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LECTURES, ORAL PRESENTATIONS AND POSTERS

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The role of autoimmune mechanisms of normogonadotropic hypo-ovarionism formation

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Introduction

Markers of ovarian reserve, plasma levels of gonadotropins, prolactin, sex steroids, adrenal hormones and free forms of thyroid hormones, as well as organ-specific antiovarian autoantibodies and anti-thyroidperoxidase autoantibodies have been studied by ELISA at adolescent-girls with normogonadotropic ovarian failure and oligomenorrhea. Autoimmune oophoritis has been diagnosed in 44.3% of patients, the diagnostics criteria of which at normogonadotropic form of ovarian failure in puberty are elevated levels of circulating Anti-Ovarian Antibodies (AOA), reduced Anti-Müllerian hormone, elevated serum levels of inhibin B, the activation of the pituitary-adrenal axis in the absence of ovarian hyperandrogenia. A violation of thyroid status at adolescent-girls with oligomenorrhea was revealed in the form of primary hypothyroidism and autoimmune thyroiditis formation, more pronounced at low Anti-Müllerian hormone rates and high serum levels of circulating antiovarian antibodies (OAO) that points to the role of co-autoimmune disorders in the formation of ovarian failure.
Sir Burnet, a creator of clonal selection theory of immunity, believed that if the cause of any disease cannot be established, it must be regarded as an autoimmune disease (1).
According to Notkins (2007) natural autoantibodies can be informative predictors of a variety of diseases and syndromes, in the months and years leading clinical manifestation of disease (2). At the present time the role of autoimmune pathology in the formation of normogonadotropic ovarian insufficiency (NOI) in adolescent-girls remains unexplored, which was the objective of this study.

Materials and methods

The research object was 88 adolescent-girls with normogonadotropic oligomenorrhea. Inclusion criteria: age ≤ 18 years; no history of sexual contacts, sex-specific gynecological inflammatory and acute infectious diseases, chromosomal or monogenic diseases; normal BMI and plasma prolactin levels. We excluded patients suffering from cancer and severe somatic diseases, and diseases that lead to a folliculogenesis disorder; having hormone treatment.
The control group included 20 healthy adolescent-girls with regular menstrual cycle. Age of the patients averaged 15.5 (16.75; 15) years and had no intergroup differences.
Serum concentrations of LH, FSH, TSH, prolactin, estradiol, testosterone, cortisol, 17 OHP, DHEAS, AOA, neopterin, Anti-Müllerian hormone (AMH) and inhibin B were investigated by ELISA. An ultrasound of the ovaries have been conducted. Statistical analysis of the results was carried out using the Statistica 6.0 application package.

Results and discussion

All the patients with oligomenorrhea were divided

TABLE 1 - THE CONTENT OF GONADOTROPINS, PROLACTIN, OVARIAN HORMONES, MARKERS OF OVARIAN RESERVE (AMH, INHIBIN B), AOA AND NEOPTERIN IN THE SERUM OF PATIENTS OF THE STUDIED GROUPS.

Indicators	I group (n=39)	II group (n=49)	Control group (n=20)
LH[2]	**3.3** (5,7; 2,5)	**8.1 (12,1**; 3,2**)**	**5.95** (7,15; 5,15)
FSH[2]	**5.5** (7,2; 3,95)	**4.7 (**6,3; 3,7**)**	**6.3** (7,13; 4,95)
Prolactin[2]	**693 (**771,5; 287,5**)**	**509 (**701; 304,5)	**468** (562,5; 378,5)
Э2[3]	**64** (89,25;22,3)	**61** (90,25; 20,25)	**47.5** (60; 39)
Cortisol[5]	**624** (858,5; 518**)**	**573** (638; 500**)**	**520** (605,25; 465,5)
17-OHP[4]	**1.4 (**1,5; 1,3**)** •	**0.9 (**1,5; 0,8**)** •	**0.81** (0,95; 0,69)
Testosteron[4]	**0.6 (**0,7; 0,4)	**1.5 (**1,8; 0,98**)** •*	**0.47** (0,66; 0,4)
AMH[4]	**1.4** (3,3; 0,96) •	**5.95** (7,1; 3,5) *•	**3.55** (4,13; 3,1)
Inhibin B[3]	**128.5** (162,8; 90,4) •	**45.43** (118,3; 36,7) *•	**38.61** (88,33; 24,9)
AOA(u/ml)	**19** (29,35; 11,9) •	**3.05** (4,5; 1,4) *	**4.4** (6,2; 2,8)
Neopterin[5]	**6.99** (9,11; 6,45)•	**11.0** (25,1; 3,6)•*	**5.89** (7,29; 3,5)

[1] The data in the study groups in all the tables are presented in the following format: ME (Kv75%; Kv25%).
[2] мМЕ/л[3]pg/ml[4]ng/ml[5]nmol/l.
Legend statistically valid differences (p <0.05): * with group I • with the control group

into 2 groups according to the presence of AOA in diagnostic titer (>10 U/ml). I group: 39 adolescent-girls with elevated AOA levels (≥11 IU/ml).II group: 49 patients with normal AOA levels (Table 1).
We found that patients of group I showed 6.2 fold increase of the serum level AOA as compared to the values in the control group ($p<0.05$) and 4.2 fold increase as compared to the values in the group II ($p<0.05$).
Functional status of ovaries is determined by follicular reserve, which is evaluated by the content of serum inhibin B and AMH (4).
Patients in group I were averages AMH 2.5 times lower ($p <0.001$), and the rate of inhibin B - 6 times higher than in the control group ($p <0.001$) and 4 times higher than in group II ($p <0.001$) (Table 1).
These results testify to deceleration of output of primordial follicles from rest in a phase of active growth.
AMH level in group II was higher than in group I by 1.8 times ($p <0.001$), and higher than in the control group by 1.7 times ($p <0.001$), and left the upper limit of regulations. It is known that the level of AMH above 5.0 ng/ml may be one of the diagnostic criteria for PCOS (4). The level of inhibin B in the group II was higher than in the control group - 1.2 times ($p <0.05$), but did not go beyond the standards (40-100 pg/ml).
17 OHP level did not differ in both groups ($p> 0.05$), but exceed the value of the control group, indicating activation of the adrenal axis. High levels of testosterone and 17 OHP in group II showed a mixed - ovarian and adrenal genesis of hyperandrogenia. The presence of ovarian component in the genesis of the HA in group II confirmed correlation between testosterone and a maximum diameter of the follicle ($\rho = 0,52$, $p <0.05$).
Anovulation can be caused by multiple small follicles production of estrogen and inhibin B, which inhibits FSH secretion and disrupts the rhythm of production of gonadotropins (5). Elevated values of serum inhibin B against amenorrhea may indicate only the presence of the growth and maturation of follicles set, but not to achieve their ovulatory maturity. This is confirmed by our established correlations between inhibin B and the size of the follicles ($\rho = 0.6$, $p <0.05$).
Thus, the diagnostic criteria for autoimmune oophoritis at normogonadotropic form of ovarian insufficiency in adolescent-girls is to increase the level of circulating AOA, decline in AMH, elevated serum inhibin B, activation of the pituitary-adrenal axis in the absence of ovarian hyperandrogenia.
Comparison of biometrics ovaries showed no dif-

ference between the group I and the control groups (p> 0.05). The size of the ovaries in patients of group II were higher than healthy adolescents (p <0.05). The number of follicles in group I was lower than in the group II (p <0.05), but in both groups the number of small follicles exceed the value of the control group (p <0.05), which is a universal manifestation of ovarian failure occurring at any etiology of development (5).

Thus, inhibin B level was increased in group I. However, as a result of autoimmune destruction affecting the theca cells, disrupted interaction theca and granulosa, and, according to the two-cell theory, this leads to a decrease in estrogen synthesis and impaired follicular maturation at the stage of "predominant". This led to the multifollicular ovarian transformation, anovulation and oligomenorrhea in our patients.

Because of the hidden flow of autoimmune diseases, the determination of a biological marker that directly trigger the formation of auto-antibodies is difficult in the laboratory and is often tardy character (6, 7). Neopterin is the most informative parameter reflecting the activation of cellular immunity in a number of diseases. In this regard, of particular interest to study serum levels of neopterin in patients with oligomenorrhea that might help determine its diagnostic value and role as an AO marker. Serum neopterin in patients of group II has a maximum value in comparison with group I and the control group. Since non-specific clinical signs appear before the specific, this indicator can be considered as an earlier marker of autoimmune diseases, organ-specific antibodies than what was shown in 1993 in the works of Fuchs et al. (7). Consequently, oligomenorrhea patients of group II can be regarded as a manifestation of the autoimmune disease, diagnosed before specific antibodies seroconversion. It should be noted that this assumption is debatable and requires further research for its confirmation. The results of the correlation analysis which establish a close relationship of this indicator with levels of cortisol ($\rho = 0.71$ $p = 0.046528$) and 17 OHP ($\rho = 0.64$ $p = 0.008\ 577$) in patients of group II indicate for the benefit of our opinion on the participation of neopterin in the genesis of oligomenorrhea.

The debut of the reproductive pathology at puberty as NOI manifested by oligomenorrhea and amenorrhea, accompanied by the activation of cellular immunity, as indicated by the increase in the concentration of neopterin in the serum. This indicator can be considered as a predictor of clinical manifestations of autoimmune process, and as a marker of autoimmune diseases and the criterion of gravity in the seronegative form of the disease.

Our results confirmed the involvement of the immune system in the pathogenesis of ovarian dysfunction, as diseases to autoimmune mechanisms of development. It is possible to select 2 variants of the shift of the immune response – at the Tx-1 type, revealed in patients of group II, as indicated by the normal level of antibodies and high neopterin level; and Th-2 type, which was found in group I - the low level of neopterin, elevated titers of autoantibodies.

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Clinical significance of atypical squamous cells of undetermined significance in detecting preinvasive cervical lesions in pre and post menopausal Albanian women

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Introduction

Cancer of the cervix uteri is the 4th most common cancer among women, with an estimated 527,624 new cases and 265,653 deaths in 2012. Worldwide, mortality rates of cervical cancer are substantially lower than incidence with a ratio of mortality to incidence to 50.3% (GLOBOCAN 2012). The majority of cases are squamous cell carcinoma followed by adenocarcinomas. Up to 80-85% of cervical cancer-related deaths occur in low-income countries.

The link between cervical cancer and HPV infection has been well established. From the more than 100 types of HPV described, about 40 are known to infect the genital tract and about 20 have been classified as oncogenic to humans. Persistent infection with high-risk HPV has been considered as the necessary condition for malignant transformation of the cervical epithelium. In most studies, HPV16 and HPV18 are the predominant genotypes: they cause about 70% of precancerous lesions and cervical cancer.

Different Authors have shown a low abnormal rate of Pap smears in older women. Although the positive pressure ventilation (PPV) for neoplastic lesions of squamous atypia in women age > 40 years was reported to be lower than that in women age ≤ 40 years in a study by Kaminski et al. (6.3% *vs* 23%), 2 other more recent studies showed a PPV for SIL of ASCUS in older women (33 and 22%, respectively) that was comparable to the value in postmenopausal women in the current study (30%). The Authors of the latter two studies concluded that squamous cell abnormalities in postmenopausal women could be significant and carry a real risk of an underlying intraepithelial lesion. Therefore, overcalling ASCUS (squamous atypia) in older women most likely is no longer an issue. Instead, the under-diagnosis of SIL may occur because of over-adjustment for age-related changes in these women.

Based on the results of a new study by Keating et al., overcalling ASCUS now appears to be a problem in perimenopausal women. The slide review in the current study showed that cells are designated as ASCUS for a number of reasons. Although the majority of these "abnormal" features were present in comparable percentages among the three age categories, a significantly higher percentage of ASCUS smears in the premenopausal group showed an increased N:C ratio and irregular nuclear membranes in the abnormal cells, features that are associated with SIL. Conversely, abnormal cells in perimenopausal women were most likely to be metaplastic (i.e., atypical squamous metaplastic cells of undetermined significance). Atypical squamous metaplastic cells on Pap smears are known pitfalls. Although they may represent high grade SIL, they also may represent atrophy. In perimenopausal women, adjustment for age-related changes may not be made as often as in postmenopausal women, thus accounting for the apparent overcalling of ASCUS as manifested by the increased ASCUS-to-SIL ratio and low PPV for SIL reported in the current study.

Management of ASCUS includes: repeat cytology, colposcopy and/or HPV DNA testing. In pre-menopausal and post-menopausal age women atrophic changes due to hypoestrogenism often diagnosed as ASCUS and resulting in over diagnosis and over treatment.
The aim of this study is the evaluation of clinical significance of atypical squamous cells of undetermined significance in detecting preinvasive cervical lesions in post-menopausal women among the women of the region of Albania.

Materials and methods

This case control study was conducted in "Mbretëresha Geraldinë" Hospital, Tiranë, Albania in the time period between January 2012 and August 2014. Health services in this hospital are provided by the government.
In the study participated 500 patients with ASCUS, who were evaluated with ASCUS cytology and divided in two groups: 194 post-menopausal women (group 1) and 306 pre-menopausal women (group 2). From the study were excluded women on hormonal replacement therapy, previously treated for CIN, women who were pregnant, women who were treated with chemotherapy or women that had smoked for more than 5 years.
Pap smear was performed by using the cytobrush technique and evaluated according to the 2001 Bethseda, while cervical brush specimens were collected in Cell Collection Media System for HPV Testing. DNA was purified with Magna Pure DNA isolation kit I on the MagNa Pure LC System. After the nucleic acid isolation, the samples were analyzed by Roche Linear Array HPV Genotyping Test (Roche Diagnostics, IN, USA). Polymerase chain reaction (PCR) was used to assess HPV DNA by amplifying the viral DNA followed by the detection of the presence and types of HPV nucleic acid hybridization using a reverse hybridization system.
All of the patients in both groups underwent immediate colposcopy and endocervical curettage and conization was performed to all women with a result suggestive of CIN 2-3. On all women was assessed HPV DNA and the smoking status. Histopathological results and demographic features of patients were compared between the two groups.

Results

A total of 500 women with the cytological diagnosis of ASCUS was included in the study. There were compared 194 post-menopausal women and 306 pre-menopausal women. Mean age of the patients was 54.6±6.5 years in group 1 and 38±6.6 years in group 2. Some 19 (9.4%) of post- menopausal women and 49 (15.9%) of pre-menopausal women were current smokers. None of them was suffering from another disease. Totals of post-menopausal and pre-menopausal women were assessed for HPV-DNA. HPV-DNA testing was performed by polymerase chain reaction (PCR) with pU1M/pU2R primers in GENOMA Laboratory, Rome, Italy.
High risk HPV was detected in 27 (14%) and 85 (28%), respectively (p=0.029).
Final histopathological results recorded were normal cervix, low grade cervical intra-epithelial neoplasia (CIN 1), and high grade cervical intra-epithelial neoplasia (CIN2-3).
In group 1, results were 84.8, 12.2 and 1.8%, respectively, and in group 2 were 71.9, 23.2 and 4.9%.
Women with a suggestive result for CIN2-3 underwent conization.
Two cases were detected as micro invasive carcinoma in premenopausal group (1%); two cases were detected as endometrial carcinoma in the menopausal group (0.6%) (Table 1) (Figure 1).

Discussion and conclusion

In this study, we compared clinical features of atypical squamous cells of undetermined significance (ASCUS) in PAP test in post-menopausal women with women that have yet to reach menopause and are still reproductive. In the study participated a total of 194 post-menopausal women (group 1) and 306 pre-menopausal women (group 2). It was detected that preinvasive lesions were statistically significantly higher in pre-menopausal women than post- menopausal women with ASCUS. Also, High risk HPV were significantly higher in premenopausal women. Meanwhile, cervicitis was more common in menopausal women.
ASCUS is defined as presence of abnormal, but not precancerous squamous cells in the Pap smear. The

TABLE 1 - FINAL HISTOPATHOLOGIC RESULTS FOR THE PATIENTS.

Final diagnosis	Postmenopausal	Premenopausal	p
Normal	54.8%	71.9%	0.1
HPV	14%	28%	
CIN1	12.2%	23.2%	
CIN 2-3	1.8%	4.9%	
Microinvasive cancer	0	1%	
Endometrial cancer	0.6%	0%	

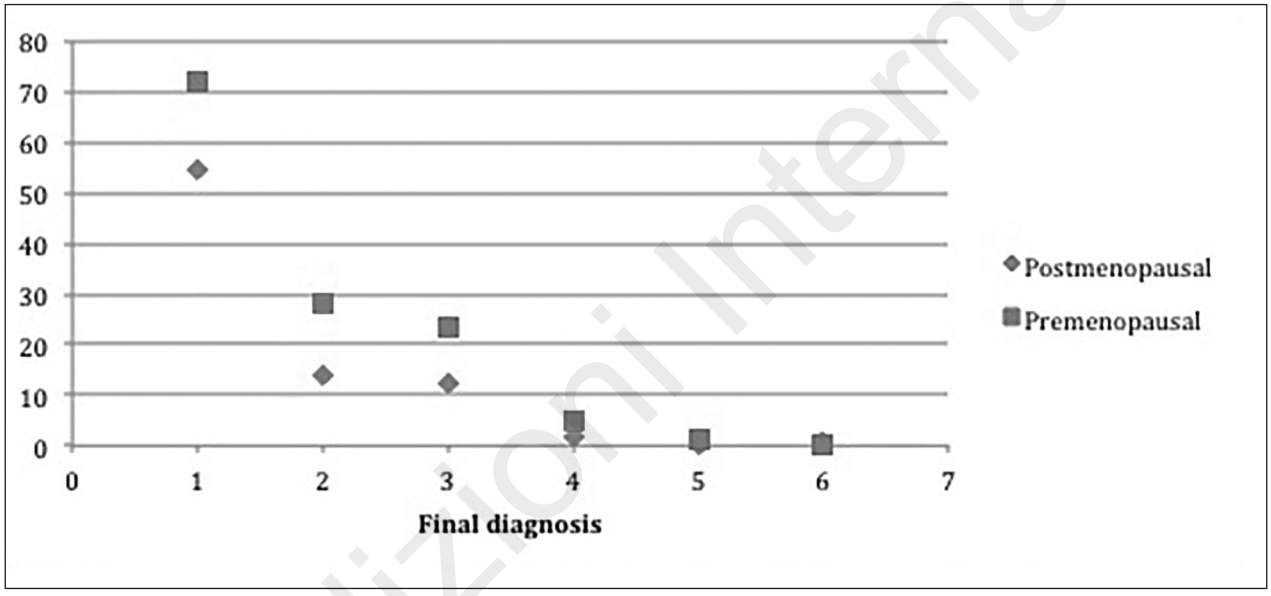

Figure 1 - Final histopathologic results for the patients displayed in a chart for easier comparison.

management of these pathologies, in adult women include an immediate colposcopy, a repetition of the Pap test every 4 or 6 months, or having a reflexive HPV DNA test. A reflexive HPV DNA test utilizes the sample used for the Pap smear and eliminates the need for another sampling. An HPV DNA test is performed just like a Pap smear. The test detects the presence of a high risk HPV infection that could potentially lead to cervical pre-cancer or cancer if left unmonitored or untreated. On the other hand, Pap smear has a sensitivity of 85% and reduces the cost of unnecessary colposcopy but also may result in delay in diagnosis of preinvasive cervical lesions.

Previous studies have reported that HPV DNA has a favor of decline as the age of the women increase (Johnstone and Logani, 2007). In another study in 2005, it was reported that in cervical HPV DNA positive women with newly apparent infections decreased, whereas persistence increased with age. In a previous study Authors found that with increasing pathological grade, the positive rate of high risk HPV also increased (Wang et al., 2013). Kececioglu et al. (2013) reported that HPV DNA testing results in more cases with high grade preinvasive lesions than immediate colposcopy in women who had ASCUS on first PAP test. We also found similar results to these studies. In our post-menopausal group women HPV DNA positivity and preinvasive lesions were lower than the premenopausal age women.

But, while this approach reduces the referral rate to colposcopy, it could increase the cost of management.

Hormonal changes in post-menopausal women, especially hypoestrogenism that results in vaginal and

cervical atrophy, are a risk factor for Pap smear abnormalities. To prevent unnecessary treatments in these women, Piccoli et al. (2008) recommend a single dose of estrogen replacement therapy. In a study held in 2013, it was proposed that hypoestrogenism should be treated before the invasive procedures take place in a woman's body. They also found that these women had more benign histological changes than pre-menopausal women.

It was also recommend managing post-menopausal women with ASCUS in the same manner as pre-menopausal women.

Some of the limitations in our study include: lack of follow-up, retrospective design, biopsies in inadequate smear and/or unsatisfactory colposcopy, conventional cytobrush technique for Pap smear, and no treatment to atrophy. Another possible limitation is that although using primary data from a randomized trial reduces concerns of bias, some of the ALTS trial conditions do not mirror community practice.

In conclusion, we think that management of post-menopausal women with ASCUS should be similar to those in pre-menopausal age women. These strategies prevent over diagnosis and over management.

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The potential benefits of Ovaleap® (follitropin alpha) for the management of female infertility in Spain: a budget impact model

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Objectives

In Spain, 12% of the population is affected by infertility. There were 1,250 cycles of assisted reproductive technology (ART) treatment per one million residents in 2010, well below the 1,500 cycles required each year; consequently, long waiting lists in the public sector may lead to women seeking private care at a personal cost. Ovaleap® (follitropin alpha), a Gonal-f® biosimilar, is a recombinant human follicle-stimulating hormone (r-hFSH) indicated for the treatment of anovulation or stimulation of multifollicular development in ART. A budget impact model was developed from the Spanish payer perspective to evaluate the economic impact of managing women affected by infertility with Ovaleap® instead of other r-hFSH – Gonal-f® (follitropin alpha) and Puregon® (follitropin beta) – over 5 years.

Methods

The eligible patient population was calculated using data from the National Statistics Institute of Spain, an epidemiological review of infertility in Spain, and the Spanish Infertility Registry. The current treatment pattern and drug acquisition costs were based on market research. The drug acquisition cost of Ovaleap® was based on the price difference between previously-launched biosimilars and their originators in Spain. The costs of administration and healthcare practitioner time were taken from publicly-available sources.

Results

The model estimated that 116,646 and 144,541 women seek treatment for anovulation and stimulation of multifollicular development in ART each year, respectively, and are thus eligible for treatment with r-hFSH. Assuming a hypothetical uptake of Ovaleap® reaching 30% in years 3-5, the model predicted cost savings totalling €49.5 million over five years. These savings could potentially enable 45,316 additional women to be treated for infertility with a full course of Ovaleap®.

Conclusions

Ovaleap® may offer cost savings compared with other r-hFSH used to treat anovulation or for ART, potentially allowing more women to receive r-hFSH for the management of infertility.

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The effect of dydrogesterone on placental angiogenesis and pregnancy outcomes in women with threatened miscarriages

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Introduction

Miscarriage is one of pregnancy complication associated with early placental dysfunction which is characterized by premature excessive maternal blood perfusion of the intervillous space at the 1st trimester, deficient trophoblast invasion, failure of maternal spiral artery remodeling, and abnormal placental angiogenesis at the 1st and the 2nd trimesters (1). If the pregnancy was prolonged such morphological changes of placental bed and placenta will clinically manifest at the 3rd trimester as preeclampsia, intrauterine growth restriction (IUGR), placental abruption, preterm birth, fetal distress and stillbirth (2). During normal pregnancy the placenta undergoes dramatic vascularization to enable feto-maternal circulation. These processes require a delicate balance of pro-angiogenic and anti-angiogenic factors (3). The method of placental blood flow visualization *in vivo* based on 3D power Doppler at the 1st and the 2nd trimesters of pregnancy can be an important tool for predicting placental disorders, the basis for evaluation of therapeutic possibilities of timely correction of these states (4, 5).
Pharmacologic therapies for women with confirmed threatened abortion include uterine muscle relaxants, human chorionic gonadotropin, progesterone, and dydrogesterone (retroprogesterone) (6). Numerous studies have showed that the treatment of threatened miscarriages at the 1st trimester with dydrogesterone appeared to be efficacious in preventing of pregnancy loss at the 1st and the 2nd trimesters (7-11). But it's unclear how can dydrogesterone therapy during the 1st trimester reduce the incidence of pregnancy complications in the 3rd trimester (preterm delivery, gestational hypertension, fetal distress) and improve perinatal outcomes (12, 13).
The aims of the study were to investigate the effect of dydrogesterone on placental angiogenesis (placental vasculature, angiogenic balance) in women with threatened miscarriages.

Material and methods

A total of 121 women with singleton pregnancies was included. Of these, 93 women were found to have vaginal bleeding or subchorionic hematomas within the first 13 weeks of gestation and were randomized to receive oral dydrogesterone (group A, n=48) or no dydrogesterone therapy with bed rest and vitamin supplements (group B, n=45). Dydrogesterone treatment (10 mg twice a day) in the group A was started after the confirmation of viable pregnancy in cases of subchorionic hematomas or vaginal bleeding. The therapy continued for 2 weeks after the bleeding had stopped or hematomas reduction. The patients were included into the group A and the group B if they had vaginal bleeding or subchorionic hematomas, normal size of gestation sac, presence of fetal heart activity from 7 weeks. Patients were excluded from the study if they had anembryonic pregnancy, no fetal heart activity, abnormal fetal nuchal translucency value be-

tween the 11th and 14th weeks of gestation, abnormal fetal karyotype and malformations, history of recurrent miscarriages, severe vaginal bleeding accompanied by the expulsion of retained products of conception. Women with clinically normal pregnancies and outcomes were included into the control group (n=28).

All women underwent 2D- and 3D-ultrasound scans (at 8-12, 10-14, and 18-22 weeks of gestation) using Voluson 730 Expert (GE Medical Systems, Austria). Evaluation of placental vasculature and placental blood flow were performed using 3DPD. Placental vascularization index (VI, i.e. vascular density) and placental flow index (FI, i.e. blood perfusion) were obtained automatically using the VOCAL™ program.

