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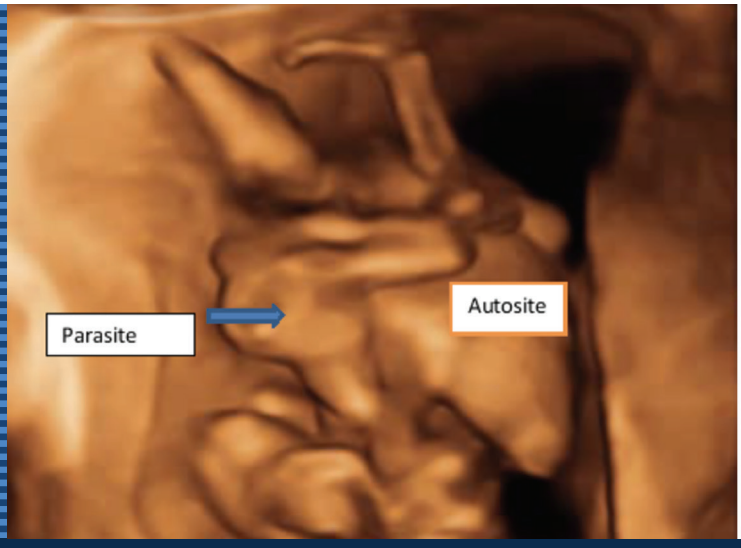

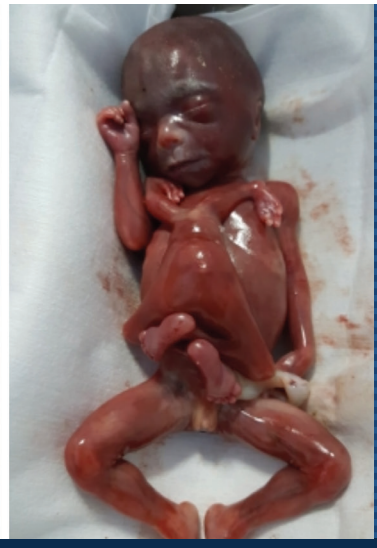

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Cover Picture: Saravanan et al. Epigastric heteropagus twins

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Contents

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Editorial

Dear Readers,

Welcome to the December 2019 issue of the Journal of the Turkish-German Gynecological Association *(J Turk Ger Gynecol Assoc)*.
Here we share some of our favorite articles that were published in this issue of the journal. In this issue, Spiliotis et al. discussed the possible differences in survival between residual and recurrent disease in patients with ovarian cancer presenting with disease relapse. Besides, Rabiepour et al. determined the relationship between maternal stress during pregnancy and cortisol plus maternal serum leptin concentrations as well as pregnancy outcomes. Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase and is part of the phosphatidyl inositole-3-kinase (PI3K)/AKT signal pathway. It plays a critical role in cellular development, metabolism, and the cell cycle of cancer cells. Güralp et al. investigated the role of mTOR in human granulosa cell ovarian tumors and the therapeutic effect of rapamycin in COV434 mitotic granulosa cell lines.
We are also pleased to introduce our new journal feature "Video article", where we encourage readers to follow. Watch all of the latest videos here (http://www.jtgga.org/video).

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You've helped our publication to become one of the busiest on the platform. As you know, every paper that appears in this journal is published first electronically online where it is freely accessible. It is worth mentioning here that the journal has been included in PUBMED central and is available to all of its readers.
I am very glad and satisfied to say that the seventh Social Responsibility Project of Turkish German Gynecological Education and Research Foundation (TGGF) held on September 6-7, 2019, in Ordu, Turkey was a great success with more than 100 participants, public awareness meeting with participation of the locals, the scientific meeting with participation of health professionals, medical examination/screening of local women, and finally a medical device donation to a local hospital.
I would like to wish you a happy new year in 2020 and we are looking forward to receiving your valuable submissions, thank you in advance for your contributions.

Sincerely,

Prof. Cihat Ünlü, M.D.
Editor in Chief of *J Turk Ger Gynecol Assoc*
President of TGGF

Secondary debulking for ovarian carcinoma relapse: The R-R dilemma – is the prognosis different for residual or recurrent disease?

John D. Spiliotis[1,2], Christos Iavazzo[3], Nikolaos D. Kopanakis[4], Athina Christopoulou[5]

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Abstract

Objective: To analyze the kind of ovarian cancer relapse by separating residual from recurrent disease and correlating them with patient survival.

Material and Methods: This was a retrospective study of 200 women with ovarian carcinoma relapse between 2005 and 2017.

Results: The main sites of residual disease included the great omentum, epiploic appendices, liver round ligament, gallbladder, and cervical/vaginal stump. The median survival for women with residual disease treated with cytoreductive surgery (CRS) + hyperthermic intraperitoneal chemotherapy (HIPEC) + systemic chemotherapy was 38 months compared with the control group, which reached 23.8 months. The morbidity rates were 18% vs 7%, respectively, and the mortality rates were 2.5% vs 1.3%. The main sites of recurrent disease included the mesenterium, pelvic floor, diaphragm, and Glisson's capsule. Women with recurrent disease treated with CRS + HIPEC + systemic chemotherapy had median survival rates of 26 months vs 16 months in the control group. The morbidity rates were 22% vs 15%, respectively, and the mortality rates were 3.3% vs 0%.

Conclusion: Patients undergoing secondary debulking plus HIPEC for ovarian carcinoma relapse have a different prognosis when compared with patients with residual and recurrent disease. A different prognosis is presented in women undergoing secondary debulking plus HIPEC for ovarian carcinoma relapse when comparing patients with residual and recurrent disease. (J Turk Ger Gynecol Assoc 2019; 20: 213-7)

Keywords: HIPEC, ovarian carcinoma, relapse, residual, recurrence, management, survival, prognosis

Received: 4 January, 2019 **Accepted:** 10 June, 2019

Introduction

Epithelial ovarian carcinoma (EOC) accounts for 2% of female cancer cases with high mortality rates and a five-year survival falling at 46%. Although the use of bevacizumab and poly adenosine diphosphate ribose polymerase inhibitors as well as an ultra-radical surgical approach to achieve zero residual disease were recently added in the current management, no satisfactory results can be achieved regarding progression-free survival (PFS) and overall survival (OS). However, ultra-radical debulking in combination with hyperthermic intraperitoneal chemotherapy (HIPEC) was revealed to be a safe and effective alternative approach. Around 70% of all women with ovarian carcinoma relapse after primary debulking and first-line chemotherapy.

The objective of our study was to discuss the possible differences in survival between residual and recurrent disease in patients with ovarian cancer presenting with disease relapse.

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Material and Methods

Two hundred patients with EOC relapse were retrospectively studied using our database. All patients with ovarian carcinoma relapse underwent surgery in three different hospitals by the same surgical group from 2005 to 2017.

During secondary cytoreduction, remaining abdominal disease after suboptimal or optimal primary or interval debulking was characterized as residual disease, and new disease found in patients who had primary or interval complete cytoreduction was considered as a recurrence. One hundred forty of 200 patients were detected as having residual disease compared with 50/200 with recurrent disease and 10/200 with splanchnic metastases (Figure 1).

