. In conclusion, udder firmness can be a useful indicator for CM.

P 209 | Expression of mRNA and protein of coactivator (PCAF) and corepressor (NCOR1) of progesterone receptor (PGR) and acetyltransferase and deacetylase activity in the reproductive tract in cows

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The final step in progesterone receptor (PGR) activation is recruitment of coactivators and corepressors, which modulate receptor action. Coactivators have an intrinsic histone acetyltransferase (HAT) activity and can induce euchromatin formation and facilitate the gene transcription. In contrast, corepressors have an activity of histone deacetylase (HDAC) which increases chromatin condensation and inhibits gene transcription. Changes in the expression of mRNA and protein of coregulators may affect the PGR function and thus regulate the effect of progesterone (P4) on the reproductive tract. Luteal and endometrial tissue from days 1–5, 6–10, 11–16, and 17–20 of the estrous cycle were collected in the local slaughterhouse. The mRNA expression of PCAF and NCOR1 genes was determined by means of Real Time PCR, while the protein levels by western blot analysis. Activity of HAT and HDAC were determined by the commercial kits. Correlated ($p < 0.05$) expression of mRNA and protein for PCAF and NCOR1 were found to be highest in CL on days 6–16, while in the uterus on days 1–10 of the estrous cycle. Activity of HAT and HDAC in CL were lowest ($p < 0.05$) on days 1–5 compared to the other days analyzed. No significant changes in the activity of these enzymes were found in the uterus. It can be assumed that, P4 influences the reproductive tract function via the regulation of mRNA and protein coregulators of PGR expression. (Supported by Grant: 2015/17/B/NZ4/02440)

P 210 | Productive and reproductive parameters of Nili-Ravi buffalos receiving rbST

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Farmers concerns for negative impact of recombinant bovine somatotropin (rbST) on reproductive performance of postpartum Nili-Ravi buffaloes were investigated. Fifty lactating buffaloes, 57–70 days postpartum, were randomized into two groups (500 mg rbST or placebo; n = 25). The injections were repeated at 14 day intervals for a period of 140 days. Productive performance in terms of milk weight and milk analysis were recorded. After estrus expression, the respective animals were monitored for antral follicular count at day 3, 5, 7 and 9 post-estrus. Serum profile of estradiol and progesterone were also monitored at the same days of scanning. The rbST treatment resulted in a 25.93% increase ($p < 0.001$) in milk production with no change in milk protein (3.96 ± 0.01vs 3.96 ± 0.02) or fat percentage (6.94 ± 0.09 vs. 6.98 ± 0.01). The treatment improved the insulin like growth factor 1 (IGF-1) concentration in blood serum (77.4 ± 3.7 vs. 56.6 ± 1.8 ng/ml; $p < 0.001$) at 10 days after treatment. The increase in ovarian follicular population (10.65 ± 0.71 vs. 8.23 ± 0.61; $p < 0.05$) was also recorded due to increased small size (3 > 5 mm) follicle count. For estrus expression pattern, no difference was observed. Treatment group has higher progesterone concentrations (ng/ml) at day 5 (2.9 ± 0.9 vs. 1.9 ± 0.2) and day 9 (5.4 ± 0.14 vs. 4.9 ± 0.15) of the estrus cycle ($p < 0.05$). Estrogen concentrations (pg/ml) at day 3 post estrus (5.3 ± 0.4 vs. 4.1 ± 0.4) were significantly higher ($p < 0.05$) compared to other days. Effect of treatment on conception rate was calculated by pregnancy confirmation after artificial insemination and there was no difference between these two groups (76% rbST vs. 70% control). Therefore, we conclude that rbST treatment has no negative effect on reproductive performance in Nili-Ravi Buffaloes.

P 211 | Determination of superovulatory response by B-mode ultrasonography in sheep

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This study aims to verify the accuracy in determining the superovulatory response by B-mode ultrasonography (US) in sheep. Santa Inês ewes (n = 24) were subjected to synchronization of estrus by using, on Day 0, an intravaginal progesterone device (CIDR®) that remained until Day 8, when 37.5 µg of D-cloprostenol (i.m.) was administered. The superovulation treatment started 48 h before the CIDR removal, while ewes received different total doses of exogenous porcine FSH (200, 133 or 100 mg of pFSH). Total doses were administered in eight applications at 12 h intervals. On Day 6 the ewes also received i.m. injection of 300 IU of eCG. On the embryo collection day (Day 15), B-mode US and laparoscopy were conducted to quantify corpora lutea (CL) and anovulatory follicles (diameter ≥5 mm). The Pearson correlation test was performed using SAS ($p < 0.05$). The mean (±SEM) number of CL and anovulatory follicles by B-mode US were 15.91 ± 1.23 and 0.46 ± 0.17, whereas by laparoscopy were 12.50 ± 1.16 and 1.54 ± 0.26, respectively. The correlation coefficient between the techniques for determining the number of CL was 0.49 ($p = 0.01$) and for anovulatory follicles was 0.33 ($p = 0.12$). In summary, the determination of the number of CL by B-mode US was efficient; however, the quantification of anovulatory follicles was divergent from that evaluated by laparoscopy, possibly because of the impossibility of measuring the diameter of the structures by laparoscopy and thus overestimation of this variable. (Financial support: FAPESP no 2015/13350-5, EMBRAPA no 02.13.06.026.00.00 and PROPE no TC1288/2015)

P 212 | The effects of administration of melatonin in uterine torsion

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In this study, the effects of melatonin administration on spontaneous myometrial contractions in pregnant rats with induced uterine torsion were investigated. Uterine torsion was induced experimentally in thirty-five rats pregnant 18–19 days. The animals were randomly split into five groups (n = 7 rats per group). The rats in group 1 underwent anesthesia alone. In the rats in group 2, 360 degrees of uterine torsion was induced and corrected 6 h later. In the rats in group 3, 360 degrees of uterine torsion was induced and corrected 6 h later, and melatonin (10 mg/kg IP) was administered at the same time. In the rats in group 4, 360 degrees of torsion was induced, melatonin (10 mg/kg IP) was administered, and the torsion was corrected 6 h later. In group 5, on days 15–16 of pregnancy the rats were given melatonin (10 mg/kg/day IP), and on days 18–19 of the same pregnancies, 360 degrees of uterine torsion was induced and corrected 6 h later. All animals underwent ovariohysterectomy the first day after giving birth and samples were taken from the uterine horns. The amplitude, frequency, and area under the contractile curve of spontaneous uterine contractions were determined in all groups. As a result of the organ bath experiments, more myometrial contractions were found in the animals that were administered melatonin together with detorsion (group 3) than in the other groups (except for the control group). (This study was supported by the Scientific and Technological Research Council of Turkey, TUBITAK - 115O381)

P 213 | Using flipped classroom to teach clinical animal reproduction

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The classical way of teaching in veterinary medicine follows a teacher-centered approach = the teacher speaks and students listen and take notes. Disadvantages: significant points may be missed, level of

attention fluctuates and students are generally unable to retain all the material presented. Flip Classroom approach = student-centered pedagogical model in which lecture and homework are reversed. Advantages: focus of instruction shifted from the teacher to the student, with the latter developing greater autonomy, engaging in independent problem solving and focusing on practices that enable lifelong learning (Roach, Int Rev Economics Educ 17:78–84, 2014; Davies et al., Educ Tech Research Dev 61:563–580). The Flipped Classroom was used during the 2015–2016 academic year for senior year vet students taking the class of Small Animal Reproduction at the University of Padova. Students received the entire set of lectures at the beginning of the course. In class they were presented with clinical cases on which they worked in groups of four. In-class use of textbooks, class notes, internet and mobile phone was allowed. Level of participation by students was evaluated and counted towards the final grade. Findings reveal: (a) excellent attendance and active participation by students; (b) clinical skills improved (based on comparison to prior year students), and (c) student satisfaction in general was higher than previous years. The Flipped Classroom can be an effective teaching method in Veterinary Medicine.

P 214 | Miasthenia gravis in the kitten - a rare congenital disease

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Myasthenia gravis (MG) is a rare occurrence in cats. MG is an immune-mediated disorder in which antibodies are directed against postsynaptic nicotinic acetylcholine (ACh) receptors of skeletal muscle, resulting in impaired neuromuscular transmission. The immunoprecipitation radioimmunoassay is used to demonstrate the existence of serum autoantibodies against muscle ACh receptors. The assay is both sensitive and specific and false positive results are rare. Clinical signs in kittens are often atypical, connected with weak muscles. A 5-months-old cat was presented with recurrent symptoms of respiratory infection, the first clinical signs reported by the owner at 12 weeks of kitten's life (cat flu, bronchitis). Standard treatment was ineffective. The patient became progressively lethargic and has been regurgitating all food and water soon after the ingestion. A clinical examination showed tachypnea (100 breaths/min) with a normal heart rate, and fever (40°C). On X-rays, megaesophagus and aspiration pneumonia (possibly secondary to megaesophagus) were found. The ACh receptor antibody test was positive (cell line TE671 > 600; positive at >450). A complete blood cell count revealed erythropenia and neutrophilia. The cat was treated with a combination of pyridostigmine and prednisolone. After 8 months due to repeated aspiration pneumonia and poor prognosis, the cat was euthanized. It has been reported a greater incidence of MG in Abyssinian and Somali cats, and generalized weakness is the most common clinical sign. Spontaneous immune remissions are not characteristic for MG in cats compared to dogs, which originates a high rate of euthanasia in cats, because the effective treatment is rarely achieved.

P 215 | Clinical examination of Japanese quail *Coturnix japonica* eggs with a use of 128-row computed tomography

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Medical imaging is a rapidly developing field in biological sciences. Computed tomography (CT) scanners can now be found in numerous veterinary clinics, making clinical examination more efficient. They allow to determine precisely the phase of forming egg structures, as well as to manage interdisciplinary research in poultry reproduction. With the use of CT and three-dimensional reconstruction, the topography of the egg has been determined in examined birds and the linear measurements of the eggs have been done. An access to the modern medical imaging techniques enables rapid estimation of normal and pathological egg structure, as well as its location. Nine quails have been examined; one of the birds had an egg located in the uterus. Obtained measurements were 18.06 mm in length and 17.27 mm in width. It has been observed that the eggshell was irregularly calcificated in that the eggshell thickness varied from 0.97 to 2.13 mm. The application of this technique in poultry large scale farming could be economically profitable. Determining the precise location of the egg and its structure may lead to more relevant diagnosis and treatment. Early detection of any pathological change would prevent its spreading among other individuals, thus improving flock's productivity.

P 216 | Effect of parity on serum nonesterified fatty acid, HDL-cholesterol and apolipoprotein A-I levels and paraoxonase-1 activity in Holstein cows during the transition period

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The transition from the non-lactating to lactating stage causes nutritional and metabolic stress in dairy cows. Parity is an important factor that could influence the ability of cows to cope with demanding energy and metabolic conditions during the transition period. The aim of this study was to assess the effect of parity on lipid metabolism, apolipoprotein A-I (ApoA-I) and paraoxonase-1 (PON1) activity in transition dairy cows. The study included 24 dairy cows assigned to two groups according to the number of lactations: primiparous (n = 12) and multiparous cows (n = 12). Blood samples were collected on days −30, −10, −2, 0, 5, 12, 19, 26 and 60, relative to calving. Serum nonesterified fatty acid (NEFA) concentration was measured using the Randox NEFA kit on the biochemical analyzer SABA 18 (AMS, Rome, Italy),

while high-density lipoprotein-cholesterol (HDL-C) concentration was assayed by the method based on selective inhibition of non-HDL fractions by means of polyanions on Beckman Coulter AU 680 (Beckman Coulter Biomedical Ltd., Ireland). ApoA-I was assayed by the bovine ELISA kit (NovaTeinBio, USA). PON1 activity was measured by the spectrophotometric assay with paraoxon as a substrate on Beckman Coulter AU 680. Statistical analysis was performed using the SAS 9.3 Software. There were no significant differences in NEFA, HDL-C, ApoA-I and PON1 between primiparous and multiparous cows during the transition period ($p > 0.05$). Nevertheless, in all cows, PON1 and HDL-C were significantly lower ($p < 0.05$) at parturition, while NEFA was significantly elevated at calving ($p < 0.05$). Results indicated that lipid metabolism, ApoA-I and PON1 activity were not influenced by parity. However, NEFA, HDL-C and PON1 were greatly influence by the transition period.