Maternal serum concentrations of vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and soluble VEGF receptor 1 (sFlt-1) were determined by immunoassays (R&D Systems, USA) in samples from all women at 18-22 weeks of gestation.

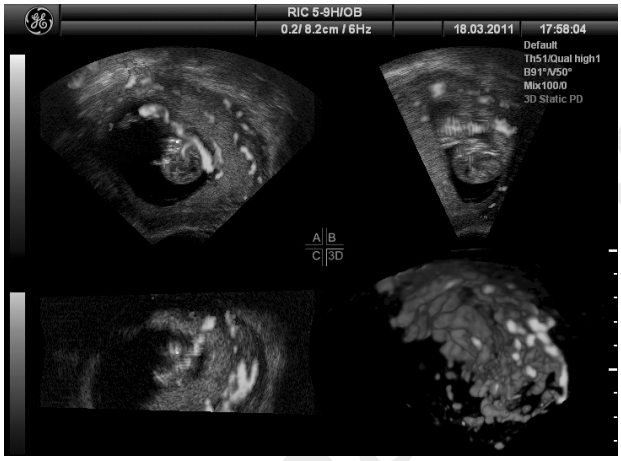

Figure 1 - 3D power Doppler ultrasound of placental bed in women at 11 weeks' gestation (group A) demonstrates flow in spiral arteries with placental indices: VI=21.7%, FI=32.1.

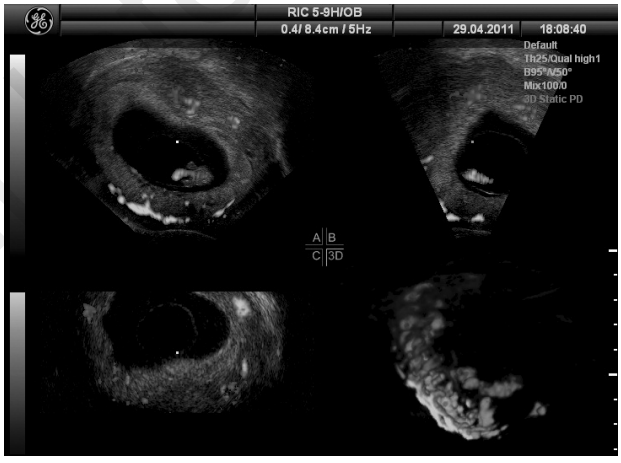

Figure 2 - 3D power Doppler ultrasound of placental bed in women at 11 weeks' gestation (group B) demonstrates flow in spiral arteries with placental indices: VI=10.9%, FI=38.4.

Results

Most women in the group A and B reported a previous miscarriage (81.3 and 91.1% respectively, p=0.18). Dydrogesterone therapy was started in 80.9% patients of the group A during the 7th or 8th week of gestation and in 19.1% patients after the 8th week of gestation. It was continued for 5.3±3.2 weeks. Dydrogesterone had no unwanted effects on pregnancy outcomes in women with threatened miscarriages.

The group A and the group B were associated with low VI values of placenta at 8-12 weeks' gestation compared with controls (9.1 [7.0-19.4]% and 9.7 [7.7-18.9]% *vs* 25.6 [18.5-29.9]% respectively, p<0.008 for all). The rate of increase in VI values in the group A was 1.6 times higher than in the group B at 8-12 and 10-14 weeks of gestation. No significant differences in VI values of placenta at 10-14 weeks' gestation between the group A and controls (21.1 [14.3-25.9]% *vs* 26.2 [22.2-30.3]%, p=0.07) were observed (Figure 1).

The group B was associated with a 1.9-fold decrease in VI values of placenta compared with the controls at 10-14 weeks' gestation (13.6 [10.9-18.9]% *vs* 26.2 [22.2-30.3]%, p=0.01) (Figure 2).

The group A was associated with low FI values of placenta after the dydrogesterone therapy with compared with controls at 10-14 weeks' gestation (31.0 [28.1-33.2] *vs* 43.6 [41.1-51.2], p=0.002). There were no significant differences between FI values of the group B and controls (p=0.24).

In the group B compared to the control group VEGF (54.8 [27.9-70.1] pg/ml *vs* 20.1 [7.2-38.8] pg/ml, p<0.05) and sFlt-1 (7435.8 [1680.3-10349.6] pg/ml *vs* 749.2 [503.3-1251.7] pg/ml, p<0.01) were significantly higher, and PlGF was significantly lower (61.2 [49.5-88.1] pg/ml *vs* 256.1 [223.1-283.7] pg/ml, p<0.01) at 18-22 weeks of gestation. There were no differences in serum levels of VEGF, sFlt-1, and PlGF in the group A and

controls (27.5 [14.0-34.9] pg/ml, 1585.7 [854.4-2454.7] pg/ml, and 186.8 [118.2-333.8] pg/ml respectively, p>0.05 for all).

The frequency of perinatal asphyxia was less in the group A than in the group B (2.1 *vs* 15.6%, p=0.03), while there were no significant differences between the groups A and B with regard to IUGR (18.8 *vs* 35.6%, p=0.11). The group A had significantly lower rate of perinatal hypoxic-ischemic encephalopathy than the group B (12.5 *vs* 44.4%, p=0.001). The incidence of preeclampsia was significantly lower in the group A than in the group B (0 *vs* 15.6%, p=0.005). The frequency of early-term cesarean delivery in the group A was lower than in the group B (25.0 *vs* 51.1%, p=0.017).

The pathology study of placentas after delivery revealed numerous chorionic villi with little intervillous space in the group A. Placental villi were characterized by few syncytial knots and numerous capillaries (Figure 3).

Of the placental lesions that we studied, hypermaturity of villi with numerous syncytial knots (40.0%), intervillous thrombi (11.1%), fibrinoid necrosis of villi (13.3%), and intervillous fibrinoid (35.6%) were found to have significantly higher rates in the group B (Figure 4).

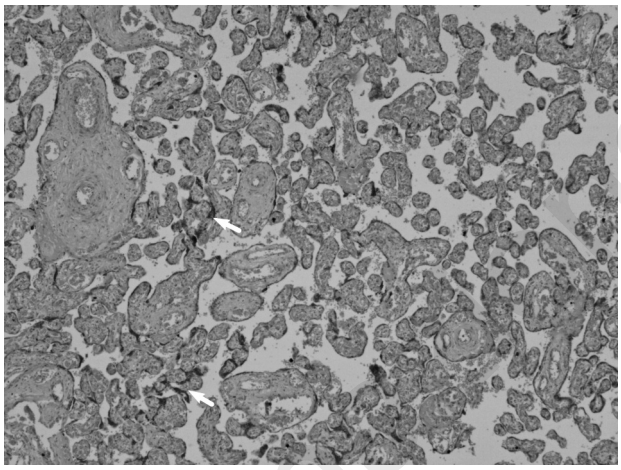

Figure 3 - Microphotograph of placenta at 38 weeks (group A). The arrows point numerous chorionic villi with few syncytial knots. Hematoxylin-Eosin, ×100.

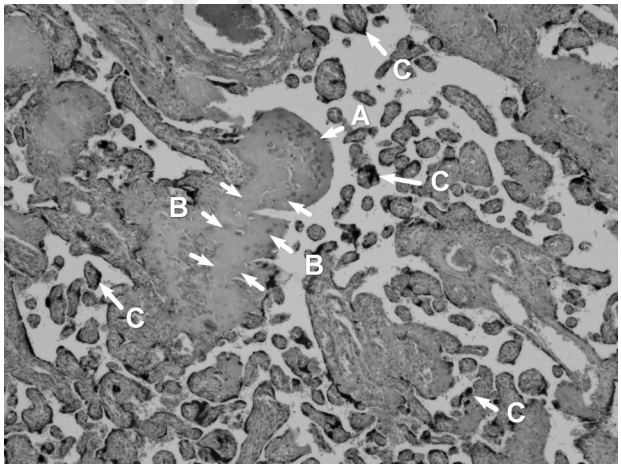

Figure 4 - Microphotograph of cross section of placental villi in woman with preeclampsia and IUGR with umbilical artery absent end-diastolic velocity at 31 weeks (group B). Avascular chorionic villi with an area of fibrinoid necrosis (arrow A) and intervillous fibrinoid (arrows B). The arrow C indicates numerous syncytial knots. Hematoxylin-Eosin, ×100.

Conclusion

Pregnancies complicated by threatened miscarriages are characterized with reduced placental vascularization at the 1st and the 2nd trimesters (p<0.008), an imbalance of circulating angiogenic factors with the predominance of the anti-angiogenic state at the 2nd trimester (p<0.01). Placental vasculature examination using 3D power Doppler at the 1st and the 2nd trimesters can identify pregnant women at-risk of placental dysfunction to optimize a management of these patients.

Dydrogesterone therapy as hormonal supplementation in cases of threatened miscarriages increases placental vasculature and balances pro-angiogenic and anti-angiogenic factors at the 1st and the 2nd trimesters. According to our data, dydrogesterone reduces the perfusion of the placental intervillous space by maternal blood at the 1st trimester. Probably such mechanism maintains low oxygen level in placenta, inhibits placental ischemia-reperfusion, and protects trophoblast and fetus from disruption by reactive oxygen species. These effects improve the milieu of placental villous tree development and influence favorably on pregnancy at the 3rd trimester and on perinatal outcomes.

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Analysis of the results of an anemia preoperative's circuit correction as technique to save blood in major gynecological surgery (2010-2015)

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Objective

Preoperative anemia is a negative prognostic factor for morbidity and mortality. Iron deficiency anemia is the most common anemia in major gynecological surgery.
According to the database provided by the Gynecology Department of our hospital, this surgery presents a transfusion rate of 14% and secondary to perioperative anemia without bleeding complications of 13%.
Since late 2010, with the creation of a multidisciplinary group of saving blood (PAS) formed by Anesthesiology, Hematology, Obstetrics and Nursing. We implemented a circuit for the correction of preoperative anemia in patients scheduled for this surgery with the aim to reduce the rate transfusional.

Material and methods

After performing from the pre-anesthesia consultation a study of anemia in the proposed patients for major gynecological surgery using hemoglobin (Hb) <12 g/dl, we programed, in susceptible cases, administration of intravenous iron (sucrose and carboxymaltose) for 2-4 weeks before surgery, creating a computer diary in order to adapt the prescribed treatment to the time of the intervention.
The treatment was administered in our Post-Anesthesia Recovery Unit (PACU), making its programming by the time zone of lower workload in order to use all available means without increasing costs.
The day before the intervention, we monitored a preoperative blood count to assess treatment response and we measured the proportion of transfused patients.
103 patients were included in our blood saving program until February 2015 (Figure 1).
Only 5 patients were transfused:

- 2 due to hemorrhagic postoperative complications
- 3 due to preoperative anemia (the treatment was not effective).

Results

Hemoglobin of the patients, which were included in our protocol, an average of 2.46 g/dl and 5.42% hematocrit increased, being the response to treatment statistically significant. 5 people of the treated group of patients were transfused: 2 of them due to postoperative bleeding complications and 3 of them due to preoperative anemia (no effective treatment).
We study the evolution of the number of red blood cells concentrates transfused in the gynecology before and after the implementation of our strategy.
We observed a decrease of 34.8% in 2010, 22.6% in 2011, 38.4% in 2012, 23.6% in 2013 and 18% in 2014 compared to 2009 (Figure 2).
Regarding the incidence of perioperative transfusion, the percentage of patients transfused included in the PAS is 4.9%, while in the historical cohort is 26.5% and this difference being also statistically significant ($p < 0.001$).

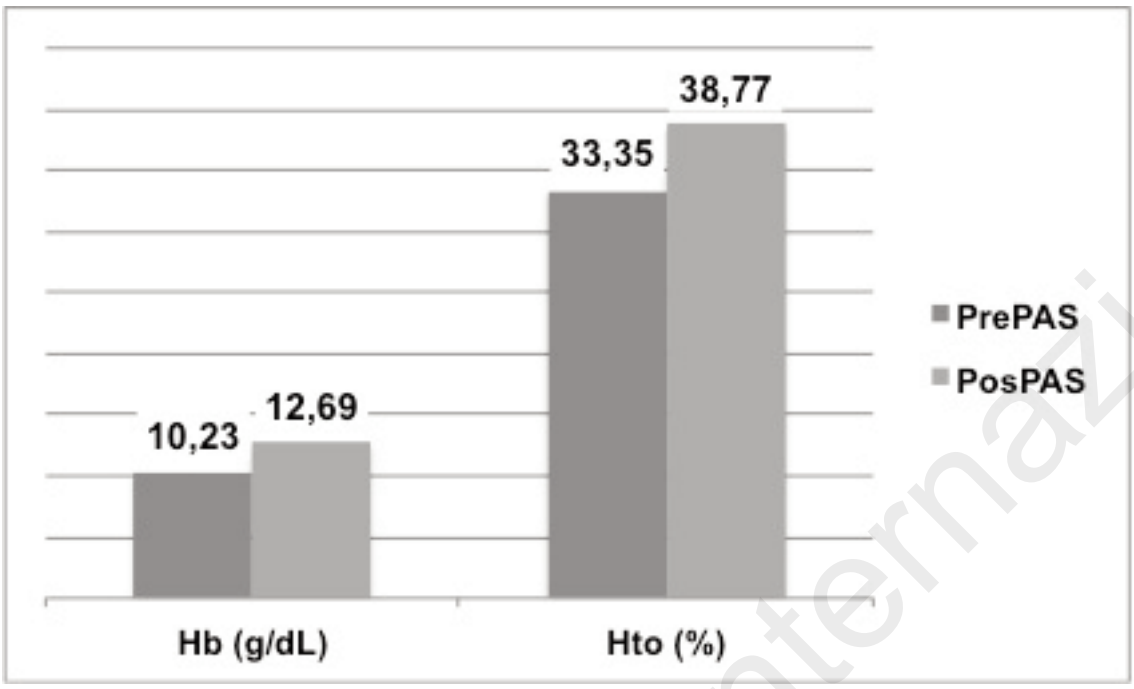

Figure 1 - Blood saving program.
p <0.001. PrePAS= previous to PAS. PosPAS= after PAS

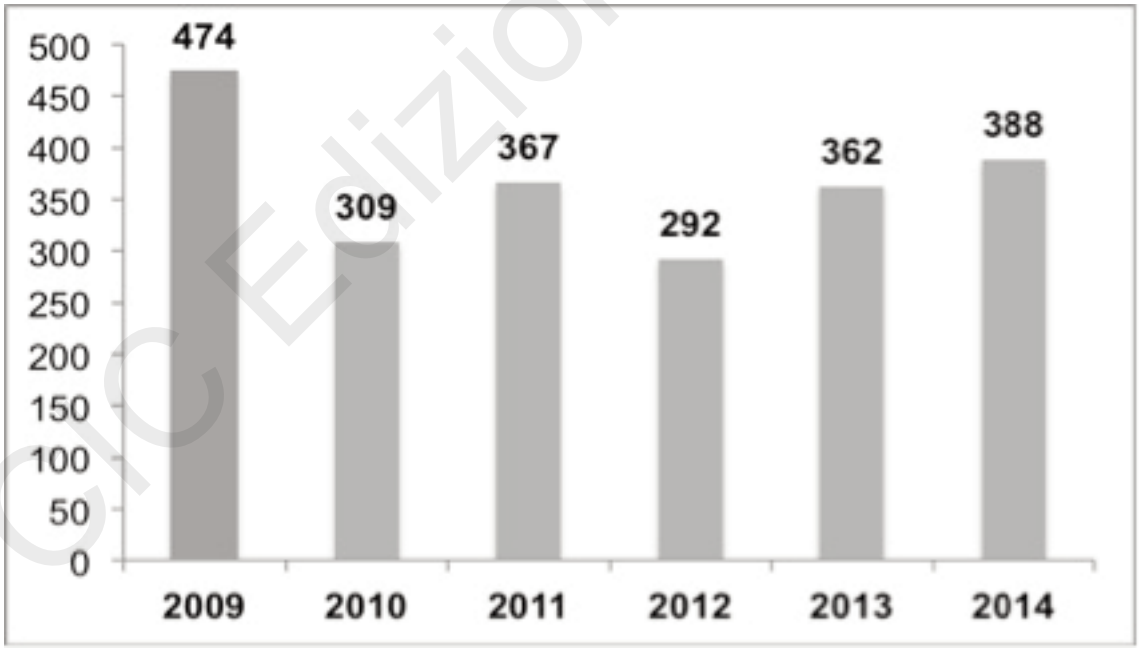

Figure 2 - Evolution of the number of red blood cells concentrates transfused in the gynecology.

Conclusions

This circuit is effective to correct the preoperative anemia of the patients proposed for major gynecological surgery. This protocol has improved hemoglobin levels and preoperative hematocrit of these patients. The results were statistically significant.

This measure seems effective in reducing the transfusion risk anemia of the patients proposed for major gynecological surgery. This protocol has improved hemoglobin levels and preoperative hematocrit of these patients. The results were statistically significant. This measure seems effective in reducing the transfusion risk.

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Pelvic exenteration in elderly patients

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Introduction

When Brunschwig reported it for the first time at the end of '40s, pelvic exenteration was a palliative procedure for locally invasive recurrent cervical cancer. As Brunschwig himself declared, although it had a high rate of postoperative complications, pelvic exenteration provided a significant alleviation of symptoms, most patients stating that they do not regret the choice they had made: "Because of the advanced stage of their disease, it is not to be anticipated that many, if any, of these patients will survive for very prolonged periods.... On the other hand, of those surviving at this writing, not one has expressed the feeling that they would have preferred to have remained as they were and not to have had the operation" (1). In the next decades attention was focused on the patterns of spread and on the possibilities to provide a complete macroscopic resection, aspects directly responsible for the transformation of the exenterative procedure from a palliative gesture into a curative one (2). Improvement achieved in the last few decades regarding association of neo-adjuvant chemo-irradiation, surgical technique and postoperative management leaded to a significant improve of the outcomes with decreased rates of postoperative complications and a 5-year survival rate which can exceed 50% (3). All these aspects, associated with the improvement of the life expectancy worldwide encouraged the surgeons to perform pelvic exenteration in elderly patients too.

Material and method

We present the case of a 83-year-old patient who was presented for pelvic pain, hematuria and constipation and was diagnosed with a stage IVA well differentiated squamous cell cervical cancer with rectal and urinary bladder invasion. The preoperative cystoscopy revealed the presence of a vesicovaginal fistula so we decided to submit directly to surgery the patient.

Results

The patient underwent a total pelvic exenteration, a total radical hysterectomy with bilateral adnexectomy en bloc with total cystectomy, rectosigmoidian resection, pelvic and para-aortic lymph node dissection being performed (Figures 1-5). The two ureters were exteriorized in right urostomy while the left colon was exteriorized in terminal left colostomy. The patient developed a febrile syndrome of uncertain origin which was remitted under conservative treatment and was discharged the 14th postoperative day. The histopathological findings confirmed the results of the preoperative biopsy and revealed the presence of negative resection margins of the specimen. In the meantime 7 of the 14 pelvic lymph nodes and 5 of the 12 para-aortic lymph nodes were positive, so the patient was submitted to adjuvant oncologic treatment. At 2-year follow-up the patient is free from any recurrent disease.

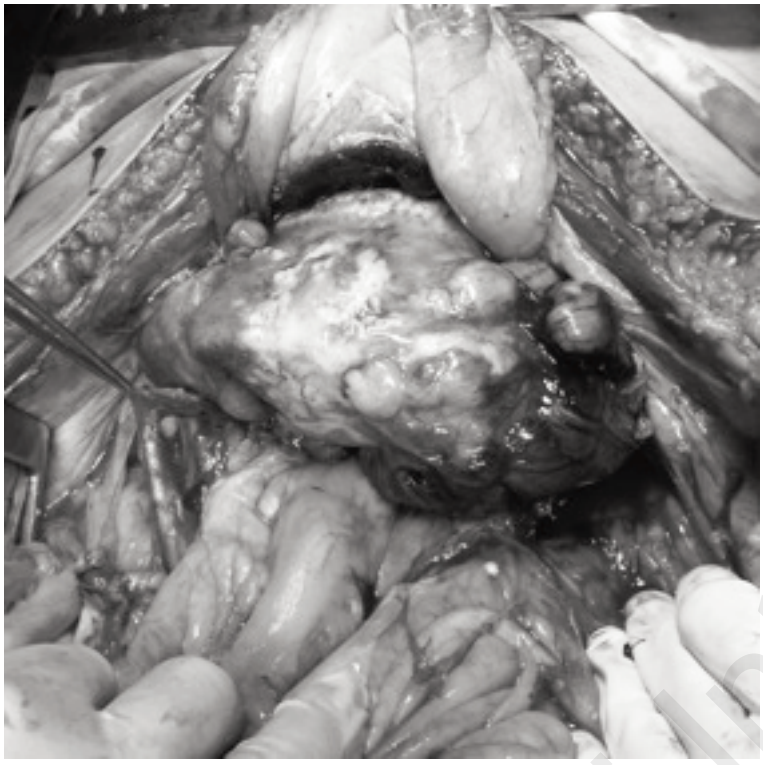

Figure 1 - The aspect after mobilization of the tumor en bloc with the urinary bladder and the rectosigmoidian colon.

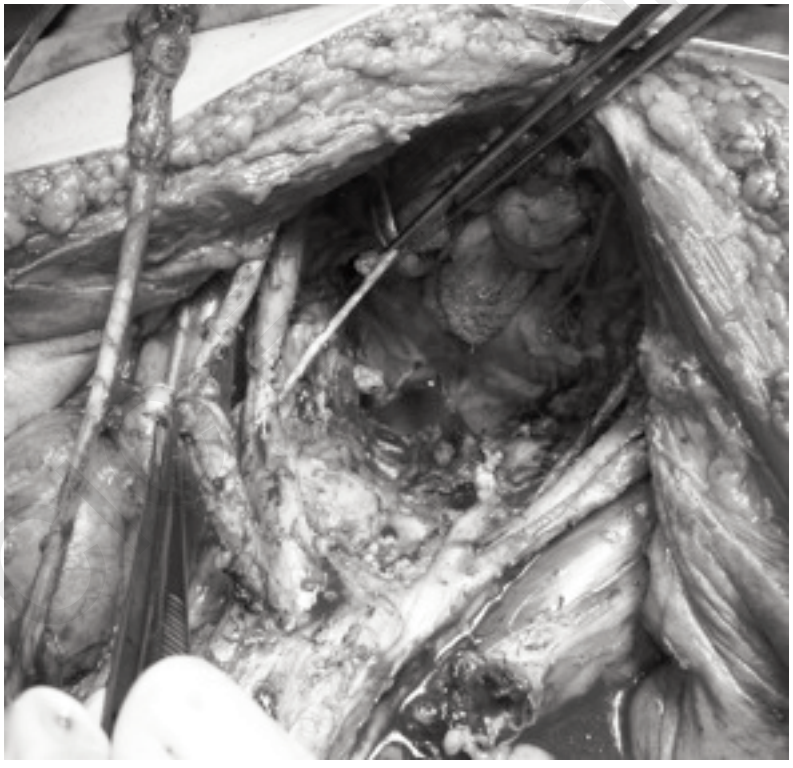

Figure 2 - The final aspect after resection of the tumor and pelvic lymph node dissection.

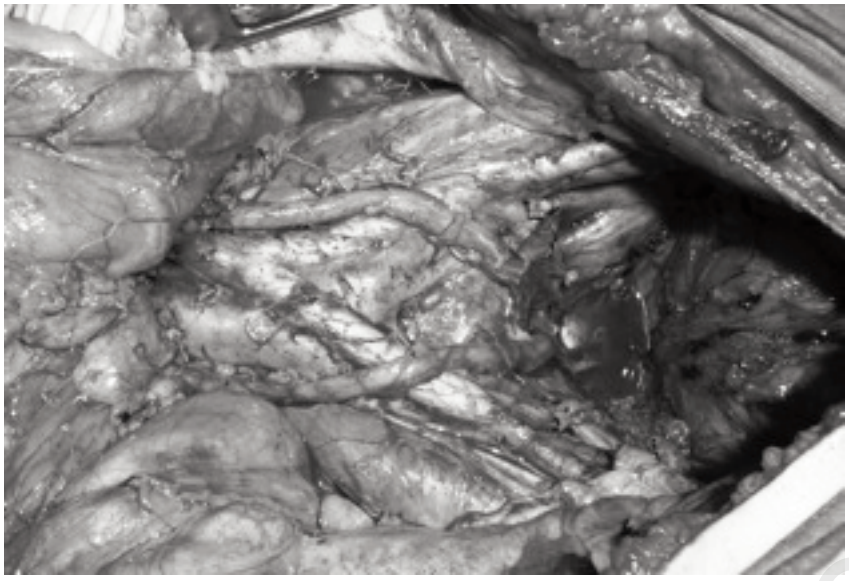

Figure 3 - Para-aortic lymph node dissection.

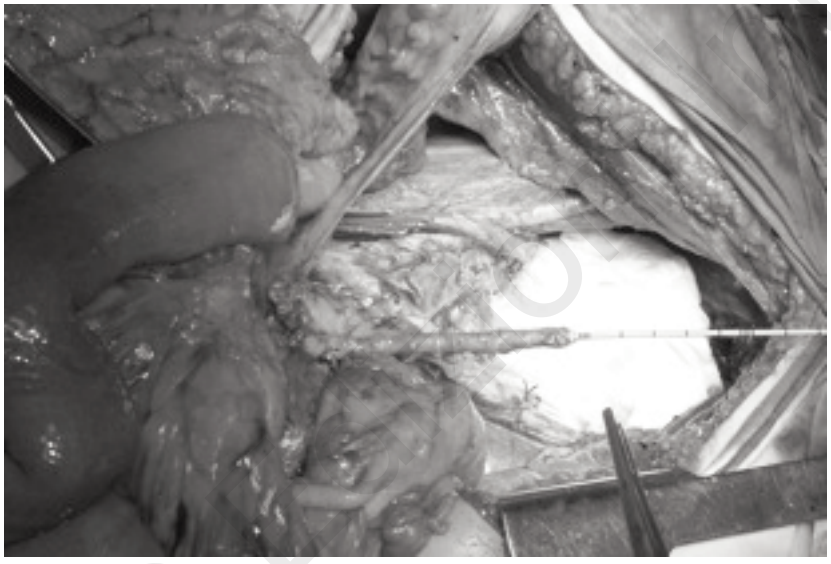

Figure 4 - Insertion of the urinary stents.