Both groups of patients with recurrent and residual disease were divided in two subgroups: CRS + HIPEC followed by systemic chemotherapy, and a second subgroup receiving CRS + systemic chemotherapy alone. The ten patients with splanchnic metastases received systemic chemotherapy (Figure 2, 3).

Results

The mean age of the patients was 69 (range, 42-83) years. The mean body mass index was 31 (range, 24-43) kg/m^2. Thirty-four patients had a family history of ovarian cancer. No information was available regarding their BRCA status.

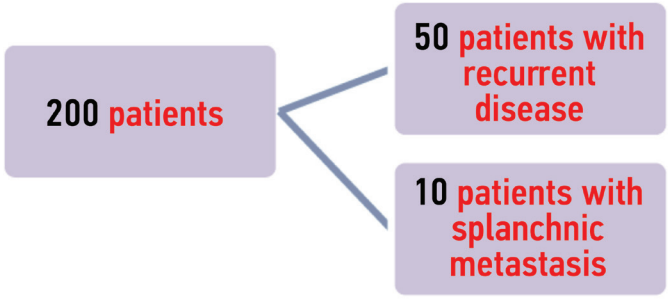

Figure 1. Flow chart of the patients' cohort

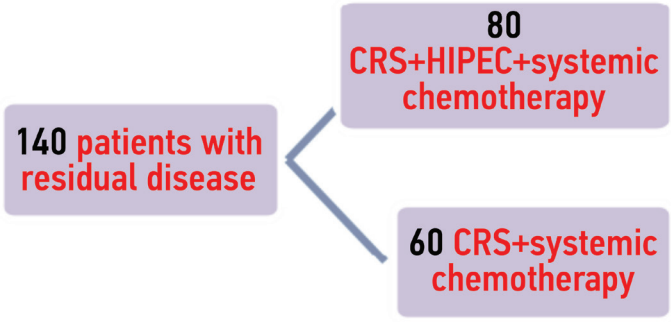

Figure 2. Division of patients with residual disease
CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemotherapy

All patients had initially received 6 cycles of carboplatin and taxol. The platinum-free interval was more than 6 months in all cases ranging from 10 months to 22 months. A difference was found between the sites of recurrent and residual disease. The main sites of residual disease included the great omentum (67%), epiploic appendices (33%), liver round ligament (55%), gallbladder (33%), and the cervical/vaginal stump (30%). The recurrent disease sites in the residual disease group were at the same sites seen in the primary surgery. The median preoperative peritoneal cancer index (PCI) was 18 and we achieved complete cytoreduction in 75%; 20% of the women experienced grade 3 and 4 complications. The median OS for women with residual disease treated with CRS + HIPEC + systemic chemotherapy was 38 months compared with the control group, which reached 23.8 months (Table 1). In this group of patients, the morbidity rates were 18% vs 7%, respectively, and the mortality rates were 2.5% vs 1.3%. The main sites of recurrent disease included the mesenterium (50%), pelvic floor (40%), diaphragm (60%), and Glisson's capsule (40%). The median preoperative PCI was 22 and we achieved complete cytoreduction in 64%; 14% of the patients experienced grade 3 and 4 complications. In the recurrent disease group, the median OS rates reached 26 and 16 months, respectively (Table 2). In this group of patients, the morbidity rates were 22% vs 15%, respectively, and the mortality rates were 3.3% vs 0%.

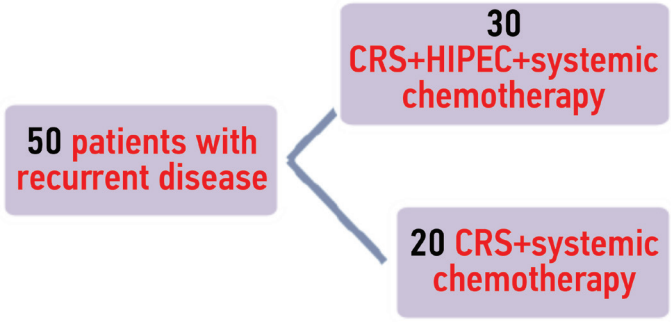

Figure 3. Division of patients with recurrent disease
CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemotherapy

Table 1. Survival, morbidity and mortality rates in patients with residual disease

Residual disease group	Median survival	Morbidity/ Mortality
CRS+HIPEC+System chem	38 months	18%/2.5%
CRS+System chem	23.8 months	7%/1.3%

CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemotherapy

Table 2. Survival, morbidity and mortality rates in patients with recurrent disease

Recurrent disease group	Median survival	Morbidity/ Mortality
CRS+HIPEC+System chem	26 months	22%/3.3%
CRS+System chem	16 months	15%/0%

CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemotherapy

Discussion

Recurrent ovarian cancer is treatable but rarely curable. The recurrence rates depend on the stage at diagnosis reaching 10%, 30%, 70-90%, and 90-95% for stages I to IV, respectively (1). One of the main factors affecting the patient's risk of recurrence is the completeness of primary/interval debulking. The majority of women with ovarian cancer have recurrence in the peritoneal cavity independent of the primary/interval debulking extent and/or type of chemotherapy (2). Rose et al. (3) proposed a nomogram for predicting individual survival after ovarian cancer recurrence, which included time to recurrence after initial chemotherapy, clear cell or mucinous histology, performance status, stage IV disease, and age. A recent retrospective study revealed that peritoneal recurrence was found in 75% of patients with advanced disease, and relapse was found at both treated and untreated sites. Nodal relapse was found in 38% of all cases, and isolated distant metastases were identified in 8% of patients (4). According to Ushijama (1), around 55% of women have recurrence at the primary site and the rest present with distant metastases including retroperitoneal nodes, liver or spleen, brain, and bone. In our study, the main areas of relapse included the great omentum, epiploic appendices, liver round ligament, gallbladder, and the cervical/vaginal stump in the residual disease group compared with the mesenterium, pelvic floor, diaphragm, and Glisson's capsule in the recurrent disease group. Women with recurrent ovarian cancer may be eligible for secondary cytoreduction (1). The DESKTOP trial suggested the main selection criteria of operability for patients with recurrent ovarian cancer, including good performance status, absence or small volume of ascites at recurrence, and completeness of primary surgery (5). Recently, DESKTOP III revealed that secondary cytoreduction led to improved PFS (19.6 months vs 14 months) compared with second-line chemotherapy in 407 relapsed patients after a progression-free interval period of more than 6 months as well as a positive the Arbeitsgemeinschaft Gynaekologische Onkologie-score performance status, Eastern Cooperative Oncology Group 0, ascetic volume of less than 500 mL, and zero residual tumor at initial debulking (6). Regarding OS rates, the results remain immature and are not yet published (6). Another study proposed that the main predictors for complete cytoreduction in women undergoing secondary cytoreduction included stage of disease, complete primary/interval debulking surgery, PFS, CA125 values and presence of ascites at recurrence (7). Based on the above, Zang et al. (8) suggested a prognostic model to predict survival benefit from secondary debulking including four parameters (progression-free interval, presence of ascitic fluid at recurrence, extent of recurrent disease, and completeness of secondary cytoreduction based on the residual disease. More specifically, the median survival after secondary debulking for women with progression-free intervals >23.1 months was 45.0 months compared with 21.0 months in women with progression-free intervals of <23.1 months. The cut-off level of CA125 at recurrence was found as 251.0 $U mL^{-1}$. Median survival was found as 43.9 months in women with local disease compared with 20.0 months in patients with multiple areas of recurrence (8). Zero residual disease after secondary cytoreduction was the strongest prognostic factor. More specifically, the median survival was 57.7 months in women achieving R0 during secondary cytoreduction compared with 27.0 months in the R1 group, and 15.6 months in the R2 group (8,9). Furthermore, Laga et al. (10) confirmed that DESKTOP score and the Tian model were the main predictors of candidate selection for complete secondary cytoreduction. However, in their study, 61% and 70% of the patients were debulked to R0 independently of the negative preoperative scores. For this reason, they suggested that other anatomic and metabolic imaging criteria should be evaluated to recognize eligible patients for HIPEC plus secondary cytoreduction (10).