P 217 | Scriptaid is a novel agent that can be used for the epigenetic transformation of in vitro maturing pig oocytes providing the source of recipient cytoplasm for somatic cell nuclear transfer (SCNT)

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This study was undertaken to examine whether the epigenomic modulation of in vitro maturing oocytes that was triggered via new-generation HDAC inhibitor (scriptaid; SCPT) affects their capability to acquire the meiotic maturity and to support the ex vivo development of porcine cloned embryos reconstructed with fetal fibroblast (FF) cell nuclei. Nuclear recipient oocytes had been matured for 20 to 21 h in TCM 199 supplemented with 1 mM db-cAMP, 5 mIU/ml pFSH, 0.1 IU/ml hMG, 10% FBS, 10% pFF, 10 ng/ml rhEGF, 5 ng/ml rh-bFGF and 0.6 mM L-Cys. Subsequently, they were cultured for a further 23 to 24 h in db-cAMP- and pFSH+hMG-depleted medium enriched with 350 nM SCPT. Sequential IVM in the SCPT-deprived and SCPT-enriched medium contributed to reaching the MII stage by 155/164 (94.5%) oocytes as compared to 134/162 (82.7%) oocytes in the SCPT-untreated group. The frequencies of dividing embryos (125/143; 87.4%), morulae (96/143; 67.1%) and blastocysts (51/143; 35.7%) that developed from SCNT-derived oocytes exposed to SCPT during IVM were found to be significantly higher than in the SCPT-untreated group (85/124; 68.5%, 53/124; 42.7% and 27/124; 21.8%, respectively). Summing up, increased competences of cloned pig embryos to complete their development to the morula/blastocyst stages suggest the improved epigenetic reprogrammability of FF-inherited genomic DNA in an epigenomically-matured host ooplasm that has undergone exposure to SCPT. (The work was supported by The National Centre for Research and Development (grant number INNOMED/I/17/NCBR/2014).)

P 218 | Effect of donkey sperm selection before semen cooling on motile sperm parameters after 24 and 48 h of cold-storage

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The aim of this study was to assess the effect of sperm selection by colloid single-layer centrifugation in comparison to sperm washing and no centrifugation prior cooling, on donkey sperm parameters after 24 and 48 h of cold-storage. Eleven ejaculates from three different Andalusian donkeys were collected by artificial vagina. After collection, semen were divided into three aliquots and submitted to three different treatments; centrifuged control samples (UC), sperm washing at 400 g/7 min (SW) and colloid single layer centrifugation (300 g/20 min) using Androcoll-E (SLC). After that, semen samples were diluted in INRA96, slowly cooled to 5°C and stored for up to 48 h. Total (TM, %) and progressive sperm motility (PM, %) were assessed by CASA after 24 and 48 h of cold-storage. The results were expressed as mean ± standard error. Different letters indicate significant differences ($p < 0.05$). At 24 h of cold-storage, SLC showed the highest values followed by SW and UC for TM (49.99 ± 7.39^a vs. 40.26 ± 4.55^b vs. 29.47 ± 5.06^c) and PM (45.38 ± 7.95^a vs. 34.79 ± 4.76^b vs. 25.00 ± 4.91^c), respectively. Additionally, after 48 h of cold-storage SLC also showed the best results in comparison to SW and UC for TM (47.13 ± 5.75^a vs. 25.12 ± 3.04^b vs. 18.52 ± 3.60^b) and PM (41.91 ± 5.66^a vs. 19.32 ± 3.04^b vs. 13.44 ± 3.13^b), respectively. In conclusion, sperm selection using SLC before cooling improved donkey sperm motility after 24 and 48 h of cold storage when compared to sperm washing and uncentrifuged control samples.

P 219 | In vitro survival of bovine embryos cultured singly or in groups after vitrification and warming

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In cattle, group embryo culture normally improves in vitro development. However, single embryo culture is useful for modelling human embryo culture, tracebility and sanitary purposes. It is unknown whether benefits of group persist in culture beyond vitrification. We retrospectively analyzed in vitro survival of 2,974 Day-7 and Day-8 expanded blastocysts (EXB) vitrified in DMSO + ethylene-glycol in fibreplugs. Embryos were produced in vitro from slaughterhouse ovaries in the course of 9 experiments, concerning 32 experimental groups and controls. Post-warming culture was performed in droplets of 25 µl mSOFaaci + 6 g/l BSA + 10% FCS under mineral oil. Droplets (N = 402) containing 1, 2–3, 4–5, 6–7, 8–9 and >10 embryos were cultured for 48 h under 5% CO_2 + 5% O_2 and 37.8°C. Data were analyzed

by GLM and REGWQ test ($p < 0.01$). Re-expansion rates after 24 h were reduced in embryos cultured singly (71.6 ± 6.3) vs. embryos cultured in groups (N = 2–3: 80.1 ± 3.9 to N > 10: 86.1 ± 3.2). Hatching rates were also lower in embryos cultured singly vs. group culture at 24 h (N = 1: 3.1 ± 5.5 vs. N = 8–9: 18.7 ± 3.6 to N = 2–3: 26.3 ± 3.5, respectively) and 48 h (N = 1: 21.8 ± 6.9 vs. N > 10: 45 ± 3.5 to N = 6–7: 57.6 ± 4.3). Single culture reduces in vitro survival after vitrification and warming. However, survival is not affected by embryo density, suggesting that cooperative effects persist after vitrification/warming. (Funding: MINECO (AGL2012-37772) and FEDER; COST Action FA1201 (Epiconcept); Principado de Asturias, Plan de Ciencia, Tecnología e Innovación 2013–2017 (GRUPIN 14-114))

P 220 | Effects of the antimicrobial peptide PMAP-37 on boar sperm quality and its effectiveness against bacterial load

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The increasing resistance of bacteria to the antibiotics present in semen extenders formulation has led to the search of alternatives. One of the most promising is the use of antimicrobial peptides (AMP). However, AMP can impair boar sperm quality, so that their deleterious effects on sperm quality may be higher than their effectiveness against bacteria. So, the aim of this study was to determine whether the porcine myeloid antimicrobial peptide 37 (PMAP-37) had any effect upon boar sperm quality and bacterial load. For this purpose, three different concentrations of this peptide (0.5 μM, 1 μM and 3 μM) were added to extended semen without antibiotics (Abs); two controls, one without PAMP-37 and Abs, and other with only Abs were used. Total (TMOT) and progressive (PMOT) sperm motility, sperm viability, osmotic resistance (ORT) and bacterial concentration were assessed before the addition of PAMP-37 or Abs and at 1, 3, 6, 8 and 10 days post-addition. Results revealed that, despite a drop in the TMOT and PMOT in the treatments and controls, PMAP-37 was more effective in keeping sperm viability than controls ($p < 0.05$). However, PMAP-37 at 3 μM produced a significant reduction ($p < 0.05$) in ORT values when compared to controls and other concentrations. Finally, PMAP-37 at 3 μM was the most efficient treatment in controlling bacterial load. In conclusion, these results suggest that PMAP-37 could be a suitable candidate to replace Abs in semen extenders. Nevertheless, further studies are still required to improve its effectiveness.

P 221 | Proposal to minimize the difficulty of recording abortion data in sheep

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Background: The most frequent viral diseases which can cause abortion in sheep are Blue tongue virus, Border disease virus and Schmallenberg virus. The diagnosis of abortion, namely virus-induced represents a challenge to field clinicians, since clinical signs of the dam are discrete, non-specific and variable. On the other hand, while some fetuses reveal characteristic and visible malformations, others do not reveal any lesions. To improve the diagnostic procedure an appropriate case history is needed and fresh samples (fetus, placenta and umbilical cord) should be sent to a specialized laboratory. Objectives: The authors suggest a registration method of all mandatory data, in order to further assist the diagnosis of viral diseases at the laboratory, including the most frequent congenital malformations reported in sheep abortions. Methods: Abortion samples of suspected viral origin were collected and all data were recorded, and in worksheet tables optimized for this purpose. Results: The authors documented malformations in abortions using macroscopic images, emphasizing the importance of maintaining good records of each case, and proposing practical and effective worksheet tables to assist the fieldwork. Conclusions: Economic losses may be diminished if the awareness of the importance of early detection of viral diseases causing abortion is raising. Clinicians in the field are stimulated to use an appropriate method of data collection to register each case of abortion, in order to contribute to a precise diagnosis and to plan subsequent epidemiological studies. (Funding: CECAV (UID/CVT/00772/2013); CITAB; FCT (UID/AGR/04033/2013 e POCI-01-0145-FEDER-006958); CI&DETS, Ovislab ICT-2013-05-004-5314 ID-6475)

P 222 | Integrin αvβ3, E-cadherin and β-catenin immunoexpression in the canine trophoblast in apposition and invasion stages

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In early canine pregnancy, embryo apposes and invades the upper layers of the maternal endometrium. Embryo-maternal interactions depend on the plasticity of the Intercellular junctions. This study intends to describe and compare by immunohistochemistry the expression of Integrin αvβ3, and of E-cadherin and β-catenin in the trophoblast during the apposition and invasion stages of canine implantation. Five samples of canine pregnant uterus for each apposition and invading implantation stages (pregnancy days 12–15 and 17–19; day 0 = ovulation; blood progesterone levels >5 ng/ml) were stained with the following monoclonal antibodies: Integrin αvβ3 (clone LM609; Chemicon International, USA), E-cadherin and β-catenin (Clones 4A2C7 and CAT-5H10, respectively; Invitrogen,

USA), at 1:100 dilution. Strong trophoblast positive staining was observed in the apposition phase, with reinforcement in the outer apical membrane. Trophoblast cells in the labyrinth cords showed strong positivity but no membrane reinforcement. The trophoblast presented higher intensity scores for both E-Cadherin and β-catenin during the apposition phase but progressively lost the membrane immunostaining, particularly for β-catenin, when the trophoblast participates in the labyrinth cords. Loss of the integrin αvβ3 polar labeling and a reduction in the strength of E-cadherin junctional complexes, as observed during the invasion phase, would contribute to a higher plasticity of the trophoblast lining and, therefore, may facilitate embryo migration through the maternal endometrium. Loss of E-cadherin membrane immunolabeling may also play a protective role against apoptosis (Gallegos et al. 2014, Dev Cell, 30:3–4). (The Project UID/CVT/00772/2013 (FCT) sponsored this work.)

P 223 | Prevalence and diagnosis of hydrocephalus and hydronephrosis in kittens

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The occurrence of congenital malformations in kittens is rare (about 1–2% of the population). Breeders selection have played an important part in the evolution of genetic diseases in cats. Simultaneous occurrence of congenital defects of the nervous system and urinary tract is extremely rare. This study aimed to evaluate a litter of only two, male cats (100% litter), aged 5 weeks from a healthy 4 year old mixed, breed queen. Kittens had clinical symptoms of poor appetite, dehydration weakness, hair loss, nystagmus, and ataxia. Blood tests showed slight anemia and increased creatinine and urea levels. The ultrasound examination revealed hydronephrosis with an ectopic ureter. Previously undiagnosed and untreated urinary tract infection led to renal failure. The CT scan of the brain showed hydrocephalus. The samples of cerebrospinal fluid (CSF) macroscopically and in basic laboratory tests were normal. Despite treatment, the two kittens died. Post-mortem examinations confirmed the earlier diagnosis. Incompatibility between production and absorption of CSF led to its accumulation and progress to hydrocephalus. The diagnosis can be facilitated by the change in the kitten's head contour and its magnification. Moreover, the histopathological examination of the kidney showed chronic inflammation of the renal parenchyma and numerous necrotic foci. Congenital malformations of the urinary tract in young kittens are often recognized accidentally. They may result in progressive destruction of renal parenchyma, leading to chronic renal failure. Especially pathogenic are malformations which cause urinary retention and predispose to renal infections. Unfortunately, the conservative treatment of this type of malformations is tough and usually results in fatal outcome.

