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Benefit and safety of 28-day transdermal estrogen regiment during vaginal hysterectomy (a controlled trial)

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Abstract

Objectives: assessment of benefit and safety of 28-day transdermal 17-βestradiol regiment during vaginal hysterectomy.
Methods: Two-hundred and sixty-nine postmenopausal women, undergoing vaginal hysterectomy were divided into: transdermal estrogen hormone replacement therapy (TEHRT) group ($n = 119$) with 28-day transdermal 17-βestradiol 50 mg/day, 14 days before and after operation; and vaginal estrogen hormone replacement therapy (VEHRT) group ($n = 150$) with 14-day preoperative vaginal conjugated estrogen 0.625 mg/day. The effect on: endometrium, wound healing, infection, recurrent organ prolapse were evaluated.
Results: Pain symptoms, vaginal fetid discharge, swelling, crusting ($p < 0.001$); visible wound opening on the 4 week control ($p < 0.01$); patient assessment of outcome ($p < 0.001$) were in favor of TEHRT. On the fifth postoperative day, VEHRT group showed: higher leukocytes increase ($p < 0.01$); more patients with leukocytes 'count higher than $15 \times 10^9\ L^{-1}$ ($p < 0.001$) and afternoon body temperature higher than 38 °C ($p < 0.01$). On the last follow-up control (VEHRT-28.3 months and TEHRT-24.5 months) TEHRT group had more patients with stage 0 of the apical segment ($p < 0.05$). Point C was higher and total vaginal length longer in TEHRT group ($p < 0.01$; $p < 0.05$). Frequency, constipation, painful coitus, incontinence during intercourse were more frequent in VEHRT ($p < 0.001$; $p < 0.05$; $p < 0.05$; $p < 0.05$). Endometrium with a thickness between 2 and 4 mm, was more frequent in the TEHRT group ($p < 0.05$). There were no significant differences in occurrence of more thickened endometrium and more significant morphological changes (endometrial polyp, simplex hyperplasia) between the groups. In none of the patients from the both study groups complex hyperplasia, atypical hyperplasia or endometrial carcinoma were observed.
Conclusions: The 28-day transdermal 17-βestradiol regiment seems to be safe and effective procedure.

Keywords: Pelvic organ prolapse; Vaginal hysterectomy; Hormone replacement therapy

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1. Introduction

The estrogen receptors are already identified in female urethra and bladder wall [1], in the basal and intermediate layer of the vaginal epithelium and muscular vaginal layer [2]. In postmenopausal age they have similar quantity and distribution as those which can be found in the estrogen phase of one ovarian cycle. The synthesis of collagen type I, which is the principal constituent of the pelvic ligaments and fasciae, is seriously disturbed in a condition of estrogen deficit, and becomes improved during estrogen hormone replacement therapy (EHRT) [3]. Regarding the formonal effects on collagen, parametrium, pelvic prolapse, stress urinary incontinence and pelvic vascularization, there are several controversies, especially about the effects of estrogen on collagen synthesis. Moalli et al. [4], who estimated the impact of estrogens on the structural components of the arcus tendineus fasciae pelvis, reported for 75%-decrease in collagen I and change in collagen I/collagen (III + V) ratio in postmenopausal versus premenopausal women, but these changes were absent in women on HRT. Tomaszewski et al. [5], found that fibroblasts from pubocervical fascia taken from women suffering from urinary stress incontinence (USI), in vitro showed increased cell proliferation after exposure to 17–β E_2 and this their proliferation capacity was higher than that of skin fibroblasts. According to them this is an indirect rationale for local estrogen treatment in case of female SUI. Ewies et al. [6], in their immunohistochemical study performed on paraffin-embedded section of the cardinal ligaments, found that the ligaments of the prolapsed uteri are characterized by a higher expression of collagen III and tenascin, and lower quantities of elastin. According to them, it appears that the use of HRT in postmenopausal women can reverse some of the changes observed in cases of prolapse. Collagen I expression is directly related to the age and menopausal status rather than to prolapse, but collagen III expression is directly related to the presence of prolapse rather than age or menopausal status and is suppressed with the use of HRT. The trauma itself may have been initiated by events such as childbirth, and the lack of estrogen following the menopause can result in decompensation. A supplementary drug therapy may help along with estrogens to rebuild these ligaments. Reay Jones et al. [7], measuring uterosacral ligament resilience (UsR) by tensiometry of the forty-five of these ligaments, which were examined for ligament thickness, muscle/collagen ratio, and estrogen and progesterone receptor, found a significant decrease in UsR ($p = 0.02$) in symptomatic uterovaginal prolapse, in UsR with vaginal delivery ($p = 0.003$), menopause ($p = 0.009$) and older age ($p = 0.005$). The uterosacral ligaments were significantly thinner and contained fewer estrogen and progesterone receptors after menopause, but this did not affect UsR. Otto et al. [8], in their experimental study with histologic specimens prepared from the paravaginal attachment of 13 oophorectomized rhesus macaques, of which: 3 animals were treated with estradiol; 6 animals were treated with estradiol and progesterone, and 4 animals were untreated, found that the fibroblasts of the paravaginal attachment are estrogen and progesterone receptor positive, and the receptors are hormone responsive. Chen et al. [9] assessed the extracellular metalloproteinase and tissue inhibitors of metalloproteinase in fibroblasts from continent and incontinent women cultured with increasing concentrations of estradiol (0–500 pg/mL) and found that periurethral vaginal tissues from incontinent women expressed less tissue inhibitors of metalloproteinase when compared with tissue the control subjects; and tissue inhibitors of metalloproteinase expression from fibroblasts of continent women significantly increased with increasing estradiol concentrations, but no significant dose response has been seen in fibroblasts from an incontinent woman, which suggests that estrogen may inhibit collagen degradation. Dundar et al. [10], in their animal study investigated the effect of estrogen on urethral connective tissue in ovariectomized rats and did not found significant differences in percentages of collagen fiber content between the groups with or without estrogen therapy. Jackson et al. [11], in a double-blind, placebo-controlled trial of 49 women treated with estradiol valerate 2 mg once daily or placebo during 6 months found that the estrogen treatment resulted in significant decreases in total collagen ($p = 0.0054$), the mature cross-link HHL ($p = 0.0009$) and the advanced glycation end-product NFC-1 ($p = 0.0009$) in the periurethral tissue and a significant rise in the immature cross-link HLKNL ($p = 0.0191$) and concluded that estrogen stimulate collagen degradation via increased proteinase activity and a new collagen is synthesised. Falconer et al. [12], analysing the connective tissue in stress urinary incontinent women after menopause with

and without estrogen replacement therapy, found that estrogen replacement therapy resulted in a lower collagen concentration both between the controls ($p = 0.02$) and between the incontinent women (0.02).

Estrogen receptors have been demonstrated in nucleus and cytoplasm of various cells: cells in human skin [13], macrophages [14], fibroblasts [15] and endothelial cells [16], all of which play vital roles in the healing process. *The inflammatory phase of wound healing* is consisted in: platelet accumulation, coagulation, increased permeability of the vessels and leukocyte migration into the wound bed. Baker [17] found that the administration of estradiol tended to suppress the infiltration and activity of macrophages, indicative of a reduced inflammatory response. Nyman at al. [18], investigating the vascular permeability in the inflammatory phase of healing by measuring the penetration of intravenously injected J^{125}-labelled human serum albumin, found a significantly lower amount of exudate in estradiol treated rabbits, but no differences in the serum albumin penetration. Lundgren [19], analysing the influence of estradiol on the inflammatory cell migration following the subcutaneous implantation of Teflon cylinders found that the estrogen-treated animals had a smaller numbers of polymorphonuclear leukocytes (PMNLs) within the cylinder than the controls. Gouveia et al. [20] and Murthy et al. [21] found separately that estrogens inhibited vascular permeability in inflammatory conditions. Rosenblum et al. [22] reported that estradiol enhances platelet aggregation in mice at the site of microvascular injury. Estrogens have been found to enhance the oxidative metabolism of activated human PMNLs and their enzyme and phagocytic activity during in vitro and in vivo studies [23,24]. Miyagi et al. [25] demonstrated that estrogens significantly reduced the chemotaxis of PMNLs. Both, monocytes and macrophages play a crucial role in the wound healing, generating chemotaxic and growth factors for co-ordination of granulation tissue formation in the next, proliferative phase of repair. Calvin et al. [26] found a significantly reduced number of mature tissue macrophages in the ovariectomised rats compared to the controls. It is possible that the low levels of estrogens affect the ability of the monocytes or immature macrophage to differentiate into their mature form. *The proliferative phase of repair* is characterised by: re-epithelisation, angiogenesis, fibroplasia, and wound contraction. Taylor et al. [27] reported a reduced degree of fibroplasia in the rats receiving estrogens compared to the controls. Murthy et al. [21] reported a significantly reduced fibroblastic response and retarded collagen synthesis in estrogen treated rats. Portugal et al. [28] observed an inhibited fibroblast reaction, but Fisher and Paar [29] showed that injection of stilbestrol resulted in increased fibroblast infiltration and collagen production. Nyman [30] reported for a markedly reduced granulation tissue following the estrogen administration in ovariectomised animals. Pallin et al. [31] observed a decreased granulation tissue and reduced quantity of collagen, determined by hydroxyproline levels, in estrogen treated rats. According to Lindhe et al. [32] estrogens have no effect on vascular proliferation in vitro, but Lundgren [33] observed a significant suppression in vascularisation in the estrogen treated group. Hu et al. [34] found that ovariectomy in rats led to reduced synthesis of interleukin-1 (IL-1) by macrophages, a growth factor which is directly involved in the granulation tissue formation and collagen deposition. The EHRT administrated to the rats effectively increased IL-1 synthesis. Shanker et al. [35] showed that estrogens can modulate platelet derived growth factor-A (PDGF-A), which is mitogenic and chemotactic for fibroblasts. According to Morales et al. [36] in vitro estradiol-treated cells migrated into the wound three times faster than untreated control cells and in vivo angiogenesis increased with estrogen. It seems that estrogens also have an influence on the *remodeling phase of wound healing*. Jorgensen and Schmidt [37] demonstrated that administration of estradiol to female rats significantly increased the tensile strength of the wounds on the 10th day. Sato et al. [38] observed that estradiol decreased the level of procollagenase produced by fibroblasts. Ashcroft et al. [39] showed that postmenopausal women who had taken HRT had markedly increased levels of collagen deposition on the 7th and 84th post-wounding day.

