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Editorial

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POSTER PRESENTATIONS

P1

Effects of ultrasonication on damaged spermatozoa and mitochondrial activity rate in Merino ram

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The aim of this study was to investigate the effects of different duration and repetitive application of sonication on ram spermatozoa. Ejaculates were divided into equal groups (8 ejaculates per group) and sonicated (SONIC Vibra Cells; net power output 130 W, frequency 20 kHz, amplitude 100%) 3, 6 or 10 s for 1, 3, 5 and 8 repetitive times. Samples were cooled for 30 s (on ice) between each sonication. After sonication, sperm concentration (1×10^6/ml), damaged spermatozoa rate (%), mitochondrial damage rate (% JC1-PI high activity, %JC1-PI low activity), lipid peroxidation and antioxidant potential levels were determined and differences between groups were analyzed using ANOVA and Duncan post hoc tests. In the groups; 3, 6 and 10 s, with the increasing number of repeated applications, damaged spermatozoa rates increased ($p < 0.05$) and mitochondrial activity rates decreased ($p < 0.05$). In relation with sonication duration time total antioxidant potential levels ($p < 0.05$) increased in single term groups compared to the control groups whereas levels were differently affected within repeated application. Conclusion: The most effective results (100% damaged spermatozoa and 100% decreased mitochodrial activity) were obtained in 8 repetitive and 10 s duration group.

P2

The effect of cooling to different sub-zero temperatures on dog sperm cryosurvival

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To test whether slow cooling to sub-zero temperatures before freezing may improve dog sperm crysosurvival, semen from 6 dogs (Belgian) was centrifuged, resuspended in a standard freezing medium, and slowly cooled from 23 to 5°C. Then, a second fraction of extender was added to obtain a final concentration of 5% glycerol and 200×10^6 sperm/ml. Diluted semen was packaged in plastic straws (0.5 ml) and kept at 5°C during 16 h. Then, 1) freezing point was determined by exposing the straws to nitrogen vapours; 2) straws were cooled from +5°C to −3, −5, and −7°C, and immediately rewarmed to 37°C; 3) straws were cooled from +5°C to −3, and −5°C, frozen over nitrogen vapours, and stored in liquid nitrogen; straws were thawed in a water bath at 37°C. Progressive motility, viability (Eosin/Nigrosine), plasma membrane integrity (HOST, SYBR14/PI, Nucleo Counter®), capacitation status (CTC assay), and acrosome integrity (PSA-FITC) were assessed. Freezing point was −13.7 ± 4.8°C (mean ± SD); in the cooling/rewarming step there were no differences between treatments in any of the variables. In the freeze/thawing step there were no differences between cooling treatments in any of the variables, although there was a tendency for −5°C to produce better sperm quality: HOST, 34, 34, and 36 (%); Nucleo Counter, 27, 28, and 30 (%); acrosome integrity, 62, 65, and 69 (%); capacitated sperm, 66, 60, and 60 (%), +5, −3, and −5°C, respectively. In conclusion, cooling of dog sperm to either −3 or −5°C, before ice formation, did not improve sperm cryosurvival. (Supported by UNAM PAPIIT IN213815 & PIAPIVC03.)

P3

Autophagy is involved in the regulation of the acrosome reaction in equine sperm

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Autophagy is a tightly regulated process requiring different proteins that leads to the conversion of LC3I to LC3II. This conversion is a final event of the process and is commonly used as an autophagy marker. Previously we have shown that LC3I can be processed to LC3II in stallion spermatozoa and that LC3II is localized in the acrosome region. Thereby, we aimed to study whether autophagy participates in the acrosome reaction (AR) in these cells. AR was induced with ionophore A23187 of 1, 2.5, 5 and 10 μM for 1, 2.5 and 5 h at 37°C and in presence of STF-62247 (50 μM) or chloroquine (50 μM). AR was measured by flow cytometry using PNA-FITC and EthD-1. In parallel, LC3II/LC3I ratio was measured by Western blotting. Subcellular distribution of PNA-FITC and LC3 was also studied. Our results showed that LC3II/LC3I ratio significantly increased in a time- and concentration-dependent manner when AR was induced, and that there was a positive correlation between the LC3II/LC3I ratio and the percentage of acrosome-reacted cells. We also found that LC3II co-localized with PNA-FITC in the acrosome. The inhibition of autophagy with chloroquine significantly reduced the percentage of acrosome-reacted cells, while autophagy activation with STF-62247 significantly increased it after a 2.5 h incubation. In conclusion, autophagy participates in AR regulation in stallion sperm showing a positive role. (Supported by Gobierno de Extremadura-FEDER, DE12006, GR10010 and PCE1002, and Ministerio de Economia y Competitividad-FEDER, BFU2011-30261.)

P4

Survival of bovine split embryos under tropical conditions in Mexico

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The use of reproductive biotechnologies has been implemented to increase animal populations. The aim of the study was to evaluate

the survival of split embryos. The study was conducted in a cattle farm located in Tabasco, Mexico. The area has a humid tropical climate with an annual average temperature of 27°C and 2550 mm of rain. Twenty Gyr-Holando multiparous donor cows were superovulated and inseminated with frozen semen to produce the embryos. The cows were flushed 7.5 days after oestrus using non-surgical procedures. After embryo searching using a stereomicroscope, the embryos were morphologically classified (80×) according to the IETS Manual and only grade 1 expanded blastocysts were used. The embryos were split using commercial splitting media, a mechanical micromanipulator and a micro blade. One hundred Brahman cows synchronised with CIDRs were used as recipients. The embryos were transferred fresh using non-surgical embryo transfer methods as whole (T1; n = 60) or split (T2; n = 40) embryos, within two hours after flushing and fifty minutes after splitting. The cows were pregnancy tested 40 days after transfer by ultrasound using a 5.0 MHz lineal probe. The results were analysed by Chi-square test. The pregnancy rate was 58.33% (35/60) for whole embryos and 50% (30/60) for split embryos. The results although were not different ($p > 0.05$), when we consider the survival of split embryos as a whole (100%) there is an increase of 41.67%. In conclusion embryo splitting can be used under field conditions to improve the efficiency of traditional embryo transfer programs.