P 224 | Adrenocorticotrophic hormone, aldosterone y cortisol concentrations during estrous cycle in healthy Spanish Purebred mares

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The aim of present study was analyze the relationship between ACTH, CORT and aldosterone (ALD) during estrous cycle of healthy Spanish Purebred mares. Daily blood samples from 24 cycling mares aged between 4 and 17 years were taken from day 3 (day −3) prior to ovulation, until day 5 of diestrous. Serum ACTH, CORT and ALD concentrations were analyzed by competitive immunoassay. ALD concentrations decreased progressively from the day of ovulation (535.3 ± 71.49 ng/ml) until day 2 of diestrous (495.2 ± 14.75 ng/ml), although subsequently increased to a peak on day 5 (576.1 ± 26.71 ng/ml) ($p < 0.05$). Compared with day −3 (64.79 ± 2.015 ng/ml), CORT levels increased in the day of ovulation (87.68 ± 10.71 ng/ml), and decreased after that ($p < 0.05$). Mean value of ACTH was 17.68 ± 6.71 pg/ml and showed no variations. Significant correlations between ACTH and CORT ($r = 0.33$) during follicular phase and ACTH and ALD during luteal phase ($r = 0.66$) were obtained ($p < 0.05$). These results suggest an increase in the stimulation of ACTH secretion and in the synthesis of CORT at the time of ovulation. However, the close relationship between ACTH and ALD might suggest a greater participation of this mineralocorticoid in the development of the corpus luteum and synthesis of P4 during diestrus, despite the involvement of the renin-angiotensin system in the synthesis ALD.

P 225 | Effect of number of $PGF_{2\alpha}$ doses in 5-d CO-Synch progesterone-based synchronization protocol on fixed-time artificial insemination pregnancy rate in Holstein heifers in Cukurova Region in Turkey

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The objective of the study was to determine the effect of 3 different $PGF_{2\alpha}$ treatments in a 5-day CO-Synch progesterone-based synchronization protocol on AI pregnancy rates in Holstein heifers in Cukurova region, Turkey. We hypothesized that two doses of $PGF_{2\alpha}$ (PGF) administered concurrently or 6 h apart would result in greater AI pregnancy compared to single dose of PGF on Day 5 at CIDR (Controlled internal drug release) removal. Heifers were randomly assigned to one of these groups: 1 PGF - received 25 mg IM of Dinoprost at CIDR removal; 2 CoPGF - received 50 mg IM of Dinoprost at CIDR removal,

and 2 PGF - received 25 mg IM of Dinoprost at CIDR removal and an additional 25 mg IM of Dinoprost 6 h later. All heifers received a CIDR (1.38 g of progesterone) and 10 μg IM of Buserelin on Day 0. The CIDRs were removed on Day 5, and each heifer was given PGF based on the assigned treatments. On day 7, each heifer was given Buserelin (10 μg IM) and artificially inseminated at 56 h after CIDR removal. Heifers were examined for pregnancy status 45 days after AI. No significant differences were found in pregnancy rates after AI in heifers treated with 2PGF at 6 h apart compared to heifers treated with 2Co-PGF [45% (9/20); $p > 0.05$] or 1 PGF [55% (11/20); $p > 0.05$]. No difference was observed between 2Co-PGF and 1 PGF groups ($p > 0.05$). In conclusion, pregnancy rates obtained after the protocol of 2PGF at 6 h apart on Day 5 at CDIR removal in a 5-Day CO-Synch + CIDR were similar but not greater than the single dose protocol of PGF.

P 226 | Desquamation of germ cells in the seminiferous epithelium during testicular regression due to exposure to short photoperiod in the Syrian hamster (*Mesocricetus auratus*)

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Testicular regression due to exposure to a short photoperiod in the Syrian hamster (*Mesocricetus auratus*) causes both a loss of testicular volume and a shortening of the seminiferous tubule. This is due to the massive loss of germ cells resulting from both a decrease in cell proliferation and an increase in apoptosis. In other seasonally breeding species, it has been observed that cell loss is mainly due to the loss of round spermatids through desquamation. The aim of this study was to determine the possible role that the desquamation of germ cells may play during testicular regression due to short photoperiod in the Syrian hamster. For this, 20 hamsters were used (15 treated with a photoperiod of 8:16 L-D for 12 weeks and 5 controls). Testes were fixed in methacarn, embedded in paraffin and stained with hematoxylin-eosin. Three testicular regression groups were established (Mild Regression (MR), Strong Regression (SR) and Total Regression (TR)). For each animal, four random testicular sections were studied and a total of 80 tubular sections were analyzed. In each tubular section, the number of desquamated germ cells in the lumen was counted. The results pointed to a significant ($p < 0.05$) increase in the percentage of tubular sections undergoing desquamation in the TR group compared with the other groups. In conclusion, this initial study shows that germ cell desquamation occurs at the end of the testicular regression process and is probably an additional factor in the maintenance of epithelium depletion when it is already fully regressed. (Funded 19892/GERM/15; Fundación Seneca; CARM)

P 227 | Investigation of nuclear factor erythroid 2-related factor 2 (NRF2) target genes in the ovine endometrium during early pregnancy

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Reactive oxygen species (ROS) involve in several physiologic and pathologic processes of reproduction system. Physiological levels of ROS play a regulatory role at follicle and oocyte development and implantation. Transcription factor NRF2 is activated by ROS and controls expression of antioxidant proteins. Kelch-like ECH-associated protein 1 (KEAP1) and Cullin 3 (CUL3) have roles in activation and ubiquitination of NRF2. Aim was to determine the expression profiles of NRF2 target genes during the early pregnancy in the ewe. Endometrial tissues were collected on days 12 (n = 5), 16 (n = 5), 22 (n = 5, artificially extended cycle by exogenous progesterone) of estrous cycle and 12 (n = 5), 16 (pre-attachment phase, n = 5), and 22 (post-attachment phase, n = 5) of pregnancy from ewes. Total RNA was isolated, converted to cDNA and qRT-PCR was employed to analyze expression levels. Expression of glutathione S-transferase 1, glutamate-cysteine ligase catalytic subunit, thioredoxin reductase-1, catalase, glucose-6-phosphate dehydrogenase, superoxide dismutase-1, CUL3, and glutathione reductase did not differ among the pregnancy and cycle days. Compared to cyclic ewes, expression of heme oxygenase-1 increased on days 16 and 22 of pregnancy. Expressions of KEAP1, NAD(P)H quinone oxidoreductase-1, glutathione peroxidase-2 decreased in both cyclic and pregnant endometria after day 12. NRF2 expression increased on day 16 of pregnancy. Pregnancy, to some extent, seems to regulate NRF2-related antioxidant system pathway during embryonic attachment in the ovine pregnancy. It can be concluded that embryonic exposure to different levels of ROS could be justified by NRF2 related proteins in the endometrium. (This study was partially funded by TUBITAK grant 214O643 to M. Kose.)

P 228 | The outlook of genomic associations for daughter fertility traits detected on *Bos taurus* X chromosome in Russian Holstein population

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Reproduction features in dairy cattle have a complex nature and depend on cow productivity level, environmental factors as well as semen quality performances. In this regard, our work was aimed at analyzing the X chromosome in genome-wide association studies of daughters' fertility traits. The GEBVs for days open (DO), number of inseminations per calving (IC), calving interval (CI), gestation

length (GL), age at first insemination (AI) and age at first calving (AC) were calculated by GBLUP approach using information about 256 genotyped Holstein sires (Illumina Bovine SNP50 v2 chip). After quality check, the total number of SNPs was 41730 (Plink 1.9). The threshold for FDR test (by Benjamini & Hochberg, 1995) was set to 10%. We found some significant associations on BtX with the studied traits: rs41571070 (MOSPD2, $p = 1.1 \times 10^{-4}$) and rs41625051 (MSL3, $p = 1.6 \times 10^{-4}$) for IC; rs108974058 (MAGEB16, $p = 9.7 \times 10^{-5}$) for CI; rs41609659 (ADGRG4, $p = 1.2 \times 10^{-5}$) for AI as well as rs41662092 (LOC104976397, $p = 3.8 \times 10^{-5}$) and rs43710081 (LOC101905348, $p = 9.7 \times 10^{-5}$) for AC. The FDR for DO and GL varied from 0.14 to 0.18, but we detected the conservative mutations in COL4A6 (rs110304690) and TLR8, TLR7 (rs41659076) genes which were associated with these traits. The total additive effect of SNPs on fertility traits determined by coefficient determination was from 4.4 to 7.5%. Our findings indicate the applicability of genomic evaluation in improvement of Russian Holstein cattle reproductive performance. (Supported by the Russian ministry of education and science, unique project no. RFMEFI60414X0062.)

P 229 | Oxide-antioxidant status of dairy cows under postpartum pyo-catarrhal metritis

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The aim of the research was to evaluate the activity of lipid peroxidation (LPO) and the level of antioxidant protection system (AOPS) in dairy cows with clinical pyo-catarrhal metritis (n = 9) and in cows with normal postpartum uterine involution (control, n = 9). The following parameters were determined: malonic dialdehyde (MDA) content, sum of stable metabolites of nitric oxide (NOo), activity of catalase, glutathione peroxidase (GPx), glutathione reductase (GR) in animals' venous blood; superoxide dismutase (SOD) in erythrocytes and concentrations of vitamin E and carotene in blood serum. MDA concentration in cows with metritis exceeded the concentration of it in healthy animals by 76%, NO◦ - 2.9 times, SOD activity – by 46%, catalase activity – by 45,7% ($p < 0.001$). This reflects the development of the oxidative stress syndrome. The increase of GPx activity level by 65.8% and GR activity level by only 14.6% indicates an imbalance in the glutathione link of AOPS. Low concentrations of vitamin E (lowered by 35.3%) and carotene (lowered by 36.4%; $p < 0.01$) show a decreased capacity of AOPS nonenzymatic link. So, uterine inflammatory processes in cows with a high level of LPO intensity are associated with an increased NO◦ synthesis and with an imbalance in AOPS. The results obtained demonstrate new aspects in comprehension of postpartum metritis pathogenesis and broaden the possibilities of increasing the effectiveness of their treatment and prophylaxis.

P 230 | Novel lytic peptide-GnRH treatment leading to sterility: proof of concept study in rats

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We propose that lytic peptide (Phor14) conjugated with GnRH will compete with naturally occurring GnRH for binding to receptors localized in the anterior pituitary, and will damage only the pituitary cells that express GnRH receptors (GnRHR). When administered intravenously (iv) or intramuscularly (im), the conjugate will selectively bind to GnRHR and ablate the gonadotrophs, leading to sterility. Forty Wistar rats 12 weeks of age were divided into five groups according to the Phor14-GnRH treatment. Each group consisted of 4 males and 4 females. Group one: control; group two: 1.2 mg of Phor14-GnRH/1 kg body weight iv; group three: 6 mg of Phor14-GnRH/1 kg bw iv; group four: 6 mg of Phor14-GnRH/1 kg bw im; group five: 12 mg of Phor14-GnRH/1 kg bw im. After 21 days, rats were euthanized. Blood samples were collected for subsequent FSH and LH profile measurements. From all animals the pituitary, uterus with ovaries or testes with epididymides and prostate, heart, thyroid, adrenal glands and liver were collected for routine (hematoxylin/eosin staining) histopathological analyses. FSH serum level was the lowest in females that had received 6 mg of Phor14-GnRH/kg iv ($p < 0.05$) or 12 mg of Phor14-GnRH/kg im ($p < 0.01$). The lowest numbers of small, mid and mature ovarian follicles were observed in group five compared with the control ($p < 0.01$) and all other groups ($p < 0.05$). Single colloidal cysts were observed in the pituitary of 3 rats that had received the last treatment/12 mg of Phor14-GnRH/1 kg bw im. However, none of the treatments affected the number of spermatids and spermatocytes during the 21 days of experimentation. The last treatment/12 mg of Phor14-GnRH/1 kg bw im was selected for further, long-term experiments.