The relationship between estrogens and human endometrium as a target organ has been subject of observation of many authors. In contrast to estradiol, estriol is thought to have a specific vaginotropic and a non-uterotropic influence. However, there are discrepancies in the data from clinical, experimental and pharmacological studies regarding the effects of estriol and estradiol on the uterus and vagina [40].

The purpose of this study was an assessment of benefit and safety of the 28-day transdermal 17-βestradiol

regiment (14 days before and 14 days after operation) versus the 14-day preoperative vaginal conjugated estrogen 0.625 mg/day regiment during vaginal hysterectomy regarding their effect on: endometrium, wound healing, infection and recurrence of organ prolapse.

2. Material and methods

1. Eligibility criteria for participants: at least 1-year long postmenopausal period, e.g. the period between the last menstrual cycle and admitting day at the hospital; and presence of genital prolapse required vaginal hysterectomy.
2. The setting, location and timing where and when the date were collected: the Department for Urogynecology and Pelvic Floor Disorders in the Clinic of Gynecology and Obstetrics, Medical Faculty of the "Saint Cyril and Methodius" University in Skopje in the period from the 1st of January 1998 to the 1st of June 2003. The study was designed according to the CONSORT statement [41].
3. Precise details of the interventions for each group, how and when they were actually administered: the experimental arm, transdermal estrogen hormone replacement therapy (TE HRT) group ($n = 119$) was treated with the preoperative/postoperative 28-day transdermal estrogen hormone replacement therapy regiment with 17-β estradiol 50 mg/day (FEM 7, Merck), 14 days before and 14 days after the vaginal hysterectomy; the control arm, vaginal estrogen hormone replacement therapy (VE HRT) group ($n = 150$) was treated with the preoperative 14-day vaginal estrogen HRT regiment with conjugated estrogens 0.625 mg/day (cream premarin 0.625 mg/g, Wyeth). The both therapeutical protocols were included into the Standard Urogynecological Protocol for Uterine Prolapse of the Department for Urogynaecology and Pelvic Floor Disorders and the study was approved by the local research ethics committee (LREC) of the University Clinic for Gynecology and Obstetrics, Medical Faculty, Skopje.
4. How sample size was determined and the method used to generate the allocation sequence, including details of any restriction: every postmenopausal patient with genital prolapse required vaginal hysterectomy admitted at our Department for Urogynecology in the period from the 1 January 1998 to the 1 June 2003, assessed for eligibility for this study ($n = 329$). Those patients who were admitted at our Department in the period from the 1 January 1998 to the 1 January 2000 were treated with preoperative 14-day regiment with local estrogen vaginal therapy and formed the control group (VE HRT group). At the 1 January 2000, we introduced our new preoperative/postoperative 28-day transdermal estrogen HRT regiment for all patients admitted in order to undergo a vaginal hysterectomy. All patients that were admitted from this period to the 1 June 2003 formed the experimental group (TE HRT group). Fourteen patients were excluded from the study because they refused to participate. So, 315 patients were randomised. Twenty-eight of them were excluded because of the presence of some contraindication for HRT: 10 with a previous history of breast cancer, 6 with a presence of biliar calculosis and 12 with a great disturbance of haemostasis and elevated plasminogen inhibitors and decreased protein C and protein S. Eighteen additional patients dropped out from the study because they were not able to return for a follow-up visit. A total of 269 patients completed the study: 119 as participants of the experimental TE HRT group and 150 as participants of the control VE HRT group. All subjects were given an explanation of the study and written informed consent was obtained. Women who were unhappy to be randomized were excluded. This was a controlled trial with no allowance for patient preference.
5. Specific objectives and hypotheses: the purpose of the study was an assessment of the benefits and safety of the preoperative/postoperative 28-day regiment with transdermal 17-β E_2 during vaginal hysterectomy versus the preoperative 14-day regiment with local transvaginal application of conjugated estrogens. The hypothesis was: the preoperative/postoperative 28-day regiment with transdermal 17-β E2 is more effective in wound healing, inflammatory reaction and prevention of recurrence of the genital prolapse and stress incontinence, but similarly safe regarding endometrial changes, versus the preoperative 14-day local transvaginal conjugated estrogens.

6. Clearly defined primary and secondary outcome measures, any methods used to enhance the quality of measurements:

   The preoperative evaluation

   1. *Demographic data*: age, duration of the postmenopausal age, parity, habits of smoking and alcohol consuming, body mass index (BMI).
   2. *Complete evaluation for urinary incontinence* included: a structured questionnaire for urinary symptoms with standardized terminology, based on the International Continence Society recommendation [42,43]; complete multichannel urodynamic examination (which is an obligatory step in the preoperative evaluation of patients with genital prolapse according to our Standard Uroginecological Protocol and includes: retrograde provocative multichannel urethrocystometry, passive and dynamic urethral pressure profilometry, cough, and Valsalva leak point pressure, simple uroflowmetry with postvoid residual urine volume) and Marshall's coughing test in upright position, lithotomy and lithotomy position with artefitial apex reposition for detection of the potential urinary stress incontinence after bladder filling with 300 mL 3% boric acid by transurethral catheter.
   3. *Complete evaluation for genital prolapse*: a structured questionnaire with standardized terminology and pelvic organ prolapse quantification according to the International Continence Society's Pelvic Organ Prolapse Quantification (POPQ) system [44]. All patients had pelvic examination performed in the supine position in a birthing chair while performing the Valsalva maneuver (Vm), with maximal effort and during Pozzi maneuver (Pm), with pulling down of the uterine cervix or vaginal cuff (on the postoperative controls) with Pozzi clamp, which allows the full development of the prolapse. The bladder was empty by catheterization and rectum too, by morning defecation.
   4. In all patients of both groups the *ultrasound examination* of the genital organs and preoperative measurement of the endometrial thickness were performed. If the endometrium was thicker than 4 mm, an explorative curettage was done.
   5. On the admitting day, and on the third postoperative day hemoglobin and haemathocrit were analysed as comparative parameters for blood loss during the operation; the day before and 5th day after the operation were analysed: leukocytes count and PMNLs/lymphocytes ratio, as laboratory signs of body infection; the day before the operation and the 3rd, 5th and 7th postoperative day the body temperature was evaluated, as objective signs of the body infection. Also, some parameters of the coagulation, such as: prothrombin time, activated partial tromboplastin time, thrombin time and number of platelets were evaluated on the admitting day and on the 3rd postoperative day.

Surgical technique: All patients received routine perioperative antibiotic prophylaxis and sequential compression boots for deep vein thrombosis prophylaxis. Patients were placed in dorsal lithotomy position while they were under spinal anesthesia. The standard surgical procedure was vaginal hysterectomy with or without oophorectomy that obligatory included the combination of the anti-incontinence procedure: The endopelvic fascia plicaturation according to Lazarevski [45] at the level of the urethro-vesical junction in a form of three slingoidal layers just underneath the bladder neck with three mattress 1–0 delayed-absorbable sutures. This procedure forms a pyramidal supporting wedge just underneath the bladder neck, which functions as a support only during the increased abdominal pressure.

Postoperative care: All patients of both groups received standard postoperative prophylaxis against infection (3 days cephalosporine of the third generation) and standard postoperative thromboprophylaxis (low-molecular Heparin, Frahepan 3000 UI/day applied subcutaneously) till withdrawal. The patients, who required higher heparinization, were excluded from the study.

Histopathological examinations: Diagnostic explorative curettage was performed in all the patients with preoperative ultrasound thickness of the endometrium more than 4 mm. The curettage materials, as well as operative specimens were fixed in 10% neutral buffered formalin for 24–48 h and routinely processed in paraffin wax. The endometrial thickness was measured in the operative specimen during dissection. The operative specimens of the patients were examined by light

microscopy by the same pathologist, who was not informed of the patient group. The endometrial samples were graded using the following standard morphological criteria: atrophy, cystic atrophy, disordered proliferative (resembling findings caused by anovulatory cycles), endometrial polyp, hyperplasia (simplex, complex or atypical) and endometrial carcinoma. Estimation of the estrogenic influence on the endometrium was done as in routine histological dating of normal cycling endometrium. Emphasis was not on mitotic counts, but on crowding and/or architectural changes of the endometrial glands and on the epithelial proliferation (degree of stratification of nuclei).