Discussions

Although initially advanced age was considered as contraindication for ultra-radical exenterative surgery, it has been widely demonstrated that it can be safely performed with comparable rates of postoperative complication and 5 year survival rate to younger patients. One of the first studies which came to demonstrate this aspect was the one conducted by Matthews et al. at the University of Texas M. D. Anderson Cancer Study. The Authors reviewed the outcomes of 63 patients older than 65 years submitted to pelvic exenteration with the outcomes of 363 patients younger than 65 years submitted to the same surgical procedure in the same period of time. The early postoperative mortality rate was similar between the two groups (11% among elderly patients and 8,5% among younger patients, p=0.51) while the long term outcomes evaluated through the 5 year survival rate was 46% among elderly patients and 45% among younger patients (p=0,52) (4). The fact that pelvic exenteration is a safe surgical procedure with good oncologic

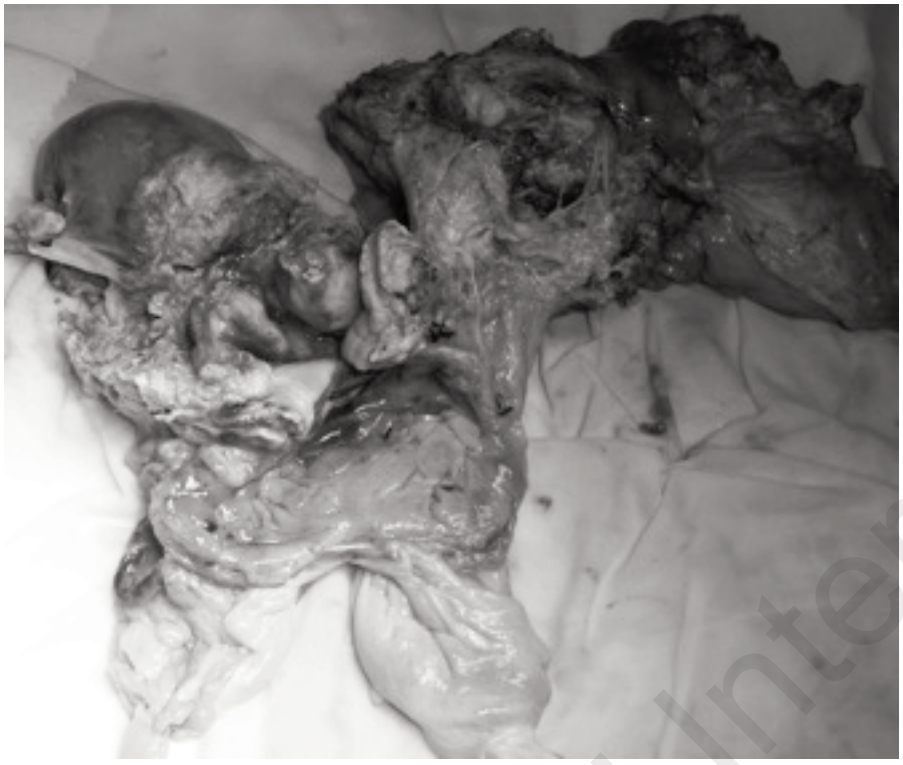

Figure 5 - The specimen of total pelvic exenteration.

outcomes independently of age is also demonstrated by Carballo's study which included 34 consecutive patients submitted to pelvic exenteration between June 2006 and December 2013 with a median age of 62 years (ranging 40-82 years). The main performed procedures included supralevator exenteration (in 61,7% of cases) and infralevator resections (in 38,2% of cases) while the main reconstructive procedures consisted of Bricker diversion and sigmoidostomy in 50% of cases, Bricker diversion and colorectal or coloanal anastomosis in 32,3% of cases and double barreled wet colostomy in 17,6% of cases. In the meantime histopathological examination confirmed an R0 resection in 70,6% of cases while in 26,5 and 2,9% of cases respectively an R1 and R2 respectively resection was achieved. The postoperative mortality rate was null while the postoperative morbidity rate requiring reoperation was 14,6%. The reported overall survival was 39 months, the only significant prognostic factor being the achievement of an R0 resection (5).

In a similar study conducted by Johannes de Wilt et al. regarding the role of pelvic exenteration in the treatment of primary and recurrent gynecologic malignancies the reported 5 year overall survival rate was 48% and was not significantly influenced by the patients' age (6).

In a more recent study conducted by Huang et al. regarding the impact of age on surgical outcomes in patients submitted to pelvic exenteration women submitted to this type of surgery for gynecologic malignancies were stratified into groups based on age: young <50 years, middle: 51-64 years and senior >65 years. There were 58 young patients, 62 middle age patients and 41 senior patients with similar body mass indexes between the three groups (p=0,5616). While cervical cancer was the main indication for pelvic exenteration in younger patients, elderly women were more likely to have a diagnosis of vulvar or vaginal cancer. In the meantime elderly patients reported a significantly higher incidence of arterial hypertension (p<0,0001) and pulmonary disease (p=0,04). When it comes to the intraoperative and histopathological aspects, there was no significant difference regarding the type of exenteration (anterior/posterior/total) between the three groups (p=0,847), the lymph node status (p=0,237) lymphovascular invasion (p=0,746) or margin status (p=0,7797) while vaginal and urinary reconstructive procedures were more frequently performed in younger patients (p=0,046 and p=0,0001 respectively). In the meantime surgery

length was significantly higher in younger patients (p=0,009) especially due to a higher grade of the surgical complexity of the procedure. The overall incidence of postoperative complications was similar between the three groups (p=0,8863). Although the recurrence rate was higher among younger patients (p=0,0165) time to recurrence was shorter in middle and senior groups compared to younger cases (p=0,017). However the overall survival rate was similar between the three groups (p=0,376) (7).

Conclusions

Pelvic exenteration can be safely performed in elderly patients, with acceptable rates of postoperative complications and similar long term outcomes. Although a higher incidence of co-morbidities might be found in advanced age patients, curative surgery should be performed whenever the patient has a good biological status, regardless of the chronological age.

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Climacteric syndrome and microcirculation

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Blood is not a homogeneous (Newtonian) liquid and it is a suspension having suspended particles, it has exceptional features and it is studied by hemorheology. Bloodhemorheology plays a significant role in blood circulation and ensuring its trophic function. Changes in rheological properties of blood may cause a slowdown in the flow, establishment of stasis, therefore tissue hypoxia, which is accompanied by a multi-disease and vice versa, the particular structure of blood depends on a local hematocrit, axial flow of erythrocytes and the existence of plasma layer, blood flow structure changes lead to disorders of its rheological properties. The rheological features of blood are mainly determined by the erythrocytes, as the volume of leukocytes is much smaller (800 times), than the number of erythrocytes and platelet (however they are numerous than erythrocytes, they are of a significantly small size). The volume of the platelet composes only 1/10 of the volume of erythrocytes. Therefore, the rheological properties of blood is determined by erythrocytes and the features of its actions (resilience, agglutination, movement, etc.). According to the concept of our research group, erythrocytes value the microcirculation and homo-rheologic condition, so called homorheological status. We were interested in how the homorheological status has been changing in woman with climacteric syndrome. According to the obtained results it turned out that during climacteric syndrome the rheological features are disordered compared to the control (status deteriorated by 25%). From our point of view climacteric syndrome and the dysfunction of rheological status developed in parallel.

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Assisted reproductive technology (ART) and changing reproductive behavior in Europe

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If we look at the human reproduction from global perspective we are witnessing historical changes in reproductive behavior that were made possible by scientific achievements in reproductive medicine: in 1959 for the first time in the history of mankind a really safe and effective contraception was brought to the market making possible sex without (the risk) of reproduction. Only 19 years later Lousie Brown was born after fertilization of human egg in laboratory conditions and embryotransfer back to the uterus. This techniques of *in vitro* fertilization (IVF) separated reproduction and sex. There is no doubt that the classic picture of sex for reproduction is increasingly being replaced by reproduction separate from sexual aktivity. It is undeniable that in the modern world, sexual activity will play a decreasing role in reproduction (1). Some scientist even think that biorythms will slow down and that the first child in the future will be delivered at the grandparents age in previous generations. It is undeniable that two fertility trends of the 21 century are already evident in the Western countries: women are having fewer children and they are delaying births to a later age than in previous centuries. This shift towards the late transition to parenthood appears to be the most prominent feature of contemporary fertility trends in advanced societies. This process has important consequences for fertility trends and demographic change in general, which in turn have many societal implications. Postponing childbearing is not a new phenomenon. Fertility postponement has taken place in all European countries. The late start of childbearing became a common strategy enabling men and women to reconcile different interests and roles with family life, mainly due to changing external factors: an expansion of university education took place since the late 1960s in many 'Western' countries and since the early 1990s in the post-communist societies. After 1973, 'oil shocks' had brought recession to many 'Western' societies; similarly, the early 1990s were characterised by the collapse of the previous economic system in Central and Eastern Europe. Both events led to high unemployment rates, pronounced among young adults. Today the four main "preconditions for parenthood" to which most men and women adhereare: finishing education, establishing oneself on the labour market, having a stable relationship, and accumulating enough resources. In the same time partnerships have become more fragile, making the decision to have a child even more difficult. In the mid 1980s about 8% of those women who got pregnant were over 40 whereas now that figure has more than doubled to 19%. Hand in hand with this growing age at the first delivery a sharp decline in total fertility rates could be documented, in some countries even with the arrival of lowest-low fertility [the term "lowest-low fertility" to denote a total fertility rate (TFR)] of 1.3 or below. Having children later was not exceptional in the past, when families were larger and women often continued bearing children until the end of their reproductive age. The problem of today is the age at which women give birth to their first child, leaving an ever more constricted window of biological opportunity for second and subsequent child-

ren should they be desired.
Delaying pregnancies means also higher risk of fertility problems or delaying the diagnosis of subfertility. As a result of this, IVF clinics are treating older and older women. With IVF we can compensate fertility decline until the age of 35, but then the pregnancy rates go down year by year. The excellent pregnancy rates with egg donation – irrespective of recipient age – are the proof of oocyte factor as the main cause of decreasing fertility in aging women. Not just women, but also men should be aware of the "reproductive consequences" of postponing parenthood, because a man's semen quality and fertility also worsens with age. In addition, babies born to fathers of "advanced paternal age" – defined as 40 and older at the time of conception – are at increased risk of genetic disorders. Postponing pregnancies also means that many women come too late for the treatment with already depleted ovarian reserve. The demand for egg donation is increasing and the restrictive legal situation in many countries is leading to the phenomenon of transborder reproductive care – in Europe the estimated annual number of treatments outside the country of residence is 24.000-30.000 (2). This strategy of deferring pregnancies has broad individual and social implications: loir fecundity, langer waiting time to conception, higher frequency of miscarriages and stillbirths, more pregnancy complications and fetus abnormities, higher number of multiple pregnancies. For the society this means increased need for intensit health care during pregnancy, a boxing demand for costly reproductive technologies, potentially worsening indicators related to infant health and mortality and finally changing character of intergenerational support and redefinition of family obligations. The implications for reproductive medicine are already visible: increasing number of subfertile couples, increasing age of women (and men) seeking help, increased medici risks of delayed childbearing, increasing demand for egg donation and newly social freezing (oocyte freezing for non medical reasons). The rational od social freezing is that oocyte donation, ineffective fertility treatments and chromosomal abnormalities could be avoided in the future. However, there are ethical issues (reproductive autonomy *vs* well-being of the child), financial issues (expensive procedure), there is no guarantee of final success and potential new problems in future are not excluded. In any case social freezing is not a solution for underlying societal problems, it only delays the existing problems.

Conclusion

The norms related to childbearing have changed considerably postponing pregnancies has important consequences for fertility trends and demographic change in general, which in turn have many societal implications. This shift towards the late transition to parenthood will be a great challenge for reproductive medicine. To avoid all these risks couples should be encouraged not to delay pregnancy past the age of 35.

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Follicular and other characteristics with Ovaleap® (follitropin alfa) in a phase 3 study of infertile women using assisted reproductive technology (ART)

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Context

Ovaleap® is a recombinant human follicle-stimulating hormone used in ovarian hyperstimulation, ovulation induction, and spermatogenesis stimulation. A phase 3 study demonstrated clinical bioequivalence of Ovaleap® to Gonal-f® with respect to the primary endpoint of number of oocytes retrieved (Gertz & Strowitzki. ESG 2013, abstract 920).

Objective

Assess secondary endpoints, including changes in follicular characteristics, after treatment with Ovaleap® *vs* Gonal-f®.

Methods

Multinational, multicenter, randomized, controlled, assessor-blind, parallel-group phase 3 study.
Patients: infertile, downregulated, ovulatory women aged 18-37 years using ART.
Interventions: one cycle of Ovaleap® or Gonal-f® (self-administered, 150 IU daily for 5 days, followed by dose adaptation for up to 15 days).
Main outcome measures: secondary endpoints included follicle size distribution, serum estradiol levels, endometrial thickness, and oocyte grading (maturity and quality).

Results

A total of 153 women were randomized to Ovaleap® and 146 to Gonal-f®. On Stimulation Day 6 prior to dose adaptation, the follicle size distribution and endometrial thickening were comparable between treatment groups. Serum estradiol levels were variable and the mean concentration was higher with Ovaleap® than with Gonal-f® (650.2 *vs* 516.3 pg/mL, respectively). On the day of hCG administration, the count for large follicles (diameter >14 mm), estradiol concentration, and endometrial thickness were all comparable between groups. Oocyte maturity and quality in the 2 treatment groups were similar. Overall, approximately 20% of the 1,689 retrieved 2 pronucleus oocytes were graded as Z1 (best quality), 40% as Z2, 30% as Z3, and 10% as Z4 (worst quality).

Conclusions

Changes in follicular and other characteristics were similar and as expected after one cycle of treatment with Ovaleap® compared with Gonal-f®.

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The role of preconception management in pregnancy outcomes in women after myomectomy

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Uterine fibroids revealed in 25-30% of women over the age of 35 years and in some cases require surgical or/and pharmacological treatment (1). In the reproductive age is preferred treatment that preserves the uterus, as an organ of fertility (2). On the other hand, today there is no universal protocol for preconception management in women who have had different methods of uterine fibroid treatment (3-5). The aim of the study was to evaluate the effectiveness of preconception management in prevention of complications of pregnancy in women after myomectomy.

Material and methods

During the period 2010 to 2014 carried out a prospective, non-randomized case-control study of 300 pregnant women with uterine fibroid after laparoscopic myomectomy. Inclusion criteria: reproductive age; uterine fibroids; laparoscopic myomectomy before pregnancy; spontaneous pregnancy; history of infertility or miscarriage due to uterine fibroids; late reproductive age (limited time for attempts to implement fertility). The average age of women - 33.4±3.2 years; disease duration of uterine fibroid 4.25±1.4 years. The clinical Group I included 150 women, which enrolled into the study from the time of surgical treatment (myomectomy) and had preconception management after myomectomy. Myomectomy carried out routinely with the imposition of endoscopic sutures, juxtaposition maximum wound surfaces on the uterus, using antiadhesion barriers. Women of Group I after the myomectomy in order to improve regeneration of the myometrium and endometrium, as anti-recurrent treatment of uterine fibroid prescribed combined oral contraceptives (COCs) for a period of 9-12 months. Preconception management also included by individual indications – polyvitamins, folic acid, progesterone.
In clinical case-control Group II were included 150 women, who have become pregnant after a myomectomy without a preconception management. Information on the amount and volume of removed fibroids, laparoscopy features compiled based on existing protocols of laparoscopic myomectomy.

Results

Initial fibroids size (up to myomectomy) in women comparison Groups were comparable, reaching a maximum diameter of 110 mm, on average 45.82±20.76 mm. The number of nodes per woman - from 3 to 11 on average 5.18±2.5. Among them: subserous-interstitial - 4.8±0.51, subserous - 1.46±0.34, interstitial - 1.0±2.05. The time interval between the end of treatment and the onset of pregnancy (Group I after stopping COCs, in Group II after myomectomy) in Group I was 2.8 (1 to 5) months, in Group II - 6 (1 to 14) months. Ultrasound (US) examination during preconception period in the myometrium at the zone of the uterine scar after myomectomy in Group I visualized structure without clear contours, moderate echogenicity with point hyperechoic inclusions (presumably - suture),

loci with or without blood flow. After the myomectomy this loci determined blood flow to 3 months, have not been determined after 6 months and further.

In the 1st trimester of pregnancy vascularization index of placental site (VI) in women in Group I was 6.4 in the central zone, 6.0 in paracentral zone, 6.2 in the peripheral zone. In women of Group II Doppler parameters were as follows: in the central zone VI was 5.7; in paracentral zone - 5.0; in the peripheral zone - 4.52. This difference regarded as a lack of vascular tissue heteromorphism emerging as the placenta ultrasound marker of a risk of a primary placental insufficiency (2). At US examination noted that the volume of chorion was comparable in both Groups: at 8 weeks of pregnancy in Group I - 10.8 cm^3 *vs* 10.5 cm^3 in Group II; at 13 weeks of pregnancy in Group I - 98.5 cm^3 *vs* 97 cm^3 in Group II.

In assessing the complications of gestation revealed that in the 1st trimester of pregnancy, ultrasound signs chorionic detachment of varying severity in both Groups revealed a comparable rate. Clinical signs of incipient abortion had 10% of women of Group II. In the 2nd trimester of pregnancy ultrasound signs of placental abnormalities revealed in 2% of women of Group II. In the 3rd trimester of pregnancy threatening preterm labor, premature birth, mild pre-eclampsia marked increasingly by more than 2 times in women Group II. 10% of women in Group II had clinical signs of placental abnormalities: 7% of women placenta accreta, 1% placenta increta, 2% placenta percreta. All cases occurred in women whose pregnancy occurred less than 3 months after myomectomy. Ultrasound signs of chronic placental insufficiency observed in the absolute number of women of Group II, and 86% of women in Group I. In the 3rd trimester of pregnancy ultrasound examination of the myometrium of the front wall of the uterus demonstrated in 4 women of Group II local thinning of the myometrium (1-2 mm with diameter 30 mm), which is regarded as a failure of the uterine scar after myomectomy. Indeed, by Caesarean section was visualized portion of thinning the uterus as a "niche" to 1-2 mm thick. During Caesarean section local thinning of the myometrium visualized only in 5% of women in Group I and in 20% of women in Group II, others - myometrium was no visible marks of myomectomy.

Massive blood loss during delivery of more than 20 ml/kg had only 2% of women of Group II due to placental abnormalities (placenta percreta). The causes of bleeding (without massive blood loss) of 5% of women in the II Group were placental abruption, 10% placenta previa, 11% placental abnormalities. The other reasons for the observations were hypotension, bleeding of the uterus and combines factors.

Conclusion

The continuing relevance of the problem of effective recovery of reproductive function, of preconception management, prevention of complications of gestation in women with uterine fibroid and associated infertility. In the absence of preconception management marked by a higher risk of primary placental insufficiency, complicated pregnancy, placental abnormalities, and due to massive blood loss. Cesarean delivery only because of the uterine scar after laparoscopic myomectomy can be a subject of discussion. However, the lack of reliable non-invasive methods, allow us during pregnancy to guarantee the consistency of the uterine scar (especially in the localization of at the back of wall of the uterus) causes the clinician to refer these as a high risk of uterine rupture, fetal death, bleeding. A clear need to improve the system of improving the outcomes of pregnancy and childbirth for mother and fetus in women with uterine fibroid.

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Blood thicker than water: a case report on familial ovarian cancer

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Introduction

Ovarian cancer is extremely hard to diagnose in its early stages, and those afflicted at the time of diagnosis are typically asymptomatic and in the late stages of the disease, with metastasis to other organs. Fleming et al. (1) reported that patients are usually diagnosed at the median age of 63 years and in more than 70% of cases, patients present with advanced disease. The National Cancer Database (2) also reports a 23-64% chance of having ovarian cancer in the 50-69 age group with only 13% occurring in the 40-49 age group.

Recent statistics from the Centers for Disease Control and Prevention (3) show that ovarian cancer is the eighth most common cancer and the fifth leading cause of cancer deaths in the United States. It causes more deaths than any other cancer of the female reproductive system, but accounts for only about 3% of all cancers in women. In 2002, Redaniel et al. (4) reported the incidence rate of ovarian cancer among Filipino women in the United States to be 11.5 per 100,000 as compared to 10.3 for Caucasians and 8.9 for Asian and Pacific Islanders (API) with mortality rates highest among Filipino women at 6.3 per 100.000 and 6 and 3.3 per 100,000 for Caucasians and APIs respectively. In 2010, the Department of Health reported that ovarian cancer ranks as the 8th leading cause of cancer deaths in the Philippines, and the 5th most common cancer among Filipino women (5).

A person's age at ovulation or the lifetime number of ovulatory cycles, is an index of a woman's ovarian cancer risk. Thus, nulliparity and refractory infertility increases risk, while multiparity is protective. Oral contraceptives, and tubal ligation or hysterectomy are likewise protective (6).

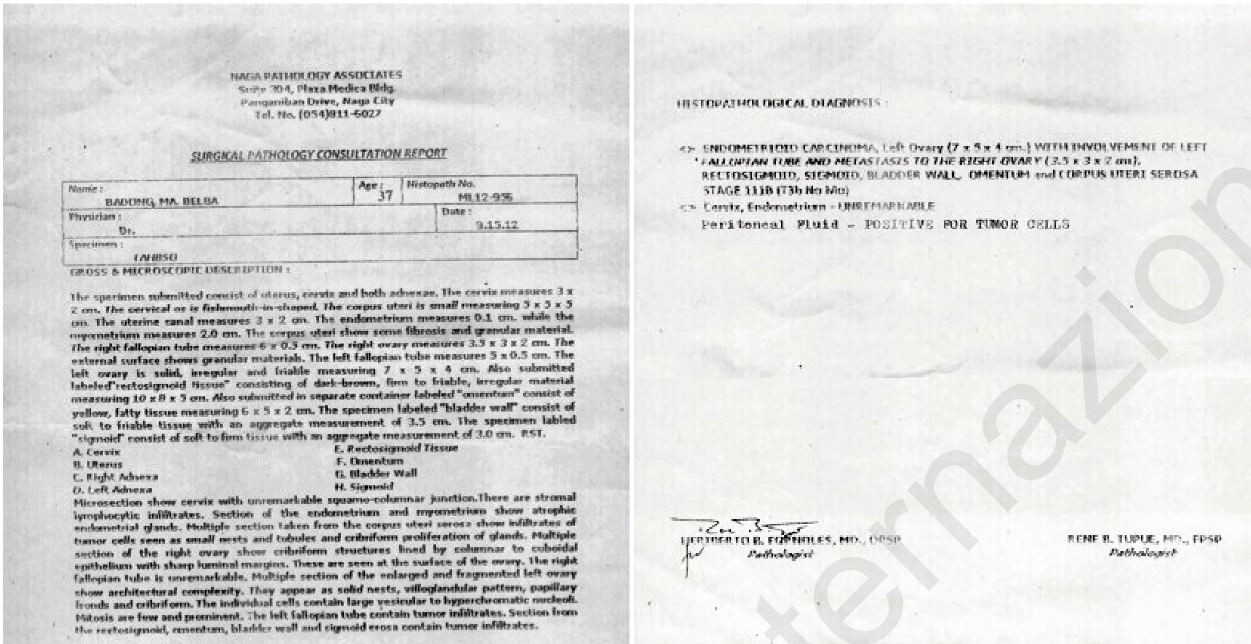
Mutations in the BRCA1 and BRCA2 tumor suppressor genes have been found to be responsible for the majority of hereditary ovarian cancers (7).

Malignant Mixed Müllerian Tumor (MMMT) is an aggressive type of ovarian neoplasm, accounting for only 1% of all ovarian cancers. MMMT usually occurs in postmenopausal women and responds poorly to treatment (8). Histologically, carcinosarcoma tumors are composed of both carcinomatous and mesenchymal components, which may be either homologous (composed purely of ovarian tissues) or heterologous (containing tissues found in other organs). They have a poor overall survival rate (9).

A case of ovarian MMMT managed in this institution is reported and it is of particular interest for the following reasons: 1) MMMT comprises only 1% of all ovarian neoplasms; 2) since patient has two sisters who succumbed to ovarian cancer, this case could involve a hereditary genetic mutation, which comprise only 5% of MMMT; 3) patient has no other risk factors such as advanced age and prolonged exposure to ovulation; 4) patient has two other sisters who are still asymptomatic and unscreened for ovarian cancer.

Case report

This is a case of a 43-year old female, G3P3 (3003),



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Panganiban Drive, Naga City
Tel. No. (054)811-6027

SURGICAL PATHOLOGY CONSULTATION REPORT

Name : BADONG, MA. BELBA	Age : 37	Histopath No. ML12-956
Physician : Dr.		Date : 9.15.12
Specimen : TAHBSO		

GROSS & MICROSCOPIC DESCRIPTION :

The specimen submitted consist of uterus, cervix and both adnexae. The cervix measures 3 x 2 cm. The cervical os is fishmouth-in-shaped. The corpus uteri is small measuring 5 x 5 x 5 cm. The uterine canal measures 3 x 2 cm. The endometrium measures 0.1 cm. while the myometrium measures 2.0 cm. The corpus uteri show some fibrosis and granular material. The right fallopian tube measures 6 x 0.5 cm. The right ovary measures 3.5 x 3 x 2 cm. The external surface shows granular materials. The left fallopian tube measures 5 x 0.5 cm. The left ovary is solid, irregular and friable measuring 7 x 5 x 4 cm. Also submitted labeled"rectosigmoid tissue" consisting of dark-brown, firm to friable, irregular material measuring 10 x 8 x 5 cm. Also submitted in separate container labeled "omentum" consist of yellow, fatty tissue measuring 6 x 5 x 2 cm. The specimen labeled "bladder wall" consist of soft to friable tissue with an aggregate measurement of 3.5 cm. The specimen labled "sigmoid" consist of soft to firm tissue with an aggregate measurement of 3.0 cm. RST.