P 231 | Left flank unilateral ovariectomy in cattle under distal paravertebral block and standing ketamine stun

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Although general anesthesia is used in cattle, there are some risks. Local or regional anesthesia is safe, effective, and still the most desirable procedure in many situations. Surgical procedures including unilateral ovariectomy can be performed safely in cattle using a combination of physical restraint, mild or stun sedation, and local

or regional nerve blocks. The aim of the study was to obtain the luteal tissue from ovaries while at defined estrous cycle stage for identification of early pregnancy factors using precise molecular biology tools to allow development of early embryonic death prophylaxis. Ten Holstein-Frisian type cows during an induced estrous cycle were enrolled into the study. The elective, surgical procedure under distal paravertebral nerve block combined with standing ketamine stun sedation was performed in all animals. The distal paravertebral nerve block (between Th13 and L3 vertebra) following pharmacological stun sedation with xylazine 5–30 mg/100 kg (Xylapan 20 mg/ml, Vetoquinol Biowet), ketamine 0.04 mg/kg (Bioketan 100 mg/ml, Vetoquinol Biowet), butorphanol 0.01 mg/kg (Butomidor 10 mg/ml, Richter Pharma AG) combined with physical restraint was performed. The ovaries were removed to obtain luteal tissue for further detailed examinations. Postoperative antibiotic therapy with procaine benzylpenicilin 1 ml/30 kg (Penillin 30%; 300 mg/ml; ScanVet Poland) in combination with analgesia flunixine 2 ml/45 kg (Flunimeg 50 mg/ml; Scanvet Poland) continued during the following 3 days. No scoliosis and pelvic limb weakness were observed during surgery. Moreover, no recumbency occurred. Distal paravertebral nerve block provides great quality, unilateral analgesia of the paralumbar fossa for flank laparotomy being alternative to the inverted L block.

P 232 | Usefulness of the double biopsy for diagnosing endometritis in the mare

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Histological examination of the endometrium is considered to be the reference standard to diagnose endometritis in mares. The aim of this study was to evaluate if there are changes in the polymorphonuclear cells (PMNs) infiltration and Kenney-Doig score and in expression of cyclooxygenase-2 (COX-2) and fibronectin between two biopsy samples taken from one mare. The research was conducted on 53 subfertile mares, Icelandic horses, which had been unsuccessfully inseminated during three estrus cycles. All of the mares had no discharge and no intrauterine fluid. Two endometrial biopsies were taken from base of either uterine horn with Kevorkian biopsy forceps and fixed in 4% formalin. The biopsies were examined for the presence of PMNs infiltration within the luminal epithelium, stratum compactum and stratum spongiosum, as well as for the Kenney-Doing score. The biopsies were considered as evidence of acute endometritis if there were more than three PMNs per five fields of high magnification (×400). The biopsies were also stained for COX-2 and fibronectin expression. We found statistically significant differences in the Kenney-Doig score, the COX-2 expression in the superficial epithelium and fibronectin expression in stroma between the left and the right horn. To conclude, our study has showed, that one single biopsy can be insufficient for diagnosing endometritis.

P 233 | Effect of IGF-I on DNA fragmentation and in vitro maturation of bovine oocytes subjected to heat shock

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The aim of this study was to evaluate the effect of different concentrations of IGF-I added to the IVM medium on DNA fragmentation and maturation of oocytes subjected to heat shock (HS). Immature bovine oocytes aspirated from ovaries obtained from slaughterhouse were selected and randomly allocated in factorial experiment design 3 × 2. IGF-I (0; 25 and 100 ng/ml - Sigma) was added to the IVM medium and two incubation conditions (conventional (CON): 24 h at 38.5°C and 5% CO_2; or HS: 12 h at 41°C followed by 12 h at 38.5°C and 5% CO_2) were tested. After IVM, the oocytes were denuded and fixed in 4% paraformaldehyde in PBS in order to evaluate by the TUNEL assay (Promega) the percentage of DNA fraTUNEL positive oocytes (DNA fragmentation) the percentage of DNA fragmented oocytes and nuclear maturation (percentage of oocytes in metaphase II). Four replicates were performed with a total of 835 oocytes. Data were analyzed considering the Binomial distribution using the Proc Genmod (SAS). Values are shown as mean ± SEM. There was no interaction ($p > 0.05$) between IGF-I concentration and incubation conditions. The percentage of TUNEL positive oocytes was not affected ($p > 0.05$) by the addition of IGF-I (10.4 ± 3.6%; 10.7 ± 4.0% and 6.7 ± 3.0% with 0, 25 and 100 ng/ml, respectively); however, it was increased ($p < 0.05$) by the HS (CON = 7.0 ± 2.2%; HS = 13.8 ± 3.2%). The percentage of nuclear maturation was not affected ($p > 0.05$) by IGF-I (62.5 ± 5.7%; 67.5 ± 4.1% and 67.7 ± 4.5% with 0, 25 and 100 ng/ml, respectively) and HS (CON = 77.7 ± 3.9%; HS = 70.6 ± 3.6%). In conclusion, the addition of IGF-I to IVM medium of bovine oocytes subjected to HS, even at low doses, had no effect on DNA fragmentation and maturation.

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P 234 | Hemolytic and non-hemolytic *Escherichia coli* strains induce differential immune response in canine endometrial epithelial and stromal cells

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Canine pyometra *E. coli* isolates display several virulence factor genes. α-hemolysin (hlyA), was detected in 48% of pyometra *E. coli* strains (Mateus et al. 2013, Vet Microbiol 166:590–594). This study evaluated the effect of HlyA in the early inflammatory response

of canine endometrial epithelial and stromal cells. Primary culture of endometrial epithelial and stromal cells were in vitro stimulated with Pyo14 (non-hemolytic *E. coli*), Pyo18 (hemolytic *E. coli*) and Pyo18ΔhlyA (isogenic mutant of Pyo18) for 1 and 4 h. qRT-PCR was used to evaluate the transcription levels of genes coding for cytokines resulting from TLR4 activation (Myd88-dependent and independent pathways). In epithelial cells, the three *E. coli* strains induced similar transcription levels of cytokines at each endpoint of incubation. In stromal cells, after 1 h of incubation and compared to Pyo18ΔhlyA and Pyo14, respectively, Pyo18 induced a lower transcription level of IL-1β (0.99 vs. 152.0 vs. 50.9 fold increase (FI), $p < 0.001$), TNFα (3.2 vs. 49.9 vs. 12.9 FI, $p < 0.05$) and IL-10 (0.4 vs. 3.6 vs. 2.6 FI, $p < 0.001$). Incubation with Pyo18 for 4 h induced death of all stromal cells and a decrease by half of epithelial cell numbers. In conclusion, the cytotoxic effect of HlyA is more severe in endometrial stromal than in epithelial cells. Hemolytic *E. coli* induces down-regulation of IL-10, TNFα, and IL-1β transcription in stromal cells, which delay the activation of the immune response. These features may be relevant in the pathogenesis of hemolytic *E. coli* canine pyometra. (Funding: PTDC/CVT/66587/2006; UID/CVT/00276/2013)

P 235 | Impact of deslorelin as inductor of ovulation for timed-artificial insemination in primiparous *Bos taurus indicus* cows

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The aim of the study was to verify the efficiency of deslorelin (DES) as inductor of ovulation in timed-artificial insemination (TAI) protocols with frozen semen on pregnancy rate (PR, %) in primiparous *Bos taurus indicus* cows. Hundred seventy six animals were divided into three groups as follows: GNDES (Group No Deslorelin; n = 59) which received on day 0 (d0) an intravaginal device with 0.558 g of progesterone (P4) + 2.0 mg estradiol benzoate (EB) (IM); d8 P4 removal + 0.5 mg of estradiol cypionate (EC) + 150 μg of cloprostenol + 400 IU of equine chorionic gonadotropin (eCG); in d10 TAI was performed; GDES (DES group; n = 60) the same as GNDES + 1.0 mg (IM) DES acetate in TAI day; GDES6 (DES applied 6 h before TAI, n = 57) the same as GDES, but DES injected 6 h before TAI. The pregnancy rate was analyzed by logistic regression using the mixed GLIMMIX procedure of the SAS system, considering a 5% significance level. The pregnancy rate (PR) in TAI was 40.6; 53.3; 43.8% and at the end of the breeding season (BS) was 72.9; 81.7; 70.2% for GNDES, GDES and GDES6, respectively. In conclusion, the DES brought an indicative of improvement in the PR for TAI, in the treated groups (3.2–12.7% more) than control, however without significance; the BCS, open days and the reproductive status of anestrous or cyclicality, did not influenced the PR in TAI at 60 days or at the end of the BS.

P 236 | In vitro production of cattle-wisent hybrid embryos

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The aim of this research was to study processes of in vitro fertilization and the subsequent development of embryos produced using cattle (*Bos Taurus*) oocytes and the epididymal sperm of wisent, also called the European bison (*Bison bonasus*). Therefore, slaughterhouse-derived cattle oocytes were subjected to a standard in vitro maturation procedure. The frozen/thawed ejaculated sperm from a Russian Black Pied bull was used as a positive control. The sperm from both species was prepared by the swim-up method using Sperm-TALP medium. Matured oocytes were fertilized in vitro with homologous (n = 191 oocytes) or heterologous (n = 202 oocytes) sperm in TALP containing 10 μg ml^{-1} heparin, PHE (20 μM penicillamine, 10 μM hypotaurine, 1 μM epinephrine), and 0.1% MEM non-essential amino acids. After an 18-h co-incubation period, a part of oocytes was examined for fertilization characteristics. The remaining oocytes were cultured for 7 days in CR1aa medium and evaluated for cleavage and blastocyst rates. The rates of sperm penetration, normal fertilization, cleavage and the blastocyst formation were similar in both sperm species (respectively, 90.0 ± 0.3, 78.3 ± 1.7, 72.4 ± 2.5, and 26.7 ± 1.9% for cattle sperm and 93.3 ± 1.7, 73.2 ± 2.3, 77.1 ± 2.5, and 27.8 ± 3.5% for wisent sperm). However, the proportion of oocytes with signs of polyspermy was higher in the case of heterologous fertilization than in the case of homologous one (21.6 ± 0.8 vs. 8.5 ± 1.8%, $p < 0.05$). Our findings demonstrate that the developmental capacity of cattle-wisent hybrid embryos is similar to that of cattle embryos despite of a higher level of polyspermy after heterologous fertilization. (The research was supported by Program of Presidium of the Russian Academy of Science, project no. IV.13.3.)

P 237 | Comparison of canine sperm characteristics and bacterial contaminations in egg yolk and soy lecithin extenders during 4 days chilled storage

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Two semen extenders were included into the study; (1) a Tris egg yolk and (2) Tris soy lecithin. Sperm motility, viability and membrane function were evaluated. For the sperm characteristics, the semen samples were collected from 18 clinically healthy dogs, of proven fertility, randomly divided into six groups. Each group was divided into four aliquots; samples were diluted either in Tris egg yolk extender or Tris soy lecithin extender in a concentration of 0.04%, 0.1% and 1%. The

diluted semen aliquots were preserved at 4°C. For the investigation of bacterial contamination, semen samples were collected from 21 healthy and fertile dogs then, randomly divided into seven groups (3 dogs per group). Each group was divided into two aliquots; samples were diluted in 20%Tris egg yolk extender or 0.04% Tris soy lecithin extender. The diluted semen aliquots were preserved at 4°C and processed for bacterial culture at 1st and 4th day of storage. The results showed no significant difference between groups, as similar rates of motility, viability and spermatozoal membrane function in all four periods; after dilution, at day 1, 2, 3 and day 4 of cold storage. Although no significant difference, canine semen extended in Tris soy lecithin extender tended to have lower bacterial contamination. In conclusion, soy lecithin is a valid alternative substance to egg yolk for the canine semen chilling extender. Increasing soy lecithin concentration did not improve chilled semen quality. Therefore, the low concentration of soy lecithin (0.04%) in Tris extender can be alternatively used to chill canine sperm with some advantages. Furthermore, instant soy lecithin commercial product is cheap, convenient, consistent and hygienic for capable of a canine semen

P 238 | Reproductive efficiency of Simmental cows under rump conformation indexation

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The aim of the study was to determine the possibility of using indexes and indicators of rump conformation to predict the success of insemination and the course of parturition. We included n = 631 Simmental cows kept in 7 herds in the Subcarpathian region. The analysis of milk (protein, fat, SCC) was performed by CombiFoss 6000 and Fossomatic. The study used production data of cows in the 1st to 6th lactation. Zoometric measurements were performed within 15–180 days of calving. Indicators and indexes of conformation, which define the overall phenotype of the tested animals, were calculated. Insemination with semen frozen in straws with a capacity of 25 ml and a concentration of 20 million sperm alive; after thawing, 10 million. Statistical analyses were performed using the SAS package based on multi-way analysis of variance. The basic measurements of height and width, including those identifying the rump conformation, increased up to the fourth lactation. The rump conformation index decreased with successive calvings. The age of animals (number of lactation) determines the number of insemination and the course of parturition. As the difference between height at hips and height at pins increases, the number of inseminations and the easy calving index is increasing. The higher the hip height to wither height index and the pelvis to chest index, the more difficulties in calving are observed and the higher the number of inseminations. Easy parturition and low consumption of semen occurred in cows with a high index of massiveness (chest girth/height at withers ×100).