The postoperative evaluation included:

1. *Operative details*: duration of the operation, blood loss (with measurement of the used gauzes before and after the operation and changes in hemoglobin and haemethocrit), intraoperative complications as: lesion of the bladder, rectum, ureter.
2. *In the early postoperative period* were evaluated: (A) indirect signs for local infection, such as: vaginal bleeding, vaginal discharge, fetid vaginal discharge, swelling, crusting, pain, clearly visible wound opening on the vaginal vault and vaginal wall suture sites according to postoperative questionnaire and vaginal examination by the operator on the 7-th postoperative day and (B) duration of the postoperative hospitalization period: till 7 day, or prolonged hospitalization due to presence of local or systemic body infection.
3. *Follow-up analyses*: initial follow-up occurred in all patients of both groups (269 subjects) at the 4-week postoperative visit, when the wound healing signs, such as: duration of pain symptom in the postoperative period, duration of the vaginal postoperative discharge, duration of dysuria after removal of a urethral catheter, degree of suture-sites re-epithelization according to postoperative questionnaire and vaginal examination by the operator were evaluated. Subsequent follow-up occurred in all 269 patients (100%), with a median follow-up of 28.3 months (range, 12–52 months) for VE HRT group, and of 24.5 months (range, 9–48 months) for TE HRT group. At the follow-up visit patients underwent: (1) *complete evaluation for urinary incontinence*; (2) *complete evaluation for genital prolapse*; (3) *the patient assessment of outcome* regarding the results of surgery on pelvic prolapse, and the time of renewal and satisfaction of sexual intercourses were evaluated.

3. Statistical methods

The Student's paired test was used to compare: demographic data, signs of wound healing, signs of postoperative infection, intraoperative blood loss, status of coagulation, preoperative and postoperative quantitative description of pelvic organ position with anatomic landmarks according to POPQ system in both groups. The Mantel-Haenzel's X^2-test was used to compare: demographic data, preoperative and postoperative functional symptoms and urodynamic diagnoses, signs of wound healing, signs of postoperative infection, preoperative and postoperative stages of prolapse according to POPQ system, morphological features of the endometrium in both groups, according to the formula:

$$X^2 = \frac{n([AD - BC] - n/2)^2}{(A + b)(C + D)(A + C)(B + D)}$$

4. Results

The operative time in the whole series averaged 60 min, blood loss averaged 240 ± 80 mL (range, 120–380 mL). There were no intraoperative injuries to the bladder, ureter, rectum or small bowel. All patients were discharged in good condition.

There were no significant differences in demographic data between the groups (Table 1).

Regarding the effects on the wound healing of the two proposed therapeutical schemes, assessed by postoperative questionnaire, vaginal examination by the operator on the 7th postoperative day and on the first (4 week) control; and prolonged hospitalization due to presence of local or systemic body infection, we found significant differences in favor of the preoperative/postoperative 28-day regiment with TE HRT: (1) vaginal fetid discharge, swelling and crusting, requiring of additional antibiotic therapy, postoperative hos-

Table 1
Demographic data: age, duration of the postmenopausal age, parity, habits of smoking and alcohol consuming, sport, diet, body mass index, systolic/diastolic blood pressure, profession

Variable	TE HRT ($n = 119$)	VE HRT ($n = 150$)	t/x^2	p
Age (years) (mean ± S.D.)[a]	62.5 ± 12.8	64.3 ± 14.2	0.09	NS
Duration of postmenopausal period (years) (mean ± S.D.)[a]	14.2 ± 3.3	16.1 ± 3.8	0.36	NS
Parity (mean ± S.D.)[a]	3.6 ± 2.1	3.3 ± 2.0	0.10	NS
Height (cm) (mean ± S.D.)[a]	158.8 ± 4.5	160.1 ± 3.8	0.22	NS
Weight (kg) (mean ± S.D.)[a]	61.2 ± 7.5	64.1 ± 7.8	0.27	NS
Body mass index (mean ± S.D.)[a]	24.6 ± 3.0	25.1 ± 3.2	0.11	NS
Smoker[b]	10/119	12/150	0.011	NS
Alcohol consumer[b]	3/119	2/150	0.07	NS
Factory worker[b]	15/119	20/150	0.002	NS
Farmer[b]	21/119	23/150	0.12	NS
Clerk/teacher[b]	4/119	7/150	0.05	NS
Housewife[b]	50/119	68/150	0.90	NS
Retired person[b]	29/119	32/150	0.20	NS
Sport/diet[b]	4/119	6/150	0.0002	NS
Diastolic blood pressure (mmHg)[a]	92.13 ± 13.20	94.22 ± 18.40	0.09	NS
Systolic blood pressure (mmHg)[a]	145.65 ± 22.33	148.12 ± 18.95	0.08	NS

[a] Student's t-test.
[b] Mantel-Haenzel's X^2-test with d.f. of 1.

pitalization longer than 10 days ($p < 0.001$); (2) vaginal discharge on the first (4 week) control ($p < 0.05$); (3) clearly visible wound opening on the suture sites in the first (4 week) control ($p < 0.01$); (4) patient assessment of outcome regarding the results of surgery on pelvic prolapse at the first (4 week) and last follow-up control ($p < 0.001$) (Table 2).

In Table 3, we present the postoperative blood analyses and changes in the afternoon body temperature as signs of postoperative body infection. The VE HRT group showed: significantly higher PMNLs/lymphocytes ratio, bigger increase of leukocytes count, less patients with leukocytes count lower than$10 \times 10^9\,L^{-1}$ and more patients with leukocytes count between $10 \times 10^9\,L^{-1}$ and $15 \times 10^9\,L^{-1}$ and higher than$15 \times 10^9\,L^{-1}$ on the 5th postoperative day ($p < 0.001$; $p < 0.01$; $p < 0.001$; $p < 0.001$; $p < 0.001$, respectively). Additionally, the number of patients with the afternoon body temperature between 37and 38 °C or more than 38 °C on the 5th postoperative day, as well as the number of patients with the afternoon body temperature between 37 and 38 °C on the 7th postoperative day were significantly higher in the same group ($p < 0.05$; $p < 0.01$; $p < 0.001$, respectively) (Table 3).

Regarding the postoperative analyses of blood picture as signs of intraoperative blood loss, we did not find significant differences between the groups. (Table 4).

Regarding the effects of the two proposed therapeutical schemes on the postoperative status of coagulation, we found that on the 3rd postoperative day: the platelets count showed decrease versus preoperative values ($p < 0.001$); and prothrombin time, prothrombin time-INR, aPTT pathromtin SL and thrombin time were longer ($p < 0.01$, $p < 0.01$, 0.002 and $p < 0.001$, respectively) versus preoperative values in TE HRT group comparing with VE HRT group (Table 5).

As to the stage of the prolapse on the last follow-up control: (1) there were no significant differences in the prolapse of the anterior segment between both groups, but in the TE HRT group the number of patients with stage 0 apical segment was higher ($p < 0.05$) (Table 6); (2) the analysis of the quantitative description of pelvic organ position with anatomic landmarks according to the POPQ system showed that: point C was significantly higher ($p < 0.01$) and total vaginal length longer in the TE HRT ($p < 0.01$; $p < 0.05$, respectively) (Table 7).

In regard to the presence of the functional symptoms and urodynamic diagnoses on the last follow-up control, the symptoms such as: frequency, postoperative constipation, painful coitus and incontinence during the intercourse were more frequent in the VE HRT ($p < 0.001$; $p < 0.05$; $p < 0.05$; $p < 0.05$, respectively) (Table 8).

Table 2
Effects on the wound healing assessed by: postoperative questionnaire, vaginal examination by the operator, prolonged hospitalization due to presence of local or systemic body infection and patient assessment of the results of surgery on pelvic prolapse

Variable	TE HRT ($n = 119$)	VE HRT ($n = 150$)	t/x^2	p
Number of patients with presence of pain symptoms in postoperative period[a]	42/119	93/150	17.87	0.001
Duration of pain symptoms In the postoperative period (days)[b]	6.25 ± 1.24	7.05 ± 1.43	2.01	0.05
Number of patients with fetid vaginal discharge in postoperative period[a]	8/119	33/150	10.83	0.001
Number of patients with swelling and crusting in the first seven postoperative days[a]	9/119	36/150	11.72	0.001
Number of patients with clearly visible wound opening on suture sites in the first postoperative week	2/119	19/150	9.65	0.01
Number of patients who required additional antibiotic therapy[a]	2/119	24/150	13.99	0.001
Number of patients who required vaginal washing with 3% H_2O_2[a]	0/119	15/150	10.78	0.01
Duration of dysuria after removal of a urethral catheter (days)[b]	3.6 ± 2.1	3.3 ± 2.0	0.10	NS
Number of patients with postoperative hospitalization less or equal to 7 days[a]	105/119	50/150	9.61	0.01
Number of patients with postoperative hospitalization between 7 and 10 days[a]	11/119	41/150	12.79	0.001
Number of patients with postoperative hospitalization longer than 10 days[a]	3/119	59/150	48.65	0.001
Patient assessment of outcome at the first (4 week) control[a]				
Satisfied of the results of surgery on pelvic prolapse	112/119	105/150	23.23	0.001
Not satisfied of the results of surgery on pelvic prolapse	7/119	45/150	23.23	0.001
Re-epithelization of the suture sites on the first (4 week) control[a]				
Complete	113/119	125/150	7.69	0.01
Incomplete	6/119	26/150	7.69	0.01
Number of patients with presence of granulations on the suture sites on the first (4 week) control[a]	15/119	30/150	2.10	NS
Number of patients with vaginal discharge on the first control (4 week)[a]	11/119	30/150	5.14	0.05
Number of patients with clearly visible wound opening on suture sites on the first (4 week) control[a]	0/119	3/150	0.93	NS
Patient assessment of outcome at the last follow-up control[a]				
Satisfied of the results of surgery on pelvic prolapse	114/119	121/150	14.13	0.001
Not satisfied of the results of surgery on pelvic prolapse	5/119	29/150	14.13	0.001
Time of renewal of sexual intercourses (week)[b]	6.32 ± 1.23	8.72 ± 1.12	1.44	NS

[a] Mantel-Haenzel's X^2-test with d.f. of 1.
[b] Student's t-test.