A. Cervix
B. Uterus
C. Right Adnexa
D. Left Adnexa
E. Rectosigmoid Tissue
F. Omentum
G. Bladder Wall
H. Sigmoid

Microsection show cervix with unremarkable squamo-columnar junction.There are stromal lymphocytic infiltrates. Section of the endometrium and myometrium show atrophic endometrial glands. Multiple section taken from the corpus uteri serosa show infiltrates of tumor cells seen as small nests and tubules and cribriform proliferation of glands. Multiple section of the right ovary show cribriform structures lined by columnar to cuboidal epithelium with sharp luminal margins. These are seen at the surface of the ovary. The right fallopian tube is unremarkable. Multiple section of the enlarged and fragmented left ovary show architectural complexity. They appear as solid nests, villoglandular pattern, papillary fronds and cribriform. The individual cells contain large vesicular to hyperchromatic nucleoli. Mitosis are few and prominent. The left fallopian tube contain tumor infiltrates. Section from the rectosigmoid, omentum, bladder wall and sigmoid erosa contain tumor infiltrates.

HISTOPATHOLOGICAL DIAGNOSIS :

<> ENDOMETRIOID CARCINOMA, Left Ovary (7 x 5 x 4 cm.) WITH INVOLVEMENT OF LEFT FALLOPIAN TUBE AND METASTASIS TO THE RIGHT OVARY (3.5 x 3 x 2 cm), RECTOSIGMOID, SIGMOID, BLADDER WALL, OMENTUM and CORPUS UTERI SEROSA STAGE IIIB (T3b No Mo)
<> Cervix, Endometrium - UNREMARKABLE
Peritoneal Fluid - POSITIVE FOR TUMOR CELLS

HERIBERTO B. FORNOLES, MD., DPSP
Pathologist

RENE B. TUPUE, MD., FPSP
Pathologist

Figure 1 - Histopathology Report of the patient's sister.

who was admitted in this institution due to dyspnea. She had a 2-month history of difficulty of breathing and sudden weight loss described as loosening of clothes. One month prior to admission, there was observed progressive abdominal enlargement. No abdominal pain, changes in urinary and bowel habits were noted. Past medical, social and sexual history were unremarkable. She had 3 uncomplicated pregnancies carried to term and delivered via normal spontaneous delivery attended by a midwife. There was no history of oral contraceptive use. Family history revealed that 2 of the patient's siblings were both diagnosed with ovarian cancer. Her eldest sister had sudden abdominal enlargement in 2007, and surgical findings in another institution showed an ovarian malignancy. She died in the same year at age 49. Her younger sister, who also presented with abdominal enlargement for which an exploratory laparotomy with surgical staging was done, had a histopathology report of endometrioid carcinoma, left ovary, measuring 7 x 5x 4 cm, with involvement of left the fallopian tube and metastasis to the right ovary (which measured 3.5 x3 x 2 cm) rectosigmoid, sigmoid, bladder wall, omentum, and corpus uterine serosa (Figure 1). She succumbed in 2013 at the age of 36 in a provincial tertiary center. She still has two older sisters, both are currently asymptomatic.

Upon admission, BP was 120/80, with cardiac rate of 96 beats per minute, tachypneic at 32 cycles per minute, and febrile with a temperature of 38.7. Chest examination showed asymmetrical chest expansion, with lagging, dullness on percussion and decreased breath sounds from T7 and below on the right lung. Abdominal examination revealed a globular abdomen, with normoactive bowel sounds, soft, with a palpable solid, slightly tender, non-movable, regularly shaped pelvoabdominal mass with distinct borders measuring approximately 10 x 10 cm; there was also noted shifting dullness and fluid wave. Internal examination revealed a firm, short, closed cervix, normal-sized uterus, and a solid, slightly tender, non-movable left adnexal mass, measuring approximately 10 x10 x 8 cm; there was also a right adnexal mass palpated measuring 3 x 2 x 2 cm, which was described as solid, non-movable, non-tender with regular borders. No cervical motion tenderness was appreciated. Admitting diagnosis was ovarian new growth, bilateral, malignant; pleural effusion secondary to malignancy, G3P3 (3003). Ultrasound showed that the uterus is retroverted with smooth contour and homogenous echopattern measuring 5.29 x 3.44 x 7.7 cm; endometrium is isoechoic measuring 0.2 cm with intact subendometrial halo; cervix measures 3.41 x 3.12 x 2.08 cm; a left adnexal mass measuring

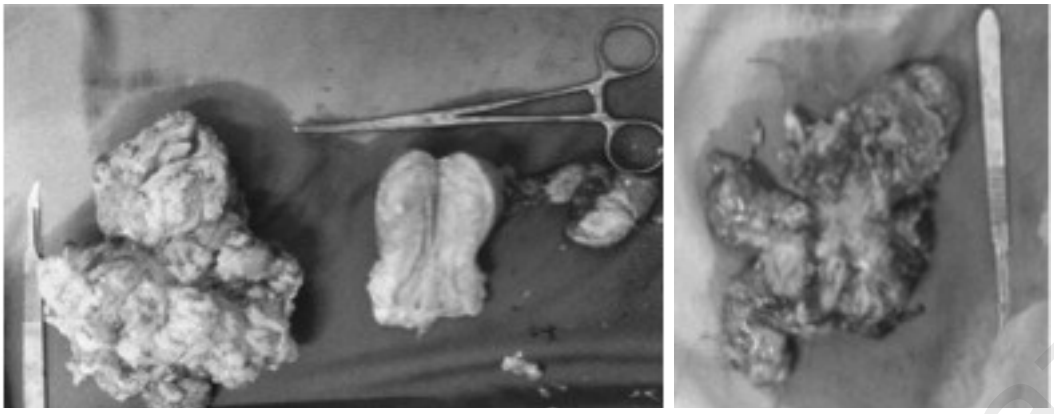


Figure 2 - Cut section of left ovary, uterus with right ovary; colorectal mass.

11.83 x 9.19 x 8.78 cm, and a right adnexal mass measuring 3 x 2 x 2 cm were also seen, which were assessed to be bilateral ovarian new growth, probably malignant. Masses were given a Sassone Score of 13 because the lesions were mostly solid, with the presence of papillary excerences, thick septa (4 mm), and mixed echogenicity. Minimal ascites was also noted. Chest tube thoracostomy was immediately performed. Pleural fluid was sent for cytology and showed absence of malignancy. Once the patient was stabilized, total abdominal hysterectomy with bilateral salpingooophorectomy, infracolic omentectomy, peritoneal fluid cytology, and bilateral lymph node dissection were performed. Intraoperatively, a red, turbid peritoneal fluid amounting to approximately 500 ml was noted. The pelvic cavity was occupied by a firm, irregularly shaped left ovary measuring 13 x 10 x 5 cm which on cut section had a soft to friable surface with yellow border; while the soft to rubbery right ovary measuring 3.5 x 2.5.x 2.0 cm had a convoluted surface, which on further sectioning showed a well encapsulated tan-white surface. The uterus was smooth, firm, and symmetrical, measuring 6.0 x 5.5 x 4.0 cm. Sectioning of uterus showed a tan-white, firm to rubbery myometrial wall. The anterior and posterior myometrial walls measured 2.2 cm and 2.0 cm respectively, while the endometrial thickness was 1.5 cm. There was a colorectal mass noted in the serosa which was described as fixed and nodular measuring 13 x 12 x 6 cm (Figure 2). The omentum was tan-yellow and described as soft to rubbery. Subdiaphragm was smooth; liver was firm, smooth with no palpable metastatic seedings; para aortic nodes were not enlarged.

Microscopic sections from the left fallopian tube, left ovary (Figure 3), right ovary (Figures 4 and 5), and colorectal mass (Figure 6) show a malignant mixed müllerian tumor characterized by admixture of malignant carcinomatous components composed of pleomorphic tumor cells lined by cuboidal to columnar epithelium exhibiting glandular complexity, some with branching papillary architecture, and some with clearing of cells, displaying nuclear atypia, increased nuclear-cytoplasmic ratio, vesicular nuclei, and prominent nucleoli which are infiltrating the stroma. Sections from colorectal mass (Figure 6) also show areas with cartilaginous elements admixed with red blood cells and necrotic tissues. Microscopic sections from the omentum (Figure 7) show invasion of the malignant tumor. Microscopic sections from the endometrium show round endometrial glands lined by columnar cells with pencil-shaped nucleus, surrounded with densely cellular stroma. The findings seen in the microscopic sections from left fallopian tube, left and right ovaries, as well as colorectal mass, were all consistent with the features of malignant mixed Müllerian tumor, since MMMT is composed histologically of malignant epithelial and sarcomatous elements. Jin et al. (10) reported that MMMT can be homologous, when the specimen contains malignant elements native to the ovary, or heterologous, when MMMT contains sarcomatous tissue not normally found in the ovary, such as bone or cartilage. The colorectal mass found in this case also showed areas of cartilaginous elements consistent with heterologous MMMT. Cytologic evaluation of the peritoneal fluid (Figure 8) showed hypercellular smears composed of abundantly scattered neutrophils, lymphocytes, and reactive mesothelial cells, admixed with enlarged crowded sheets and

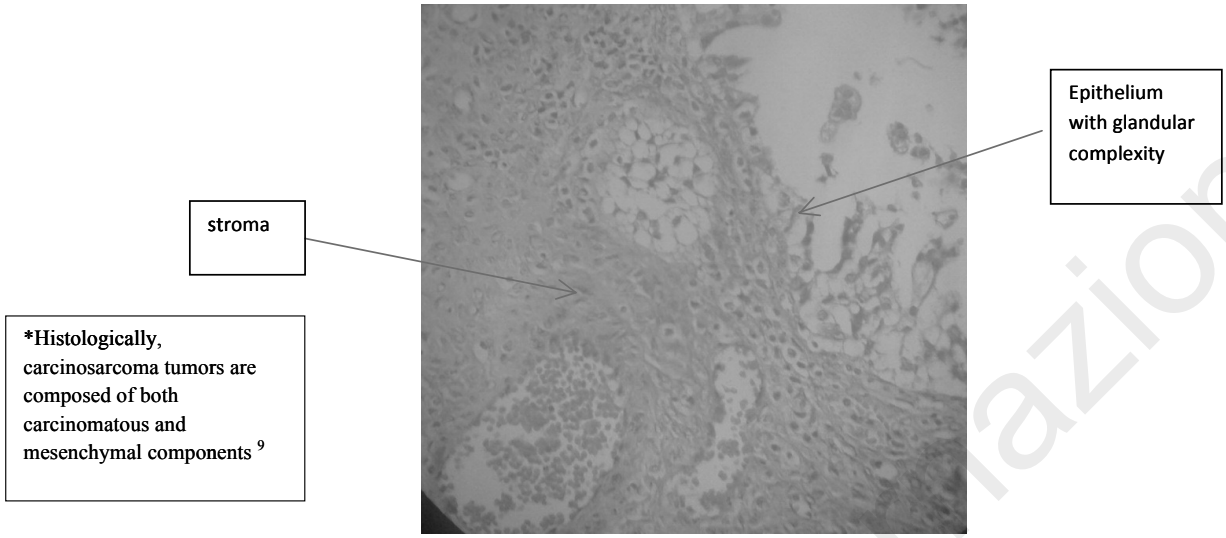


Figure 3 - Left ovary (high power field).

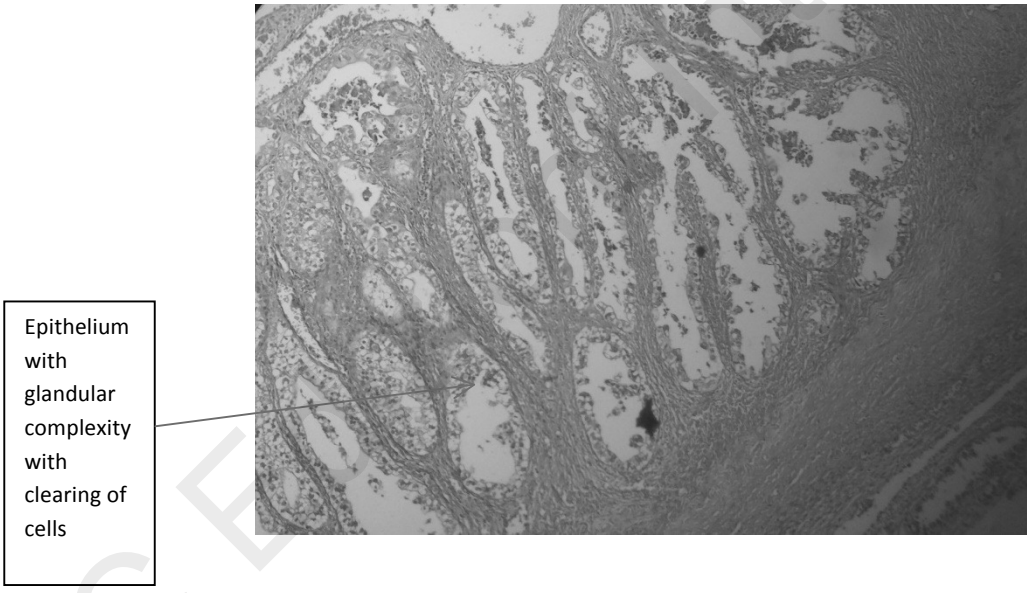


Figure 4 - Right ovary (low power field).

balls of malignant cells exhibiting pleomorphism, hyperchromatic nuclei, and increased nuclear to cytoplasmic ratio set in a background of red blood cells.

The patient was stable until 2 days post-operatively when she developed dyspnea, with absence of CTT output. Chest ultrasound revealed loculations, for which Video-assisted thoracostomy (VATS) was contemplated, so patient was transferred to another institution. During the patient's stay in the said institution, loculations resolved and VATS was no longer needed. Chemotherapy was offered but patient refused due to financial constraints.

Case discussion

Malignant mixed Müllerian tumor of the ovary, recently called Ovarian carcinosarcoma, is a very rare tumor, comprising only 1% of all ovarian neo-

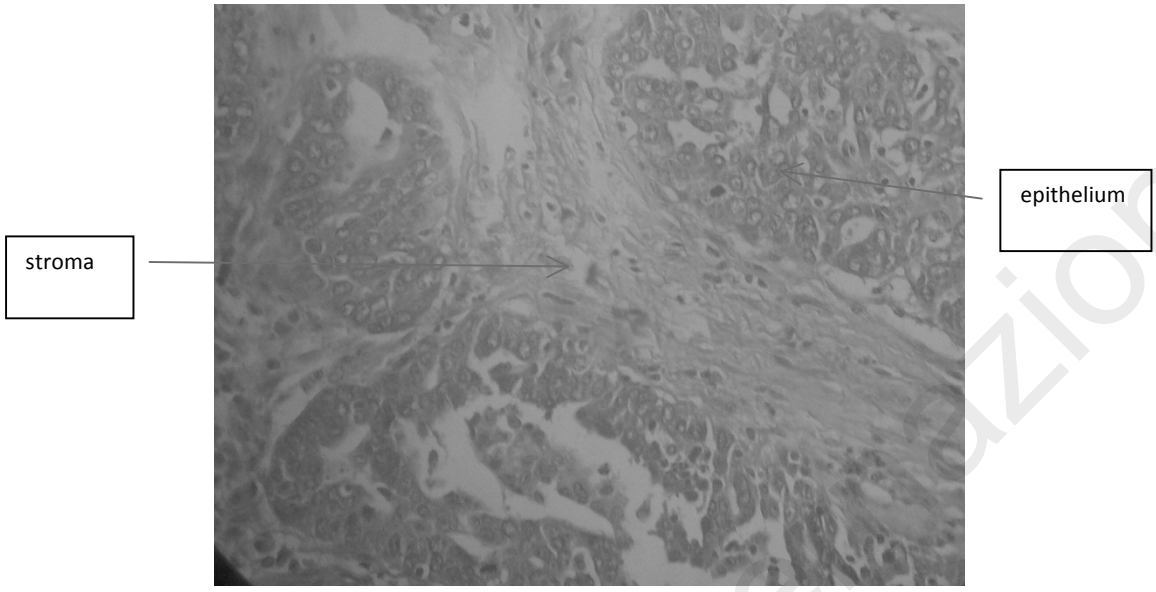


Figure 5 - Right ovary (high power field).

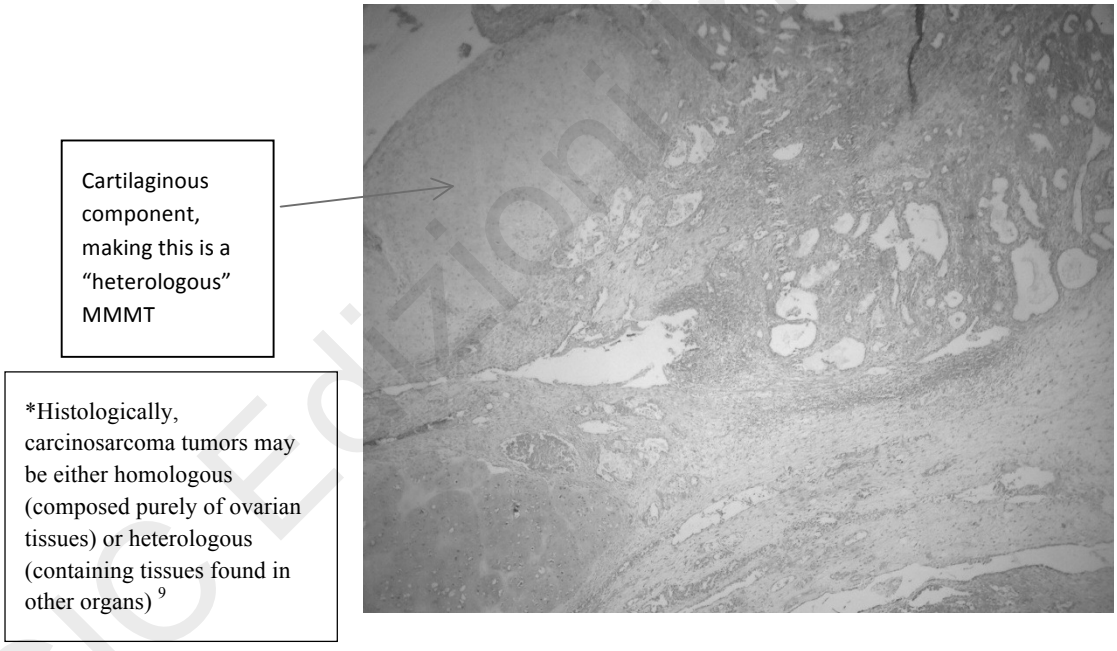


Figure 6 - Colorectal mass (low power field).

plasms (11). It is not common to arise in the female genital tract, and when it does, it usually affects the uterine corpus, followed by the cervix, vagina, ovary, and fallopian tube (12). Ovarian carcinosarcoma is an extremely aggressive subtype of epithelial ovarian cancer that is often advanced at diagnosis (13).

In women with family histories of ovarian cancer, the risk of ovarian cancer is higher compared with the general population (14). While approximately 90% of ovarian cancers occur sporadically, this patient falls under the 5-10% of women with ovarian cancer who have inherited genetic changes that predispose them to ovarian cancer, since she has two

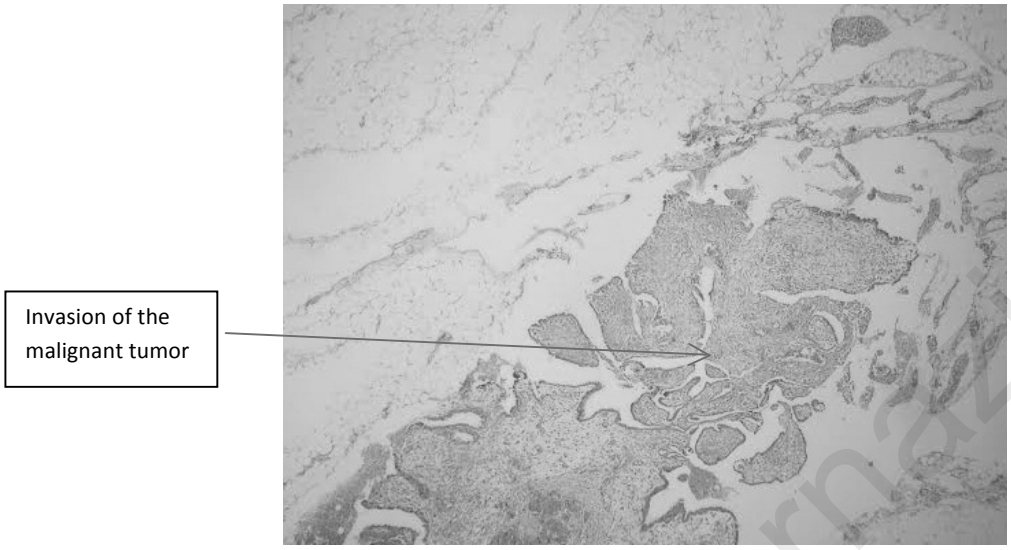


Figure 7 - Omentum.

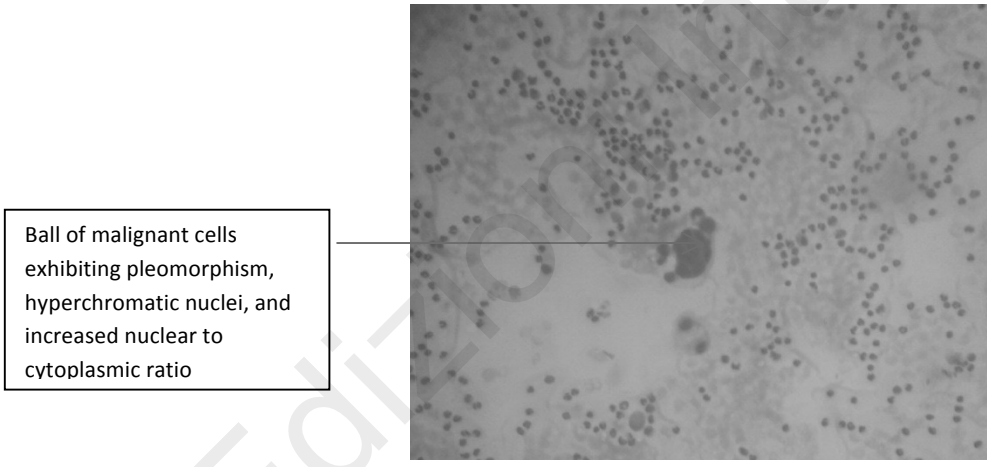


Figure 8 - Peritoneal fluid.

sisters who both died shortly after being diagnosed with ovarian cancer. Recent literature says that familial ovarian cancer confers a 4.6 relative risk (RR) of this disease in the proband's mother and a 1.6 RR in the proband's sister. This translates to a lifetime risk estimate of about 7.0% risk for the patient's mother, and 2.5% for the sister of an ovarian cancer patient, such as this patient (15).

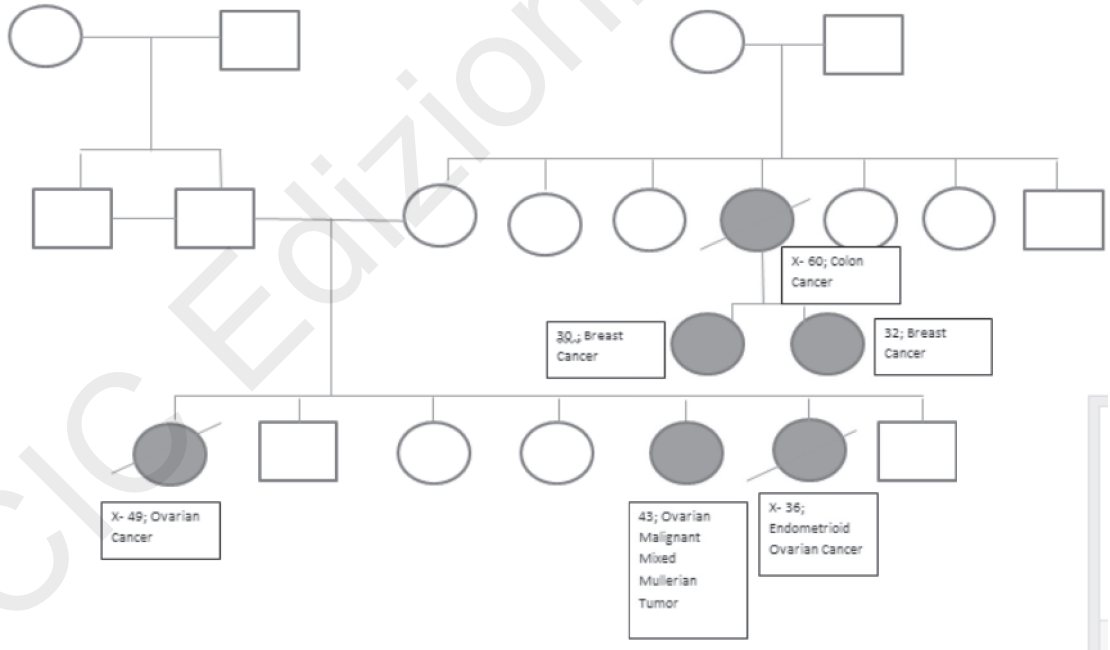
Two inheritable genetic mutations are known to predispose to ovarian cancer. 90% of the ovarian cancers in the hereditary breast-ovarian cancer (HBOC) syndromes involves mutations mostly in the breast cancer-associated genes BRCA1 located on chromosome 17, and small proportions have mutations in BRCA2, located on chromosome 13; while mutations in at least four mismatch repair (MMR) genes (MLH1, MSH2, MSH6 and PMS2) in the Lynch II syndrome (also known as hereditary nonpolyposis colorectal cancer syndrome) account for another 10-15% of hereditary ovarian carcinomas (16).