HIPEC following secondary cytoreduction is an alternative approach for patients with recurrent ovarian disease. Harter et al. (5) concluded that "HIPEC remains experimental in ovarian cancer patients but it can be used inside prospective controlled trials". A recent meta-analysis showed better OS rates for patients with recurrent ovarian cancer when adding HIPEC to secondary cytoreduction and traditional chemotherapy. Additionally, a positive correlation between completeness of debulking and survival was found. In the same analysis, morbidity and mortality rates were similar (11).

It should be highlighted that in high-volume centers with HIPEC specialists, morbidity and mortality has drastically improved (12,13). The published results from our center showed that women with advanced ovarian carcinoma recurrence had a mean survival benefit of around 13.3 months when HIPEC is offered (26.7 months vs 13.4 months in the non-HIPEC group) (14). Hotouras et al. (15) showed that in women with ovarian carcinoma recurrence undergoing debulking plus HIPEC administration, the OS ranged between 26.7 and 35 months,

with PFS varying between 8.5 and 48 months. The role of HIPEC in patients with ovarian cancer was recently confirmed in a randomized controlled trial that highlighted a better PFS (15 months vs 11 months) as well as OS (46 months vs 34 months) in patients with stage III EOC undergoing interval cytoreduction plus HIPEC administration (16). The results of other randomized trials in the field are awaited.

The questions raised by our study related to whether disease recurrence refers to relapse or residual disease post initial surgery, and whether secondary cytoreduction followed by HIPEC has a different effect on PFS and OS in the two different groups. This was actually confirmed from our results because the median survival for women with residual disease treated with CRS + HIPEC + systemic chemotherapy was 38 months compared with the control group, which reached 23.8 months. In addition, patients who presented with recurrent disease had median survival rates of 26 months and 16 months, respectively. To summarize, the addition of HIPEC improves survival rates in both patients with residual as well as recurrent disease, and such rates were obviously better in the residual tumor group compared with the recurrent disease group. Such findings also highlight the need of major cytoreductive effort/ultra-radical surgery at the moment of primary/interval cytoreduction.

This study has some limitations that have to be addressed, including the small patient population and the retrospective nature of the study. It is a well-known fact that maximal and optimal cytoreduction have better prognosis than suboptimal debulking. One hundred forty patients had residual disease in our study. This number could be considered quite high, but we should clarify that all these patients were referred to our group for further management in our tertiary centers after undergoing surgery either by non-subspecialists or in cases where neoadjuvant chemotherapy had not been considered an option prior to primary debulking. Unfortunately, because the majority of patients were initially treated by non-subspecialists, we are unable to subdivide optimal and suboptimal cytoreduction categories in the residual disease group.

Our retrospective study shows that HIPEC improves survival rates in both patients with residual as well as recurrent disease. Better survival rates were found in women with residual disease treated with HIPEC – rates that were are actually longer compared with the recurrent group. Prospective randomized multicenter studies are essential to further empower our findings.

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The relationship between stress during pregnancy with leptin and cortisol blood concentrations and complications of pregnancy in the mother

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Abstract

Objective: Pregnancy is one of the most stressful periods a woman experiences in her life. The present study was an attempt to determine the relationship between maternal stress during pregnancy and cortisol plus maternal serum leptin concentrations as well as pregnancy outcomes.

Material and Methods: This longitudinal study was conducted on 90 pregnant women in Miandoab city between 2015 and 2016. The samples were chosen from mothers with a gestational age of 24 to 28 weeks. The participants were asked to complete Cohen's Perceived Stress Scale (PSS) and a demographic questionnaire and blood samples were taken from them. The mothers were then tracked with four-week intervals until the time of delivery and were asked to complete Cohen's PSS each time along with a questionnaire related to maternal outcomes. Again, a blood sample was taken at the time of delivery. Data analysis was performed using SPSS 16. Descriptive statistics, Pearson's correlation coefficient, and the t-test were employed for analysis.

Results: A significant relationship was found between maternal stress and preeclampsia (p=0.008). The relationships between preterm childbirth and maternal cortisol concentrations in weeks 24-28 (p=0.015), and between preterm childbirth and maternal leptin concentrations at the time of delivery (p=0.007) were also found to be significant.

Conclusion: Pregnancy and labor, as physically and mentally stressful events, can affect women's physiologic and psychological indicators. As a consequence, during pregnancy, the cortisol and leptin index changes in response to the activity of the hypothalamic-pituitary axis and autonomic nervous system under stress. (J Turk Ger Gynecol Assoc 2019; 20: 218-23)

Keywords: Stress, cortisol, leptin, pregnancy complications

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Introduction

Pregnancy is associated with significant physiologic and psychological changes, which sometimes lead to pathologic changes (1). Stressful situations can have adverse effects on women's psychological status. Indeed, stress is the most obvious symptom in pregnant women's behaviors and clinical symptoms (2). For instance, the prevalence of pregnancy stress in England and Sweden was reported as 33-37% and 5-7% respectively (3). Evidence suggests that mothers' reaction to stress changes during pregnancy (4), which is directly correlated with pregnancy outcomes (5,6). Mothers' stress also predicts delivery problems and complications (7). A pregnant woman's stress affects her and leads to negative perceptions towards delivery and birth, unnecessary fear of childbirth and motherhood, self-medication with alcohol, and activity restriction (8). Studies suggest that preterm birth and gestational hypertension are more likely to occur in mothers with stressful pregnancies (9,10).

Pregnancy stress increases the chance of unplanned cesarean and labor complications (11,12). To measure the amount of stress during pregnancy, different methods such questionnaires

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(13) or measurement of biochemical markers, which are indicators of stress (14), are used. Today, cortisol and other hormones produced by the sympathetic nervous system activity, which can be measured from blood samples, are measured as indicators of stress (14). Cortisol, which is called the stress hormone, is considered as a decisive indicator in stressful situations (15,16). The hypothalamic-pituitary-adrenal axis is activated in response to stress and releases cortisol in the organism (17). The level of cortisol is a function of actual and perceived stress (18). In a study, Edwards et al. (19) examined the effects of stress during pregnancy on changes in hormonal and behavioral responses affecting serum concentrations of cortisol and leptin. They found that adipose tissue had an effect on the response to stress with secretion of leptin. Glucocorticoids and probably adrenocorticotropic hormone stimulate synthesis and secretion of leptin (20).