P 239 | Immunolocalization of the tyrosine kinase III receptor CD117 and vimentin positive cells in the reproductive tract of the mare

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Vimentine and tyrosine kinase type III receptor - c-kit/CD117 are currently the sole practical markers for Interstitial cells of Cajal (ICC) in gastrointestinal (GI) tract and Interstitial Cajal-Like Cells (ICLC) in all tissues contained smooth muscles outside GI. ICLC in myometrium are suspected to have dual functions as pacemakers and conduction pathways for the active propagation of electrical slow waves. Since regulation of uterine contraction processes is critical to equine reproductive success, localization of ICLC was evaluated by immunofluorescent (IF) methods in oviductal and uterine tissues from healthy mares. The fresh whole thickness samples were collected from 25 slaughtered mature mares. Afterwards the collected material was stained with hemotoxylin-eosin, labeled with primary (anty vimentine and anty CD117) and secondary antibodies and imaged by using light and confocal microscopy respectively. The cells with morphologic and immunologic phenotypes similar to the ICC of the gastrointestinal tract were identified inside the oviductal and uterine walls of mares tissues. The CD117 and vimentine positive cells were localized in the muscle layers as fusiform, triangular or starlike-cells with dendritic processes formed a cellular network and were classified as ICLC. The fusiform ICLC were the predominant cell type in oviduct isthmus while triangular or starlike-cells predominated in uterine horns and corpus uteri. Vimentin reactivity was mainly localized within the cell processes, and CD117 has a patchy pattern in cell body. The relative accumulation of ICLC was found in the isthmus and the uterine horn tip. Furthermore, ICLC were localized predominantly near the small blood vessels. The ICLC in reproductive tract of mare can be identified by IF methods.

P 240 | Highly successful method for generation of nuclear-transferred goat embryos using adult blood-derived fibroblast-like cells undergoing sodium valproate-mediated epigenetic transformation

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The purpose of our study was to evaluate the impact of sodium valproate (SV)-based epigenomic modulation of adult peripheral blood-derived fibroblast-like cells (APB-FLCs) on the ex vivo developmental capacity of caprine cloned embryos produced using

this novel type of nuclear donor cells. The enucleated in vitro-matured oocytes were reconstructed with the cell nuclei of serum-starved APB-FLCs that either had been exposed to 3 mM SV for 24 h (Group I) or had not been exposed to SV (Group II). Efficiently reconstituted and activated clonal cybrids were intended to be in vitro cultured. In Group I, from among 146 cultured nuclear-transferred (NT) embryos, 122 (83.6%) were cleaved. The proportions of embryos that developed to morula and blastocyst stages were 85/146 (58.2%) and 51/146 (34.9%), respectively. In Group II, out of 134 cultured embryos, 83 (61.9%) were able to divide, but 48 (35.8%) and 26 (19.4%) reached the morula and blastocyst stages, respectively. Cumulatively, SV-dependent epigenomic modification of APB-FLCs resulted in both cleavage and morula/blastocyst formation rates of NT goat embryos that were found to be enhanced remarkably as compared to those of NT embryos created using SV-untreated nuclear donor cells. This appears to be related to improved ability of donor cell nuclei to undergo epigenetic transcriptional reprogramming in blastomeres of caprine cloned embryos. (This study was supported by the Polish Ministry of Science and Higher Education as the statutory activity No. 02-011.1, which is realized from 2015 to 2017.)

P 241 | PPARs expression profiles in the bovine corpus luteum throughout the estrous cycle

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Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptors, comprising three isoforms: PPARα, PPARβ/δ, and PPARγ. PPARs agonists are arachidonic acid metabolites. Therefore, we hypothesized that the expression profiling of PPARs in corpus luteum (CL) might show different expression patterns during the estrous cycle. The aim of this study was to determine the PPARs at mRNA and protein levels in the bovine CL during stages of the estrous cycle. Corpora lutea were dissected from post mortem cows as following: early luteal I (n = 8), early luteal II (n = 8), mid luteal (n = 8), late luteal (n = 8), and CL regression (n = 8) phases of the cycle. The mRNA and protein expression profiling of PPARs were evaluated, by qReal-Time PCR, Western blotting as well as ELISA, respectively. Results were statistically analyzed by one-way ANOVA followed by Bonferroni test. The expression of PPARA and PPARD mRNAs increased throughout the estrous cycle ($p < 0.05$), and the highest mRNA expression was observed during CL regression phase ($p < 0.05$). PPARG mRNA expression and concentration revealed the highest level during mid luteal phase ($p < 0.05$). Collectively, results have shown different expression pattern of PPARα, PPARβ/δ, and PPARγ at mRNA as well as protein levels in bovine CL ($p < 0.05$), which could play a vital role during the estrous cycle. (This research was supported by National Science Centre 2014/15/N/NZ9/02428.)

P 242 | Stimulating the onset of early mating season in horses

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Spontaneous resumption of the cyclic ovarian function starts by middle of April. The aim of our present study was to compare the stimulatory effect of a novel light treatment (Equilume®) with the long term gestagen treatment. Additional light, starting in December, can hasten the first ovulation of the year. Light of 446 – 477 nm proved to be the most effective to prevent melatonin production, which is responsible for winter anestrus. Fifteen Lipizza problem mares were allotted into three groups. They were checked daily for heat and weekly blood samples have been collected for P4 analysis. (1) Equilume® group: five mares were fitted with light masks (from Dec 3rd until March 26th) emitting a blue light to one eye of the mare between 16 and 23 p.m. every day. (2) Regumate® group: from Feb 5th, five mares received 22 mg altrenogest daily for 15 consecutive days, followed by a single luteolytic dose of $PGF_{2\alpha}$. (3) Untreated control group: five mares received no treatment at all. Results and Discussion: In Group 1 all mares started cycling, three of them middle of March, and the other two in April. They have all been inseminated, four became pregnant. In Group 2, four mares started to cycle early March, one did not show cyclic ovarian activity until end of April. By then, all mares were cycling regularly, have been inseminated and conceived. In Group 3, two animals were cyclic already in February (one conceived), the third started to cycle middle of April and the other two did not show heat at all. Our preliminary experiment indicates that both the light mask and the altrenogest treatment are able to trigger the cyclic ovarian function early in the transition period. Further studies started to refine the protocols and elucidate their physiological background. (9877-3/2015/)

P 243 | Harmful effects of T-2 and Fumonisin B1 co-occurrence on preimplantation stage mouse embryos

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The worldwide contamination of foods and feeds with mycotoxins is a significant problem. In the temperate region, most common pathogens in cereals are the species of Fusarium genus which produce fusariotoxins. Instead of the common occurrence of two or more fusariotoxins in the same sample, co-contamination studies are available in a few amounts, especially in the field of reproduction. The aim of our study was to assess the effect of two fusariotoxins, T-2 and Fumonisin B1 on early embryo development in vitro.

Zygotes were produced in vivo in BDF1 female mice, flushed 6 h after mating and cultured for 96 h in vitro in media contaminated with T-2 (0.5, 0.75 and 1 ng/ml), FB1 (1, 2 and 10 ng/ml) or both toxins (0.5 ng/ml T-2 and 1, 2 or 10 ng/ml FB1). Blastocyst rate, proportion of late blastocysts, cell number and rate of blastomers with micronuclei were assessed. Blastocyst rate in single-dose groups (78.8–86.7%) did not differ from control embryos (86.4%). Contrary, co-occurrence of the two toxins significantly (based on Chi-squared test) decreased the blastocyst rate (14.5, 33.6 and 22.8%, respectively; $p < 0.001$ in all cases). The same tendency was found in the rate of late (expanded and hatched) blastocyst forms. Compared to control blastocysts, all of the toxin treatments decreased the cell number (85.92 ± 3.28 vs. 44.62 ± 4.91 to 69.48 ± 5.61). However, tendencious decreasing of blastomer number was found between co-contamination treatments and corresponding FB1-exposition. Difference in micronucleus rate was not found neither between control (8.9 ± 1.4) and toxin-treated blastocysts (5.2 ± 1.2 to 8.3 ± 1.3). In conclusion, T-2 and FB1 co-occurrence could harmfully affect the development of early embryos in more significant way than single doses.

P 244 | The "male effect" stimulates the estrus behavior in lactating long-days treated goats

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We investigated if the male effect can stimulates the estrus behavior in lactating anovulatory goats previously exposed to 3 months of long-days (14 h light/day). The mean (±SEM) date of parturition of all goats was on December 31st. For the male effect, photo-stimulated bucks were used (n = 4). Seventeen goats were exposed to natural short days during first 3 months of lactation and thereafter were submitted to the male effect (control). Other 22 goats were submitted to artificial long-days during the first 3 months of lactation and thereafter were submitted to the male effect (treated). In 11 control and 12 treated goats the milk yield on a 24-h period were measured at day 16 and 60 postpartum. Initial milk yield did not differ between control and treated goats (1.5 ± 0.2 kg, in both groups). However, at 60 days postpartum, milk yield was higher in treated (2.5 ± 0.3 kg) than in control goats (1.8 ± 0.2 kg, $p < 0.05$). The latency to estrus behavior was not different ($p > 0.05$) between control (56 ± 4.0 h) and treated (62 ± 5.0 h) goats. In both groups, the 100% of goats displayed estrus behavior during the 10 days that were in contact with the bucks. It was concluded that male effect stimulates the estrus behavior in anovulatory lactating goats that are previously exposed to 3 months of long-days.

P 245 | Sphenoid fontanelle in English bulldog puppies with cleft palate

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In adult animals and human skull is characterized by the presence within its area fibrous joints which are sutures joining the individual bones of the skull. Based on today's modern imaging diagnostic we are able to show very accurately changes in bone and cartilage located within the skull. The subject of this study were four dead 1-day old, English bulldog puppies with cleft palate. Imaging diagnostic has been performed using 64-row CT scanner. Thickness of layers amounts to 0.5–3 mm. Volumetric reconstruction of the skull bones was created. Shape and surface of sphenoid fontanelle have been described. Sphenoid fontanelle is localized at the junction of the parietal, temporal and frontal bones. Surface area of left fontanelle is from 17 to 28.6 mm^2, and fontanelle on the right side of the skull is 18–38 mm^2. A difference has been observed in surface area of the left and right fontanelle of 9.4 mm^2 to the right side. Puppies with such pathologies refer to disturbances in the process of skull bones formation and its subsequent overlapping. In the future, need to focus on the difference in overlapping of detected fontanelle. It can cause increased intracranial pressure and consequently damage to the brain structure. Knowledge of the presence of sphenoid fontanelle and its possible differences between individuals will avoid damage to the brain of the fetus during parturition assistance.

P 246 | The role of proinflammatory cytokines in the progression of equine endometrosis

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Endometrosis is a degenerative chronic process, defined as an active or inactive fibrosis that develops around the endometrial glands and/or in the stroma in equine uterus. Generally, inflammation is often a prelude to fibrosis, with disorders in innate and adaptive immunity involved in both the initiation and regulation of the fibrotic process. The proinflammatory mediators can initiate a cascade within the cellular milieu that leads to the accumulation of extracellular matrix components (ECM). Myofibroblasts are one of the most important cellular components of fibrosis, which are characterized by excessive ECM deposition and de novo α-smooth muscle actin (α-SMA) expression. The effects of IL-β and IL-6 on the expressions of α-SMA, collagen type I (COLI), COLIII and fibronectin (FN) in progression of endometrosis were investigated. The endometrial strips from diestrus (category

I, II A/B, III of endometrosis) were incubated for 24 h with 10 ng/ml of IL-1β or IL-6. Interleukin 1β affected α-SMA expression on mRNA and protein levels only in category III endometrium ($p < 0.05$). IL-6 upregulated the mRNA and protein expressions of α-SMA in category I, II A/B, III endometria ($p < 0.05$). The COLI, COLIII and FN mRNAs transcription and concentration in response to IL-6 and IL-1β differed depending to the severity of endometrosis. The overall results suggest that IL-6 plays important roles in myofibroblasts differentiation and ECM deposition in the pathogenesis of endometrosis. (Supported by "Iuventus plus" Polish Ministry of Science and Higher Education (0463/IP1/2015/73))

P 247 | Prediction of calving day

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The farmer should be present at the moment of calving in order to intervene in case of dystocia. In the case of beef rearing establishments, the farmer should wake up every 3 h during the night to check his cows at term; but in dairy farming, due to the low value of the new born, the supervision is no longer assumed or even neglected. Prediction of the calving date was for a long time limited to the observation of the relaxation of the sacrosciatic ligaments, then completed by taking the temperature twice a day. Calving supervision can now be done by a camera or by using a warning device based on the raising of the tail. In this study, n = 17 Prim'Holstein cows at term, aged from 4 to 6 years, and 3 heifers aged of 30 months (embryo transfer recipients) were included. Every morning 5 ml blood was collected from the tail vein until the day of calving in order to measure the plasma progesterone level. Progesterone level determination was performed from 200 µl of plasma using a compact automatic analyzer (miniVIDAS®) developed by bioMerieux laboratories, designed initially for human medicine and based on the ELFA principle, with immunofluorescence reading at 450 nm. In the days preceding parturition, the plasma progesterone level varied between 4.5 and 6 ng/ml. The day prior to calving, progesterone dropped down to less than 1 ng/ml, except for the 3 embryo transfer recipient heifers in which it dropped to between 1.16 and 1.95 ng/ml. All animals calved in the afternoon or during the night following this drop of progesterone. Daily progesterone analysis allows the prediction of the day of calving. Cows should henceforward be under better surveillance.