Autosomal dominant inheritance has been established in these syndromes. The lifetime risk of ovarian cancer in those with BRCA1 gene mutation may be as high as 28 to 44%, while for those with BRCA2 mutation, the risk is 27% (17).

It is also important to note that hereditary ovarian cancers, in general, occur in women approximately 10 years younger than those with nonhereditary tumors (14). As stated in the National Cancer Database (2) report, in the general population, those who are 50-69 years of age are 23-64% more likely to have ovarian cancer, while only 13% of the general population with age 40-49 is predisposed to develop ovarian cancer. In this case, the patient was diagnosed with ovarian cancer at the age of 43 years. The same can be said about her sister, who was diagnosed and died at the age of 37 due to endometrioid ovarian carcinoma.

Pedigree analysis is also necessary in determining the risk for hereditary ovarian cancer. The risk of carrying a mutation that predisposes to ovarian cancer depends on the number of first and second degree relatives with a history of epithelial ovarian carcinoma or breast cancer, and on the number of malignancies that occur at an earlier age (14).

Below is the pedigree analysis of the patient:



Since our patient has two sisters who both died because of ovarian cancer, this patient has a 35 to 40% risk. The same goes for their two remaining sisters who are still asymptomatic and unscreened.

CA 125, a monoclonal antibody, is used to diagnose ovarian cancer, even though the sensitivity is only 50% (14). It has a 96% positive predictive value in identifying malignancy in postmenopausal patients presenting with an adnexal mass and an elevated serum CA125 of more than 200U/mL. Its specificity in premenopausal patients is low because it tends to be elevated in common benign conditions (14). The addition of transvaginal sonography has enhanced its specificity. In current practice, CA 125 is still requested, not for diagnostic purposes, but for monitoring tumor response (17). In this patient, the test was requested but not performed due to financial constraints.

The American Cancer Society states that no screening test has proven to be effective and sufficiently accurate for early detection of ovarian cancer. However, for women who are at high risk, the combination of a thorough pelvic examination, transvaginal ultrasonography, and a blood test for the tumor marker CA-125 may be offered (18). Since these recommendations were not done for this patient earlier, these can be offered to her 2 sisters who are still unscreened.

Combining CA125 with other markers in tumor marker panels has been shown to increase sensitivity by 5-10%; however, specificity is decreased (19). Initial analysis of a tumor marker panel that included CA125, leptin, prolactin, osteopontin, insulin-like growth factor II, and macrophage inhibitory factor was reported to significantly improve sensitivity and specificity.

Surface-enhanced laser desorption ionization time-of-flight (SELDI-TOF) technology is a new approach in identifying ovarian cancer by using proteomic patterns. Although this study is still in the early phase, this seems to be promising as it has the sensitivity of 100% and specificity of 95% (20).

The measurement of plasma DNA levels and allelic imbalance by digital single nucleotide polymorphism analysis is another new approach, which is 87% correlated with stage I and II patients and 95% correlated with stages III and IV patients (21).

Like an advanced epithelial ovarian neoplasm, ovarian MMMT patients usually present with a pelvic mass, abdominal distention, and belching (11). This patient consulted due to dyspnea and an enlarging abdomen. Dyspnea was secondary to the malignant pleural effusion. This is expected as 75% of ovarian MMMT is diagnosed in stage III or IV, where in 90% of cases, have already metastasized to extragonadal sites (22).

It has also been suggested that expression of CD10 should be examined – it may be one of the characteristics of MMMT (23). However, the significance of CD10 expression needs to be elucidated by further studies.

Ultrasound is also an indispensable inexpensive diagnostic tool, as it can image a complex ovarian mass, with both solid and cystic components, internal echoes and/or septations, and ascites or evidence of peritoneal metastases in the presence of an ovarian mass, are highly suggestive of ovarian malignancy (24). One must also take note of the size and laterality of the lesion, as cystic masses more than 8 cm in diameter are more likely to be neoplastic. In this patient, a predominantly solid mass measuring 9.02 x 8.78 x 7.72 cm was seen on the left adnexa, and 3 x 2 x 2 cm solid mass on the right adnexa, with Sassone score of 13. Minimal ascites was also noted. Other procedures like CT scan and MRI may be requested to help in staging and planning the surgery (17).

Microscopically, specimens from left ovary and fallopian tube, right ovary, and colorectal mass all exhibited an admixture of malignant carcinomatous component composed of pleomorphic tumor cells lined by cuboidal to columnar epithelium exhibiting glandular complexity, some with branching papillary architecture, and some with clearing of cells, displaying nuclear atypia, increased nuclear-cytoplasmic ratio, vesicular nuclei, and prominent nucleoli which are infiltrating the stroma. This is consistent with the histologic criteria of MMMT, which must show a biphasic differentiation with malignant epithelial components and malignant sarcomatous components (17). Sections from colorectal mass (Figure 6) also showed areas with cartilaginous elements admixed with red blood cells and necrotic tissues.

A study done by Reed (17) shows that the diagnosis of a metastatic ovarian carcinosarcoma can be problematic, as when MMMT metastasizes, the sarcomatous component is either absent or is present in very minimal amount. This has not been the case in this patient, as both the epithelial and sarcomatous components were seen in the colorectal mass. There is no statistical difference in survival rates between homologous and heterologous MMMT.

Because this tumor is rare and there are very limited reports in literature to date that define the prognostic factors and optimal treatment strategies associated with survival in women with ovarian carcinosarcoma, treatment has still not been well established.

The goal of surgery in ovarian MMMT is not to leave any residual disease. Once ovarian cancer is suspected, total abdominal hysterectomy, bilateral salpingooophorectomy, omentectomy, and peritoneal washings should be done. Lymphadenectomy and blind biopsies from diaphragmatic surface can be included (22). These were all performed in this patient. Excision of the colorectal mass was also done. The patient was assessed to have ovarian malignant mixed Müllerian tumor stage III-C since the patient had a colorectal metastasis involving the serosa, and pleural fluid cytology was negative for malignant cells. The FIGO 5-year survival rate for this patient is 23% for stage IIIC. But since this patient's histopathology revealed MMMT, it is decreased to 18%.

A study by Jarnigan et al. (25) supports the concept that no gross residual disease is associated with improved survival outcomes over traditional "optimal" cytoreduction to ≤1 centimeter. In their analysis, even when controlling for age and stage, the as-

sociation between cytoreduction to no gross residual disease and survival remains.

Chemotherapy, which the patient refused, should have been in the form of platinum-based regimens used in epithelial ovarian tumors. It has been hypothesized that the sarcomatous and carcinomatous components both arise from a single malignant epithelial precursor which has undergone metaplastic change to a sarcomatous form, which contributed to the presence of both histological types (9). Recent literature shows a 68% overall response rate compared with 23% response rate in the non-platinum containing regimens (26).

The prognostic factors of ovarian MMMT are reported to be the age at presentation, insufficient surgical removal, and the stage. The recurrence rate is 50% in stage I and up to 90 to 100% in stage II or greater.

By understanding the hereditary ovarian cancer syndromes and its relation to this patient, it cannot be overemphasized that a comprehensive family history is really fundamental for early diagnosis. It is a useful tool to arrive at a sound clinical judgement to prevent disease progression in genetically susceptible individuals, like the patient's two remaining sisters. They should also be tested for BRCA1 and BRCA2.

Conclusion

In conclusion, the clinicopathological features of a patient with primary ovarian malignant mixed Müllerian tumor, a rare malignancy comprising only 1% of ovarian neoplasms are discussed. The familial association of the disease, given that the patient has two sisters who were diagnosed with an advanced stage of ovarian cancer and succumbed to the disease at a much earlier age than what is reported in the general population, points to a possible hereditary syndrome which occurs in only 5% of ovarian neoplasms.

The following recommendations put forward by Berek et al. (14) for the management of women at high risk for ovarian cancer will be useful in clinical practice: 1) patients who are high risk should undergo genetic counselling and may be offered genetic testing for BRCA1 and BRCA2; 2) those who want a conservative management to preserve their reproductive organs can have a transvaginal sonography every 6 months; 3) young women should be offered oral contraceptives before they attempt to have a family; 4) prophylactic bilateral salpingooophorectomy should be offered to those with completed family or pregnancy; 5) annual mammographic screening should be offered to those with strong family history of breast and ovarian cancer from age 30 and beyond; 6) periodic screening mammography, colonoscopy, and endometrial biopsy should be offered to those with hereditary non polyposis colorectal cancer syndrome.

However, in a low-resource setting, the following will be recommended for monitoring and/or screening women who are at high risk for developing ovarian cancer:

1. physical examination focusing on the breast, abdomen, and rectal area every 6 months
2. transvaginal sonography every 6 months
3. mammography annually
4. CA125 for postmenopausal women.

Genetic testing for BRCA1 and BRCA2 will be reserved for those who are financially capable.

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Immune histochemical research of matrix metalproteinase 9 and cyclin D1 in hyperplasia of endometrium of women of reproductive age

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According to modern representations, hyperplasia of endometrium (HE) is the most frequent form of pathology of mucous uterus body and it is considered as not physiological proliferation of endometrium which is followed by restructuring rebuilding and to a lesser extent of stromal component of tissue. According to the standard point of view, in development of HE the leading role belongs to excess estrogene stimulation in combination with insufficiency of progesterone influence. However it is necessary to mark that there appeared new facts which are outside such point of view (1, 3, 9). Nowadays the role of molecular and biological factors in pathogenesis of HE is actively studied. According to different Authors, essential value in violation of a tissue homeostasis of endometrial tissue has the imbalance of processes of apoptosis and proliferation with the relative dominance of the latter (2, 6). Nevertheless, the modern ideas of molecular and biological development mechanisms of proliferative processes assume existence of one more important factor of regulation of tissue reorganization – extracellular matrix which is under the influence of specific proteolytic enzymes – matrix metalproteinases (MMP). It is known that these enzymes express in all tissues at all stages of ontogenesis, and their expression is activated in the conditions of intensive tissue reorganization. The main result of proliferative signal stages is strengthening of cellular proliferation, i.e. increase in quantity of cells in tissue (14).
Besides, it is known that regulation of a cellular cycle is carried out by means of reversible phosphorylation/dephosphorylation of special regulatory proteins to which belong serin/treonin-cyclin-dependent kinases (CDKs), or kinases of a cellular cycle. CDKs are activated in case of incovalent linking with the appropriate proteins of cyclin. So, cyclin D1 which is synthesized in the phase G1 and defining transition of the sharing cage to the phase S that finally leads to a mitosis. And the result of mitotic division is a doubling of number of cages during the cellular cycle. Therefore the disregulation of a normal progression of a cellular cycle is the fundamental biological phenomenon which is the cornerstone of any hyper plastic process (12, 15).
Therefore scientifically and practically justified the study of activity of MMP-9 and cyclin D1 for assessment of course of cyclic changes in endometrium in case of reconstruction of an intercellular matrix during the menstrual cycle, and also for establishment of dynamic interaction of these components in formation of HE and its recurring.

Research objective

Assessment of activity of components of extracellular matrix in hyperplasia of endometrium of women of reproductive age.

Materials and methods

97 women of reproductive age have been examined, among them 61 have HE. Group of control: 36 patients without pathology of endometrium in a proli-

feration phase to whom examination was carried out because of infertility. Two main groups according to the character of examination have been formed: 1 - women with simple HE (41 patients), 2 - with complicated one (20 patients). The groups were comparable on age (on average 28,4 ± 1,6 years).

Criteria of inclusion were: reproductive age, performance of research in the 1st phase of a menstrual cycle, morphologically confirmed diagnosis of HE, lack of hormonal therapy for the last 3 months, absence of an infection. Obligatory condition of participation in the research was patients' informed.

Criteria of exception were: malignant new growths of genitals and precancer of endometrium, the combined benign diseases of uterus which are recommendation for hysterectomy, contraindication for carrying out invasive intrauterine interventions.

To all women was carried out hysteroscopy ("Karl-Storz", Germany) by a standard technique. By curettage were received separate scrapes of a mucous membrane of the cervical channel and of the uterus body, the material was fixed in 10% solution of neutral formalin. After filling in paraffin they prepared 5-6 microns thick cuts and painted them with hematoxylin and eosin. At histologic diagnostics were used the criteria recommended by WHO (15).

Immune histochemical research has been done according to the standard technique with unmasking of antigenes in the microwave oven on the serial paraffin cuts of endometrium placed on the glasses covered with poly-L-lysine. As primary specific antibodies were used monoclonal antibodies to MMP-9, D1 cyclin ("LabVision", the USA). Results of immunohistochemical reactions were estimated in points (from 1 to 6) by a semi-quantitative method on percent of the painted cages: 0 point – lack of the painted cages, 1 point – 5-10%, 2 points – 10-20%, 4 points – 20-40%, 6 points – more than 40% of positive cages (2).

Statistical processing was carried out with the use of computer programs Biostat, Statistica 6.0 for Windows.

Results

Patients with HE had the main complaint which consisted of abnormal uterine bleedings ($\chi 2=59,98$, $p=0,0001$). Recurring process of HE dominated ($\chi 2=3,99$, $p=0,046$) with the greatest frequency at the age from 36 till 39 years (46,7%). During the analysis of clinic data depending on the fact of recurring of HE were revealed the following differences: the increase in the index of body weight ($U=211$, $p=0,020$), the high frequency of abnormal uterine bleedings ($\chi 2 =13,28$, $p=0,0003$), increase in number of medical and diagnostic scrapes ($U=98$, $p=0,0001$) at patients with recidivous HE is revealed.

According to ultrasonography HE signs (raised echogenicity, non-uniform structure, thickening of endometrium) have been noticed at most of the patients. Statistically significant tendency to prevalence of follicular cysts of ovaries is found ($p=0,084$). Informational content of hysteroscopy in diagnostics of hyperplasia of endometrium (on compliance to the histologic conclusion) made 93,6%.

The analysis of anamnestic data revealed the increased level of somatic pathology of patients with HE: every fourth woman had a hypertensive illness,vasoneurosis on hypertensive type, every fifth woman had a varicose illness of veins of the lower extremities; chronic diseases of digestive tract and hepatobiliar system had a third of patients, chronic diseases of urinary tract – 13% of patients; pathology of a thyroid gland – 9% of patients.

In case of histologic research of scrapes of a mucous membrane of uterus body all the patients had HE without atypia: 41 women had simple and 20 women had the complicated one, among them 11 patients with simple HE had a recuring process, and also 9 women with complicated HE had a hospitalization because of recurrence of HE.

For the purpose of study of the molecular and biological mechanisms involved in development of HE was carried out the research of activity of components of extracellular matrix of mucous uterus body. According to the obtained data, hyperplasia of endometrium is characterized by the increased level of metalproteinase activity in comparison with endometrium of proliferation stage. MMP-9 expression level at HE made 3,6 ± 1,0 points that authentically differed from this indicator in not changed endometrium (1,3 ± 0,7 points; $p<0,05$). At complicated HE the tendency to increase in the maintenance of MMP-9 in comparison with simple HE is revealed (3,8 ± 0,7 and 3,5 ± 0,9 points respectively), and at recidivous HE we haven't received reliable distinctions in the relevant groups of the examined women ($p>0,05$). When determining the maintenance of cyclin D1 at HE increase of its expression in

comparison with endometriun of proliferation stage is revealed ($p< 0,05$): the average value in samples of HE made 3,8 ± 0,6 points, in not changed endometrium 2,2 ± 0,9 points. The expression made 3,6 ± 0,5 and 4,1 ± 0,2 points at simple and complicated HE respectively. However it should be noted that the expression of cyclin D1 in the tissue of hyperplasia of endometrium at recidivous HE averaged 4,1 ± 1,1 points: at simple HE 4,0 ± 1,2 points, at complicated 4,3 ± 1,0 points.

Discussion

According to medical literature, MMP represent group related on structure cynk-dependent endopeptidases, having essential value in processes of tissue remodeling (4). In physiological conditions these proteins realize degradation of basal membrane and components of the extracellular matrix playing a dynamic role in the metabolic processes influencing proliferation of cells, their differentiation, migration, apoptosis and neoangiogenesis, and also growth of depositing biologically active factors (7, 16). As a result of numerous researches Authors received data on substrate specificity of MMP. In particular, gelatinase of MMP-9 lease only the denatured collagens and interacts with the collagen IV and elastin which are components of basal membrane (7, 15, 16). It is known also that MMP realize also proteolytic degradation of the proteins connecting insulinoid and transforming growth factors, modulating their activity (16). The results obtained by us testify to increase in activity of MMP-9 in comparison with not changed endometrium in a proliferation stage that confirms a specific role of these proteins in violation of processes of cyclic growth, differentiation and loss of cells of mucous uterus body.

Literature data concerning the maintenance of MMP in hyperplasium of endometrium are contradictory. According to data of some Authors (15, 16), unlike the endometrium of proliferation stage HE is characterized by the high level of expression of MMP-1, MMP-2, and also MMP-7, MMP-9. However in other researches essential distinctions depending on a type of HE were not revealed (11, 13). At the same time it is reported about increase in metalproteinase in case of atypical HE and especially in case of cancer of endometrium (8). In our judgement, the results of the research conducted by us testifying to increase of metalproteinase activity in case of HE explain development mechanisms of the progressing violation of ferriferous and stromal ratio and disruptions of links of numerous cellular elements while forming hyperplastic process.

Besides, according to our data, development of HE recuring is associated with sharp increase of expression of cyclin D1, thus is marked also not such expressed increase in level of its expression in case of simple and complicated HE, revealed for the first time. It is known that protein regulator of a cellular cycle cyclin D1 activates cyclin-dependent kinases, activated (phosphorylated) forms which are components of intracellular signal stages which regulate processes of cellular proliferation, survival (apoptosis) and differentiation (13). According to literature, the involvement of kinases in regulation of cellular survival (apoptosis) substantially depends on the type of cells and the type of the incentive inducing a signal. In one cells such signal causes cellular loss, and in others – the same kinases stimulate cellular survival, growth and differentiation (9). The research conducted by us showed that recuring of HE is connected to more deep cellular reorganization at the molecular level, and the expression of cyclin D1 can be a prognostic marker of recuring of HE.

Thus, in pathogenesis of endometrial HE an important role can be played by violation of dynamic operation of MMP-9 and cyclin D1 that promotes change of cyclic processes of growth and degradation of endometrium.

The data concerning cyclic change of activities of MMP in endometrium during the menstrual cycle testify to hormonal regulation of their expression. In particular, powerful inhibitor of secretion of MMP is progesterone. During the lutein phase of the menstrual cycle when concentration of progesterone is high, the level of expression of the majority of MMP decreases, while the highest level of their activity is marked when there are intensive processes of degradation of endometrial tissue (11). It is possible to assume that change of expression of MMP-9 in endometrium is the result of change of hormonal relations in case of HE. Besides, the data obtained by us indicate one more important aspect, namely the molecular development mechanism of the uterine bleedings which are a characteristic symptom of HE. Increase in activity of MMP in case of HE promotes proteolytic degradation of mucous uterus body and its rejection. On the other hand, absence of changes of expression of cyclin

D1 in case of the first time appeared HE, can significantly influence duration of uterine bleeding, decelerating process of reepitelization of mucous uterus body, and increase in its expression can predict recurred episodes of the disease.

Thus, molecular and biological features of processes of tissue remodeling in hyperplasia of endometrium of women of reproductive age are characterized by the expressed changes of components of an extracellular matrix that leads to violation of processes of growth, differentiation and death of cages of mucous uterus body, starting the process of recurring.

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Clinical and hormonal indicators of PCOS developed during non classical adrenal hyperplasia

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Introduction

Polycystic ovary syndrome occurs in as many as 5-10% of women of reproductive age (1, 2). Polycystic ovaries are detected in a higher percentage of women with oligomenorrhea (85%), hirsutism (95%) and congenital adrenal hyperplasia (75%) (3). One of the major biochemical abnormalities seen in PCOS is a state of hyperandrogenism. It is mainly of ovarian origin with adrenal contribution since certain percentage of PCOS patients show a mild steroidogenetic defect in adrenal glands (4, 5). Adrenal involvement in the PCOS has long been recognized (6). The important clinical question is the following: is excessive androgen secretion by the adrenal gland a primary disorder in women with PCOS or is it a secondary reaction to the hormonal changes associated with anovulation?

Non classical adrenal hyperplasia (NAH), caused by incomplete deficiency of enzymes (the most common enzymes to be deficient are 21-hydroxilase and 3β-hydroxysteroid dehydrogenase) is now recognized to be the most common autosomal recessive disorder and appearing later in life. The clinical presentations is extremely variable and is greatly influenced by stress. NAH leads to PCOS quite often (5, 7, 8). Suppression of ACTH secretion by glucocorticosteroids through feedback mechanism finally decreases adrenal hyperadrogenemia and corrects clinical manifestations like irregular menses, hirsutism, acne, seborrhea, infertility, recurrent pregnancy loss (5, 9).

The aim of the study was to detect clinical and hormonal indicators of PCOS in the women of reproductive ages with non classical adrenal hyperplasia.

Materials and methods

Case-control study included 105 female patients aged 20-34 with non classical adrenal hyperplasia, confirmed by clinical, ultrasound and hormonal investigations. Patients were divided into two groups according to the sonographic picture: I group- 58 patients (50,4%) with PCO (the presence of 12 or more follicle in each ovary measuring 2-9 mm in diameter and/or increased ovarian volume >10ml), II group-57 patients (49,6%) with normal ovaries. 18 same age healthy volunteers served as a control. The results were analyzed statistically by method of variance (ANOVA). All data are expressed as average ±SD. Student's t-criterion was used to compare the groups. The value of $P<0.05$ was considered as statistically significant.

Results and discussion

The majority of PCO patients (Igr)-62% had oligomenorrhea and amenorrhea, the rest ones-38% - anovulatory cycle. Hirsutism existed in 82,7%, acne in 74,1%, seborrhea in 81%. There were not differences in frequency of hirsutism, acne and seborrhea between PCO and normal ovary patients (IIgr: hirsutism-78,9%, acne-71,6%, seborrhea-73,6%), but patients with normal ovaries in 7%

had normal biphasic cycle, in 28%-insufficiency of lutheal phase, the rest ones- 65%- anovulatory cycle, amenorrhea and oligomenorrhea. In both groups most patients (Igr- 65.3%, IIgr- 70%) had normal BMI, only 6.3% in Igr and 5,3% in II gr had BMI >30. There were not differences between groups.

The average level of LH was significantly higher in I gr-6,49±2,9 IU/I, as compared with II gr-4.50±2,64 IU/I and control-4,25±2,42IU/I (P<0,05, p<0,005). No difference was revealed among II gr and control (P>0,2). Average level of FSH not differ between I gr- 2,39±0,98 IU/I and II gr-2,42±0,98 IU/I (P<0,2), but was significantly low than in control-3,37±1,63 (P<0,01, P<0,05). LH/FSH ratio were elevated in I gr -3,13±1,22 compared to the II gr - 2,09±1,11 and control-1,25±0,36 (P<0,05, P<0,001). There were no statistically significant differences between the I and II groups regarding to the average levels of DHEA-S (387,20±115,18ng/ml; 390,39±130,3ng/ml),17αOHP (1,67±1,18ng/ml; 2,95±4,37ng/ml),T(0,9±0,3ng/ml; 1,0±1,69ng/ml) (P>0,20), but they were significantly higher compared to the control (DHEAS - 147,43±54,41ng/ml;17αOHP - 0,4±0,23ng/ml;T-0,41± 0,23ng/ml) (P<0,001). Average level of androstendione was higher in I gr- 4,30±1,64ng/ml compared to the II gr-2,27±1,44 ng/ml and control- 1,65±2,79ng/ml, but statistically significant was difference between Igr and control (P<0,05), no difference was revealed between I gr and II gr (P>0,05). Cortisol level didn't differ between groups: I gr-377,29±133,81nmol/l; II gr-422,78±124,67nmol/l; control- 462,32±168,23nmol/l (p>0.2).

Polycystic changes in the ovaries developed during non classical adrenal hyperplasia is one of the clinical manifestation of the disease. Clinical picture includes: oligomenorrhea, amenorrhea, anovulatory cycle, hirsutism, acne, seborrhea. Obesity is not characterizing feature of the disease. The endocrinological aberrations include excess of androgens - DHEAS,17α OHP, T, with normal cortisol level. Elevation of ΔA_4 is seen only in patients with PCOS, that indicates to secondary inclusion of ovaries in the process. Specific hormonal diagnostic indicators are: high LH and low FSH levels, that altered LH/FSH ratio (5). It is higher than 2,5, but lower than 3.5.

Conclusions

PCOS developed during non classical adrenal hyperplasia may be discussed as a one clinical forms of the disease. Adrenal hyperandrogenemia has primary, key role in this process, breaks normal modulation of gonadotropins, increases the secretion of LH, that induces hypersecretion of androgens by the ovaries. Hence, the elevated androgens, both ovarian and adrenal, contribute within the ovary preventing normal follicular development and inducing premature atresia. The end point being the emergence of the typical polycystic ovary.

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Comparison of endometrial histology and safety after an oral four-phasic contraceptive and a non-oral sequential estro-progesterone regimen in healthy peri and early postmenopause women

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Introduction

Peri/postmenopause Hormone Therapy (pHT) rised many questions, worries, and controverses regarding type of hormones, routes of administration, and estrogen therapy timing on promoting midlife health in clinical practice parallel with ageing population, and cancer fear in Western and Northern countries from Europe, and America, facts that are more and more present in European Central and Eastern countries.