A number of studies have investigated the relationship between maternal stress and concentrations of cortisol, and have obtained contradictory results. On the other hand, no studies were found to have only investigated the relationship between perceived maternal stress during pregnancy plus labor and leptin concentrations. Accordingly, the present study examines the relationship between stress during pregnancy and leptin plus cortisol concentrations as well as complications of pregnancy in pregnant women.

Material and Methods

The present study is a longitudinal study conducted between November 2015 and the end of April 2016 in Miandoab city. The study population consisted of pregnant women of 24 to 28 weeks' gestation. The samples were chosen through available sampling. The sample size was calculated as 90 participants using the correlation coefficient formula a d based on the study conducted by Alavian et al. (21) and considering the correlation coefficient between stress and cortisol concentration (0.312), confidence level of 5%, and statistical power of 80%.

The criterion for inclusion in the study was not having any underlying disease during and before pregnancy. The required information was collected from the reference laboratory where all pregnant women of the city referred to for routine check-ups within weeks 24-28 of pregnancy. The reference laboratory was selected so that the samples would have the same conditions. This laboratory had lower costs because it was public, and all pregnant women from all over the town (including villages) were referred to this center.

The objectives and method of study were explained to the participants and their written consents were obtained. Cohen's perceived stress scale (PSS) and a questionnaire developed by the researcher about the demographics of the participants were completed by the participants. Also, mothers' blood samples taken for routine pregnancy tests at 24-28 weeks of pregnancy were used to test the leptin and cortisol concentrations of mothers' blood.

Subsequent follow-up of the mothers in the study was performed three times with 4-week intervals at 28-32 weeks and 34-36 weeks, and at the time of delivery. The first and second follow-ups were performed by telephone with the PSS completed by the participants; for the third follow-up, they were asked to call the researcher, via the number they were provided with, in case they went to the delivery room having gone into labor. Also, according to the first day of the last period, the delivery time was estimated and the researcher was present in the delivery room at the time that was determined for the delivery of the participants, even if the participants did not call the researcher. Thus, the third follow-up was performed through presence in the delivery room. At the time of labor, again the PSS and a questionnaire related to maternal outcome were completed. In this stage, the mothers' blood samples that were taken as a routine at the time of reception were used to test the leptin and cortisol concentrations of the mothers' blood. Due to the circadian rhythm of cortisol and in order to minimize the impact of this rhythm on cortisol concentrations, the time of maternal blood sampling in the first stage was within 8-10 a.m, while in the second stage it depended on the time of delivery. The blood samples were centrifuged for 10 minutes at 2500 rpm and their serum samples were separated. Serum samples were placed in closed and coded Eppendorf tubes and were kept in -20 °C in the laboratory. All samples were analyzed under the same conditions (environmental, time, place, and analyst). To measure leptin and cortisol concentrations, Bio Vendor kits with sensitivity of 0.2 ng/mL and specificity of 100% for leptin, and DiaMetra kits with sensitivity of 2.44 ng/mL and specificity of 100% for cortisol were used, respectively.

Tools

1. Cohen's PSS; the PSS includes 14 expressions investigating the participants' feelings and thoughts as well as their general perceived stress during the last month. The 14-item questionnaire including seven negative items and seven positive items was used here. The items were rated on a 5-point Likert scale ranging from 'Almost Never' to 'Almost Always.' Items 4, 5, 6, 7, 9, 10, and 13 were reverse coded. The lowest and highest scores were zero and 56, respectively (13,22). Cronbach's alpha for American and Iranian populations was found by Ghorbani et al. (23) as 0.86 and 0.81, respectively. The questionnaire's construct validity was established at 0.63 and was significant at $p<0.05$ (24).

2. Demographic questionnaire; this questionnaire was developed by the researcher and had three parts: demographic information (age, address, pregnant women

and their husbands' level of education, and job), information about fertility (gravidity, type of delivery, parity, abortion, the pregnancy planning, neonate's sex, desirability of the neonate's sex, mother's interest in pregnancy), and maternal outcomes (preeclampsia, preterm delivery, dystocia, excessive bleeding in childbirth, and spotting during pregnancy).

Data analysis

Data analysis was performed using the SPSS 16 software package. Further, the relationship between factors ($p<0.05$) was determined using Pearson's correlation coefficient and the t-test.

Results

In the present study, the average age of the mothers was 27.48 (range, 15-40) years. The general and reproductive characteristics of the sample are displayed in Table 1.

Some psychological issues and problems were also investigated in this study: 86.7% had planned pregnancy, the pregnancy in approximately 91.1% of the mothers was intended, 86.7% were happy with their child's sex, and 91.1% were interested in their pregnancy.

Table 1. General and reproductive characteristics of the sample

Variable		n (%)
Locality	City	35 (38.9)
	Village	55 (61.1)
Mother's education	<High school	37 (41.1)
	High school graduate	41 (45.6)
	College graduate	12 (13.3)
Father's education	<High school	35 (38.9)
	High school graduate	46 (51.1)
	College graduate	9 (10)
Mother's job	Housewife	87 (96.7)
	Employed	3 (3.3)
Father's job	Unemployed	7 (7.8)
	Employed	83 (92.2)
Gravida	First	24 (26.7)
	Second	45 (50)
	Third or more	21 (23.4)
Type of delivery	NVD	61 (67.8)
	C/S	29 (32.2)
Parity	Non	28 (31.1)
	1	48 (52.3
	2 or more	14 (15.5)
Miscarriage	1	13 (14.4)
	2 or more	3 (3.3)
Sex of the fetus	Boy	54 (60)
	Girl	36 (40)

Maternal PSS scores at different time points of pregnancy, as well as cortisol and leptin concentrations (ng/mL) in mother are shown in Table 2.

According to Table 3, there was no significant relationship between maternal perceived stress during pregnancy and the time of delivery, the two conditions (yes or no) related to preterm birth, dystocia, spotting during pregnancy, and bleeding at the time of delivery. Although there was no significant difference between yes or no conditions, the PSS was higher in the yes state in almost all cases. There was a significant relationship between PSS scores at the time of delivery and preeclampsia ($p=0.028$) (Table 4).

Cortisol concentrations at the delivery were significantly higher with score of perceived stress in 24-28 weeks ($p=0.019$, $r=0.246$) and total stress score ($p=0.046$, $r=0.211$) and there were non-significant correlations between maternal cortisol concentrations and maternal leptin concentrations and PPS scores in pregnancy and at the time of delivery (Table 5).

At the time of delivery, the leptin concentrations showed a significant and negative relationship with preterm childbirth ($p=0.007$), i.e. the leptin concentrations were lower in preterm childbirth. However, the relationship between preterm child birth and cortisol concentrations in weeks 24-28 was significant and positive ($p=0.015$), but there were no significant relations between maternal leptin and cortisol concentrations and complications of pregnancy in the mother.

Discussion

The findings revealed a significant relationship between perceived maternal stress at the time of delivery and preeclampsia. Shamsi et al. (25) evaluated the risk factors of preeclampsia in Pakistani women and reported a higher level of stress in women with preeclampsia. In another study, it was found that stress had a significant relationship with preeclampsia. Further, stress had a significant relationship with the severity and worsening of preeclampsia (26). This is also confirmed by the findings of Black who suggested that women with severe preeclampsia had higher stress concentrations compared with those with a mild preeclampsia (27). The findings of the present study in this area agree with those of previous research.