P5

A novel computer-assisted sperm analysis system for determination of the fundamental characteristics of spermatozoa

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The main target of this study was developing a novel computer assisted sperm analysis system to analyze objective motility and motion characteristics of bull sperm cells. For this aim, first of all the hardware components of the system were determined and procured. These included a phase-contrast microscope (Olympus BX-43) with warm plate, a c-mount adaptor (Olympus x0.35), a fast camera (Basler Ltd. Corp., pilot 210 gc, 480 × 640 px) and a computer. Secondly, the motion parameters including the average pathway velocity (VAP μm/s), straight line velocity (VSL μm/s) and curvilinear velocity (VCL μm/s) were determined using image processing and pattern recognition techniques computationally via the developed software. Three μL samples from frozen-thawed (100×10^6 sp/ml) were transferred to slide and closed with cover-slip. The videos recorded from microscope areas via ESAS hardware system were transferred to ESAS software module (AriTek ArGe Ltd. Corp.) and analysed in computer environment. The sperm cells were identified via image processing and their paths were extracted using pattern recognition and object tracking techniques. The mean subjective motilities of investigation areas were recorded as 65% and the mean (objective) ESAS motility were determined as 71.4% ($p > 0.05$). In this study, mean VAP (um/s), VSL (μm/s) and VCL (um/s) of motile cells were determined as 134, 167, 175 μm/s respectively. In conclusion, a new computer assisted sperm analyses system capable of estimating the progressive motility, VAP, VSL and VCL has been developed during this study. Moreover, a new electronic sperm analysis system (ESAS) was designed. (Supported by TUBITAK, 2130042)

P6

Effect of N-acetylcysteine (NAC) on post-thaw semen quality of Tushin rams: higher doses of NAC may be toxic

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The aim of the present study was to investigate the effect of N-acetylcysteine (NAC) on freezability of Tushin ram semen. Ejaculates from four Tushin rams were collected with artificial vagina and then pooled. Pooled semen was divided into four aliquots to be diluted with skim milk-based-egg yolk-glycerol (SEG) extender supplemented with various concentrations of NAC (0, 0.25, 0.5 and 0.75 mM). The semen was loaded into 0.25 ml straws, equilibrated (at 4°C for 2 h), frozen in liquid nitrogen (LN) vapour (at −120°C for 15 min) and stored in LN (−196°C). After thawing (at 37°C for 1 min), sperm motility, viability, morphology, acrosome and membrane integrity (HOST) were evaluated. Results showed 0.75 mM NAC to have some detrimental effects on motility, compared to the other three NAC doses evaluated ($p < 0.05$). Membrane integrity was higher in 0.25 and 0.5 mM NACs. There were significant differences ($p < 0.05$) in semen viability among NAC doses. In conclusion, higher doses of NAC, especially used with SEG extender, may have some detrimental effects on freeze-ability of ram semen. Moreover, although modest doses of NAC slightly improved freeze-ability of Tushin ram semen.

P7

Linoleic and linolenic fatty acid content in the blood and/or in the follicular fluid are associated with follicular dynamics after $PGF_{2\alpha}$ induced luteolysis

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The aim of the present study was to examine the association between linoleic (C18:2n6) and linolenic (C18:3n3) fatty acid (FA) content of the serum and follicular fluid (FF) with the follicular dynamics (FD) after induced luteolysis in dairy cows. Twenty nine Holstein dairy cows bearing a CL > 25 mm and a follicle ≈15 mm at the start of the experiment (d0) were submitted to ultrasound guided trans-vaginal follicular aspiration for FF collection from the largest follicle and injected with 500 μg of cloprostenol. Follicular development was monitored daily by transrectal ultrasonography starting at d0 until the dominant follicle reached the size of ≈15 mm and was on his turn punctured (d1). Blood samples for determination of FA in both serum and FF were taken simultaneously with FF collection at d0 and d1. No significant differences were observed in total PUFA content neither between the serum and th FF neither between days 0 and 1. However, on both days the C18:2n6 level in the serum was significantly higher than in the FF, while C18:3n3 levels showed a tendency to be lower in the serum than in the FF on both d0 and d1 ($p = 0.08$ and $p = 0.05$), respectively. Additionally, there was a strong positive correlation between the serum and FF C18:3n3 ($r = 0.61$, $p < 0.0001$) on both days. On d0, there was a tendency of both serum and FF C18:2n6 content to be negatively correlated with the daily follicular growth rate (DGR, $r = -0.44$, $p = 0.05$ and, $r = -0.39$, $p = 0.09$, respectively) while on d1, a strong negative correlation between the serum C18:2n6 and the DGR was recorded ($r = -0.71$, $p = 0.0006$). The serum and FF C18:3n3 levels were not correlated with the DGR. In conclusion, the present data suggest the PUFA content in the blood and in the FF to be associated with the FD after induced luteolysis.