P 248 | Progesterone detection in hair from dairy cattle by EIA: protocol validation

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Hair accumulates hormones during all its growth. Therefore, it provides an integrative measure of long-term retrospective hormonal levels that can comprise from days to months. This study validated the use of a methanol-based extraction protocol and a commercial enzyme immunoassay kit (progesterone ELISA KIT; Neogen Corporation) for the quantification of progesterone levels in hair from dairy cattle. Hair samples (250 mg) from 25 cows were used. Three washes of 2.5 ml of isopropanol and 2.5 min of vortex each one were applied to each sample. Then, hair samples were left to dry for 36 h at room temperature. Next, samples were powdered using a ball mill (22 Hz; 5 min) and 50 mg of powder were weighed and 1.5 ml of methanol was added. Samples were incubated under moderate shaking for 18 h at 30°C. Afterward, extracted samples were centrifuged at 7000×g for 2 min at 25°C and 0.75 ml of supernatant was placed in an oven at 38°C. Once the methanol was evaporated, the dried extracts were reconstituted with 1 ml of buffer. The extracts were stored at −20°C until analysis. All extracts were pooled and used for biochemical validation. Intraassay coefficient of variation was 8.64 ± 6.41%. The linearity of dilution showed a R2 = 0.98. The recovery percentage from spike-and-recovery test was 103.80 ± 10.37%. The standard and pool curves showed parallel displacements. According the results, successful hair progesterone detection can be performed using this protocol. The use of hair could open a new window in long-term hormonal monitoring for future endocrinology or reproductive research.

P 249 | Cardiac changes in pregnant Shih Tzu bitches by echocardiography

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Adjustment in the cardiovascular system is necessary for proper development of fetuses during pregnancy. Nevertheless, these changes in the pregnant bitch are still poorly understood. To assess cardiac changes during the gestational process, ten healthy pregnant Shih Tzu were evaluated by M-mode echocardiography every 15 days from estrus (D0) to parturition (D60) and also 45 days after parturition (D105). The evaluation focused the interventricular septum thickness in diastole (IVSd) and systole (IVSs), the left ventricular end diastolic (LVDd) and end systolic (LVDs) diameter, the left ventricular free wall in diastole (LVFWd) and in systole (LVFWs), the shortening fraction (%SF) and the ejection fraction (%EF). Comparison of the results among the evaluation moments was performed by Friedman and Dunns post-hoc tests ($p < 0.05$). The LVDd, %SF and %EF were higher ($p = 0.036$; 0.024; 0.016, respectively) in D60 (25.3 ± 3.2 mm; 77.7 ± 2.9%; 44.8 ± 1.9%) compared to other moments. These functional

changes in late pregnancy could be related to systolic function improvement due to blood volume increase that occurs during pregnancy, leading the ventricular diameter and the shortening and ejection fraction to increase. We believe that volume expansion might occur due to neuroendocrine activation secondary to vascular resistance decrease. The decrease in peripheral resistance is correlated with a reduction in resistance index (RI) of the uterine artery, which guarantees appropriate uterine blood flow for fetal development. Similarly to previous studies in another dog breeds, our investigation showed that Shih Tzu pregnant bitches showed no obstetric problems reinforcing the idea that maternal cardiac adaptation during gestation plays a major role to support fetal development.

P 250 | Placental mRNA expression during aglepristone induced midterm pregnancy in the queen

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This study aimed to assess placental mRNA expression during aglepristone (AGL) induced abortion and after AGL+Prostaglandin (PG) $F_{2\alpha}$ induced abortion. A total of 24 mature queens of different breeds at days 25–35 of pregnancy were included. Pregnancy diagnosis and determination of gestation day were performed by ultrasonography scan. Twenty queens were injected with AGL 10 mg/kg SC twice, 24 h apart. Considering the day of 2nd AGL injection as day 0, ovariohysterectomy was performed on days 1 (n = 4), 2 (n = 4), 3 (n = 4) and immediately after completion of abortion (n = 4). Four cats were injected a single dose SC cloprostenol (5 μg/kg) 24 h after 2nd AGL and ovariohysterectomized immediately after complete abortion. Four cats served as controls. Serum estradiol (E2), progesterone (P4), cortisol, oxytocin and PGFM concentrations were measured on the day of surgery. Placental estrogen receptor-alpha, progesterone receptor, oxytocin receptor, PG F receptor, PG E receptor 2 (PTGER2), PG E receptor 4 (PTGER4) and cyclooxygenase-2 (COX-2) mRNA expression levels were determined using qRT-PCR. Data analyzed using the ΔΔCt method, using RT2 Profiler PCR Array Data Analysis software version 3.5. Serum E2, oxytocin and PGFM levels increased on the day of abortion induced both by AGL and by AGL+$PGF_{2\alpha}$. Serum P4 levels initially increased then decreased during the course of abortion and levels were lower in AGL+ $PGF_{2\alpha}$ induced abortion ($p < 0.05$). No differences were observed in serum cortisol levels. Significant increase in mRNA levels of PTGER2, PTGER4 and COX-2 was observed on the day of AGL+$PGF_{2\alpha}$ induced abortion ($p < 0.05$). In conclusion, AGL+$PGF_{2\alpha}$ combination in termination of feline pregnancy modulates reproductive hormone concentrations and placental mRNA expression.

P 251 | Follicular development during superovulation induced with p-FSH in Lesvos ewes

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Follicular development and ovarian steroid concentrations were studied in Lesvos ewes during superovulation. The ewes received FGA (20 mg) sponges for 12 days, 200 mg p-FSH, in either 8 (SOV1, n = 6) or 6 (SOV2, n = 6) decreasing doses, 200 IU eCG at sponge removal (SR) and 8 μg GnRH 24 h later. Embryos were collected 8 days after SR. Transrectal ovarian ultrasonography (TROVU) was performed every 24 h, starting before the 1st FSH dose and ending 24 h after GnRH. The number (NF) and total diameter (TDF) of follicles ≥3, ≥4 or ≥5 mm in diameter were recorded (TROVU sessions 1–4, 5 or 6, respectively). Blood samples were collected at sponge insertion and every 12 h starting before the 1st FSH dose, ending 72 h after SR. Serum progesterone (P4) and oestradiol-17β (E2) concentrations were determined using a radioimmunoassay. Based on P4, E2 concentrations and TROVU findings, no difference was observed between groups at almost any time. Significant positive linear relations were revealed between NF or TDF recorded during each TROVU session and the E2 concentrations 12 h ($p < 0.05$) or 24 h ($p < 0.001$) later. The NF and TDF increased in a quadratic manner over the observation time ($p < 0.001$). In total, 175 follicles of ≥4 mm diameter were recorded 24 h after SR, representing 80.65% of the corpora lutea observed on the ovaries during embryo collection; 83.43% of these follicles had ovulated 24 h after GnRH injection. (Financed by SEE-ERA.NET Plus (ERA83))

P 252 | Ovarian characterization of gonadotropins and steroid hormone receptors expression in diet-induced obese mice

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Obesity is a worldwide problem with relevant impact on reproductive tract. We conducted an in vivo study on C57BL/6J mice (n = 8/group), in which one group was fed chow (CD) vs. high-fat diet (HFD) for 4 and 16 weeks (wk). The animals were sacrificed and the ovaries were collected for further analysis. The level of folliculo-stimuline hormone receptor (FSHR), luteinizing hormone receptor (LHR), estradiol receptor (ER) α and ERβ, and progesterone receptor (PR) A and PRB were assessed at mRNA and protein levels. Both FSHR and LHR mRNA levels after 16 wk of HFD were decreased, comparing to CD ($p < 0.01$), while the protein of both receptors was decreased already after 4 wk

HFD ($p < 0.05$). A similar pattern was observed for PRA ($p < 0.01$) and PRB mRNA and protein ($p < 0.05$). Whereas ERα mRNA was not changed, its protein level was elevated after 4 wk HFD ($p < 0.05$), as well as after 16 wk HFD ($p < 0.05$), comparing to CD. The ERβ mRNA was augmented after 4 wk HFD ($p < 0.01$), but showed no change after 16 wk HFD. Conversely, ERβ protein presented the opposite profile, being decreased after 4 wk and 16 wk HFD ($p < 0.05$). In conclusion, gonadotropin hormone receptors are decreased during HFD, which might hamper ovarian functionality. Furthermore, the rise in ERα protein level soon after the introduction to HFD might represent a relevant trigger for pathology establishment in the ovary; however, further studies are needed to understand the role of ERα in the ovary of obese mice. Finally, the decrease in ERβ and PRA and PRB should account for the functional impairment of the ovary of obese females. (Work supported by National Science Centre (2014/15/D/NZ4/01152))

P 253 | Prediction of the caesarean section day in dogs

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In the brachycephalic dog breeds, in particular in French and English bulldogs, the natural birth of puppies is sometimes virtually impossible as their heads are too big. The solution is a systematic caesarean section, which is also recommended for other breeds presenting dystocia. Daily progesterone measurements carried out in the morning during the final days of gestation, allows the programming of the caesarean section for when the surgical team is complete, matching the fall in progesterone level below 5 ng/ml. Thirty-two dogs were subjected to progesterone measurements; 24 were brachycephalic. Blood collection was performed from 9 to 12 h. Progesterone measurement was performed using 200 µl of plasma on a compact automatic analyzer (miniVIDAS®) developed by bioMerieux laboratories, designed initially for human medicine based on ELFA principle, with immunofluorescence reading at 450 nm. Based on the progesterone results, either the caesarean was programmed in the hours following the results, or the bitch was asked to come back 24 or 48 h later for a new blood sample. During late gestation, progesterone levels fluctuated between 13 and 16 ng/ml. The progesterone level decreased rapidly in the 24 h before full-term to less than 5 ng/ml. A caesarean section was then performed successfully in the 2 or 3 h following the progesterone results. Three dogs were presented in the morning with a progesterone level markedly decreased but still above 5 ng/ml. In such cases, the caesarean section was programmed for the evening around 18 h. When dog blood progesterone levels decline to less than 5 ng/ml, according to miniVIDAS measurement, a caesarean section can be performed with no risk for the newborns. Indeed, the peripartum mortality rate was only 5% (6/126 puppies).