A significant relationship was found here between cortisol concentrations at the time of delivery and perceived stress scores. Similarly, the relationship between perceived stress and cortisol concentrations in late pregnancy was found to be significant in another study, though no significant relationship was established between cortisol concentrations and early pregnancy (28). On the other hand, the findings revealed

no significant relationship between perceived stress scores during pregnancy and plasma cortisol concentrations (29). A significant relationship was reported between severe stress based on visual analogue scale at the time of labor and salivary cortisol (21). In addition, Pluess et al. (30) reported a significant relationship between mothers' state anxiety and salivary cortisol concentrations in early and late pregnancy. The findings of our study are in agreement with most previous studies in this regard. The difference between our findings and some other studies can be attributed to the use of different instruments to measure stress concentrations during pregnancy.

We found a significant relationship between preterm childbirth and maternal plasma cortisol concentrations in weeks 24-28 of pregnancy. Previous studies suggested that the mean concentrations of maternal plasma cortisol in women with preterm labor were higher compared with their counterparts. It

Table 2. Maternal perceived stress score and cortisol and leptin concentrations (ng/mL) of the sample (mean ± standard deviation)

Time	24-28 week mean ± SD	28-32 week mean ± SD	32-36 week mean ± SD	Delivery time mean ± SD
Mean score of perceived stress	25.54±4.33	24.50±4.35	26.01±4.34	31.35±5.04
Cortisol (ng/mL)	295.44±9.16	N/A	N/A	298.38±16.62
Leptin (ng/mL)	34.77±12.24	N/A	N/A	29.94±12.68
SD: Standard deviation; N/A: Not applicable				

Table 3. The relationship between perceived mother's stress score and complications of pregnancy (p value)

	24-28 weeks	28-32 weeks	32-36 weeks	Delivery time
Preeclampsia	0.799	0.727	0.722	0.028*
Preterm birth	0.165	0.198	0.295	0.249
Dystocia	0.427	0.346	0.525	0.727
Spotting during pregnancy	0.358	0.221	0.283	0.968
Bleeding in delivery	0.380	0.271	0.806	0.238
*The mean of groups were compared using Student's *t*-test. Statistically, p<0.05 was significant				

Table 4. The relationship between maternal perceived stress and cortisol and leptin concentrations of the mother

	24-28 weeks	28-32 weeks	32-36 weeks	Delivery time	Total stress score
Cortisol (24-28 weeks)	p=0.619 r=0.053	p=0.855 r=0.020	p=0.368 r=-0.096	p=0.623 r=-0.052	p=0.755 r=-0.033
Leptin (24-28 weeks)	p=0.229 r=-0.128	p=0.608 r=-0.055	p=0.496 r=-0.073	p=0.899 r=-0.014	p=0.314 r=-0.107
Cortisol (delivery time)	p=0.019* r=0.246	p=0.141 r=0.157	p=0.073 r=0.190	p=0.626 r=-0.052	p=0.046* r=0.211
Leptin (delivery time)	p=0.771 r=-0.031	p=0.564 r=0.062	p=0.477 r=0.076	p=0.213 r=-0.133	p=0.861 r=-0.019
*Pearson test was used. Statistically, p<0.05 was significant					

Table 5. The relationship between maternal cortisol and leptin with complications of pregnancy (p value)

	Cortisol (24-28 weeks)	Leptin (24-28 weeks)	Cortisol (delivery time)	Leptin (delivery time)
Preeclampsia	0.530	0.056	0.454	0.418
Preterm birth	0.015*	0.912	0.676	0.007*
Dystocia	0.737	0.318	0.511	0.873
Spotting during pregnancy	0.597	0.614	0.622	0.441
Bleeding in delivery	0.675	0.420	0.209	0.904
*The mean of groups were compared using Student's *t*-test. Statistically, p<0.05 was significant				

indicates that cortisol plays a significant role in the mechanism of preterm labor in some women (31). In another study, plasma cortisol concentrations in women giving preterm birth were found higher than in cases of normal delivery, implying the importance of maternal hypercortisolemia in preterm labor (32). According to these findings, the risk of preterm delivery grows with high blood cortisol concentrations (31,33,34).

In the present study, a negative significant relationship was observed between preterm childbirth and maternal plasma leptin at the time of delivery. The mechanism of leptin in preterm birth is generally unknown in the literature. Some studies claim that increased concentrations of leptin in preterm delivery are closely linked to antenatal exposure to corticosteroids (35).

The literature suggests that the risk of preterm labor before week-34 of gestation decreases with higher concentrations of leptin. Wuntakal et al. (36) reported that induced myometrium contraction was determined by the availability of leptin and might prove helpful in preventing preterm birth. In a study on 1304 pregnant women in weeks 16-27 of gestation, Palchevska et al. (37) showed that the amount of maternal leptin was higher in women who delivered at term than in those with premature delivery. The difference was still observed after controlling for diabetes, blood pressure disorders, and pre-pregnancy body mass index. In the same vein, Palchevska et al. (37) studied 110 neonates and found that that leptin concentrations were higher for term infants. This was further confirmed in the study by Laivuori et al. (38).

Study limitations

Environmental conditions and circadian rhythm affect cortisol concentrations. This was controlled, as much as possible, by taking samples in the morning in weeks 24-28 of pregnancy. However, at the time of delivery, due to its unexpected and emergency nature, it was beyond the researcher's ability to control this condition.

The present study found a significant relationship between preeclampsia and average stress scores in pregnancy. In addition, there was a significant relationship between leptin and cortisol concentrations in maternal serum and preterm childbirth. These findings indicate the negative and undesirable impact of stress on pregnancy outcomes. Other studies can be conducted to discover the possibility of predicting pregnancy outcomes by measuring cortisol and leptin concentrations in blood serums at other stages of pregnancy. Note that in order to draw safer conclusions, there is a need for more longitudinal studies with larger samples.

Ethical Issues: Information about the participants remained confidential thought the study and the results were disseminated collectively.

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Uncommon borderline ovarian tumours: A clinicopathologic study of seventeen patients

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Abstract

Objective: To evaluate uncommon types of borderline ovarian tumors (BOT) and define the clinical, surgical, and pathologic features.

Material and Methods: Seventeen patients who were treated in our hospital between 1990 and 2017 were identified. Patients' data were collected from the gynecologic oncology clinic electronic database, patients' files, and pathology reports. Conservative surgery was defined as preservation of the uterus and at least part of one ovary.

Results: The mean age was 47 (range, 22-70) years. Based on histopathologic tumor type, there was mixed tumor in five (29.4%) patients, endometrioid-type in nine (52.9%), seromusinous-type in two (11.8%), and Brenner-type in one (5.9%). Conservative surgery was performed in 4 patients. Two patients with endometrioid BOT had synchronous endometrial pathology, including one (11%) patient with endometrial cancer, one (11%) with endometrial hyperplasia without atypia, and 3 (33%) patients had endometriosis. The median follow-up was 19 (range, 1-137) months. No recurrence was observed during the follow-up period.