Objective

Endometrial histology and safety after an oral four-phase contraceptive comparative to a non-oral sequential HT in perimenopause, and early postmenopause healthy women.

Materials and methods

Prospective endometrial assessment by microscopy at pre-study, after 12, 24 months treatment in 30 healthy perimenopausal, and early postmenopausal women, who were analyzed in two patients groups from two in-patient gynecology departments - "Dr. I. Cantacuzino" and "St Pantelimon": 1) 10 cases on a registered four-phasic oral contraceptive (estradiol valerate, estradiol valerate plus dienogest - Qlaira® - during 28 days/monthly; 2) 20 patients on 0.5g/day 17-β-oestradiol gel-Oestrogel® x 28 days plus 200 mg/day vaginal micronized progesterone (VMP) during 12 days from the 14th day of 17-β-oestradiol.

The endometrial of the first patient group was collected by biopsy (Pipelle de Cornier/ Novak curette) and assessed between days 12 and 19 of the cycle, at screening, 12, 24 months. Women from the second group underwent endometrial biopsy (Pipelle de Cornier/ Novak curette) between the 9-12th days of progesterone (days 22- 25 of therapy), at screening, 12, and 24 months.

Patients are enrolled if accomplished inclusion and exclusion criteria (Table 1).

Collected endometrial specimens are processed, being fixed in 10% formalin and paraffin embedded; sections are done at 2 μm, stained with the commune technique of haematoxylin- eosine.

Endometrial pathological assessment is done by a single, independent pathologist. The endometrial samples are classified according to World Health Organization classification (1994, 2003), and the criteria of Endometrial Collaborative Group (1, 2) for endometrial hyperplasia (EH) and endometrial intraepithelial neoplasia (EIN) or endometroid neoplasia [European Study – (3)] (Table 2).

Histologic analysis of endometrial structures

- *Architecture* and *cytological* analysis of glandular epithelium is done looking for atrophic, weak proliferative, secretory endometrium, hyperpla-

TABLE 1 - CRITERIA OF INCLUSION/EXCLUSION.

Inclusion criteria	Exclusion criteria
• age: 40- 55 years • intact uterus • at least 3 months of amenorrhoea • negative pregnancy test • moderate/ severe climacteric syndrome • no previous at least 3 months of HT • signed informed consent	• submucous leiomyomas • endometrial hyperplasia with atypia • history of endometrial, breast cancer or other hormone dependent tumor • ovarian cysts • suspect mammographic changes one previous year • actual/history of thromb-embolism • severe liver disease, jaundice or generalized pruritus during pregnancy • Dubin Johnson/Rotor Syndrome • congenital disturbances of lipids metabolism • history of gestational herpes • pregnancy aggravated othosclerosis • no cardiovascular pathology • intolerance to estradiol, progesterone or progestogens

TABLE 2 - CLASSIFICATION OF ENDOMETRIAL BIOPSY SAMPLES (ADAPTED FROM WHO 2002; MUTTER GL, 2000; 2002; DIETEL M, 2001).

0= no tissue of technical reasons;

1= insufficient tissue for diagnosis;

2= atrophic/inactive endometrium;

3= secretory endometrium;

4= normal proliferative endometrium;

5= endometrial hyperplasia (EH): simple and complex without and with atypia

6= endometrial intraepithelial neoplasia (EIN)

7= carcinoma (endometrioid type, non- endometrioid type)

sia: mitosis for proliferative effect, subnuclear glycogen accumulation and intraluminal secretions for secretory changes. Mitosis *per se* were not quantified, the categories 2-3 do not have mitosis by definition. The regulation effect of progesterone is analyzed for the degree of proliferation suppression – mild, moderate, strong – when is not possible an accurate assessment of secretory transformation treatment induced.

- *Stroma* analysis: characteristic aspects for perimenopause and menopause/postmenopause: the fibroblast – fibrocytic proliferation – marked (MP) and moderate (mP), the fibrosis, and the presence of inflammatory elements (I). In order to differentiate and discriminate between EH and EIN – a premalignant lesion, it is specially assessed the stromal volume. EIN diagnosis is based on the presence of cytological demarcation, crowded gland architecture, minimum size of 1mm, careful exclusion of mimics, and diminution of stromal volume to less than approximately half of the total sample volume (a new architectural criterion for EIN) (Mutter GL, 2000, 2002; Dietel M, 2001). Most EIN lesions have been diagnosed as atypical endometrial hyperplasias in the WHO system.
- *Vasculature* analysis: vessels' number on examined fields: high (HV) and low number (LV), and sub-epithelial hemorrhages (H).

Endometrial safety is assessed in terms of estrogen-induced hyperplasia prevention.
The therapeutic effect was appreciated on epithelium, glands, stroma, and vessels; and histologic aspects considered were mitosis for proliferative effect, and subnuclear glycogen accumulation and intraluminal secretions for secretory changes.
Mitosis *per se* are not quantified, the categories 2-4 do not have mitosis by definition.
The progesteronic effect was analyzed for the degree of proliferation suppression – mild, moderate, strong – when it was not possible an accurate assessment of secretory transformation therapeutic induced.

Statistic analysis

It was based on the paired-sample *t* test, and differences between oral – contraceptive medication and non-oral, sequential hormone therapy considered significant at $p < 0.01$.

TABLE 3 - ANALYZED PATIENTS.

Patients	Group 1: 10 cases	Group 2: 20 cases
Type of pHT	a registered four-phasic oral contraceptive (estradiol valerate, estradiol valerate plus dienogest - Qlaira®- during 28 days/monthly	transdermal 0.5g/day 17-β-oestradiol gel-Oestrogel®, x 28 days plus 200 mg/day vaginal micronized progesterone (VMP) during 12 days from the 14th day of 17-β-oestradiol

Results

There were analyzed 30 patients from two groups (Table 3).

Table 4 is presenting the patients' characteristics – demographic, parity, and habits as smoking, and the cases are comparable according age, weight, height, smoking, and thromboembolism/cardiovascular risks, with the exception of parity (more multiparous patients between patients treated with the contraceptive regimen). All patients accomplished inclusion/exclusion criteria.

The endometrial assessment at enrollment (screening), 12 months and 24 months are listed in Table 5.

At enrolment microscopy registered similar endometrial state in both groups: hyperplasia (6 cases/group 1, and 7 cases/group 2, with complex hyperplasia without atypia: one case of in group 2), weak proliferative (3 *vs* 12 cases- 0.3%/group 1 *vs* 60%/group 2), and secretory (one case in group 2).

The analysis of the endometrial changes from baseline induced by the oral combined contraceptive method, and by sequential non-oral hormone treatment in perimenopausal, and early postmenopausal women (Table 5 for epithelial/glandular, and Table 6 for stroma and vessels) is focusing on secretory and atrophic changes, appreciated as safe for endometrium health.

After 12 and 24 months of treatment there are:

- atrophy/ inactive endometrium: 20%, respective 40%;
- 74%, and respective 49% cases with low grade secretory changes, characterized only by cytoplasmic vacuolization;

TABLE 4 - PATIENTS' CHARACTERISTICS.

Characteristics	Group 1 Qlaira ®	Group 2 E2 trd + VMP
Number	10	20
Age (yrs)		
Average	47.3	48
Variation	45-52	46-55
Ethnicity		
Caucasian	10	20
Weight (kg)		
Average	59.6	59.7
Variation	44-91	50-79
Height (cm)		
Average	160.8	160.6
Variation	135-168	154-164
Parity		
Nuliparous	1	5
Multiparous	34	10
Smokers	2	5

Legend: Qlaira ® = four phase contraceptive;
E2 trd : Oestrogel ® transdermal + VMP (Vaginal micronized Progesterone)

TABLE 5 - ENDOMETRIAL ASPECTS AT SCREENING (0), 12 MONTHS, AND 24 MONTHS OF THERAPIES.

Endometrial Pathological Aspects	Group 1 Qlaira ®			Group 2 E2 trd + VMP		
	0	12 months	24	0	12 months	24
Insufficient tissue	0	0	0	0	0	0
Atrophy/inactive	0	4	9	0	3	3
Proliferative	3	1	0	12	1	1
Secretory	0	6	1	1	16	9
Hyperplasia	6	0	0	7	0	0
Others	1	0	0	0	0	0
Carcinoma	0	0	0	0	0	0
Total	**10**	**10**	**10**	**20**	**20**	**20**

TABLE 6 - STROMAL AND VASCULATURE CHANGES AFTER THERAPIES.

Endometrium	Stroma				Vasculature		
Number of Cases/ **Percentage**	MP	mP	F	I	HV	LV	H
Enrolment = 30	14/**46.6**	16/ **53.3**	4/**13.3**	24/**80**	17/**56,6**	14/**46.6**	4 /**13,3**
12 months = 30	26/**86.6**	2/**6.33**	1/**3.33**	16/**53.3**	20/**66.6**	6/**20**	7/**23.3**
24 months = 30	25/**83.3**	2/**6.33**	0	16/**53.3**	25/**83.3**	5/**16.3**	8/**26.6**

Legend: MP= marked fibroblast- fibrocytic proliferation; mP= moderate fibroblast- fibrocytic proliferation; F= fibrosis; I= inflammatory elements; HV= high number of vessels; LV= low number of vessels; H= sub-epithelial hemorrhages.

- 6 cases with inadequate progesteronic response;
- proliferative endometrium: a total of 3 cases at 12, and 1 case at 24 months;
- increase of stromal fibroblastic-fibrocytic proliferation (specially in the first year of treatment);
- increase of blood vessels number and of sub-epithelial hemorrhage (more frequent in the second year of treatment);
- no hyperplasia/ carcinoma.

Discussion

The risks revealed by HERS, WHI for oral HT imposed the analysis of endometrial effects of other regimens with: only low dose estrogens, low dose synthetic estrogens as in combined oral contraceptives, new types of progestogens (gonan, norpregnan, spironolactone derivates- in reduced doses parallel with reduced doses of estrogen) as alternatives to medroxyprogesterone acetate (MPA), norethisterone (NET) or non-oral route of administration [transdermal natural estrogens, and vaginal micronized progesterone] for prevention of endometrial hyperplasia, and carcinoma.

The ages of patients enrolled in the Romanian study were in years of menopausal transitions and the histopathological reports of endometrial pattern were in the limits of this period of women's life.

The four phase contraceptive- E2V/DNG (Qlaira®, Klaira®- estrogen step-down, progestogen step-up regimen) is studied in Europe since 2003 (4), and in USA - as Natazia® (5), recommended initial for uterine bleeding control. This contraceptive product is generating through estradiol valerate a stable estrogen serum level (6), a very important pharmacokinetic characteristic beside other benefic properties of the natural estrogen for women health in comparison to synthetic ethinyl-estradiol or to the

natural 17β estradiol, which has a poor cycle control in contraceptive pills. On the other hand, dienogest – a new progestogen, a specific progesterone receptor agonist, which combines the properties of 19-nortestosterone derivatives and progesterone derivatives, has a special pharmacokinetic profile, with higher plasma concentrations than those of the other progestogens with higher doses, and it is proved to have a high potency *in vitro*, explaining its superior capacity on endometrial structures *vs* other progestogencs (MPA, DYD, NES) in balancing the estrogen (7, 8).

We have choose Qlaira® to avoid abnormal uterine bleeding characteristic for perimenopause years (9-12), and for the control of climacteric syndrome, low/no effect on metabolisms and hemostasis (13), having also in mind other non-contraceptive effects of contraceptive drugs (14) and the estrogen timing hypothesis (15); – the estrogen administration in newly menopausal women than for older women, for long time health preservation (16).

The association of the natural estrogen "estradiol valerate" to dienogest in the step down and step up regimen was considered the revolution in contraception, and the Romanian study makes the comparison to a sequential non- oral HT regimen known and proved by many studies and by our own studies to be endometrial safe in peri- and early postmenopause (17).

It was demonstrated that oral contraceptives (commun and high potency) (Table 7) exert a predominant progestational effect on the endometrium, inducing an arrest of glandular proliferation, pseudosecretion, and stromal edema and granulocytes, thin sinusoidal blood vessels (18) (Table 7). After prolonged use it is record a progressive endometrial atrophy, and an indirect reduction of endometrial hyperplasia/carcinoma.

The endometrial protection from proliferation, hyperplasia, or carcinoma was ensured by the administration of the contraceptive method with the natural estradiol valerate step down and dienogest, step up and by the 12 days of 200 mg micronized progesterone vaginally administrated sequential to continuous transdermal estradiol. This statement is for all studied cases, and specially in those with hyperplasia without atypia at enrolment.

Endometrial microscopical assessment registered important changes after 12 and 24 months of treatment: appearance of atrophy/inactive endometrium in both groups, reduction of proliferative endometrium – more frequent in the non-oral HT group 2 ($P < 0.01$), with accentuation after 2 years, and an increased rate of secretory endometrium (60%/group 1, and 80%/group 2) – more frequent in the first year in the both groups.

The analysis regarding endometrial stroma has revealed two important aspects to be discussed:

- the increase of the fibroblast-fibrocytic proliferation in the stroma and the reduction of fibrosis in all treated cases, indirectly the maintain of the stromal volume (an important criteria when discussing about endometrial intraepithelial neoplasia);
- the increase of the granulocyte inflammatory reaction is like a pseudodecidualisation is not a sign for endometritis, which need to discover the presence of periglandular plasma cells and leukocytes. In the natural menstrual cycle the presence in the stroma of these leukocytes infiltration is normally in the premenstrual phase, when starts the decline of estrogen and progesterone.

TABLE 7 - ENDOMETRIAL HISTOLOGICAL CHANGES ON ORAL COMBINED CONTRACEPTIVES (ADAPTED FROM DELIGDISH L, 2000).

Common contraceptives	High potency contraceptives
Arrest of glandular proliferation	Glandular atrophy
Abortive secretion	Stromal hyperplasia
Low gland/stroma proportion	Pseudosarcoma
Stromal edema	Vascular thickening
Decidua, granulocytes	Stromal leiomyomas
Thin blood vessels, venous sinusoids	Decidua
Atrophy	

We consider that the histological records in the Romanian study at 12 and 24 months of Qlaira® in perimenopausal and early postmenopausal women are different from literature records; one explanation is that the examination is done after a longer period of administration, not in the first cycles after first administration, and another one is specific hormonal structure of each pill of the product (estrogen step down- progestogen step up regimen).

At Authors knowledge, there is only one multicenter European study that assessed endometrial histology and safety during 20 cycles of treatment on the four phase contraceptive- E2V/DNG in healthy women of 18 to 50 years, with BMI ≤30 kg/m^2, (19), and no study for endometrial effects and safety in elder women, in peri- or early postmenopause. The open-label, non-comparative multicenter study of the four phase contraceptive- E2V/DNG assessing endometrial safety recorded similar changes in the endometrium into a secretory/inactive or atrophic status, and no deleterious effects on histology.

Conclusions

- The four phase oral contraceptive E2V/DNG regimen (Qlaira® estrogen step-down, progestogen step-up) is endometrial safe for 24 months: no hyperplasia, no carcinoma registration.
- Atrophy is recorded predominantly after E2V/DNG regimen *vs* non-oral sequential hormone therapy – transdermal 17 β oestradiol plus VMP: P< 0.01 (40 *vs* 15% at 12 months, 90 *vs* 15% at 24 months).
- Secretory changes are more frequent after non-oral sequential hormone therapy – transdermal oestradiol plus vaginal micronized progesterone: P< 0.01 (80 *vs* 60% at 12 months, 45 *vs* 10% at 24 months).
- Proliferative endometrium is recorded on E2V/DNG regimen (Qlaira®) only at 12 months *vs* non oral pHT - P < 0.01 (10 *vs* 5%) and no case at 24 months.
- Endometrial stromal transformations are associated to both types of therapy changes are protective against endometrial intraepithelial neoplasia, and different to normal endometrium.
- Four phase contraceptive E2V/DNG regimen (Qlaira ®) and non- oral sequential hormone therapy induce similar endometrial aspects, but not identic to those of reproductive age.

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The predictive value of endometrial thickness in the diagnosis of endometrial cancer in postmenopausal women

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Background

Postmenopausal uterine bleeding is one of the most common complaints in gynecologic clinic. Postmenopausal bleeding is defined for practical purposes as vaginal bleeding occurring after twelve months of amenorrhea, in a woman of the age where the menopause can be expected (1).
There is a controversy about the best diagnostic method for evaluating postmenopausal uterine bleeding. Measurement of the endometrial thickness could be an alternative diagnostic approach. Thickening of the endometrium may indicate the presence of pathology. In general, the thicker the endometrium, the higher the likelihood of important pathology i.e. endometrial cancer being present. Transvaginal sonography (TVS) gained popularity for evaluating postmenopausal bleeding in the early 1990s due to its ready office availability and its value in ruling out significant endometrial disease. Initial studies indicated that an endometrial thickness >5 mm would identify 96% of endometrial cancers (2).
The majority of patients with postmenopausal vaginal bleeding experience bleeding secondary to atrophic changes of the vagina or endometrium. The clinical approach to postmenopausal bleeding requires prompt and efficient evaluation to exclude or diagnose carcinoma.

Objective

The objective of the present study was to evaluate the usefulness of trans-vaginal ultrasound in the assessment of the endometrial thickness in patients with postmenopausal uterine bleeding in relation to the histological results of the endometrial biopsy.

Methodology

A random selection from the medical file registration system was made. This is a retrospective study. A total of 243 medical records from January till December 2014 with the diagnosis of postmenopausal bleeding were studied. All the patients had performed the transvaginal ultrasound to measure the endometrial thickness. Endometrial thickness is measured as the maximum anterior-posterior thickness of the endometrial echo on a long-axis transvaginal view of the uterus (3). The histological results of endometrial biopsy were analyzed in relation with endometrial thickness.

Statistical analysis

Continuous data are reported as median, range and 2.5^{th} and 97.5^{th} percentiles and the chi-square test was used for univariate analysis. $P < 0.05$ was considered statistically significant.
Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) were calculated.
Statistical analysis was performed using the SPSS software package.

Results

A total of 243 postmenopausal women aged from 45-74 years with uterine bleeding were included in the analysis.
The endometrial thickness measured with transvaginal ultrasound ranged from 6.2 mm to 18.4 mm. The endometrial cancer was diagnosed in 11,3% of cases. The clinical endometrial carcinoma rate was significantly lower in cases with endometrial thickness below 6 mm. In addition, no endometrial carcinoma was observed in the patients with endometrial thickness 6 mm.
The endometrial thickness in the women with the diagnosis of endometrial atrophy was 3,1+/-1,7 mm. The endometrial thickness has a negative predictive value 96% (Table 1).
The clinical characteristics of the patients with postmenopausal bleeding like age, parity, episodes of bleeding and diseases like hypertension, diabetes and breast cancer, are shown below in Table 2.
The patients are divided into two groups depending on the diagnosis of endometrial cancer results of the biopsy.
The prevalence of endometrial cancer was higher in older women (P <0.0001a). There was no significance between groups depending on parity. The cases with endometrial cancer was higher when the

TABLE 1 - THE VALUES OF ENDOMETRIAL THICKNESS FOR PATIENTS WITH ENDOMETRIAL CARCINOMA AND ENDOMETRIAL ATROPHY.

	Endometrial thickness for patients with endometrial carcinoma	Endometrial thickness for patients with endometrial atrophy
N=*243*		
	18, 4 ± 6,2 mm	3, 1 ± 1,7mm
	P value <0.0001[a]	

TABLE 2 - UNIVARIATE ANALYSIS COMPARING CLINICAL VARIABLES AND ENDOMETRIAL ASSESSMENT BETWEEN WOMEN WITH (28) OR WITHOUT (215) ENDOMETRIAL CANCER.

Variables	**Women with endometrial cancer (%)**	**Women without endometrial cancer (%)**	**P value**
Age (years)*	28 (66-71)	215 (55-65)	<0.0001[a]
Parity			0.22[b]
Nulligravid	5 (16.6)	52 (23.9)	
Parous	23 (83.4)	163 (76.1)	
Vaginal bleeding			<0.0001[b]
Single episode	9 (33.3)	135 (63.0)	
Recurrent episode	19 (66.7)	80 (37.0)	
Hypertension			<0.0001[b]
Yes	19 (66.7)	21 (37.6)	
No	9 (33.3)	134 (62.4)	
Diabetes			0.88[b]
Yes	4 (16.6)	23 (15.2)	
No	24 (83.4)	132 (84.8)	
Breast cancer			0.097[c]
Yes	0 (0)	7 (4.3)	
No	28 (100)	148 (95.7)	
Endometrial echogenicity			<0.0001[b]
Uniform	0 (0)	56 (36.2)	
Non uniform	28 (100)	99 (63.8)	

TABLE 3 - DIAGNOSTIC PERFORMANCE WITH REGARD TO ENDOMETRIAL THICKNESS.

ET cut-off (mm)	Sensitivity (%)	Specificity (%)	PPV(%)	NPV (%)
6.0	99	100	100	96
7.0	35	98	20	88
8.0	35	90	45	87
9.0	35	87	35	88
10.0	35	85	31	96
11.0	71	89	83	88
12.0	35	86	33	96
13.0	71	90	90	88
14.0	53	91	55	94
15.0	71	91	10	88
16.0	10	92	15	84
17.0	17	89	35	64
18.0	32	96	87	91

episodes of bleeding were recurrent (P <0.0001b). There was a significant high percentage of cancer among the women with hypertension, (P <0.0001b); whereas no significance was seen for diabetes and breast cancer.

Also endometrial echogenicity has a significant difference; in all cases with endometrial cancer the endometrial echogenicity was not uniform.

The Table 3 shows the diagnostic performance of endometrial thickness from 6-18 mm in predicting endometrial cancer studied on 243 cases. The maximum of NPV is 96% and max of specificity is 100% with the endometrial thickness 6 mm.

Discussion

Endometrial cancer is the commonest gynaecological cancer mostly affecting women in the postmenopausal age. Approximately 3 out of 4 cases are diagnosed in women aged 55 years and older. Ninety percent of postmenopausal patients with endometrial cancer present with vaginal bleeding (4). In our study a total of 243 postmenopausal women aged range from 45-74 years with uterine bleeding were included in the analysis.

The study shows the presence of endometrial cancer in 11.3% of cases we found endometrial cancer when the endometrial thickness was from 18 to 6 mm. In addition no endometrial carcinoma was observed in the patients with endometrial thickness less than 6 mm.

It is likely that endometrial thickness assessment, along with further predictive factors, could provide better results in the prediction of intrauterine malignancy among high-risk women.

Different studies evaluated the merit of even thinner thresholds of endometrial thickness for further evaluation. Gupta et al. determined that the probability of endometrial cancer was reduced the thinner the endometrium on initial evaluation: 5 mm = 2.3%; 4 mm = 1.2%; and 3 mm = 0.4% (5). Timmermans et al. also found that decreasing the threshold endometrial thickness improved cancer detection, with sensitivities of 90% at 5 mm, 95% at 4 mm, and 98% at 3 mm (6). They recommended decreasing the cut off for excluding endometrial cancer to 3 mm. Clearly, decreasing the thickness that prompts further endometrial evaluation increases the sensitivity but decreases the specificity of TVS. Most studies recognize that a threshold endometrial thickness >4 mm adequately screens for >98% of endometrial cancers.

In the present study the cut off of endometrial cancer were 6 mm, the sensitivity were 99%, NPV were 96%.

Conclusion

The criterion standard of practice for gynecologists is to measure the endometrium thickness with ultrasonography in women presenting postmenopausal bleeding.

The endometrial thickness by trans-vaginal ultrasound can predict the outcome of biopsy in cases of postmenopausal bleeding. Imaging, especially ultrasonography, plays a key role in screening and diagnostic triage.

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Surgical management of adnexal torsion in children and adolescents: update and review of the literature

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This article aims to systematically illustrate the most relevant approach to follow in cases of suspected adnexal torsion in adolescents. We based our evaluation of the role of clinical features, laboratory tests and imaging on recent literature (2013-2015), till we consider the most appropriate surgical treatment which has been reached during our experience. Particularly, this study proves – through the literature review and the evaluation of a record of cases – that conservative treatment is to date the most highly recommended surgical approach for adnexal torsion. To assess the state of ovarian tissue and proceed with the most adequate surgical approach, an intraoperative frozen section analysis may be helpful in selected cases. Recent experimental studies have focused on the consequences of detorsion, which may cause ischemia-reperfusion damage and have speculated about the role of different drugs for its avoidance.

Introduction

Adnexal torsion (AT) is a surgical emergency defined as total or partial rotation of ovary, the fallopian tube or both around its vascular axis causing impairment of blood flow to adnexa (1). Its incidence in females under 20 is estimated at 4.9/100 000, with a mean age of 12 years (2). During AT compression of ovarian vessels occurs. If left untreated, the ovary becomes ischemic and then necrotic and complications (pelvic thrombophlebitis, hemorrhage, infection, and peritonitis) may occur (3, 4), including calcifications and auto-amputation of the ovary (2, 5, 6).

Up to 25% of pediatric patients with AT presents normal ovaries without malformations (7-9) but AT may also occur due to adnexal pathology such as ovarian cysts and tumors (cystic teratomas, follicular or hemorrhagic cysts, paraovarian cysts, cystadenomas or hydrosalpinx) (7, 10).

A benign mass with a size of 5 cm or greater increases the risk of AT (3) as well as ovarian hyperstimulation, polycistyc ovarian syndrome, endometriosis, adhesions, infections and congenital anomalies (9, 11-13). Preoperative diagnosis is difficult due to vague clinical presentation and aspecific imaging findings of AT; the final diagnosis is often made during exploratory surgery (14).

The best way to manage AT is early diagnosis and immediate surgery. There are conservative and radical options for AT treatment: conservative treatment consists of only detorsion or detorsion associated with subsequent cistectomy or tumorectomy; radical treatment of oophorectomy.