P 254 | A freemartin with gonadal sex reversal and extreme masculinization of the genitalia

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A referred 5-day old Holstein calf, co-twin of a male calf, showed 4 teats, an empty scrotum, a prepuce and a penis enclosing a functional urethra. The calf was reared and developed a female body phenotype. Chromosomal analysis of blood and skin revealed a blood 60,XX/60,XY karyotype and a somatic 60,XX karyotype, a blood mosaicism confirming the freemartinism. At 15 months of age the heifer detected and mounted estrous females, but progesterone and testosterone plasma concentrations were basal. Following euthanasia, the necropsy evidenced the typical male external genitalia. The glans penis had an extension similar to the ram urethral process. The sigmoid flexure was absent. Epididymis and vas deferens were absent, but a small gonad with testes-like appearance was found, loose in the abdomen. Oviducts and uterus were absent. Histologic analysis of the gonad evidenced cords of Sertoli cell-only seminiferous tubuli, and Leydig cells aberrantly distributed in voluminous aggregates. Areas of seminiferous tubuli and of Leydig cells were also located in the mesenchymal tissues surrounding the gonad, where a hypoplastic pampiniform plexus was present. At this location, there were large tubular structures apparently originating from abnormal development of the Müllerian ducts. Some glandular lobules of diffuse prostate were found in the submucous lamina propria of the urethra. (Funding: UID/CVT/00276/2013)

P 255 | The effect of failure luteolysis on the endometrial expression of estrogen and progesterone receptors in the dairy cows

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The estrogen and progesterone, acting through their endometrial steroid receptors (estrogen α-ER and progesterone-PR), influence the timing of upregulation in proper as well as failure luteolysis. This study examined the expression patterns of steroid receptors (ER, PR) in the bovine endometrium during diestrus and anestrus type III to elucidate their respective roles in the regulation of luteolysis in dairy cows. Anestrus type III representing cystic ovarian degeneration (follicular lutein cyst (FLC)). Uterine biopsy samples were collected from 32 HF nonlactating cows. The ultrasound examinations were carried out at days 9, 14 and 18 after ovulation, to confirm corpus luteum (CL) (16 cows) or the follicular lutein cyst (FLC) (16 cows). The samples

were fixed and stained based on the standard protocols. The ER and PR were localized by HE and in situ immunostaining and demonstrated using light and confocal microscopy respectively. The results were also quantified by scanning cytometry using SCAN^R screening station. For ER and PR receptors, most immunostaining was noted (staining intensity/50 cells; mean ± SEM) in the nucleus of luminal epithelium (ER: 92 ± 108 / PR: 156 ± 19), gland tubule (ER: 97 ± 44 / PR: 111 ± 50) and gland opening cells (ER: 172 ± 32 /PR: 160 ± 39). Concentration of ER was significant higher ($p < 0.05$) in epithelial cells in contrast to PR significant higher ($p < 0.05$) in the uterine stroma both in CL and FCL groups. There were no significant differences ($p > 0.05$) in steroid receptors concentration in both progesterone states. There was no effect of failure luteolysis on the endometrial expression of ER and PR in the dairy cows. However there are important differences in the steroid receptors expression among different types of endometrial cell.

P 256 | The possible role of certain glycosidase on cow reproduction – in vivo and in vitro studies

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Glycosidase (GLYC) modifies glycoprotein or glycolipid structure and thereby can affect most cell membranes. They are associated with cumulus cells expansion, sperm capacitation, sperm oviductal epithelial cells interaction, sperm zona pellucida binding and polyspermy block. The aims of our research were to study the role of certain glycosidase (α-mannosidase - α-MAN, β-N-acetyloglucosaminidase - NAGASE and β-galactosidase - β-GAL) on cow reproduction by in vivo and vitro studies. Several experimentations were performed to study GLYC activity: (i) in the cervical mucus, (ii) in the uterine luminal fluid after superovulation, (iii) in the follicular fluid and in maturation, fertilization or culture medium during IVM-IVF. Our main interest was to evaluate if: (a) oocytes or embryos release or use GLYC during their development, (b) the addition of certain GLYC in culture medium affects embryo development, and (c) GLYC may be used as markers of embryo quality or superovulatory response (SR). According to our results: (i) GLYC activity was significantly higher in the cervical mucus of spontaneous estrous compared to induced estrous cows; (ii) a high SR is related to low NAGASE, probably because of the poor quality of embryos; (iii) NAGASE affects negatively embryonic development when added to culture medium; (iv) COCs release NAGASE and use β-GAL during maturation, but differences exist between individual and group maturation; (v) embryos release NAGASE and α-MAN during their development, but they use only α-MAN; (v) degenerate embryos release less NAGASE and α-MAN compared to good, whereas NAGASE seems to be related to retarded morulae; (vi) GLYC affects the developmental competence of oocytes collected from different sized follicles during IVF, cultured either in groups or individually.

P 257 | Effect of zearalenone on qualitative sperm parameters of rabbit bucks

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The in vivo effect of zearalenone (ZEA) on male fertility is considerably discussed in literature, whereas references on rabbit bucks are confined to combined mycotoxin effects. The aim of the present study was to determine the effect of ZEA at a dose of 50 μg/kg body weight on sperm quality of rabbit bucks. In our study, 8 adult New Zealand White bucks were included. The experimental period was equally allocated into a 7-week control (C) and treatment (T) period, respectively. Stock solutions were prepared by dissolving purified ZEA into dimethyl sulfoxide up to the desired dose concentration. ZEA solution was administered daily per os during T. Semen was collected weekly using an artificial vagina and was diluted 1:1 (v/v) with a commercial extender. Samples were evaluated for sperm kinetics (CASA analysis), morphology (Spermblue®stain), vitality (Calcein-PI double stain) and DNA fragmentation (Acridine Orange stain). Data were analyzed using a Mixed model. During T, animals showed a statistically significant increase in BCF ($p = 0.05$), % of head and neck abnormalities ($p < 0.0001$), and % of DNA-damaged spermatozoa ($p = 0.04$) compared to C. No significant differences ($p > 0.05$) were found between C and T regarding the % of total and progressive motility, % of rapid and live spermatozoa, VSL, VCL, VAP, ALH, LIN, STR and WOB. In conclusion, ZEA at the studied dose affects some of the rabbit buck sperm quality parameters. However, in the light of the present literature, the observed differences are considered mild in order to impair the fertilizing capacity.

P 258 | Research on the dynamics of progesterone in the blood plasma of recipient cows

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The purpose of this research was to study the dynamics of progesterone in blood plasma of recipient cows during the estrous cycle in order to provide a method for valuating the functional activity of the corpus luteum. Embryo transfers were performed in a non-surgical way to Holstein cows on the dairy farm “Bayserke-Agro” LLC. Blood for the study was taken from the jugular vein of 57 recipient cows on the 4th, 6th and 19th days of the estrous cycle in vacuum test tubes containing activator gel. ELISA analysis of blood samples of cows were carried out on the enzyme immunoassay analyzer «ImmunoChem-2100 Microplate Reader» made in the USA, using a

set of reagents "Progesterone - ELISA", from "Hema" Ltd. (Russia). Pregnancy in recipient cows was determined by rectal palpation on the 60th day after embryo transfer. A total of 171 blood samples from 57 recipients were tested by ELISA. According to the results of the rectal palpation of 57 recipient cows, 32 were pregnant (56.14% pregnancy rate). The concentration of progesterone in the barren recipients (n = 25) was 1.547 on day 4, 2.485 Day 6, and on the 19th day 0.702 ng/ml, while in the pregnant recipient cows (n = 32) the indexes relative to the barren ones were higher: on the 4th day 2.158, on the 6th day 4.185 and on the 19th day 12.100 ng/ml. The amount of progesterone in the barren recipients and in the pregnant cows (n = 57) on the 4th and 6th days of the estrous cycle was 1.853 and 3.335 ng/ml, respectively. In conclusion, a high progesterone concentration in recipients has a positive effect on the engraftment of embryos. As a criterion for the evaluation of the physiological activity of the corpus luteum we recommend to use a method of determining the amount of progesterone in the blood plasma.

P 259 | *Solea senegalensis* spermatozoa quality: are apoptotic cells and reactive oxygen species playing a role in F1 reproductive failure?

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Solea senegalensis broodstock (F1 generation) presents a failure on spawning performance compared to wild-captured counterparts. It is known that the problem relies on males in the F1 generation. Courtship lack and low quality semen hinder the expansion of sole aquaculture. The absence of courtship in F1 individuals leads to the use of artificial fertilization protocols, which require sperm cryopreservation prior to fertility trials. Cryopreservation can generate reactive oxygen species (ROS) that could have a negative impact on spermatozoa function. The aims of this study were: (1) to evaluate ROS in males born in captivity (F1) and in wild individuals (2) to implement a selection method for optimal sperm subpopulation recovery prior to cryopreservation. The percentage of positive cells for dichlorofluorescein (DCF) and propidium iodide (PI) were determined by flow cytometry in both groups' males. The presence and distribution of H_2O_2 within the cells was analyzed by confocal microscopy. In order to select non-apoptotic cell subpopulations magnetic activated cell sorting (MACS) was used and YOPRO and caspases were determined in the recovered population. Our results indicated a decrease in viability (55%) and DCF+ cells (66%) in slow F1 spermatozoa. Cryopreservation did not affect viability neither the presence of DCF+ cells in samples from wild individuals but decreased viability in F1 samples. Confocal studies demonstrated a colocalization of H_2O_2 with active mitochondria but also with nuclear DNA. Finally, MACS significantly reduced the percentage of apoptotic cells (54% and 75% removal in wild and F1, respectively) showing a potential future application on aquaculture. (Funding: AGL2015-68330-C2-1-R (MINECO-FEDER); AQUAGAMETE FA1205 COST Action; JCyL EDU1084/2012 and FSE)

P 260 | Assessment of the effect of a new technique for laparoscopic partial closure of the inguinal canal on sperm production and testicular perfusion in stallions

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In order to simplify other laparoscopic techniques, a new standing laparoscopic technique for partial closure of inguinal canal (PCIC) has been developed. This technique uses a new anchoring device and can be performed without advanced laparoscopic skills. The aim of this study was to develop a prior evaluation of the effects of this new technique on the stallion reproductive capacity, assessing the sperm production and testicular perfusion. Standing laparoscopic PCIC was performed unilaterally in 8 experimental stallions without evidence of inguinal hernia, using the contralateral canal and testicle as controls. Pre and postoperative testicular examinations and Doppler ultrasound scans were serially performed to assess the perfusion of both testicles. After 28 days, laparoscopic examination was performed in all horses. After that, they were castrated and seminal characteristics of epididymal sperm from both testicles were evaluated. Laparoscopic procedure was quickly and easily performed in all cases without any surgical complication. Significant differences in testicular perfusion and sperm characteristics were not found between both testicles, in studied variables: PSV, EDV, RI, PI, sperm concentration, percentage of live/dead and static/slow/medium/fast spermatozoa and hypo-osmotic stress test. The simplicity to implant the device, the absence of complications and the lack of adverse effects on sperm production and testicular perfusion, makes this new system a promising candidate for laparoscopic inguinal hernia repair. Further studies would be necessary to check the device performance in clinical cases of breeding stallions in a long-term period.

P 261 | Immunohistochemical studies of the effect of ischemia on glucose transporter GLUT1 localization in porcine uterus

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Ischemia consistently causes a large increase in the extraction of glucose from the blood, reflecting a greater capacity for glucose

transport across the cell membrane. There is a lack of information about GLUT-1 in ischemic uterus. This study investigated the effects of ischemia on compartmentation in distribution of GLUT1 conducted in two different stages of physiological blood supply of the uterus during the estrous cycle. Four groups of gilts were used in this experiment: on 1–2 or 9–10 day of the estrous cycle with physiological blood supply (control groups, CG) and followed by experimental induction of a 60-min ischemia (ischemic groups, IG). Uterine samples were collected: close to the ovary (A), from the middle part of the uterine horn (B) and close to the uterine body (C). Cryostat sections were stained by immunofluorescence (rabbit polyclonal anti-GLUT-1) and Alexa 594 anti-rabbit secondary to visualize the anti-GLUT1 antibody. Immunoreactivity (IR) derived from optical density measurements (Zeiss Axio Imager.Z1) was on day 9–10 in ischemic tissues higher ($p < 0.05$–0.001) in all examined parts of the uterus in: glandular and luminal epithelium, stroma, arterial endometrial endothelial cells, venous endometrial muscular layer compared to IR in these structures in CG. IR on day 1–2 in IG was higher only in the glandular epithelium in A, B, C parts, in stroma (C), arterial endothelium (B), arterial muscular layer (A, C) and venous muscular layer (A, B). These date confirm that IR of GLUT1 in ischemic uterus depends on the region of the uterus and the stage of blood supply during estrous cycle. (Supported by statutory research founds of MSHE (GW-15), NCSci Grant NN311527040.)