Conclusion: In our small volume case series, it could be said that non-serous/non-mucinous BOT has excellent prognosis. However, endometrial pathology should be checked in endometrioid type. (J Turk Ger Gynecol Assoc 2019; 20: 224-30)

Keywords: Borderline ovarian tumors, survival, stage

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Introduction

Borderline ovarian tumors (BOT) account for 14-15% of all ovarian neoplasms (1). These tumors usually occur at an early stage, with 25% of cases diagnosed at stages 2-4 according to the staging system defined by International Federation of Gynecology and Obstetrics (FIGO). Considering all BOT cases, the overall 10-year survival rate was reported as 97%. BOTs are tumors of epithelial origin characterized by increased cellular proliferation and mild nuclear atypia; however, stromal invasion is not typical for these types of tumors (2). This group of tumors was described by Taylor in 1929 as "semi-malignant tumors" presenting with peritoneal involvement (3). In FIGO 1971, these tumors were named "low malignant potential tumours" thus separating this entity from ovarian carcinomas (4). They were defined as "atypical proliferative tumors" instead of "borderline tumors" in the 2014 World Health Organization (WHO) classification (5). Based on the epithelial cell type, six histologic subtypes of these tumors have been defined. Serous (50%) and mucinous (45%) tumors are the most common subtypes, whereas endometrioid, clear cell, seromucinous, and Brenner subtypes are seen more rarely (6).

In this study, pure serous and pure mucinous subtypes of BOT were excluded, thereby aiming to define the clinical, surgical, and pathologic features of rarer BOT subtypes, including the endometrioid, seromucinous, and Brenner tumours, and the mixed types.

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Material and Methods

A total of two hundred ninety-three eligible patients, treated between 1990 and 2017, were reviewed as to whether they qualified for inclusion in this retrospective study. The inclusion criteria were a history of surgery and confirmed pathologic diagnosis of BOT, and having had a follow-up examination at the local gynecologic oncology center. Among these, 17 (5.8%) patients with a final pathologic diagnosis of either endometrioid, seromucinous, or Brenner tumors, or a mixed epithelial borderline tumor defined as containing more than one of these five major types of cells including the serous, mucinous, endometrioid, clear cell, and Brenner cell types (7). The patient data were obtained from the computerized database of the gynecologic oncology department and from the pathology reports and patient files, including medical records.

The staging surgery was defined as "abdominal cytology ± infracolic omentectomy ± appendectomy ± peritoneal biopsy" performed at the right and left paracolic sites. Lymphadenectomy was included in the surgical procedure to include the pelvic ± paraaortic regions as per the surgeon's discretion. For women in the reproductive period desiring fertility, a fertility-sparing approach was preferred. The fertility-sparing approach was defined as the preservation of the uterus and a portion of at least one ovary. According to this definition, conservative surgery included either of a unilateral oophorectomy (UO); a unilateral oophorectomy and contralateral cystectomy [UO+contralateral cystectomy (CC)]; a unilateral cystectomy, or bilateral cystectomy. Definitive surgery was accepted to describe procedures that spared neither the uterus nor the ovaries. Staging was performed according to the FIGO 2014 system. In patients who had not undergone a standard staging surgery, the stage of the disease was determined according to intraoperative findings and pathology results. The patients were seen at the postoperative follow-up visits, which were scheduled every 3 months in the first 2 years, then every 6 months until the 5th year, and thereafter once a year. Gynecologic examination, measurement of Ca-125 levels, and whole abdominal ultrasonography were routinely performed during each follow-up visit.

The study was conducted in compliance with the principles and procedures for clinical studies after approval from the Local Ethics Committee.

Results

The median age of the 17 patients included in the study was 47 (range, 22-70) years. Histopathologic diagnosis was mixed tumor in five (29.4%) patients, endometrioid subtype in nine (52.9%), seromucinous subtype in two (11.8%), and Brenner tumor in one patient (5.9%). The main symptom of the patients was abdominal pain; six (35.3%) patients were referred to the clinic due to this symptom. In addition, four (23.5%) patients had menstrual irregularities and two (11.8%) had abdominal distention. It was found that three (17.6%) patients with no active symptoms were diagnosed during routine examinations. The median pre-operative Ca-125 level was 77 (range, 12-589) IU/mL. The median tumor size was 100 (range, 50-250) mm. The tumor was unilateral in 14 (82.3%) patients. The clinical and pathologic features of the patients are summarized in Table 1.

It was observed that standard staging surgery was performed in 12 patients. Of these patients, four were at stage 1A, one was at stage 1B, and seven were at stage 1C. Stages of the patients in whom staging surgery was not performed were stage IB in one patient and stage 1A in four patients. In three patients with bilateral BOTs, the tumor subtypes were seromucinous in one patient, endometrioid in one patient, and mixed in one patient (patients #4, #11, and #17). No invasive implantations of the tumor or extraovarian diseases were detected. Lymphadenectomy was included in the surgical procedures performed in 11 patients. Both para-aortic and pelvic lymphadenectomies were performed in 10 patients. None of the patients had pathologies in the lymph nodes. The two patients (patient no #1 and #8) who were diagnosed and underwent surgery at an external center were re-staged in our clinic. The pathologic examination results of these patients were reported as mixed tumor and an endometrioid subtype, respectively. The tumors of these two patients were both staged as 1C according to the final pathology examination reports.

Conservative surgery was performed in four patients (patients #5, #6, #7, and #16). Of these patients, three underwent a UO and one underwent a UO+CC. Detailed information about the surgical procedures is presented in Table 2. Sixteen of 17 frozen section examination reports were available, one of which was reported as a malignant epithelial tumor (patient #4) and a discrimination between BOT and malignancy could not be made in two patients (patients #8 and #10). The final reports of the pathologic examination of the biopsy specimens of these three latter patients were reported to be the mixed subtype of BOT in the former patient and endometrioid subtype BOT in the two latter patients, respectively. Two frozen section examination results were evaluated as benign tumors, the remaining 11 (64.7%) were evaluated to be BOTs.

The pregnancy records of four patients who underwent conservative surgeries were not available. Of nine patients who had endometrioid BOTs, three (33%) patients had endometriosis (patients #6, #8, and #13), one (11%) had endometrial carcinoma (patient #9), and one (11%) patient had endometrial hyperplasia (patient #14). No endometriosis or endometrial pathology was identified in the two patients

who were diagnosed as having seromucinous tumors. The presence of endometriosis and endometrial pathology in the study patients is summarized in Table 3.

According to the available postoperative follow-up records of the patients, it was determined that adjuvant treatments were not administered to 16 patients. The median duration of follow-up was 19 (range, 1-137) months. Sixteen patients were followed up at least for a period of 9 months. No recurrence was observed in the patients during the follow-up period.

Discussion

Data on non-serous and non-mucinous BOT subtypes are limited in the literature. These tumor subtypes constitute 3-4% of BOT cases (7). In the 2014 WHO classification, these rare tumor subtypes were histologically classified into endometrioid, clear cell, transitional (Brenner), and seromucinous subtypes (5). Information about the serous and mucinous subtypes is based on a retrospective analysis. In rare borderline tumors, data on the clinical behavior, treatment, and follow-up of the subtypes are very limited and most reports in the literature are case series. BOT is seen more commonly in younger people compared with the occurences of epithelial ovarian cancers. The ages of our patients ranged from 22 to 70 years and their median age was 47 years. Similar age ranges have been reported in other series in the literature (8,9). Infertility is another finding that occurs in 10-35% of BOTs (10). Retrospective data on the infertility history of our study patients were not available.