It is not always easy to determine whether patients should be treated with detorsion or oophorectomy (15). To date, conservative treatment is the most recommended surgical approach for AT in order to increase the future reproductive potential of these patients.

Materials and methods

This study is based on a literature review carried out between 2013-2015, in order to present the

most up-to-date approach to diagnosis and treatment of AT. In addition to diagnostic and therapeutic evaluation, we pay attention to the outcome and the future prospects about the surgical treatment.
Moreover, we consider all pediatric cases with AT surgically treated from 2001 to 2015 at our institutions.
Case history, clinical picture and instrumental data were examined in each patient. Surgical treatment was achieved through a radical or conservative approach, according to the type of pathology. Every child subjected to the conservative treatment was evaluated by a follow-up program.

Results

The literature review shows how to date the most adequate surgical treatment for AT is the conservative approach: the reserves described in the past about the risk of maintaining a torsed ovary or tube *in situ*, have been overcome by scientific evidence (2, 7, 8, 16-26).
In the space of 15 years, 36 children with AT were surgically treated (out of a total of 156 with ovarian disorders, Table 1). Torsion occurred in 23.1% of ovarian disorders. Mean age was 12.2 years and abdominal pain was the main symptom referred.
In 18 children of 36 (50%) a laparotomy surgery was performed; the remaining 18 (50%) underwent a laparoscopic surgery: from 2011 the latter has been applied in all children with torsion.
Among the 36 cases, 20 (55.5%) were non neoplastic lesions, 6 (16.7%) were benign lesions, 0 were malignant lesions and 10 were normal ovaries (Table 1).
In total we observe: 47.2% of patients (17 out of 36) were treated with conservative treatment; 52.8% (19 out of 36) with radical treatment. In each patient treated with conservative treatment, recovery of ovarian function was described. A qualitative analysis shows an increased trend for the conservative approach from 28 to 45%, as previously observed (21).
In conclusion (Figure 1) :

- non neoplastic lesions (TOT 20): 9 (45%) were treated with conservative approach, 11 (55%) with radical approach;
- benign lesions (TOT 6): 1 (16.7%) was treated with conservative approach; 5 (83.3%) with radical approach;
- malignant lesions: 0;
- normal ovaries (TOT 10): 7 (70%) were treated with conservative approach, 3 (30%) with radical approach.

Table 1 - HISTOPATHOLOGICAL DESCRIPTION AND EVOLUTION OF TOTAL OVARIAN MASSES.

HYSTOPATHOLOGICAL FEATURE	TOT MASSES	NO TORSION	TORSION
Non Neoplastic Lesions	78 (50%)	58 (48.3%)	20 (55.5%)
Follicolar Cysts	*43*	*35*	*8*
Hemorrhagic Cysts	*35*	*22*	*12*
Benign Lesions	63 (40.4%)	57 (47.5%)	6 (16.7%)
Mature Cystic Teratoma	*37*	*32*	*5*
Serous Cystadenoma	*10*	*9*	*1*
Mucinous Cystadenoma	*8*	*8*	
Ovarian Fibroma	*5*	*5*	
Serous Cystadenofibroma	*2*	*2*	
Malignant Lesions	5 (3.2%)	5 (4.2%)	0
Yolk Sac Tumor	*2*	*2*	
Fibrosarcoma	*2*	*2*	
Poliembrioma	*1*	*1*	
Normal Ovaries	10 (6.4%)		10 (27.8%)
TOT	**156**	**120**	**36**

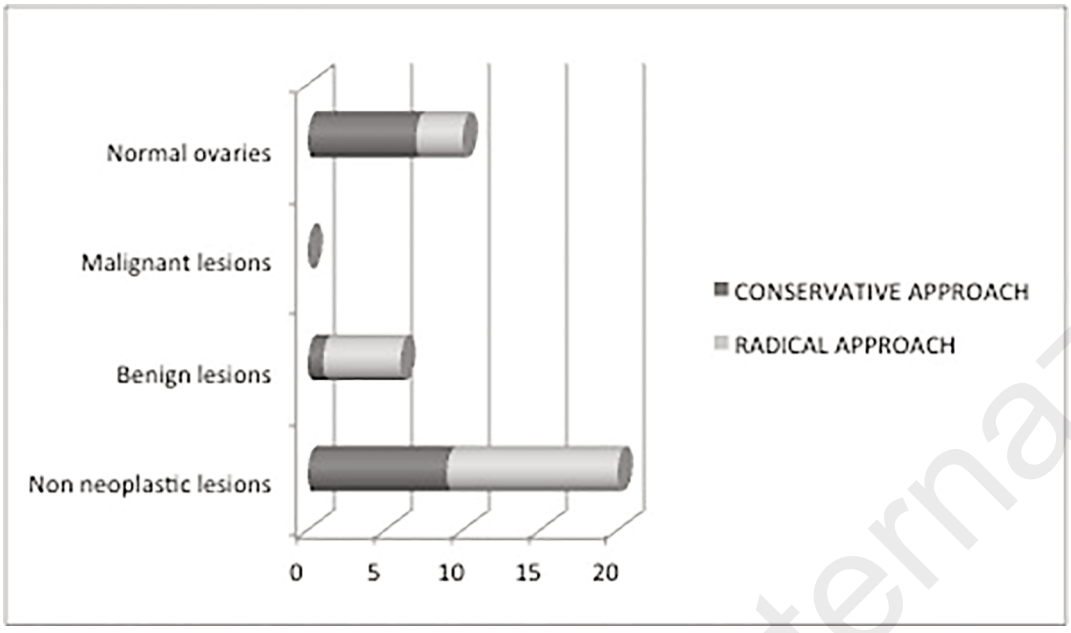

Figure 1 - Relationship between type of lesion and surgical approach.

Discussion

AT is reported to be the 5° most common gynecologic emergency encountered, with a prevalence of 2.7% (5, 16, 17, 27). The most common symptom in women with AT is acute onset of pelvic or abdominal pain, usually isolated to one side (15), which may be variable (11). It can be associated with nausea, vomiting, flank pain, anorexia, vaginal bleeding, and bowel or bladder abnormalities (2, 4, 28). AT must be differentiated from other causes of acute abdomen (3, 7, 15). Examination findings are often nonspecific (2, 29). A pregnancy test, a complete blood cell count and electrolyte values are usually determined: a slight leukocytosis may be observed (15) and occasionally a sterile pyuria is described (29). High serum levels of tumor markers such as CA125 and AFP may be correlated with AT and they return to normal levels after surgical removal of the mass (16, 30).

Pelvic ultrasonography (US) is the imaging study most commonly used to help diagnose AT (3, 15, 18). Asymmetric ovarian enlargement associated with an underlying mass, is the most common finding associated with AT (3, 4, 7, 15). Other common findings include free pelvic fluid, edematous and heterogeneous appearance of the ovary, uterine deviation towards the side of torsion and uniform peripheral follicles as they are pushed to the periphery by the ovarian stromal edema (31). A torsed fallopian tube can appear dilated and edematous; the "beak sign" can be described (32). Twisting of the vascular pedicle can produce a specific feature, the "whirlpool sign"(4, 33). The absence of vascular flow is highly suspicious for torsion but it does not occur always (3, 15). CT and MRI findings are similar to those reported for US (3, 4, 15, 34).

The best way to manage AT is early diagnosis and immediate surgery (7, 16). Laparoscopy is considered the best diagnostic and therapeutic approach, especially for the pediatric population (27, 35, 36). Conservative treatment is the most recommended surgical approach for AT (2, 7, 16-18, 20-22, 26). In the past the risk of missing an underlying malignancy, thromboembolism after detorsion and a belief that a grossly black hemorrhagic adnexa is irreversibly damaged were arguments in favor of oophorectomy (17, 20, 22, 29). Evidence proves that these risks are overestimated (7, 16, 17). The presence of edema, inflammation, congestion and ischemia leads to enlargement of the ovary and an aspect called black-bluish. This may confuse decision making intraoperatively, resulting in a more inopportune use of oophorectomy (21, 26). It has been demonstrated that a black-bluish ovary, which does not change its color during surgery, is no evidence of necrosis, and recovery is still likely to oc-

cur (15, 17, 21, 26). Most ovaries demonstrate normal follicular development on US and normal Doppler flow after only 6 weeks (29). If unfortunately the ovary appears atrophic on US follow-up, a second look is necessary (21, 26).

In view of that, it may be useful perform an intraoperative frozen section analysis in selected cases to ensure that the viability of ovarian tissue is preserved and to exclude the presence of necrosis (20, 21, 26, 37): if the ovary is ischemic but not necrotic, a conservative treatment consisting in untwisting of the adnexa is more adequate, but, if the ovary is proved to be necrotic, its removal becomes appropriate. In case of benign neoplasms and cystic lesions, a detorsion associated with synchronous or subsequent tumorectomy or cystectomy can be performed. When a suspected malignancy is confirmed by biopsy, an oophorectomy is required (7, 15, 16, 21, 22, 26, 29, 35, 38). As the salvage of ovary is evaluated a safe choice in case of torsion, similarly the salvage of a torsed tube can be recommended (13). Oophoropexy is a surgical technique aimed at limiting ovarian mobility and preventing further AT: its role is still debated (2, 8, 27).

AT produces ischemic damage (39). The treatment goal is to restore blood flow and improve tissue perfusion in ischemia through detorsion. During reperfusion, tissue damage can be more severe than during ischemia because of oxygen-derived radicals (ROS) which accumulate after detorsion (40). This process is known as "ischemia-reperfusion injury" (I/R injury) and it can determine impairment at systemic and tissue levels (41). Assuming that detorsion can provoke injury and alone is insufficient to protect ovarian reserve since the follicle count decreases [Ozler et al. (41)], several agents on rat ovaries have been evaluated to determine whether they can prevent I/R injury (39, 42-56). Gradual detorsion can also have a protective role (40). Yucel et al. (57) and Bozdag et al. (58) oppose this vision affirming that follicle reserve is not reduced after detorsion and it is not influenced by duration and intensity of ovarian damage.

The late effects of radical and conservative surgical approaches on fertility remain uncertain. Zhai et al. (59) suggest that loss of a single ovary does not adversely affect gonadal function, nor does it appear to compromise future fertility. Nevertheless, the Authors recommend a conservative approach when possible. This concern is partially shared by Bellati et al. (60). On the other hand, the concept that resection of the unilateral affected adnexa as radical treatment can have a negative impact on fertility persists (17). Many studies affirm that conservative laparoscopic surgery is the best surgical approach for preserving ovarian function permitting normal development of puberty and maximize the future reproductive potential of women of reproductive age with AT (7, 8, 16, 17, 22, 26, 29).

Conclusions

AT should be considered in female adolescents with acute lower abdominal pain since it can present as an emergency condition. Diagnosis is difficult since clinical features and imaging are nonspecific and a definitive diagnosis is made only during surgery. Surgical treatment should be conservative in order to increase the future reproductive potential of these patients. This approach is suggested by literature and is imposing itself in surgical reality. It may be useful to perform an intraoperative frozen section analysis in selected cases to assess the viability of ovarian tissue and potentially avoid a more aggressive approach. At present, experimental studies investigate the role various substances may play in preventing I/R injury and improving the outcome of these patients.

Disclosure

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Evidence for fetal neuroprotection by estrogens and progesterone

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The fetus is supplied from the placenta with estradiol (E2) and progesterone (P) in increasing amounts during gestation. After delivery of a premature infant, placental supply of both hormones is disrupted resulting in a rapid (up to 100-fold) decrease in E2 and P. Nothing is known about the consequences of the deprivation of E2 and P at this early developmental stage. However, we know the problems of extremely preterm infants, especially their impaired neurodevelopment. Many *in vitro* and *in vivo* studies show that E2 and P is neuroprotective and neurotrophic. Synaptogenesis, astrocytogenesis and oligodendrogenesis and also axonal and dendritic growth are ongoing processes during fetal neurodevelopment and can be triggered by E2 and P. First clinical data suggest that postnatal replacement of E2 and P in extremely preterm infants in order to maintain plasma levels as expected to occur in utero improves neurological outcome. This is a strong reverse argument that E2 and P in utero is neuroprotective.

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Polycystic ovary syndrome - another risk factor for gallstone disease

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Introduction

The interconnection between female gender and gallstones is well known. Women of childbearing age are 4 times more likely to suffer from this disease than men. The leading role in the pathogenesis of gallstone formation, in most cases, is played by two key mechanisms: changes in the chemical composition of bile with cholesterol super-saturation and the violation of the motor-evacuation function with onset of stagnation of bile in the gallbladder. Gender-associated factors affecting the implementation of these two mechanisms in women of childbearing age are the sex hormones: oestrogen and progesterone. In this regard, it is not surprising that the incidence of gallstone disease is directly correlated with the frequency of pregnancies and births, when the concentration of female sex hormones is maximised.

However, regardless of sex and childbearing history, other factors have now acquired greater importance in the prevalence of gallstone disease, which has been rapidly increasing all over the world, turning it into one of the "diseases of modern society". Regardless of gender, the risk of gallstone disease, in addition to heredity and age, includes a whole group of factors related to the peculiarities of lifestyle and nutrition (irregular meals; high-fat and high-carbohydrate diet with a deficit of dietary fibre; lack of exercise). Also a rapid decline in body weight and metabolic disorders (obesity, insulin resistance, type 2 diabetes, disorders of lipid metabolism) play a role in gallstone disease (1). These metabolic disorders are largely the result of lifestyle changes, and "Western" diet in developed countries too.

However, these metabolic disorders are often not only isolated primary pathology, but may be part of complex neuro-metabolic endocrine syndromes, one of which is polycystic ovary syndrome (PCOS).

Polycystic ovary syndrome - a complex of metabolic and endocrine disorders

PCOS is the most common endocrine disorder of the female reproductive system and is one of the most significant causes in the structure of endocrine-related infertility. PCOS is detected, depending on the diagnostic criteria used, in 4-25% of women of reproductive age (2). The criteria used in identifying PCOS are the presence of 2 of the following 3 signs: clinical or laboratory evidence of hyperandrogenism, ovulatory dysfunction (oligo- or anovulation) and polycystic ovaries, revealed on ultrasound examination.

Currently, PCOS is not seen as solely reproductive and endocrine pathology, but as an interdisciplinary problem in connection with the involvement in the pathogenesis metabolic disorders. Associated with PCOS, insulin resistance, atherogenic dyslipidemia, and often obesity are the basis of the formation of higher risk and an earlier manifestation, than in the general population, of type 2 diabetes mellitus and cardiovascular disease. Almost 40% of women with PCOS revealed complex metabolic disturbances

corresponding to metabolic syndrome (3). Insulin resistance is detected in approximately 70% of patients with PCOS and 10% of this pathology is combined with type 2 diabetes mellitus (4). It is noteworthy that, in nearly half of the cases, the presence of insulin resistance in PCOS is not dependent on body weight. In some cases, patients with PCOS in the early stages of the disease might not have carbohydrate metabolism disorders. However, within 3 years, 25% of them develop insulin resistance (5). In addition, the characteristic of PCOS hyperandrogenism further affects the metabolism of a variety of settings. Higher-than-normal levels of androgens are highly correlated with insulin resistance (6). It has been shown inverse effect of the insulin resistance at the increasing in the activity of androgens in PCOS. The presence of hyperinsulinemia decreases binding globulin sex steroids synthesis in the liver, which leads to an increase in the plasma concentration of free, active testosterone (7). Moreover, the risks of developing the metabolic syndrome and type 2 diabetes mellitus in women correlate directly with increased testosterone concentrations (8, 9).

Lipid disorders that often accompany PCOS are characterised by elevated total cholesterol and its atherogenic fractions – low-density lipoprotein (LDL) cholesterol, decline anti-atherogenic fraction – high-density lipoprotein (HDL) cholesterol and an increase in triglycerides. Dyslipidemia in PCOS may be a consequence of obesity, and is associated with insulin resistance (10). Less clear is the impact of androgen excess in PCOS on lipid metabolism. Testosterone reduces atherogenic lipoprotein-A, whereas Dehydroepiandrosterone (DHEA) increases insulin sensitivity (10). However, heterogeneity of the metabolic phenotype was shown in PCOS patients depending on the severity of hyperandrogenism. High androgen levels correlated with lipid disorders and impaired insulin sensitivity (7).

Thus, in the pathogenesis of PCOS is integrated a complex set of disturbances of endocrine status and metabolic disorders, which are the basis of predisposing a number of diseases that are interrelated and aggravated.

Polycystic ovary syndrome and cholelithiasis - crossing pathogenetic pathways

As already mentioned, the described disturbances of carbohydrate and fat metabolism, are also considered to increase the risk of cholelithiasis. In recent studies the relationship of hyperinsulinemia with the formation of cholesterol gallstones, diabetes and obesity was clearly demonstrated (12, 13). The component of hyperinsulinemia in the pathogenesis of the gallstone was composed of the impact on the cholesterol metabolism and the effects on the motor-evacuation function of the gallbladder. The presence of hyperinsulinemia increased activity of 3-hydroxy-3-methylglutaryl-CoA-reductase (HMG-CoA-reductase), a central enzyme in cholesterol biosynthesis, and, as a consequence, increases the synthesis of cholesterol and LDL captured by the liver, resulting in increased secretion of cholesterol with bile and its concentration in the gallbladder (14). Similarly, in patients with diabetes exogenously administered insulin increases the degree of saturation of bile with cholesterol (15). The effect of high levels of insulin on the contractile function of the gallbladder was unexpected. Insulin resistance is associated with a dysfunction of the gallbladder, even in the absence of obesity or diabetes, which indicates a direct effect on its contractile activity (16). In the presence of hyperinsulinemia in patients with type 2 diabetes, the basal and stimulated by cholecystokinin motor activity of the gallbladder is impaired (17, 18).

Studies to determine the relationship between PCOS and cholelithiasis are not numerous. In addition to lipid disorders inherent in PCOS, insulin resistance leads, through a chain of biochemical events, to violation of the rheological properties of bile and contributes to the pathogenesis of stone formation.

Hyperinsulinemia in PCOS, as in other pathological conditions involving insulin resistance, disturbs motor activity of the gallbladder. Confirmation of the importance of this factor is the improvement of the contractile function of the gallbladder by the appointment of drugs which increase insulin sensitivity (metformin) in patients with PCOS and insulin resistance (19).

Hyperandrogenism in PCOS also contributes to the dysfunction of the gallbladder. According to experimental studies, testosterone and dihydrotestosterone inhibit the contractile activity of the gallbladder due to its regulation through nongenomic pathways. Presumably, androgens violate intake of calcium into the cell from the extracellular space and increase the secretion of intracellular calcium, leading to a significant reduction of its content in

the cell, which ultimately reduces the contractile ability of the gallbladder (20). In the study by S. Isik et al. testosterone levels in PCOS positively correlated with the fasting volume of the gallbladder and negatively with indicators of the bile ejection fraction from the gallbladder, 20 minutes after receiving breakfast, which shows a decline in the contractile function of the gallbladder under the influence of androgens (19).

Hormonal contraceptives - an additional risk factor for gallstone disease

Nowadays, the first line in the algorithm of treatment associated with PCOS menstrual disorders and the treatment of symptoms of androgen dermatopathy, hormonal contraceptives are to be used, as a rule, over a long period. The relationship of the use of hormonal application, and particularly of combined oral contraceptives, with the development of cholelithiasis (CL) was repeatedly confirmed in numerous studies. Both ethinyl estradiol and progestins, as part of the OC, contribute to the formation of gallstones.

The most common gallstones are those mainly composed of cholesterol. The pathogenesis of the stone formation consists of several stages, the foundation of which is a damage of the physico-chemical properties of bile with hypersaturation by cholesterol and a decrease in the contractile function of the gallbladder. The impaired motor-evacuation function of the gallbladder and a high concentration of cholesterol in gallbladder bile make the conditions for the formation and precipitation of cholesterol crystals and their future growth with the formation of stones. A synthetic oestrogen, ethinyl estradiol, similar to endogenous oestrogens, interacts with receptors in the liver and has a number of modulating effects on cholesterol metabolism. However, unlike natural oestrogen its effect is more pronounced due to the greater affinity to oestrogen receptors and longer circulation in the bloodstream. The result of the oestrogen action is an increase of cholesterol content in the gallbladder bile caused by an aggregation of effects: induction of expression of the HMG-CoA-reductase and increasing the cholesterol synthesis in the liver (21); increased expression of LDL receptors in the liver and enlarged capture of cholesterol-rich LDL (22); activation of lipolysis in adipose tissue release with increased VLDL and further transport it to the liver (23); increasing the absorption of cholesterol in the small intestine (24). Progestins, like natural progesterone, have a muscle relaxant effect on smooth muscle cells of the gallbladder wall, reduce contractile activity and ejection fraction in response to stimulation by cholecystokinin, leading to the development of bile stagnation (25, 26). Besides affecting the function of the gallbladder, progestins increase the activity of hepatic lipase, stimulate capture and splitting in the liver of the transport particles saturated with cholesterol (VLDL, LDL, chylomicrons remnant), and thus, increase cholesterol bile secretion (27, 28). It is believed that currently used low and micro-dose OC don't increase the risk of gallstone disease significantly (29). However, according to our data, in the presence of PCOS patients with risk factors for gallstone disease, the appointment of OC may be the trigger process of gallstone formation. The factors in the observed group, of 22 patients with PCOS, and who had ultrasound signs of CL, were: a violation of lipid metabolism in 16 (72.7%) cases; reducing motor function of the gallbladder - in 13 (59%) cases; hyperinsulinemia - in 17 (77.2%) cases; obesity or overweight - in 10 (45.4%) cases and a family history of cholelithiasis - in 7 (31.8%) cases. In the study group, 15 patients (68%) were appointed to OK for a long period. The average duration of OCs taking was 21 ± 3 months. In 17 observations of CL was identified in stage of biliary sludge (BS), in 5 patients - at the stage of biliary stones.

Thus, patients with PCOS are a group that requires alertness of clinicians to the possible development of CL, since the presence of their source of metabolic disorders and hormonal contraceptives provide a set of additional risk factors for cholelithiasis as the endogenous or iatrogenic origin. In addition, dynamic monitoring of the gallbladder on the background of long-term use of OCs in this group of patients help identify gall stones at an early stage of BS. Detection of CL in the first stage of BS (heterogeneous bile, "sediment", clots) is of great practical importance because of the reversibility of the disease at this stage in most cases.

To improve the rheological properties of bile and cholesterol solubilisation in it, as well as medical litholysis of cholesterol stones, is the widely used ursodeoxycholic acid (UDCA). The UDCA litholytic effect is provided by its hydrophilicity, and the ability to form liquid crystals in a supersaturated by

cholesterol bile, reduce cholesterol absorption in the intestine and inhibition of cholesterol biosynthesis in the liver.

In our study patients with BS were appointed therapy with UDCA and cholekinetics for three months. Two patients discontinued the treatment on their own after one month. The BS regression marked in all patients after 3 months of treatment. Patients who have relapse of BS in 3 months of treatment (4 patients) were appointed repeated and then supporting courses UDCA therapy with good effect. Thus it should be noted that the preservation of etiopathogenetic factors (OCs, dyslipidaemia, hyperinsulinemia, functional disorders of the gallbladder) may require a more prolonged use of litholytic therapy (more than one-three months) with further dynamic ultrasound control and, if necessary, repeated courses of treatment.

Conclusion

Therefore, patients suffering from PCOS are at risk of developing gallstones due to the high prevalence of their metabolic disorders associated with its pathogenesis. The use of OCs this category of women significantly increases the risk of gallstones and can be a trigger factor for stone formation.

In this regard, the prescription of OCs, in this group of women, doctors should actively identify and take into account the individual risk of CL, as well as obesity; disturbance of lipid metabolism; dysfunctional gallbladder motility disorders; insulin resistance and hereditary factors. This will individualise the prescription of OC, to conduct dynamic monitoring of the gallbladder, will help to promptly identify CL at an early pre-stone stage, and implement appropriate preventive therapy against further lithogenesis progression. The use of UDCA in patients with PCOS, including OC users, is effective in the treatment and prevention of BS progression in gallstone disease.

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Effect of nutrients on ovulation and oocytes quality in mice

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The reproductive process is sensitive to the nutritional state of the mother. Some studies have shown that the ovulation process and oocyte maturation may also be directly affected. There is growing consensus that egg quality declines with age and in conditions such as polycystic ovary syndrome (PCOS). The role of nutrition and genomic factors in these processes is becoming relevant. The critical processes underlying ovulation and oocyte quality such as energy status, oxidative stress, inflammatory status and the mothers hormonal cycle may all be affected. Indeed this would represent the changes in the ovarian environment which would eventually affect the quality of the ovum. Here we show that specific nutrients like zinc, copper, iron, L-carnitine and acetyl-L-carnitine have a role in the quantity and quality of oocytes obtained from superovulated mice, and the evidence that carnitines have an important effect on embryo development.

Introduction

Recent studies are showing that the actual ovulation process and the oocyte maturation are also sensitive to the nutrient status of the mother and may also be directly affected by any deficiency in the diet of minerals, vitamins and nutrients. Around 10 to 15% of couples have trouble getting pregnant or getting to a successful delivery, in the USA according to the Centers for Disease Control and Prevention (CDC). Women who experience problems achieving a recognized conception also have elevated rates of early unrecognized pregnancy loss (1) and elevated rates of clinical spontaneous abortion (2). There is growing consensus that egg quality declines with age. This is part could be connected to the accumulation of cellular damage with time, a reduction in cellular metabolic processes, which in turn leads to a reduction in energy production and in increase in cellular oxidative stress, which in turn can trigger abnormal physiological processes especially immune and inflammation processes. Thus the role of mitochondriotropic compounds that can affect energy levels as well as antioxidant status in nutrition, together with genomic and other factors is becoming important in all process including female fertility and successful pregnancy (3-6). In particular L-carnitine (Figure 1), which is essential in fatty acid metabolism, has been shown to prevent mitochondrial damage induced in the rat choroid plexus by medium chain fatty acids (7) or by mitochondrial toxins (8, 9).