The morphological features of the endometrium in the curettage materials, as well as operative specimens in the both study groups are presented in Table 9. Diagnostic curettage was performed in seventeen patients from the TE HRT group and thirteen patients from the VE HRT group with preoperative ultrasound thickness more than 4 mm. Simplex endometrial hyperplasia was present in only two patients (2/17, 11.8%) belonging to the TE HRT group, while in all the remaining patients from both groups the presence of endometrial polyp resulted in thickened endometrium. The number of patients with endometrium in the operative specimen thinner than 2 mm was significantly higher in VE HRT group ($p < 0.05$), while there were significantly more patients with endometrial thickness between 2 and 4 mm in TE HRT group ($p < 0.01$). Nevertheless,

Table 3
Postoperative analyses of blood picture and changes in the afternoon body temperature as signs of postoperative body infection

Variable	TE HRT ($n = 119$)	VE HRT ($n = 150$)	t/x^2	p
Leukocytes on the 5th postoperative day[a] ($10^9\ L^{-1}$)	8.15 ± 1.06	11.38 ± 1.22	2.00	0.05
PMNLs on the 5th postoperative day[a] (%)	70.52 ± 9.20	93.45 ± 9.10	1.77	NS
Lymphocytes on the fifth postoperative day[a] (%)	21.82 ± 1.20	6.54 ± 0.98	9.85	0.001
PMNLs/lymphocytes ratio on the 5th postoperative day[a]	3.12 ± 0.65	14.19 ± 0.77	11.00	0.001
Increase of leukocytes'count on the 5th day vs. preoperative value ($10^9\ L^{-1}$)[a]	1.99 ± 0.65	5.38 ± 1.01	2.83	0.01
Number of patients with leukocytes count lower than $10 \times 10^9\ L^{-1}$ on the 5th postoperative day[b]	96/119	52/150	54.62	0.001
Number of patients with leukocytes count between $10–15 \times 10^9\ L^{-1}$ on the 5th postoperative day[b]	21/119	73/150	26.73	0.001
Number of patients with leukocytes count higher than $15 \times 10^9\ L^{-1}$ on the 5th postoperative day[b]	2/119	25/150	14.88	0.001
Number of patients with normal afternoon body temperature on the 3rd postoperative day[b]	89/119	65/150	25.51	0.001
Number of patients with afternoon body temperature between 37 and 38 °C on the 3rd postoperative day[b]	26/119	81/150	27.30	0.001
Number of patients with afternoon body temperature more than 38 °C on the 3rd postoperative day[b]	4/119	4/150	0.00	NS
Number of patients with normal afternoon body temperature on the 5th postoperative day[b]	84/119	66/150	17.95	0.001
Number of patients with afternoon body temperature between 37 and 38 °C on the 5th postoperative day[b]	29/119	59/150	6.09	0.05
Number of patients with afternoon body temperature more than 38 °C on the 5th postoperative day[b]	6/119	25/150	7.69	0.01
Number of patients with normal afternoon body temperature on the 7th postoperative day[b]	112/119	98/150	30.45	0.001
Number of patients with afternoon body temperature between 37 and 38 °C on the 7th postoperative day[b]	7/119	46/150	24.22	0.001

[a] Student's t-test.
[b] Mantel-Haenzel's X^2-test with d.f. of 1.

there was no difference in the frequency of occurrence of the endometrium thicker than 4 mm in both groups. Regarding to the histological features of the endometrium in the hysterectomy specimens, the cystic atrophy of the endometrium was more frequently present in the VE HRT group ($p < 0.05$), while disordered proliferative endometrium was more frequently observed in the TE HRT group ($p < 0.05$). More pro-

Table 4
Postoperative analyses of blood picture as signs of intraoperative blood loss

Variable	TE HRT ($n = 119$)	VE HRT ($n = 150$)	t	p
Hemoglobin preoperatively[a] ($g\ L^{-1}$)	128.54 ± 11.20	132.06 ± 12.05	0.21	NS
Hemoglobin on the third postoperative day[a] ($g\ L^{-1}$)	112.72 ± 12.05	115.38 ± 13.32	0.001	NS
Decrease of the hemoglobin on the 3rdpost- operative day vs. preoperative value[a] ($g\ L^{-1}$)	-15.83 ± 1.66	-16.81 ± 1.23	0.46	NS
Haematocrit preoperatively[a] ($L\ L^{-1}$)	382.9 ± 8.0	391.9 ± 6.0	0.09	NS
Haematocrit on the 3rd postoperative day[a] ($L\ L^{-1}$)	331.0 ± 4.0	337.2 ± 5.0	0.10	NS
Decrease of the haematocrit on 3rd post-operative day vs. preoperative value[a] ($L\ L^{-1}$)	51.9 ± 0.6	57.9 ± 0.7	0.30	NS
Red blood cells count preoperatively[a] ($10^{12}\ L^{-1}$)	4.38 ± 0.32	4.50 ± 0.33	0.26	NS
Red blood cells on the 3rd postoperative day[a] ($10^{12}\ L^{-1}$)	3.87 ± 0.30	3.93 ± 0.29	0.05	NS
Decrease of the red cells count on the 3rd post-operative day vs. preoperative value[a] ($10^{12}\ L^{-1}$)	-0.51 ± 0.026	-0.58 ± 0.027	1.76	NS

[a] Student's t-test.

Table 5
Effects of the two proposed therapeutical scheme on the postoperative changes in status of coagulation

Variable	TE HRT ($n = 119$)	VE HRT ($n = 150$)	t/x^2	p
Platelets count before therapy[a] (10^9 L^{-1})	270.43 ± 9.30	249.64 ± 8.42	1.65	NS
Platelets on the 3rd postoperative day[a] (10^9 L^{-1})	238.42 ± 8.40	245.14 ± 6.25	0.64	NS
Changes in platelets count on the 3rd day vs. before therapy value (10^9 L^{-1})[a]	−32.01 ± 1.62	−4.50 ± 0.80	15.28	0.001
Prothrombin time before therapy[a] (s)	10.60 ± 2.04	10.77 ± 2.20	0.06	NS
Prothrombin time on the 3rd postoperative day[a] (s)	10.73 ± 2.10	10.82 ± 1.99	0.03	NS
Changes in prothrombin time on the 3rd day vs. before therapy value (s)[a]	+0.13 ± 0.03	+0.05 ± 0.01	2.67	0.01
Prothrombin time% before therapy[a] (%)	97.68 ± 17.76	88.23 ± 18.67	0.37	NS
Prothrombin time% on the 3rd postoperative day[a] (%)	89.34 ± 16.40	83.31 ± 12.26	0.29	NS
Changes in prothrombin time% on the 3rd day vs. before therapy value[a] (%)	−8.34 ± 1.29	−4.50 ± 0.92	2.43	0.025
Prothrombin time-INR before therapy[a] (INR)	1.023 ± 0.21	1.062 ± 0.19	0.14	NS
Prothrombin time-INR on the 3rd postoperative day[a] (INR)	1.073 ± 0.19	1.068 ± 0.18	0.02	NS
Changes in prothrombin time-INR on the 3rd day vs. before therapy value (INR)[a]	+0.050 ± 0.012	+0.005 ± 0.009	3.00	0.01
aPTT Pathromtin SL before therapy[a] (s)	27.53 ± 2.32	26.89 ± 3.18	0.16	NS
aPTT Pathromtin SL on the 3rd postoperative day[a] (s)	29.18 ± .3.33	28.16 ± 3.21	0.22	NS
Changes in aPTT Pathromtin SL on the 3rd day vs. before therapy value (s)[a]	+1.65 ± 0.09	+1.27 ± 0.08	3.17	0.002
Thrombin time before therapy[a] (s)	13.07 ± 0.91	13.77 ± 0.88	0.55	NS
Thrombin time on the 3rd postoperative day[a] (s)	15.27 ± 0.85	13.86 ± 0.78	1.23	NS
Changes in thrombin time BC thrombin on the 3rd day vs. before therapy value (s)[a]	+2.20 ± 0.04	+0.09 ± 0.06	29.31	0.001

[a] Student's paired t-test.

nounced morphological changes of the endometrium (endometrial polyp, simplex hyperplasia) occurred with equal frequency at both study groups. Nevertheless, more sinister changes such as complex hyperplasia, atypical hyperplasia or endometrial carcinoma were not observed in any of the endometria of the patients from the both study groups (Table 9).