Conservative surgeries were performed in 4 patients in our study. In the literature, the recurrence rates after conservative surgeries are reported at higher rates compared with those rates of the patients undergoing radical surgeries. Morice et al. (11) reported more recurrence in patients treated with ovarian cystectomy compared with those undergoing oophorectomies. In the present study, no recurrence was observed during the median follow-up of 19 months in any of the patients who underwent definitive surgeries or conservative surgeries.

The stage of the disease is the most important factor in determining the prognosis. Unlike ovarian carcinomas, BOTs are detected at earlier stages. In this study, 17 patients with mixed serous or mucinous tumors were evaluated. Similar to the rates observed with pure serous and mucinous subtypes in the literature, all of the 17 patients included in our study were diagnosed at stage 1. In the literature, the 5-year survival rate is reported as 95% in stage 1 tumors; however, there are no specific data on the rare subtypes.

Although 30% of BOTs are asymptomatic, 50-60% of patients present with non-specific symptoms such as abdominal pain or abdominal distention, and 10% of patients have bleeding-associated symptoms (12,13). In our study, eight patients (47.1%) presented with abdominal pain and distention, four patients (23.5%) had menstrual irregularities, and three patients (17.6%) were asymptomatic.

The levels of Ca-125 are elevated in epithelial ovarian cancer and are used in monitoring the patients; however, Ca-125 is

Table 1. Clinical and pathological features of patients

Patient no	Age	Tumor type	Laterality	Menopausal status	Main symptoms	Ca-125 (IU/mL)	Tumor size (mm)
1	N/A	Mixed	Unilateral	Menopause	Absent	48	120
2	60	Mixed	Unilateral	Premenopausal	Absent	N/A	N/A
3	50	Mixed	Unilateral	N/A	N/A	250	100
4	65	Mixed	Bilateral	Premenopausal	Pain	N/A	N/A
5	24	Mixed	Unilateral	Premenopausal	Pain	316	150
6	29	Endometrioid	Unilateral	Premenopausal	Pain	130	120
7	30	Endometrioid	Unilateral	Premenopausal	Abdominal distention	12	80
8	50	Endometrioid	Unilateral	Menopause	N/A	163	60
9	42	Endometrioid	Unilateral	Premenopausal	Menstrual irregularity	N/A	50
10	52	Endometrioid	Unilateral	N/A	Absent	N/A	60
11	42	Endometrioid	Bilateral	N/A	Pain	38	150
12	63	Endometrioid	Unilateral	Menopause	Menstrual irregularity	N/A	100
13	42	Endometrioid	Unilateral	Premenopausal	Menstrual irregularity	589	70
14	47	Endometrioid	Unilateral	Premenopausal	Distention	14	70
15	N/A	Brenner	Unilateral	Menopause	Menstrual irregularity	38	100
16	22	Seromucinous	Unilateral	Premenopausal	Pain	106	250
17	45	Seromucinous	Bilateral	Premenopausal	Pain	22	50

N/A: Not applicable

not specific for BOTs in terms of diagnosis or follow-up. Ca-125 levels were negative (Ca-125 <35 IU/mL) in 53.8% of patients in a meta-analysis performed by du Bois et al. (12). The median pre-operative Ca-125 level in our study was 77 IU/mL and the available Ca-125 levels of 9 out of 12 patients were over 35 IU/mL. More than half of the patients in our study (53%, n=9/17) had endometriosis and their endometrial pathologies might explain these high levels of Ca-125. Some studies reported that endometriosis and endometrial pathologies could be comorbid with these rare subtypes of BOTs (7). In the present study, the presence of endometriosis was detected in 33% of patients and endometrial pathologies were identified in 22% of patients; however, no endometriosis or endometrial pathologies were present in seromucinosis BOTs, which are known to be associated with endometriosis.

In BOTs, the accuracy of frozen section reports is determined to be between 58-86% and 31% of the cases are diagnosed as benign tumors (14). In a previous study conducted earlier at our clinic, which included all tumor types in the study, reported that the frozen section diagnoses were consistent with 79% of the final pathology reports making a diagnosis of BOT. In that study, the accuracy of the frozen section diagnoses was determined by the tumor type and the tumor diameter. The accuracy rate was reduced if the tumor subtype was mucinous and if the

Table 2. Details of surgical procedure

Patient no	Surgery type	Surgical treatment	Frozen/Section result	Stage	Total number of lymph node	Re-staging	Follow-up time (month)
1	Definitive	TAH + BSO + BPPLND + Omentectomy + Appendectomy + Cytology	Serous cystadenoma	1C	91	+	82
2	Definitive	TAH + BSO	N/A	1A	-	-	137
3	Definitive	TAH + BSO + BPPLND + Omentectomy + Cytology	Serous BOT	1A	37	-	27
4	Definitive	TAH + BSO + BPPLND + Omentectomy + Appendectomy + Cytology	Malignant epithelial tumor	1C	21	-	57
5	Conservative	UA+ BPPLND + Omentectomy + Appendectomy + Cytology	BOT	1B	21	-	23
6	Conservative	UA + CC+ BPPLND + Omentectomy + Appendectomy + Cytology	BOT	1C	60	-	17
7	Conservative	UA	Endometrioid BOT	1A	-	-	9
8	Definitive	TAH + BSO + BPPLND + Omentectomy + Appendectomy	Borderline / malign no discrimination	1C	71	+	45
9	Definitive	TAH + BSO	Endometrioid BOT	1A	-	-	29
10	Definitive	TAH + BSO	Borderline / malign no discrimination	1A	-	-	1
11	Definitive	TAH + BSO	Endometrioid BOT	1A	-	-	72
12	Definitive	TAH + BSO + BPPLND + Omentectomy + Appendectomy	Benign	1A	46	-	9
13	Definitive	TAH + BSO + BPPLND + Omentectomy + Peritoneal biopsy	BOT	1C	73	-	1
14	Definitive	TAH + BSO + BPPLND + Omentectomy + Cytology	Serous BOT	1A	41	-	1
15	Definitive	TAH + BSO	BOT	1A	-	-	1
16	Conservative	UA + BPPLND + Omentectomy + Appendectomy + Cytology + Peritoneal biopsy	BOT	1C	N/A	-	19
17	Definitive	TAH + BSO + BPPLND + Omentectomy + Appendectomy + Peritoneal biopsy	Mucinous BOT	1C	61	-	12

TAH: Total abdominal hysterectomy; BSO: Bilateral salpingo-oophorectomy; BPPLND: Bilateral pelvic-paraaortic lymph node dissection; UA: Unilateral adnexectomy; CC: Contralateral cystectomy; BOT: Borderline ovarian tumor; N/A: Not applicable

tumor size was over 10 cm (15). Eleven (64.7%) intraoperative frozen section results were reported as BOT. One intraoperative frozen section examination reported a malignant tumor but the final histopathologic diagnosis was BOT.

Endometrioid borderline tumors

This subtype of tumor, which is defined as an atypically proliferating endometrioid tumor, accounts for 2-3% of BOT cases, being the third most common subtype after the serous and mucinous subtypes. The mean age at diagnosis is 57 years (16,17). The endometrioid subtype was detected in 3% of the patients in our BOT cohort (n=9/293). It is reported in the literature that patients are mostly diagnosed at stage 1, as it was found in our study.