Methods

Female 8 weeks old CD1 mice were divided into four groups of eight mice each (one control group and three treated groups). The animals were treated daily for 3 weeks by intragastric gavage as follows. *Group 1 Control* - gavage treatment only with vehicle; *Group 2 Carnitines* - (L-carnitine 0.4 mg and acetyl-L-carnitine 0.12 mg per mouse); *Group 3 Microelements* - (zinc: 4 ng, copper: 0.8 ng, iron: 7 ng per mouse)*; Group 4 Microelements plus Car-*

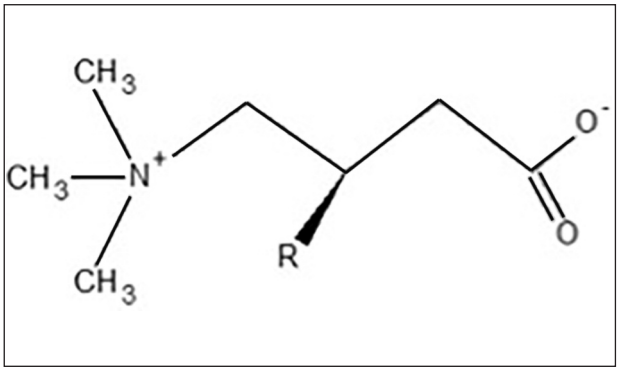

Figure 1 - Structure of L-carnitine.

nitines - (mice were treated with compounds from both group 2 and 3).

At the end of the treatment superovulation was induced, mice were sacrificed and oocytes collected to assess quantity and quality. To evaluate the preimplantation embryos development, *in vitro* fertilization (IVF) experiments were performed and the reaching of every single stage was monitored.

Results

Results showed that the mean number of oocytes was significantly higher in the group 2 (carnitines) and in group 4 (microelements plus carnitines): 32.45 and 31.28 respectively, *versus* control group: 24.50. The number of oocytes in group 3 (microelements) was not significantly affected (27.17).

The measurement if the numbers of good *versus* the degraded oocytes showed that the number of degraded oocytes in group 2 (Carnitines) and in group 4 (Microelements plus Carnitines was positively regulated: 29.10% and 19.30%, *versus* 34.28% (control group) (Figure 2).

This is an important result that showed a significant effect of microelements and carnitines to increase oocytes number and also to maintain their good quality. It is interesting to note, that in the microelements only (Group 3) a higher percentage of degraded oocytes (35.25%) was observed and this was markedly decreased by the addition of the carnitines (19.30%), suggesting the positive effect of the combination of both classes of compounds. These observations suggest that the carnitine group seems to give some benefits in both genesis and ovulation process.

The IVF experiments performed to establish the magnitude of fertilized eggs and developed embryos showed that despite the treated groups had a minor percentage of 2-cells embryos *versus* control group, the major part of them reach the blastocyst stage (86 *versus* 60%).

Discussion

These results show evidence that nutrients have an important effect on ovum numbers and also on embryo development. Further experiments are ongoing to examine the expression of genes involved in the ovulation and fertilization processes (10).

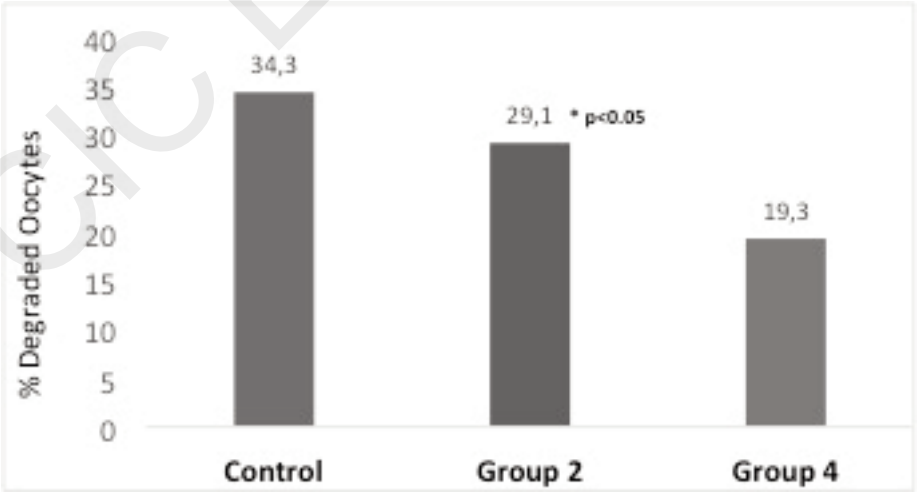

Figure 2 - CD1 mouse oocyte percentage degraded in control (untreated) *versus* group treated.

These studies also show the positive effect carnitines on increasing the quantity and quality of oocytes. Since carnitines are a significant carriers of fatty acid in the mitochondrial matrix, it should indicate the important role of mitochondria in fertility. Carnitine is a natural product synthesized in mammals from the essential amino acids lysine and methionine or obtained from dietary sources and it is necessary for cellular energy production. Dysfunctions in the carnitine system manifest in various diseases; these are related to abnormal mitochondrial function and thus reduced energy production and increased oxidative stress. Evidence is accumulating to support the control of the oxidative stress through metabolism. Studies suggest that carnitine reduces oocyte cytoskeletal damage and embryo apoptosis (11). Further it may stabilize the hypothalamic pituitary gonadal axis and protect tissue cells such as the ovary (8, 12).

Carnitine through the carnitine shuttle is a major partner in lipid beta oxidation. In the acquisition of oocyte competence the enzymes involved in lipid beta-oxidation are strongly expressed (13). Studies show a correlation between the levels of CPT2 expression and embryo developmental competence (14). Indeed the inhibition of beta-oxidation during oocyte maturation or zygote cleavage impairs subsequent blastocyst development. In contrast, L-carnitine supplementation during oocyte maturation significantly increases beta-oxidation and improves developmental competence (15).

Conclusion

A key factor in the process of conception is the whole physiological process leading to the production of the ovum. The mitochondria play a central role in cellular metabolism and mitochondrial dysfunction is strongly involved in various disease processes including infertility. The positive actions of nutrients and minerals are in part related to the classical and well known actions related to energy metabolism, but what is also important is that the right balance of that make us healthier do so in part because they induce the expression of health-promoting genes and reduce the expression of disease-promoting genes. By looking at gene expression and in particular mitochondrial DNA gene expression it is possible to understand the beginnings of a disease process and its mechanism.

The metabolic compounds, antioxidants and micronutrients may play an important role in fertility; further improved mitochondrial function could provide adequate energy for successful conception.

Targeting ovum, tubal and uterus function may provide new approaches to improve fertility diseases however further experimental studies are needed in this field.

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Safety and efficacy findings with multiple cycles of Ovaleap® (follitropin alfa) in assisted reproductive technology (ART): open-label follow-up to main phase 3 study

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Context

A phase 3 study demonstrated clinical bioequivalence of the recombinant human follicle-stimulating hormone (r-hFSH) Ovaleap® to the reference follitropin alfa, Gonal-f® (Gertz & Strowitzki. ESG 2013, abstract 920).

Objective

Assess safety and efficacy outcomes with a second and third treatment cycle of Ovaleap®.
Methods: open-label, uncontrolled, follow-up treatment period of a multinational, randomized, controlled, assessor-blind, phase 3 study.
Patients: infertile, downregulated women aged 18-37 years who did not become pregnant in the main study (first cycle) could be treated for a second or third cycle of ART using Ovaleap®.
Interventions: dosing of Ovaleap® and downregulation procedures were at investigator discretion.
Main outcome measures: primary outcomes were safety and nonimmunogenicity; secondary outcomes included total r-hFSH dose and number of oocytes retrieved.

Results

A total of 147 patients were included in Cycle 2 and 61 in Cycle 3. The overall frequency of TEAEs was low (10.9%, Cycle 2; 6.6%, Cycle 3; and 12.9%, Cycles 2/3 combined) and as expected. In Cycles 2/3 combined, overall frequencies of TEAEs were comparable in patients previously treated with Ovaleap® *vs* Gonal-f® in the main study (11.3 *vs* 14.9%, respectively). TEAEs considered possibly related to study drug were reported in 4 patients (2.7%). The total dose of Ovaleap® was 1998.2±771.0 IU for Cycle 2 and 2112.7±939.4 IU for Cycle 3. The number of oocytes retrieved per patient overall in Cycle 2 and 3 was 12.0±5.9 and 13.3±6.6, respectively. No anti-FSH antibodies were detected.

Conclusions

After a second or third treatment cycle of Ovaleap®, the safety profile was as expected. The results suggest that a course of up to 3 cycles of treatment with Ovaleap® is safe and well tolerated, regardless of previous treatment with Ovaleap® or Gonal-f®; however, further research is necessary.

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A randomized multi-center controlled study on the efficacy and safety of a new crosslinked hyaluronan gel to prevent intrauterine adhesion following hysteroscopic adhesiolysis

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Introduction

Intrauterine adhesion (IUA) is one of the common disorders in OB/GYN practice. Any procedures that result in endometrium damages could possibly lead to IUA. In a cohort study, Taskin et al. (1) find that IUA formation after resection was present in 31.3% patients with solitary fibroids and 45.5% with multiple myomas. Guida et al. (2) find that the incidence of IUA after hysteroscopic resection of myoma, polyps and septa were 33.3, 18.2 and 37.5%, respectively. As abortion procedures reached 40-50 million worldwide at 2012 (3) and association between IUA and abortion was demonstrated (4), the prevalence of IUA would likely increase. Effective and safe interventions to prevent IUA after intrauterine procedures are therefore in urgent need.
Hysteroscopic adhesiolysis is currently a widely-performed procedure to remove the adhesions, restore the shape of uterine cavity, and ideally the functionality of endometrium. The major concern for this procedure is postoperative adhesion reformation. It was reported that adhesion reformation occurs in about 60% of severe cases (5). Preventing adhesion reformation after adhesiolysis is essential for a successful adhesiolysis procedure.
It has been reported that hyaluronic acid (HA) molecules could modulate inflammatory processes, regulate secretion of cytokines by macrophages, and facilitate scar-free tissue repair. However, due to its fluid nature and rapid *in vivo* degradation nature HA was not demonstrated to achieve a satisfactory efficacy to prevent post-operative adhesion (6). Crosslinking modification is an effective way to enable that HA material has high viscosity and degrades slowly so that it would stay in the application site and cover tissue surface during the critical tissue healing processes to prevent adhesion (7, 8). MateRegen® Gel is a new crosslinked HA gel that was developed using a proprietary chemical modification to the non-animal sourced HA material.
The objectives of this study were to explore the efficacy and safety of MateRegen® Gel in reducing IUA after hysteroscopic adhesiolysis for patients with moderate to severe IUA.

Methods

This study was approved by the Institutional Review Board and Ethical Committee. In total, 120 patients diagnosed with moderate to severe IUA according to the AFS scoring system (Table 1) (9) and underwent hysteroscopic adhesiolysis for the first time were recruited. All patients were informed consent and signed the Informed Consent Forms. Patients who were allergic to hyaluronan or its derivatives, with infection or malignant tumor of reproductive organs and with systemic diseases that could cause coagulopathy were excluded.
All patients were first examined by an experienced physician under hysteroscopy; the severity of the IUA was scored. Sharp hysteroscopic adhesiolysis with blunt tipped scissors under continuous saline flow was then performed. Upon completion of the adhesiolysis, patients were randomly assigned into

TABLE 1 - THE AMERICAN FERTILITY SOCIETY SCORING SYSTEM FOR IUA (1988).

Extent of cavity involved	<1/3	1/3-2/3	>2/3
	1	2	4
Type of adhesions	Filmy	Filmy and dense	Dense
	1	2	4
Menopausal pattern	Normal	Hypomenorrhea	Amenorrhea
	0	2	4
Stage I	Mild	1-4	
Stage II	Moderate	5-8	
Stage III	Severe	9-12	

treatment group (N=60) or to the control group (N=60). For patients in the treatment group a Foley balloon catheter (Perak, Malaysia) was first placed into the uterine cavity. Through the balloon port, 3ml Normal saline was injected into the balloon; through the urine drainage port; 2 ml of MateRegen® Gel (BioRegen Biomedical Co., Ltd., Changzhou, China) was then injected into the uterine cavity. For the control group, only Foley balloon catheter was placed into the uterine; 5ml Normal saline was injected into the balloon. The Foley balloon catheter was removed at the 4th day postoperatively for all patients.

Patients were followed-up at 3 days, 1 month, and 3 months postoperatively. Second-look hysteroscopic examination was performed 3 month postoperatively at 3-7 days after the completion of menstruation. The physicians who performed the second-look hysteroscopy were not aware of the study group assignment of the patients. After the IUA was evaluated and scored according AFS scoring system and recorded. IUA, if any, was then further lysed as needed per the physician's discretion.

The primary endpoint was the percentile of patients with zero AFS total score (Zero-AFS score rate) in each group. Secondary endpoint was AFS total score and score for each subcategory. Percentage of patients with different stages in each group was calculated and compared. The safety was evaluated based on the frequency of complications and severe events.

Data was statistically analyzed with SAS9.13 software (SAS Institute Inc.). The quantitative data was compared with two-tailed *student- t* test, ANOVA, and rank test; qualitative data was analyzed with c^2 test. $P< 0.05$ was considered as statistically different.

Results

Among the 120 patients, 5 in the treatment group and 4 in the control group were dropped off due to reasons unrelated with testing materials and treatment methods. Therefore, 111 patients completed this study, 55 in treatment group and 56 in control group.

The demographic characteristics, medical history, and AFS scores at the baseline were not significantly different between two groups.

Zero-AFS score rate in the treatment group is significantly higher than the control group (38.2 *vs* 16.1%, P = 0.0078; Figure 1). Using MateRegen® Gel resulted in significantly lower total AFS score, scores for the extent of cavity involved and the menopausal pattern than the control group ($p< 0.05$) (Table 2). The treatment group had significantly lower proportion of patients with moderate to severe adhesive stages than the control group ($p <0.05$) (Figure 1).

No complications, adverse events, and SAE related to the material and treatment were observed for both groups.

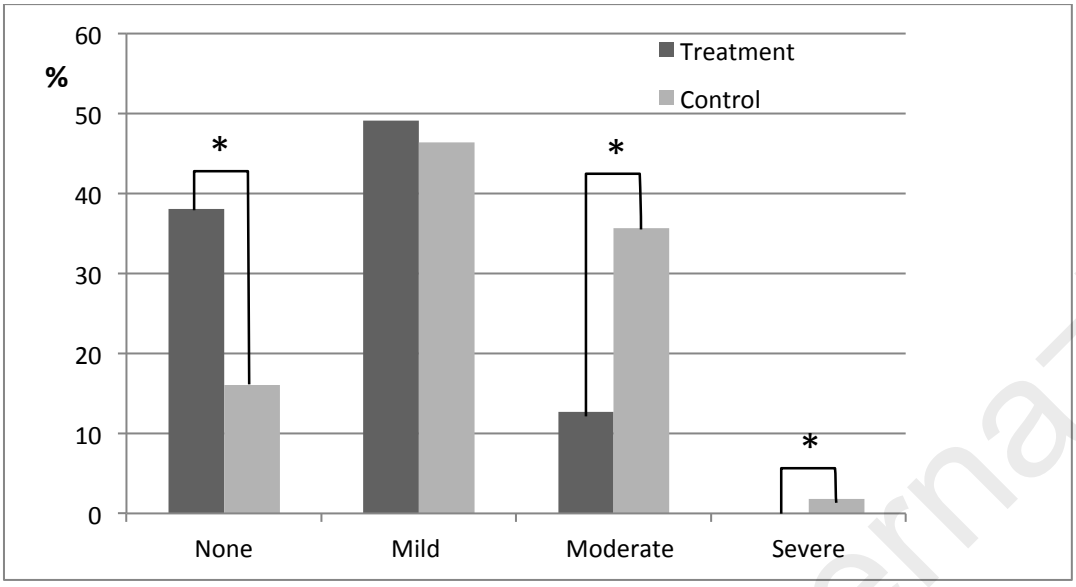

Figure 1 - Percentage of patients with different stages of IUA after treatment.
**** indicating significant differences between treatment and control group (p<0.05).***

TABLE 2 - THE SCORES OF IUA AT 3 MONTHS AFTER HYSTEROSCOPIC ADHESIOLYSIS (MEAN±SD).

Categories	Treatment group	Control group	*P* value
Extent of cavity involved	0.60 (0.74)	1.09 (1.08)	0.015
Type of adhesions	0.71 (0.85)	1.20 (1.26)	0.051
Menopausal pattern	0.80 (1.13)	1.39 (1.14)	0.005
Total score	2.11 (2.12)	3.68 (2.50)	0.001

Discussion

Only patients with moderate to severe IUA were enrolled in this study in order to evaluate the challenge situations dealt with during the clinical practice. Using of MateRegen® Gel significantly reduced reformation of IUA and its severity, and improved the endometrium function as shown by the normal menopausal pattern.

According to "Guidelines for treatment of IUAs" from AAGL (10), hysteroscopic adhesiolysis and use of anti-adhesion barrier are proposed to be the treatment choices. However, reformation of adhesion after adhesiolysis was observed in 60% of patients who initially had severe adhesion (2). Therefore, use of anti-adhesion barrier after adhesiolysis for those patients is obviously necessary. Formation of adhesion after surgery is associated with normal tissue healing processes where inflammatory reactions and re-vasculation of the repair tissue are physiological processes. HA has shown to modulate inflammatory reaction and reduce free-radicle production and scarring during tissue healing processes (11). However, nature HA material degrades too quickly *in vivo* and is not able to achieve the expected anti-adhesion efficacy.

Crosslink method was used to modify nature HA and improve its stability to prevent adhesions in abdominopelvic cavity and in uterine cavity. MateRegen® Gel is crosslinked hyaluronan that is from non-animal sources. The proprietary crosslinking technique improved the viscoelastic property meanwhile maintained the biological characteristics of natural HA molecules. This material is able to stay in the installation cavity for up to 14 days and cover the critical period of inflammatory phase during the wound healing (12). MateRegen® Gel could be also recommended for prophylactic application af-

ter intrauterine procedures that may cause injuries to endometrium.

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Prenatal screening and thyreopathies

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Risk of aneuploidy

Down syndrome (trisomy 21) is the most common significant aneuploidy. Prenatal screening has emphasized the detection of this disorder. However, it is recognized that screening tests (biochemistry and/or ultrasound) have a variable potential to detect other aneuploidies, some genetic disorders, specific foetal anatomic abnormalities, and pregnancy complications such as preeclampsia (1). Prior to undergoing prenatal screening, women should be given information on the screening process and be provided with an opportunity to discuss this with a health professional before making a personal decision to accept or decline screening (2).

Foetal aneuploidy risk is evaluated on the basis of a combination of maternal age, serum biochemical tests and foetal ultrasound markers. In the first trimester of pregnancy are measured biochemical markers-pregnancy-associated plasma protein A (PAPP-A) and free β hCG subunit and by ultrasound is examined foetal nuchal translucency (NT) (3). The combined test in the first trimester has detection rate about 80 and 3% false positive rate (4, 5). For women who only come into care after the first trimester, risk assessment testing should be made by biochemical tests in the second trimester of pregnancy (AFP, hCG, unconjugated estriol and inhibin). This second trimester test has higher false positive rate about 5% and detection rate is only 65% (6). There are also combinations of results- integrated test which use strategies incorporating measurements obtained from both of the trimesters. The efficacy of integrated test is the best of all of the screening types (more than 90%), false positive rate is about 2%. Additionally, the safety and cost efficiency is also the best (7). In those cases where ultrasonography is not available, the serum-integrated test is recommended. The risk is only calculated from the biochemical markers and the efficacy of this approach is common as the first-trimester combined screening (8). Definitive prenatal diagnosis of chromosome abnormalities can be detected through conventional chromosome analysis (karyotyping). The invasive prenatal diagnostic testing (amniocentesis or CVS procedures) carry some degree of risk for miscarriage or other pregnancy complications.

Recently, new non-invasive prenatal testing based on massively parallel sequencing of circulating free foetal DNA (cfDNA) in maternal plasma has been shown to be highly effective for trisomy 21, as well as other foetal aneuploidy detection (9). In this time is this technology commercially available. Although the detection rate of this test is very high (up to 98%), it reminds still only a screening test and positive results have to be confirm by karyotyping (10, 11).

Thyroid diseases in pregnancy

Undetected thyroid disease can have serious consequences during the course of a person's life. The sufficient level of thyroid hormones and normal thyroid gland function is necessary for physiologi-

cal reproduction, as well as being extremely important for pregnancy (12). Pregnant women with subclinical hypothyroidism seem to escape early clinical detection. The implications are staggering when one considers that there is a significant increase in intrauterine deaths, spontaneous abortions, premature births, and preeclampsia; additionally, poor development of the foetus, such as major malformations and loss of IQ. While the hyperfunction during pregnancy usually manifests itself by clinical symptoms, lowered function is much more dangerous due to its non-specific symptoms which can also accompany the physiological pregnancy (13). It has been clearly proven that even slight (subclinical) hypothyroidism can affect the neuropsychological development of the child (14). Up until the 12th to the 14th week, the embryo depends completely on the mother's thyroxine; and is even still partially dependent on it, afterwards. Even minor perturbations in foetal thyroid hormone status may have effects in terms of the neurodevelopmental outcome (15, 16).

The main laboratory parameters, for thyroid diagnosis are thyroid-stimulating hormone (TSH), free thyroxine (FT4), and anti-thyroid peroxidase antibody (TPO Ab). The suppression of TSH during pregnancy is well known; hCG produced by placenta has thyrotrophic activity. High levels of hCG in early pregnancy suppress TSH by approx. 18% (17). By using the standard reference range for serum TSH in the healthy population, one might misdiagnose as healthy those women who already have a slight elevation of TSH; conversely, one might suspect hyperthyroidism in normal women who have a lowered serum TSH value (18). FT4 is biologically active hormone not affected by the concentration of binding proteins (19), which are changed during pregnancy. Its concentration during pregnancy is partly affected both by the inflow of iodine and the duration of the pregnancy. The recommended dietary allowance for pregnancy is 220 - 250 μg/d (20).

Anti TPO antibodies are markers of an autoimmune process in the thyroid gland, and their determination is both diagnostic and prognostic important (21). The presence of TPOAb during pregnancy also alerts the medical professional to the risk of development of postpartum thyroiditis (22); therefore, it is necessary to follow-up on those women. TPOAb positivity may endanger not only the current, but also subsequent pregnancies.

Investigation thyroid disorders can be combined with basic examination in early pregnancy or blood sampling in the context of screening for aneuploidy in the first trimester of pregnancy.

Setting of pregnancy specific reference intervals for TSH is one of the basic requirements when implementing general examination of the thyroid gland in early pregnancy. Moreover, the use of different analytical systems could lead to a misdiagnosis, considering the differences among reference intervals for different analytical systems (23).

Our first study was performed between 2006 and 2008, and examined 7,530 consecutive asymptomatic pregnant women (between the 9th to the 11th week of pregnancy; 99% Caucasian) who were undergoing their first trimester prenatal screening for aneuploidy, at the same time they were having TSH, FT4 and TPOAb measured. The aim of this study was to evaluate the prevalence of thyroid disorders in pregnant Czech women, and to identify the optimal reference intervals in evaluations of maternal thyroid function during the first trimester of pregnancy (24). The analyses were performed on an automated immunoassay analyser ADVIA Centaur (Siemens). The average age in the group of pregnant women was 31.2 (+/- 4.3) years. Sufficient levels of iodine supplementation could be expected, as iodized salt has been in regular use in the Czech Republic since the 1950s (25).

We established the reference interval for TSH in pregnant women in the first trimester of pregnancy, such as the 2.5th percentile and 97.5th percentile of this group: 0.06 - 3.67 mU/L. The FT4 reference interval was determined to be the same as that of the manufacturer for adult population (9.8 - 23.0 pmol/L) The limit for TPOAb positivity was determined to be 143 kU/L (24). In 2010 change the Siemens reagent for TPOAb determination and with other testing we conclude, that for pregnant woman we can use the same positivity cut off like for other healthy population. Raised concentrations of TSH were found in 5.1% of the women, suppression of TSH was found in 2.9% of the women, and 11.5% of the pregnant women were found to be TPOAb positive.

The part of women who screened positive (n=822) were invited to a follow-up study one to three years after delivery. We found that four risk factors could identify 82% of the high-risk women: age >30 years (in our analysis, 31 years and more), a personal or family history of thyroid disease, and the presence

of goitre. Only 67.5% of all of the women had normal deliveries, 70% were treated by an endocrinologist at follow-up. Forty percent of the initially euthyroid pregnant women, positive for TPOAb, had thyroid dysfunction more than one year after delivery. There is strong agreement that after delivery TPOAb positive women should be closely monitored, even if they are euthyroid during pregnancy (26). TPOAb positivity carries a high risk of developing hypothyroidism up to one year postpartum.

In the other study we verify differences between reference intervals for TSH, FT4 and TPO Ab determined by 7 different immunoassay analytical systems in pregnant women with no history of thyroid dysfunction. The FT4 reference intervals for Architect, Centaur, Modular E170, and RIA Immunotech were slightly higher than those for Immulite 2500 and AIA 2000; the lowest being for UniCel DxI (8.13-13.2 pmol/L). For TSH, a higher interval (0.25-3.86 mU/L) is recommended for Modular E170 and IRMA Immunotech; and a lower one (0.17-2.81 mU/L) for Immulite 2500 and AIA 2000. The established cut-off limits for TPO-Ab differ according to the system used (from 7 to 94 kU/L) and are similar to those recommended by their manufacturers (27).

In conclusion, our data provides a contribution to the world guidelines for the management of thyroid disease in pregnancy and support to the implementation of general screening for thyroid disorders in pregnant women. It is very important the close follow-up after delivery in those women who screen positive.

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