5. Discussion

From our results represented in Table 2, which describes the estrogen effects on the wound healing in the postoperative period, we can conclude that the TE HRT regiment was more effective. The same conclusion we can make also from Table 3, which represents the postoperative analyses of blood picture and changes in the afternoon body temperature as signs of the postoperative body infection. Some of the up-to-date research on the influence of estrogens on the individual cell types, such as: neutrophils, macrophages, fibroblasts during injury or surgical wound is included. Batra et al. [46], in their retrospective case control study of 44 female patients who underwent combination CO2/Er full-face LSR, of which 16 were postmenopausal using oral HRT during follow-up and were compared with 16 controls without HRT, did not found significant differences in variables evaluated included: erythema, swelling, crusting, pain, assessment of outcome, time until re-epithelization between premenopausal and postmenopuasal patients, and also between groups receiving and not receiving HRT. According to Margolis et al. [47], estrogen in the form of HRT might have an effect on wound healing, because their study showed that patients who received HRT were less likely to develop a venous leg ulcer (relative risk 0.65; 95% CI) or pressure ulcer (0.68; 95% CI) than those who did not use HRT. Characteristic of both chronic and acute wounds that fail to heal are excessive leukocytosis and reduced matrix deposition. Estrogen is a major regulator of wound repair, which is characterized by a dampened inflammatory response and increased matrix deposited at the wound site. Macrophage migration inhibitory factor (MIF) is a candidate pro-inflammatory cytokine involved in the estrogen regulation of the inflammatory process. According to Ascroft et al. [48], estrogen inhibits the local inflammatory response by down-regulating MIF. Beer et al. [49], using the differential display reverse transcriptase-PCR technology, identified the gene encoding the estrogen-responsive B

Table 6
Stage of prolapse at anterior, posterior, apical and most severe segments

POPQ stage	Preoperative values		Last follow up		X_1	X_2
	VE HRT ($n = 150$)	TE HRT ($n = 119$)	VE HRT ($n = 150$) (median, 28.3 mo)	TE HRT ($n = 119$) (median, 24.5 mo)		
Anterior segment[a]						
Stage 0	1 (0.6%)	1 (0.8%)	109 (72.7)	94 (79.0%)	0.30 (NS)	1.11 (NS)
Stage I	25 (16.7%)	23 (19.3%)	10 (6.7%)	9 (7.6%)	0.16 (NS)	0.02 (NS)
Stage II	67 (44.7%)	58 (48.7%)	22 (14.67%)	14 (11.76%)	0.29 (NS)	0.26 (NS)
Stage III	42 (28.0%)	32 (26.9%)	6 (4.0%)	2 (1.7%)	0.004 (NS)	0.56 (NS)
Stage IV	15 (10.0%)	14 (11.8%)	3 (2.0%)	0 (0%)	0.07 (NS)	0.93 (NS)
Posterior segment[a]						
Stage 0	0 (0.0%)	2 (1.7%)	109 (72.7%)	98 (82.4%)	0.77 (NS)	2.98 (NS)
Stage I	22 (14.7%)	20 (16.8%)	14 (9.3%)	7 (5.9%)	0.10 (NS)	0.35 (NS)
Stage II	81 (54.0%)	55 (46.2%)	11 (7.3%)	5 (4.2%)	1.31 (NS)	0.67 (NS)
Stage III	39 (26.0%)	36 (30.3%)	12 (8.0%)	9 (7.6%)	0.32 (NS)	0.03 (NS)
Stage IV	8 (5.3%)	6 (5.0%)	4 (2.7%)	0 (0.00%)	0.09 (NS)	1.67 (NS)
Apical segment[a]						
Stage 0	0 (0.0%)	0 (0.0%)	106 (70.7%)	100 (84.3%)	0.0 (NS)	5.89[b]
Stage I	22 (14.7%)	18 (15.1%)	10 (6.7%)	5 (4.2%)	0.004 (NS)	0.37 (NS)
Stage II	89 (59.3%)	70 (58.8%)	18 (12.0%)	7 (5.9%)	0.002 (NS)	2.26 (NS)
Stage III	27 (18.0%)	22 (18.5%)	12 (8.0%)	6 (5.0%)	0.003 (NS)	0.52 (NS)
Stage IV	12 (8.0%)	9 (7.6%)	4 (2.7%)	0 (0%)	0.009 (NS)	1.66 (NS)
Stage of the most severe segment of prolapse[a]						
Stage 0	0 (0.0%)	0 (0.0%)	103 (68.7%)	93 (78.2%)	0.00 (NS)	2.56 (NS)
Stage I	13 (8.7%)	12 (10.1%)	11 (7.3%)	10 (8.4%)	0.03 (NS)	0.009 (NS)
Stage II	66 (44.0%)	54 (45.4%)	20 (13.3%)	7 (5.9%)	0.34 (NS)	3.30 (NS)
Stage III	59 (39.3%)	46 (38.7%)	12 (8.0%)	7 (5.9%)	1.57 (NS)	0.19 (NS)
Stage IV	12 (8.0%)	7 (5.9%)	4 (2.7%)	2 (1.7%)	0.19 (NS)	0.02 (NS)

POPQ: International Continence Society's Pelvic Organ Prolapse Quantification system; Stage 0, Aa, Ap, Ba and Bp are all at −3, but C is upper or equal to (tvl-2); Stage I, the most distal portion of the prolapse is >1 cm above the hymen; Stage II, the most distal portion of the prolapse is less or equal to 1 cm proximal to or distal to the plane of the hymen; Stage III, the most distal portion is >1 cm below the hymen but protrudes no further than 2 cm less than total vaginal length (tvl); Stage IV, the distal portion of the prolapse protrudes to at least (tvl-2) cm; X_1, differences between preoperative values in both groups; X_2, differences between last follow-up values in both groups.

[a] Mantel-Haenzel's X^2-test with d.f. of 1.

[b] $p < 0.05$.

box protein (EBBP). Their results suggest that the presence of EBBP in basal keratinocytes is important for the differentiation capacity of these cells. The different investigators demonstrate contradictory findings as to the effects of estrogen deficiency, or HRT on the process of wound healing. So, it is difficult to draw valid conclusions. According to Calvin [50], the influence of estrogens on the various phases of wound repair-inflammation, proliferation and remodeling, as an area of research is of paramount clinical importance both in terms of financial cost and human suffering, especially in the elderly population, of whom postmenopausal women comprise the majority. It seems that our proposed transdermal 17-β E2 regiment could be useful for preoperative tissue preparation for vaginal hysterectomy and in prevention of postoperative local infection and more effective wound healing.

The results from Tables 6–8 can be explained with the possible positive effects of estrogens on the strength of the uterine ligaments and fascial structures in female pelvis. It seems that TE HRT is more effective in the postoperative restitution of the pelvic ligaments because of the fact that the potential SI and incontinence during the intercourse were less frequent, and the stage of prolapse of the posterior, apical and most severe segments was significantly lower and the vault of the vagina was significantly higher situated in this group on the last follow-up control. All these findings

Table 7
Quantitative description of pelvic organ position with anatomic landmarks

POPQ stage	Preoperative values		Last follow up		t_1	t_2
	VE HRT ($n = 150$)	TE HRT ($n = 119$)	VE HRT ($n = 150$) (median, 28.3 mo)	TE HRT ($n = 119$) (median, 24.5 mo)		
Aa	+0.22 ± 1.02	+0.56 ± 1.03	−2.84 ± 0.77	−2.44 ± 0.61	0.23	0.41
Ba	+0.55 ± 1.02	+0.99 ± 1.03	−2.48 ± 0.44	−2.82 ± 0.69	0.30	0.41
C	2.55 ± 2.01	−1.11 ± 2.09	−6.03 ± 0.53	−8.05 ± 0.52	0.50	2.73[†]
Bp	0.62 ± 1.00	−0.12 ± 0.99	−2.53 ± 0.50	−2.79 ± 0.30	0.25	0.59
Ap	−1.78 ± 1.01	−1.37 ± 1.02	−2.45 ± 0.43	−2.79 ± 0.40	0.29	0.58
gh	5.02 ± 0.49	5.26 ± 0.41	3.56 ± 0.42	3.92 ± 0.40	0.38	1.12
pb	3.08 ± 0.34	3.32 ± 0.32	3.02 ± 0.35	3.32 ± 0.33	0.51	0.62
tvl	3.22 ± 1.08	2.68 ± 0.82	7.02 ± 0.60	8.65 ± 0.61	0.40	1.96[*]

POPQ: International Continence Society's Pelvic Organ Prolapse Quantification system. Aa, a point located in the midline of the anterior vaginal wall 3 cm proximal to the external urethral meatus; Ba, the most distal position of any part of the upper anterior wall from the vaginal cuff to point Aa; C, leading edge of the vaginal cuff scar; Bp, the most distal position of any part of the upper posterior wall from the vaginal cuff to point Ap; Ap, a point located in the midline of the posterior vaginal wall 3 cm proximal to the hymen; gh, genital hiatus; pb, perineal body; tvl, total vaginal length. t_1, differences between preoperative values in both groups; t_2, differences between last follow-up values in both groups.
[†] Student's paired test-$p < 0.01$.
[*] Student's paired test-$p < 0.05$.