P 262 | Comparative study of automatic and manual thermogram analysis of bovine udders with induced *E. coli*-mastitis

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Infrared thermography is a simple and noninvasive method to measure changes in bovine udder surface temperature and thus a helpful tool for early mastitis detection. However, the manual analysis of thermograms is very time-consuming. The aim of this study was to compare whether the automatic segmentation and evaluation of thermal images gains comparable results. Five healthy Holstein-Friesian cows were challenged with *E. coli* into the right hind quarter. In each case, acute mastitis was induced. Thermograms were taken every 2 h in a period of 24 h before and after challenge. The images were interpreted manually, using a polygon-tool (ThermaCAM Researcher Pro 2.8, FLIR Systems®). Automatic image analysis based on Active Shape Model approach was implemented by Fraunhofer Institute. For each image, values for average and maximum udder temperature were calculated and compared by boxplot-analysis. Manually determined temperatures were higher than automatically analyzed values (median = 0,80K; lower quartile (q1) = 0.76K; upper quartile (q3) = 0.87K). Both methods detected peak values for maximum udder temperature 13 and 15 h after infection: 2.20K (13 h p.i.) and 2.19K (15 h p.i.) difference from median maximum temperature (37.44°C; q_1 = 37.27°C; q_3 = 37.61°C) for manual analysis, as well as 2.33K (13 h p.i.) and 2.08K (15 h p.i.) for automatic segmentation (median 36.38°C; q_1 = 36.19°C; q_3 = 36.61°C). It is presumed that higher temperatures in manual analysis occur due to inclusion of warmer regions, e.g. udder-thigh cleft, whereas automatic segmentation leaves these regions out. Still, temperature curves for each method show similar progresses. Thus, automatic segmentation of infrared images is a quick and promising approach to enhance early mastitis detection.

P 263 | Heat expression in milk cows depending on their activity

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The aim of the study was to determine the effect of the daily activity of cows on heat expression. The study used two groups of Polish Holstein-Friesian dairy cows from the Experimental Station of the NRIAP. The first group - 10 heifers and the second group of 10 cows (1–3 lactation). Feeding and housing conditions were the same for both groups. Activity tags (Israeli AfiAct and Japanese Gyuho) were attached to the front legs of each animal. Pedometer readings were taken over 3 complete estrous cycles of each cow, at 06:00 and 12:00 for heifers and also at 19:00 for cows. These data were used to analyze the 24-h activity of animals. Average resting time in relation to total resting time, as the restlessness ratio were estimated. Statistical analyses were performed using the SAS package based on one-way analysis of variance. It has been found that the higher the hours activity of the cows, (from 9- to 138 steps/h), the longer the period of heat confirmation to 13 h. Heifers showed a longer time (to 14 h) until the estrus compared to cows (to 12 h). In 40% of the heifers estrus occurred at 5:00 am, compared to only 20% in cows. Heifer activity was 125 steps/h (355–624 steps/h in heat); they rested more but the duration of one rest was shorter (36–53 min). Cows with a higher restlessness ratio than heifers showed lower total daily activity - 100 steps/h (245–512 steps/h in heat) and rested less during the day. Confirmation of the 1st, 2nd and 3rd heat was significantly higher in heifers than in cows.

P 264 | Sequential in vitro culture that is highly efficient for the meiotic maturation of porcine oocytes turns out to be inefficient for the meiotic maturation of canine oocytes

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The efficiency of oocyte IVM in dogs remains at the extremely low level (ranging from 0% to 25%). Therefore, development of the effective strategies used for IVM in this mammalian species is still required for successful generation of canine embryos by in vitro fertilization, parthenogenetic activation and somatic cell nuclear transfer. The aim of the study was to compare the rates of canine (Group I) and porcine (Group II) oocytes reaching the MII stage under identical biochemical and biophysical conditions of sequential IVM. The medium intended for the first step of IVM was comprised of TCM 199 and enriched with 10% FBS, 10% pFF, 5 ng/ml rh-bFGF, 10 ng/ml rhEGF, 0.6 mM L-Cys, 0.1 IU/ml hMG, 5 mIU/ml pFSH, and 1 mM db-cAMP. In the second step of IVM, the oocytes were cultured in the medium depleted of hMG, pFSH, and db-cAMP. In Group I, canine COCs were matured in vitro for 22 h in the hMG-, pFSH-, and db-cAMP-supplemented medium. They were subsequently cultured for an additional 50 h in the medium deprived of hMG, pFSH, and db-cAMP. In Group II, porcine COCs that had been selected for IVM were incubated for 22 h in the hMG-, pFSH-, and db-cAMP-enriched medium, followed by 22-h culture in the medium lacking hMG, pFSH, and db-cAMP. The sequential IVM led to reaching the MII stage by 0/128 (0.0%) canine oocytes as compared to 202/221 (91.4%) porcine oocytes. In conclusion, canine oocytes failed to acquire the meiotic competence and to reach the meiotic maturity under the same conditions of two-step IVM that were used for porcine oocytes.

P 265 | Evaluation of the pregnancy status in Bulgarian Murrah buffaloes after hormonal treatment during the early postpartum period

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The study was designed to evaluate the pregnancy status in Bulgarian Murrah buffaloes after hormonal treatment during the early postpartum period. The experiment was carried out with 30 clinically healthy multiparous buffaloes, during breeding season, housed in the same management. The calves were separated from the dams immediately after parturition. The animals were randomly allocated in three groups: group I (non treated; n = 10); group II (n = 10) received 100 μg GnRH on day 14 and 25 mg $PGF_{2\alpha}$ (Dinoprost) on day 21 postpartum and group III (n = 10) injected with GnRH-$PGF_{2\alpha}$-$PGF_{2\alpha}$ with the above mentioned doses on days, 14, 21 and 32 postpartum, respectively. Fertile bulls remained in the first group permanently, while in the treated groups they were introduced after the last prostaglandin injection. Ultrasound pregnancy check was performed monthly until month six after calving. The results were processed by correlation analysis and statistical test for comparing the differences between proportions. At the first time pregnancy was registered in the hormonal treated groups II and III (30% and 20%) after the first and in the control animals (10%) after the second postpartum month. The cumulative percentages of pregnant buffaloes correlated positively ($r \geq 0.96$; $p < 0.05$) to postpartum days. The final ultrasound exams showed more pregnancies (60% and 70%) in group II and group III than in group I (30%). Significant difference ($p < 0.05$) between the pregnancy results for groups I and III was also determined. In conclusion, the pregnancy status in Bulgarian Murrah buffaloes could be improved by the used hormonal treatments during the early postpartum period.

P 266 | Association between farrowing duration and plasma oxytocin concentrations at the subsequent estrus in sows

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We investigated whether duration of farrowing affects plasma oxytocin concentrations of sows at subsequent estrus. A total of 31 crossbred sows (Finnish Yorkshire × Finnish Landrace) were allocated into two treatment groups according to duration of farrowing: 1) SHORT (159 ± 29 min): 14 sows (parity 3.8 ± 2.6) with shorter than 200 min farrowing duration, and 2) LONG (579 ± 263 min): 17 sows (parity 5.1 ± 2.5) with longer than 300 min farrowing duration. Duration of farrowing was determined by the birth interval between the first and the last piglets. After a 4 week lactation, the sows were confined in the stalls and catheterized 3 days before the expected estrus through the auricular vein following a nonsurgical catheterization procedure. Upon triggering the standing reflex by boar introduction, blood samples were collected −15, −10, −5, 0, 1, 2, 3, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50 and 60 min while the boar introduction occurred at 0 min. Plasma oxytocin concentrations were measured using ELISA kits (Enzo Life Sciences, Switzerland). Repeated measures using mixed model were used to analyze periodical oxytocin concentrations according to the boar introduction. Plasma oxytocin concentrations of estrous sows in the LONG group (22.2 ± 1.8 pg/ml) were greater than in the SHORT group (15.6 ± 2.0 pg/ml) during the whole sampling period, i.e. 15 min prior to until 60 min post boar introduction ($p < 0.05$). During the presence of the boar for 10 min, estrous sows in the LONG group (27.8 ± 3.6 pg/ml) tended to have greater plasma oxytocin concentrations than in the SHORT group (17.8 ± 4.0 pg/ml; $p = 0.07$). The results indicate that prolonged farrowing might lead to an increase in plasma oxytocin concentrations of estrous sows at the following breeding.

P 267 | Cesarean section in bitches: therapeutic plan to increase neonatal viability

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The present study tried to define the reliability of an adapted Apgar score as a tool to check newborn viability in puppies delivered by cesarean section. Data from 36 cesarean sections and 121 puppies were included. Immediately after the uterine delivery, the pups were evaluated to detect birth defects and then, a modified Apgar score (range: 0–10) was used to define neonatal viability as critical neonates (0–3), moderate neonates (4–6) and optimal neonates (7–10). Apgar score assessed the following parameters: heart rate, respiratory rate, mucous membrane color, reflex irritability and mobility. Neonatal mortality at birth was 0.8% (1/121), increasing till 4.95% after 48 h. The incidence of birth defects was 4.13% (5/121) included cleft palate as the most frequent pathology. Five minutes after birth, the incidence of critical neonates was below 5% (5/121) and this percentage decreased to 1.65% (2/121) at 1 h after birth. The percentage of neonates classified as optimal were 95.8% (116/121) after 1 h after birth. Resuscitation drugs (naloxone, heptaminol, epinephrine) were applied to 9.09% (11/121) of neonates (5 critical neonates and 6 moderate neonates). This study determined that the spontaneous neonatal mortality was not influenced by the different anesthetics protocols applied. Apgar score quickly detects the viability of neonates and identified the puppies in critical condition and led to establish a suitable protocol of neonatal resuscitation and intensive care in order to increase survival of puppies

P 268 | Reproductive performance of Bama Miniature Pig under sub-tropical monsoonal climate

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Bama Miniature Pigs, which in small size and body weight, have become important biomedical models due to their anatomical and physiological resemblance to humans. The aim of the current study was to estimate reproductive parameters of Bama Miniature Pig for providing basic data in applications on the biomedical and agricultural research. Data on 235 litters were collected from pig farmers in the Chongqing and Sichuan province, under sub-tropical monsoonal climate, and the results showed that age at puberty of female piglets was 68.04 ± 19.1 days while estrus cycle averaged 21.32 ± 0.64 days. Gestation length and litter size averaged 114.13 ± 2.03 days and 7.72 ± 2.59, respectively, with 4.47 ± 1.77 kg of birth litter weight. The weaning time averaged 41.08 ± 1.65 days with the litter weaning weight 20.75 ± 7.34 kg. According to the litter size of sows (≤5, 6, 7, 8, 9, 10 and ≥11), the piglets were divided into 7 groups, and the body weight at birth, 30 and 60 days was significantly different ($p < 0.01$) among groups. Data of semen quality from 900 boar semen samples showed that the ejaculation volume, spermatozoon density, pH value, sperm live rate, sperm resistance index and rate of abnormality spermatozoa were 95.31 ± 26.53 ml, (7.80 ± 1.60) × 10^7 cells/ml, 8.23 ± 0.40, (75.33 ± 5.18)%, 581 ± 35 and (5.13 ± 1.65)%, respectively.

P 269 | Antioxidative capacity and intensity of lipid peroxidation in the reproductive system of boars

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The aim of this study was to investigate the differences in the level of antioxidative protection and lipid peroxidation in the testes and different parts of the epididymis in reproductive boars. The study was performed on five boars of the Swedish landrace breed, 10 months of age. Tissue samples were taken of the testes and the head, body and tail of the epididymis. In the obtained supernatants of the homogenized tissues, the activities of glutathione peroxidase (GSH-Px), glutathione reductase (GSH-RD), superoxide dismutase (SOD), gamma glutamyl transferase (GGT), and concentration of malondialdehyde (MDA) were determined. Significantly higher activities of GSH-Px and GSH-RD ($p < 0.05$) were found in the testes than in the epididymis. The testes were found to have a significantly higher activity of SOD than the head and tail of the epididymis ($p < 0.05$). The MDA concentration in the head of the epididymis was significantly higher than in the testes, body and tail of the epididymis ($p < 0.05$). Simultaneously, the MDA concentration in the testes, was significantly higher than in the body and the tail of the epididymis ($p < 0.05$). In epididymis tail, a significantly higher activity of GGT was recorded than in the testes and other parts of the epididymis ($p < 0.05$). The results of the current study indicate the physiological importance of antioxidative enzymes in tissues of the reproductive system in boars. Furthermore, the obtained results may serve to better understand the mechanisms of male infertility and to improve boar semen storage.