are in favor of the possibility that TE HRT might be enough effective in the reparation of the pelvic ligaments, which have the very important role in the pelvic organs position, such as sacrouterine ligaments for the vagina after hysterectomy, or pubovesical ligaments for the bladder neck. Investigations by Brincat et al. [51] demonstrated that the administration of estrogens increases the collagen content of uninjured skin. According to Ashcroft et al. [48], this effect of estrogens might be possibly mediated by an increase in the activity of cytokine transforming growth factor-β 1. In a condition of estrogen deficit, the mitotic activity and entire function of all cells into the pelvis decrease. This atrophy of the muscular and ligamentar pelvic structures results in genital prolapse. The urinary incontinence is also very common in the postmenopausal age. According to Iossif and Bekassy [52], it is present in 29% of the women older than 60 years. From 228 women-users of HRT in Dulwich Menopause Clinic Study [53], 20% had severe urge incontinence, and even 50% of them had stress incontinence. Estrogens have very important role in: the prevention of the genital atrophy, expenditure of the pelvic blood flow and maintaining of the peripheral sensor perception. The estrogen receptors are already identified into the smooth muscle cells of the pelvic vessels walls [54], and into the peripheral and central nerve system [55]. Girao et al. [56], estimating some Doppler velocimetry parameters of periurethral vessels in 25 postmenopausal incontinent women receiving estrogen replacement, such as: the number of vessels, systolic peak, minimum diastole, resistance and pulsatility indexes and the A/B ratio, prior to estrogen replacement and after 1 and 3 months of hormone use, found that estrogen replacement alone in postmenopausal women with urinary stress incontinence increased the number of periurethral vessels, systolic peak and minimum diastole; however, a trend of no statistical significance towards the reduction of resistance and pulsatility rates of periurethral vessels was found; nor was a significant difference in the A/B ratio shown. Tsai et al. [57], analyzing the bladder neck circulation by Doppler ultrasonography in 113 premenopausal women with USI ($n = 55$) or without USI ($n = 58$); and in 31 postmenopausal women with USI and 12 without USI, all of whom received 0.625 mg conjugated equine estrogen plus 5 mg medroxyprogesterone acetate daily for 6 months, found that: (1) the postmenopausal women with USI had the highest pulsatility index (PI); (2) The presence of USI did not change the PI values in the premenopausal women; (3) after 3 months of HRT, the PI levels decreased significantly ($p < .001$) in the postmenopausal women with USI; (4) the subjective improvement of USI appeared after 3 months of HRT. Martan et al. [58] analyzed ultrasonographic parameters of the lower urinary tract

Table 8
Preoperative and postoperative functional symptoms and urodynamic diagnoses

	Preoperative values		Last follow up		x_1	x_2
	VE HRT ($n = 150$)	TE HRT ($n = 119$)	VE HRT ($n = 150$) (median, 28.3 mo)	TE HRT ($n = 119$) (median, 24.5 mo)		
Urinary symptoms						
Stress incontinence SI	55 (36.6%)	39 (32.7%)	18 (12.0%)	7 (5.9%)	0.29	2.27
Potential SI	42 (28.0%)	28 (23.5%)	12 (8.0%)	3 (2.5%)	0.48	2.82
Genuine SI	13 (8.7%)	11 (9.2%)	6 (4.0%)	4 (3.4%)	0.003	0.002
Frequency	93 (62.0%)	69 (58.0%)	55 (36.7%)	25 (21.0%)	0.28	7.06[†]
Urgency	76 (50.7%)	62 (52.1%)	61 (40.7%)	37 (31.1%)	0.56	2.23
Hiperactive bladder	56 (37.3%)	43 (36.1%)	37 (24.7%)	22 (18.5%)	0.006	1.14
Hesitancy	70 (46.7%)	55 (46.2%)	55 (36.7%)	33 (27.7%)	0.002	2.02
Nocturia	62 (41.3%)	52 (43.7%)	43 (28.7%)	23 (19.3%)	0.07	2.64
Incomplete emptying	94 (62.7%)	78 (65.5%)	23 (15.3%)	9 (7.6%)	0.13	3.12
Weak stream	83 (55.3%)	63 (52.9%)	12 (8.0%)	7 (5.9%)	0.01	1.19
Manual reposition to start voiding	54 (36.0%)	45 (37.8%)	0 (0.0%)	0 (0.0%)	0.03	0.00
Bowel symptoms						
Flatus incontinence	18 (12.0%)	15 (12.6%)	8 (5.3%)	6 (5.0%)	0.001	0.03
Incontinence of liquid stool	4 (2.7%)	3 (2.5%)	0 (0.0%)	0 (0.0%)	0.10	0.00
Urgency of defecation	13 (8.7%)	10 (8.4%)	7 (4.7%)	5 (4.2%)	0.02	0.01
Discomfort with defecation	105 (70.0%)	85 (71.4%)	12 (8.0%)	4 (3.6%)	0.01	1.79
Constipation	91 (60.7%)	73 (61.3%)	37 (24.7%)	16 (13.4%)	0.0002	4.60[*]
Digital manipulation to finish defecation	97 (64.7%)	78 (65.5%)	4 (2.7%)	2 (1.7%)	0.0004	0.02
Feeling of incomplete evacuation	65 (43.3%)	52 (43.7%)	6 (4.0%)	3 2.5%)	0.004	0.11
Rectal protrusion during defecation	28 (18.7%)	22 (18.5%)	5 (3.3%)	3 (2.5%)	0.01	0.0008
Sexual symptoms						
Pain with coitus	130 (86.7%)	101 (84.9%)	27 (18.0%)	9 (7.6%)	0.0004	5.37[*]
Unsatisfactory coitus	134 (89.3%)	104 (87.4%)	12 (8.0%)	6 (5.0%)	0.48	0.52
Decrease in orgasmic response	130 (86.7%)	100 (84.0%)	61 (40.7%)	47 (39.5%)	0.19	0.005
Incontinence during the intercourse	45 (30.0%)	38 (31.9%)	22 (14.7%)	7 (5.9%)	0.009	4.45[*]
Other local symptoms						
Vaginal pressure and heaviness	128 (85.3%)	103 (86.5%)	10 (6.7%)	5 (4.2%)	/0.01	0.37
Vaginal/perineal pain	78 (52.0%)	56 (47.1%)	16 (10.6%)	8 (6.7%)	0.47	0.83
Awareness of tissue protrusion	137 (91.3%)	106 (89.1%)	13 (8.7%)	9 (7.6%)	0.47	0.01
Low back pain	102 (68.0%)	92 (77.3%)	17 (14.3%)	7 (5.9%)	2.47	1.80
Abdominal pressure	92 (61.3%)	71 (59.7%)	7 (4.7%)	4 (3.3%)	0.02	0.05
Observation or palpation of a mass	124 (82.7%)	98 (82.4%)	17 (14.3%)	8 (6.7%)	0.009	1.20

x_1, differences between preoperative values in both groups; x_2, differences between last follow-up values in both groups Mantel-Haenzel's X^2-test with d.f. of 1.
[†] $p < 0.001$.
[*] $p < 0.05$.

in 40 women after the menopause with the stress or mixed type of urinary incontinence before and after 2-month local estriol treatment (Ovestin) and found: (1) after treatment no statistically significant differences in thickness of the urethral sphincter area, nor in the thickness of the pelvic floor muscles before and after estriol administration; (2) statistically significant differences in the mobility of the urethrovesical junction and a significant increase in the thickness of the urethral mucosa and a more abundant vascularization; (3) in women with the mixed type of incontinence after estrogen treatment a decline in the thickness of the urinary bladder. These facts give an excellent explanation why HRT results into dramatic improvement also of the sexual function. The estrogen deficit affects the nerve transmission, as well as the peripheral blood flow. Sorrel [59], investigating the mean vulvar blood flow with Doppler in postmenopausal women-users of transder-

Table 9
Morphological features of the endometrium

Variable	TE HRT ($n = 119$)	VE HRT ($n = 150$)	x^2	p
Histological features of the endometrium in patients with preoperative ultrasound thickness >4 mm, who required diagnostic curettage				
Endometrial polyp[a]	15/17 (88.2%)	13/13 (100%)	0.04	NS
Endometrial hyperplasia[a]	2/17 (11.8%)	0/0 (0.0%)	0.04	NS
Thickness of the endometrium in the operative specimen				
Less than 2 mm[a]	97/119 (81.5%)	138/150 (92.0%)	5.69	0.05
2–4 mm[a]	16/119 (13.4%)	6/150 (4.0%)	6.66	0.01
More than 4 mm[a]	6/119 (5.0%)	6/150 (4.0%)	0.01	NS
Histological features of the endometrium in the hysterectomy specimen				
Atrophic endometrium[a]	73/119 (61.3%)	87/150 (58.0%)	1.85	NS
Cystic atrophy of the epithelium[a]	24/119 (20.2%)	51/150 (34.0%)	5.64	0.05
Disordered proliferative endometrium[a]	20/119 (16.8%)	10/150 (6.7%)	5.90	0.05
Endometrial polyp[a]	6/119 (5.0%)	18/150 (12.0%)	3.14	NS
Simplex hyperplasia[a]	2/119 (1.7%)	2/150 (1.3%)	0.07	NS
Complex hyperplasia[a]	0/119 (0.0%)	0/150 (0.0%)	0.0	NS
Atypical hyperplasia[a]	0/119 (0.0%)	0/150 (0.0%)	0.0	NS
Endometrial cancer[a]	0/119 (0.0%)	0/150 (0.0%)	0.0	NS

[a] Mantel-Haenzel's X^2-test with d.f. of 1. x^2, differences between preoperative values in both groups; NS, not significant.

mal HRT, found its great improvement and, in the same time, an improvement of the sexual problems, which depended of the estrogen serum levels. Semmens and Wagner [60] report similar results in their study of postmenopausal women-users of HRT, investigating the vaginal blood flow. Blakeman et al. [61], in 59 patients undergoing urogynecological surgery of whom 23 were premenopausal, 20 were postmenopausal and taking no HRT and 16 were postmenopausal and receiving HRT, found significantly higher levels of Ki-67 expression (robust marker of cell proliferation) in the squamous epithelial tissues of the lower urinary tract and vagina in the HRT group. Pushkar et al. [62], in their study of 118 patient who underwent transvaginal operations, in 82 of them, who received local estriol treatment (Ovestin cream) for 2–3 weeks before the operation, found improvement in: duration of pain symptom, vaginal discharge, dysuria after removal of the urethral catheter, the time of renewal of sexual intercourses.

From Table 5, which represents the postoperative changes in status of coagulation, we can see that TE HRT was more effective than VE HRT. Aune et al. [63], investigating the effects of 12-month treatment with topical and oral combined HRT on the reactivity of platelets in whole blood in 32 postmenopausal women found significant reductions in the formation of thromboxane B2 and concluded that HRT reduces the cellular activation of blood platelets. According to the Writing Group for the 3rd European Conference on Sex Steroids and Cardiovascular Diseases [64], sex steroids increase thrombin and fibrin degradation products and activation markers of coagulation (prothrombin fragment 1 + 2), but these changes are minor or moderate and in normal subjects within the normal physiological ranges. Estradiol administered transdermally does not usually induce significant changes in coagulation or fibrinolysis. According to our results the transdermal estrogens might not have any influence on the hepatic synthesis of coagulating factors till the step of prothrombin formation. They might have an essential influence on the step of prothrombin transformation into thrombin, as well as on the process of megakaryocytes segregation into platelets.

Endometrial polyps were the most frequent cause of endometrial thickening in patients with preoperative ultrasound thickness more than 4 mm. Regarding the histological analysis of the operative specimens, we found that endometrium with a thickness between 2 and 4 mm, as well as endometrium with apparent morphological signs of estrogenic stimulation (disordered proliferative) was significantly more frequent in the TE HRT group ($p < 0.05$). Nevertheless, there were no significant differences in occurrence of thicker endometrium (more than 4 mm) and

endometrium with more pronounced morphological changes (endometrial polyp, simplex hyperplasia) in both study groups. Therefore, it seems that TE HRT has a more significant, although weak estrogenic effect on endometrial morphology. On the other hand, there were no significant differences in more pronounced morphological changes under estrogenic stimulation between TE HRT and VE HRT study group. Both regiments proved to be relatively safe with regards to the non-occurrence of complex, atypical hyperplasia and endometrial carcinoma in any of the patients. Von Haafen et al. [40] did not find clear differences between vaginal estradiol and estriol with regard to the effects on receptor levels in vaginal and uterine tissues, and similar signs of estrogen stimulation of the endometrium at the light microscopy. Naessen and Rodriguez-Macias [65], monitoring changes in endometrial thickness during treatment with ultralow doses of estradiol (7.5 μg/day), delivered by a vaginal ring in 60 postmenopausal women for 12 months found the maximal endometrial thickness of 2.8 mm at baseline and 2.6 mm at 12 months. According to them, this might indicate that there is a "therapeutic window" for systemic effects without apparent increase in endometrial thickness. Manonai et al. [66], in their study of 53 postmenopausal women treated with local vaginal treatment of 25 μg estradiol or 1 g of conjugated estrogen cream for 12 weeks found only 2 cases of endometrial proliferation and restoration of normal vaginal epithelium and urogenital symptoms. Fanchin at al. [67], in their study of 39 infertile women received 17-β E_2 (2 mg/day) orally or vaginally found significantly thicker endometrium on day 14 in the vaginal E_2 cycles. Cagnacci et al. [68], found that endometrial thickness increased within 3 months from 3.07 to 5.74 mm, with no further increases, and only one case of endometrial hyperplasia at 12 months in his study group of 48 postmenopausal women receiving sequential transdermal E_2 (50 μg/day) combined in the last 14 days with norethisterone (0.25 mg/day).

6. Conclusion

In recent years there has been negligible work on the effects of estrogens on the wound healing in the vaginal surgery, emphasizing the need for further experimentation in this area. Our aim with using the new TE HRT treatment during vaginal hysterectomy was to get a more rapid and effective restoration of the integrity and function of pelvic organs after the operation, which was confirmed in this our study. There were no clear differences between estrogens applied transdermally and transvaginally with regard to the effects on endometrial thickness and histological changes of the endometrium.

References

[1] Batra SC, Iosif SC. Female urethra: a target for estrogen action. J Urol 1983;129:418–20.
[2] Di Carlo F, Racca J, Gallo F, et al. Oestrogen and progesterone receptors in the human vagina. J Endocrinol Invest 1985;8:131–4.
[3] Versi E, Cardozo L, Brincat M, et al. Correlation of urethral physiology and skin collagen in post-menopausal women. Br J Obstet Gynecol 1988;95:147–52.
[4] Moalli PA, Talarico LC, Sung VW, et al. Impact of menopause on collagen subtypes in the arcus tendineous fasciae pelvis. Am J Obstet Gynecol 2004;190(3):620–7.
[5] Tomaszewski J, Adamiak A, Skorupski P, Rzeski W, Rechberger T. Effect of 17 beta-estradiol and phytoestrogen daidzein on the proliferation of pubocervical fascia and skin fibroblasts derived from women suffering from stress urinary incontinence. Ginekol Pol 2003;74(10):1410–4.
[6] Ewies AA, Al-Azzawi F, Thompson J. Changes in extracellular matrix proteins in the cardinal ligaments of post-menopausal women with or without prolapse: a computerized immunohistomorphometric analysis. Hum Reprod 2003;18(10):2189–95.
[7] Reay Jones NH, Healy JC, King LJ, Saini S, Shousha S, Allen-Mersh TG. Pelvic connective tissue resilience decreases with vaginal delivery, menopause and uterine prolapse. Br J Surg 2003;90(4):466–72.
[8] Otto LN, Slayden OD, Clark AL, Brenner RM. The rhesus macaque as an animal model for pelvic organ prolapse. Am J Obstet Gynecol 2002;186(3):416–21.
[9] Chen B, Wen Y, Wang H, Polan ML. Differences in estrogen modulation of tissue inhibitor of matrix metalloproteinase-1 and matrix metalloproteinase-1 expression in cultured fibroblasts from continent and incontinent women. Am J Obstet Gynecol 2003;189(1):59–65.
[10] Dundar M, Kocak I, Erkus M, Celasun B. Changes in urethral connective tissue after estrogen replacement therapy in ovariectomized rats. Scand J Urol Nephrol 2002;36(3):173–6.
[11] Jackson S, James M, Abrams P. The effect of oestradiol on vaginal collagen metabolism in postmenopausal women with genuine stress incontinence. BJOG 2002;109(3):339–44.
[12] Falconer C, Ekman-Ordeberg G, Blomgren B, et al. Paraurethral connective tissue in stress-incontinent women after menopause. Acta Obstet Gynecol Scand 1998;77(1):95–100.
[13] Hasselquist MB, Goldberg N, Schroeter A, et al. Isolation and characterisation of the estrogen receptor in human skin. J Clin Endocrinol Metab 1980;50:76–82.

[14] Gulshan S, McCruden AB, Stimson WH. Oestrogen receptors in macrophages. Scand J Immunol 1990;31:691–7.
[15] Malet C, Gompel A, Yaneva H, et al. Oestradiol and progesterone receptors in culturated normal human breast epithelial cells and fibroblasts: immunocytochemical studies. J Clin Endocrin Metab 1991;73:8–17.
[16] Venkov CD, Rasnkin AB, Vaughan DE. Identification of authentic oestrogen receptor in cultured endothelial cells. A potential mechanism for steroid hormone regulation of endothelial function. Circulation 1996;94:727–33.
[17] Baker BL. The connective tissue reaction around implanted pellets of steroid hormones. Anat Rec 1954;119:529–32.
[18] Nyman S, Lindhe J, Zederfeldt B. Vascular permeability in wounded areas of oestradiol and progesterone treated female rabbits. Acta Chir Scand 1971;137:709–14.
[19] Lundgren D. Influence of estrogen and progesterone on exudation, inflammatory cell migration and granulation tissue formation in preformed cavities. Scand J Plast Reconstr Surg 1973;7:10–4.
[20] Gouveia MA, Halbe HW, Schutze Filho N. The significance of female sex steroids on the vascular permeability of the traumatised uterine serosa of rats. Arch Gynecol Obstet 1987;241:121–6.
[21] Murthy CP, Prakash A, Pandit PN, et al. Effects of oestrogen on wound healing-an experimental study. Indian J Surg 1974;36:1–7.
[22] Rosenblum WI, el-Sabban F, Nelson GH. One day of oestradiol treatment enhances platelet aggregation at the site of microvascular injury without altering aggregation ex vivo. Life Sci 1988;42:123–8.
[23] Jansson G. Oestrogen-induced enhancement of myeloperoxidase activity in human polymorphonuclear leukocytes-1 possible cause of oxidative stress in inflammatory cells. Free Rad Res Commun 1991;14:195–208.
[24] Magnusson U, Einarsson S. Effects of exogenous oestradiol on the number and functional capacity of circulating mononuclears and polymorphonuclear leukocytes in the sow. Vet Immunol Immunopathol 1990;25:235–47.
[25] Miyagi M, Aoyama H, Morishita M, et al. Effects of sex hormones on chemotaxis of human peripheral polymorphonuclear leukocytes and monocytes. J Periodont 1992;63:28–32.
[26] Calvin M, Dyson M, Rymer J, et al. The effects of ovarian hormone deficiency on macrophage infiltration during the inflammatory phase of wound healing in a rat model. Wounds: Compend Res Clin Prac 1998;10:158–63.
[27] Taylor FW, Ditter TL, Porter DO. Wound healing and the steroids. Surgery 1952;31:683–90.
[28] Portugal H, Lima A, Olivera GR, et al. The effect of sex steroids upon granulation tissue. Int Arch Allergy 1951;2:274–81.
[29] Fisher R, Paar J. Carrageenin granuloma in the guinea-pig and rat. Am Med Assoc Arch Path 1960;70:565–9.
[30] Nyman S. Studies on the influence of estradiol and progesterone on granulation tissue. Granulation tissue formation and respiratory gas tensions in wound fluid in oestradiol and progesterone treated female rabbits. J Periodont Res 1971;6(suppl 7):12–3.
[31] Pallin B, Ahonen J, Zederfeldt B. Granulation tissue formation in oophorectiomized rats treated with female sex hormones: Studies on the amount of collagen and on tensile strength. Acta Chir Scand 1975;141:710–4.
[32] Lindhe J, Birch J, Branemark P. Wound healing in estrogen treated female rabbits. J Periodont Res 1968;3:21–3.
[33] Lundgren D. Influence of estrogen and progesterone on vascularization of granulation tissue in preformed cavities. Scand J Plas Reconstr Surg 1973;7:85–90.
[34] Hu SK, Mitcho YL, Rath NC. Effect of oestradiol on interleukin 1 synthesis by macrophages. Int J Immunopharm 1988;10:247–52.
[35] Shanker G, Sorci-Thomas M, Adams MR. Oestrogen modulates the inducible expression of platelet-derived growth factor mRNA by monocyte/macrophages. Life Sci 1995;56:499–507.
[36] Morales DE, McGowan KA, Grant DS, et al. Oestrogen promotes angiogenic activity in human umbilical vein endothelial cells in vitro and in a murine model. Circulation 1995;91:755–63.
[37] Jorgensen O, Schmidt A. Influence of sex hormones on granulation tissue formation and on healing of linear wounds. Acta Chir Scand 1962;124:1–10.
[38] Sato T, Ito A, Mori Y, et al. Hormonal regulation of collagenolysis in uterine cervical fibroblasts. Modulation of synthesis of procollagenase, prostromelysin and TIMPs by progesterone and oestradiol-17 beta. Biochem J 1991;275:645–50.
[39] Ashcroft GS, Dodsworth J, van Boxtel E, et al. Estrogen accelerates cutaneous wound healing associated with an increase in TGF-beta 1 levels. Nat Med 1997;3:1209–15.
[40] Von Haaften M, Donker DM, Sie-Go DM, Haspels AA, Thijssen JH. Biochemical and histological effects of vaginal estriol and estradiol applications on the endometrium, myometrium and vagina of postmenopausal women. Gynecol Endocrinol 1997;11:175–85.
[41] Moher D, Schulz K, Altman G. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. Lancet 2001;357:1191–4.
[42] Abrams P, Blaivas JG, Stanton SL, Anderson JT. The standardization of terminology of lower urinary tract function. The International Continence Society Committee on Standardization of Terminology. Scand J Urol Nephrol 1988;114S:5–19.
[43] Walters M. Description and classification of lower urinary tract dysfunction and pelvic organ prolapse. In: Walters DM, Karram MM, editors. Urogynecology and reconstructive pelvic surgery. 2nd ed. St. Luis Mosby Inc.; 1999. p. 3542.
[44] Bump RC, Mattisson A, Bo K, Brubaker LP, et al. The standardization of terminology of female pelvic organ prolapse and pelvic floor dysfunction. Am J Obstet Gynecol 1996;175:10–7.
[45] Lazarevski BM. Suburethral duplication of the vaginal wall – an original operation for urinary stress incontinence in women. Int Urogynecol J 1995;6:73–9.
[46] Batra RS, Dover JS, Hobbs L, Phillips TJ. Evaluation the role of exogenous estrogen in postoperative progress after laser skin resurfacing. Dermatol Surg 2003;29:43–8.
[47] Margolis DJ, Knauss J, Bilker W. Hormone replacement therapy and prevention of pressure ulcer and venous leg ulcers. Lancet 2002;359:675–7.
[48] Ashcroft GS, Mills SJ, Lei K, et al. Estrogen modulates cutaneous wound healing by downregulating macrophage

migration inhibitory factor. Clin Invest 2003;111:1309–18.
[49] Beer HD, Munding C, Dubois N, Mamie C, Hohl D, Werner S. The estrogen-responsive B box prote: a novel regulator of keratinocyte differentiation. J Biol Chem 2002;277:20740–9.
[50] Calvin M. Oestrogens and wound healing. Maturitas 2000;34:195–210.
[51] Brincat M, Moniz CF, Studd J.W.W, et al. Sex hormones and skin collagen content in postmenopausal women. Br Med J 1983;287:1337–8.
[52] Iossif C, Bekassy Z. Prevalence of genito-urinary symptoms in the late menopause. Acta Obstet Gynecol Scand 1984;63:257–60.
[53] Cardozo LD, Tapp A, Versi E. The lower urinary tract in peri- and postmenopausal women in the urogenital oestrogen deficiency syndrome. In: Samsoe G, Eriksan PB, editors. The urogenital oestrogen deficiency syndrome. Bagsvaerd: Novo Industri A/S; 1987. p. 10–22.
[54] Sorell MM. Sexuality and menopause. Obstet Gynecol 1990;75S:25S–6S.
[55] Horwitz KB, Horwitz LD. Canine vascular tissues are targets for androgens, estrogen's, progestinis and glucocorticoids. J Clin Invest 1982;69:750–8.
[56] Girao MJ, Jarmy-Di Bella ZI, Sartori MG, Baracat EC, Lima GR. Doppler velocimetry parameters of periurethral vessels in postmenopausal incontinent women receiving estrogen replacement. Int Urogynecol J Pelvic Floor Dysfunct 2001;12(4):241–6;
Girao MJ, Jarmy-Di Bella ZI, Sartori MG, Baracat EC, Lima GR. Doppler velocimetry parameters of periurethral vessels in postmenopausal incontinent women receiving estrogen replacement. Obstet Gynecol 2001;98(1):52–6.
[57] Tsai E, Yang C, Chen H, Wu C, Lee J. Bladder neck circulation by Doppler ultrasonography in postmenopausal women with urinary stress incontinence. Obstet Gynecol 2001;98(1): 52–6.
[58] Martan A, Masata J, Halaska M, Voigt R. Ultrasound imaging of the lower urinary tract in post-menopausal women with urinary stress or combined type of incontinence before and after intravaginal administration of estriol. Ceska Gynekol 1999;64(1):6–9.
[59] Pfaff D, Mc Ewan B. Action of estrogen's and progestinis on nerve cells. Science 1983;219:808–14.
[60] Semmens JP, Wagner G. Estrogen deprivation and vaginal function in postmenopausal women. JAMA 1982;248:445–8.
[61] Blakeman P, Hilton P, Bulmer J. Cellular proliferation in the female lower urinary tract with reference to oestrogen status. Br J Obstet Gynaecol 2001;108:813–6.
[62] Pushkar DI, D'lakov VV, Godunov BN, Gvozdev MI. Ovestin I preparation for transvaginal surgeries. Urologia 2002;5:34–6.
[63] Aune B, Oian P, Omsjo I, et al. Hormone replacement therapy reduces the reactivity of monocytes and platelets in whole blood: a beneficial effect on atherogenesis and throbus formation? Am J Obstet Gynecol 1995;173:1816–20.
[64] The Writing Group for the 3rd European Conference on Sex Steroids and Cardiovascular Diseases. The European Consensus Development Conference 2002: sex steroids and cardiovascular diseases on the route to combined evidence from OC and HRT/ERT. Maturitas 2003; 44:69–82.
[65] Naessen T, Rodriguez-Macias K. Endometrial thickness and uterine diameter not affected by ultralow doses of 17 beta-estradiol in elderly women. Am J Obstet Gynecol 2002;186:944–7.
[66] Manonai J, Thepisai U, Suthutvoravut S, Udomsubayakul U, Chittacharoen A. The effect of estradiol vaginal tablet and conjugated estrogen cream on urogenital symptoms in postmenopausal women: a comparative study. J Obstet Gynaecol Res 2001;27:255–60.
[67] Fanchin R, Righini C, Schonauer LM, Olivennes F, Cunha Filho JS, Frydman R. Vaginal versus oral E2 administration: effects on endometrial thickness, uterine perfusion, and contractility. Fertil Steril 2001;76:994–8.
[68] Cagnacci A, Arangino S, Draetta FP, Angiolucci M, Volpe A, Melis GB. Transdermal administration of estradiol and norethisterone: effect on the uterus and uterine arteries. Menopause 2000;7:117–23.

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