The endometrioid subtype is known to co-exist with endometriosis and endometrial pathologies. The reported rate of co-existing endometriosis was 63% in the study conducted by Hauptmann et al. (2) and 67% in the study by Roth et al. (17). However, Bell and Kurman (16) reported that 36% of patients with endometriosis were diagnosed with an endometrioid BOT subtype. In that study, a complex atypical endometrial hyperplasia was detected in six patients. Uzan et al. (18) reported that the endometrioid subtype of BOT was found to be associated with endometriosis in 19% of patients and with endometrial carcinoma in 6% of patients. Jia et al. (19) identified comorbid endometrial disorders in endometrioid BOT, reporting that endometrial intraepithelial neoplasia was present in five patients (25%), endometrial carcinoma was present in six patients (24%) with endometrial pathologies, and endometriosis was reported in 23% of patients. In our study, we reported endometriosis in 33% of patients, atypical endometrial hyperplasia in 11%, and endometrial cancer in 11% of patients in our endometrioid BOT series. Therefore, endometrial curettage should be recommended if a fertility preserving approach is chosen.

Table 3. Synchronous endometrial pathologies in endometrioid borderline ovarian tumor

Patient no	Age (years)	Concomitant endometriosis	Concomitant endometrial pathology
3	50	Negative	Negative
6	29	Positive	Negative
7	30	Negative	Negative
8	50	Positive	Negative
9	42	Negative	Endometrial carcinoma
10	52	Negative	Negative
11	42	Negative	Negative
12	63	Negative	Negative
13	42	Positive	Negative
14	47	Negative	Endometrial intraepithelial neoplasia

The clinical presentation of the disease and the disease symptoms are not specific, similar to the other subtypes of BOT. Pelvic masses are found in 70% of patients, and abdominal pain and distention is the main symptom in 20% of cases (20). Concomitant endometrial pathologies in the endometrioid subtype of BOT can lead to symptoms such as menorrhagia and menstrual irregularities. In the present study, the main symptom was menstrual irregularities in 33% of patients (n=3/9).

The endometrioid subtype of BOT occurs mostly as a unilateral tumor (16,18,20); however, bilateral tumors account for 3-9% of cases. The bilaterality rate was 11% in our patients (n=1/9). A small number of tumors of the endometrioid subtype of BOT have been reported after conservative surgeries. The standard management of the endometrioid subtype of BOT is bilateral salpingo-oophorectomy with hysterectomy and peritoneal staging surgery. Snyder et al. (21) reported that no recurrence occurred in four patients who underwent conservative surgeries. Uzan et al. (18) performed conservative surgeries in seven patients in their series (5 unilateral salpingo-oophorectomies and 2 unilateral cystectomies) and they reported that tumor recurred twice in one patient who underwent a unilateral salpingo-oophorectomy. In our study, no recurrence was observed during the follow-up period of 80 and 120 months, respectively, in two patients in whom conservative surgeries were performed.

Borderline Brenner tumors

Brenner tumor or the transitional cell subtype of BOT occurs rarely. It is defined as a transitional-cell tumor composed of urothelial cells arranged in solid cystic groups in the fibrous stroma. Less than 3-5% of these tumors are of borderline or invasive type (5). It is reported in the literature that 95% of Brenner tumors are benign, 3-4% are borderline, and 1% are malignant (22). Borderline Brenner tumors usually originate from benign Brenner tumors and often present with co-existing borderline and benign components. Until 2012, approximately 30 borderline Brenner tumors were reported in the literature (22).

Uzan et al. (22) reported that all 10 patients in their study had unilateral tumors, with the disease being limited to one ovary (stage 1). One of the 5 patients, who was followed-up regularly, was reported to have recurrence and died (22). The Brenner tumor in the patient in our study was a 70-year-old woman with postmenopausal bleeding. An endometrial biopsy revealed a simple atypical hyperplasia after the pathologic examination and her pre-operative Ca-125 level was found as 35 IU/

mL. A total abdominal hysterectomy and bilateral salpingo-oophorectomy was performed in this patient who had a mass of 10 cm originating from the left ovary. The frozen section evaluation revealed a BOT; however, without a definition of the tumor subtype. The final pathologic examination reported a borderline Brenner tumor and the stage was determined as IA.

Seromucinous borderline tumors

The seromucinous subtype of BOT is known as an atypical proliferative serous neoplasm and was previously described as an endocervical type mucinous BOT or as a mullerian mucinous BOT. It is classified as a separate subtype in the 2014 WHO classification (5). It accounts for approximately 5-7% of all BOT subtypes (2). Unlike other endometriosis-related tumors, seromucinous BOT is highy related with benign seromucinous tumours and seromucinous carcinomas. The seromucinous BOT has been suggested to be composed of atypical endometrioid cysts showing mucinous differentiation (23).

The seromucinous subtype of BOT usually arises in young women (age 34-44 years) (24). Bilateral tumors occur in 40% of cases and 20% have peritoneal implantations and lymph node involvements (25). Approximately 30-70% of patients with seromucinous BOT have endometriosis (5).

Prognosis is good even in the presence of extraovarian involvement. There were 2 patients with this subtype in our study and these patients were respectively aged 22 and 45 years. Conservative surgery was performed in the 22-year-old patient and definitive surgery was performed in the 45-year-old patient. Staging surgeries were performed in both patients during the primary surgery. Both patients were determined to be at stage 1C. No adjuvant treatments were given to these patients. There was no evidence of endometriosis-related pathologies or malignancies in either patient. No recurrence occurred during the follow-up periods of 19 and 12 months, respectively. The intra-operative consultation with the pathology department resulted in subtyping the tumor as a mucinous BOT in the young patient and as a serous BOT in the older patient.

Clear cell borderline tumors

The clear cell BOT subtype accounts for less than 1% of all BOTs and is usually detected in the 59-68 years age range. Most clear cell BOTs are unilateral (5). The disease is usually staged 1 at the time of diagnosis. In this subtype, the prognosis is generally good; therefore, conservative treatment can be offered to selected patients. However, the endometrium should be evaluated with endometrial sampling.

The non-serous/non-mucinous subtypes of BOT present with good prognostic outcomes. However, endometrial pathologies should be screened in the endometrioid subtype. Although the common subtypes of BOT are well-examined, the data are limited for the rare subtypes, usually being reported as case series. Like the frequent borderline ovarian tumours, the uncommon subtypes represent different biologic behavioral patterns and their malignant potential is still uncertain.

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Double balloon catheters: A promising tool for induction of labor in multiparous women with unfavorable cervices

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Abstract

Objective: To compare the effectiveness and safety of oxytocin and a cervical ripening balloon in women with unfavorable cervices for inducing labor.

Material and Methods: A total of eighty pregnant women between 37-41 gestational weeks having singleton pregnancies and intact membranes with unfavorable cervices were randomized into two groups, cervical ripening balloon (n=40) and oxytocin infusion (n=40). The primary outcomes were the labor time and the route of delivery. Secondary outcomes were the effect of parity on time of labor, and obstetric and perinatal outcomes.

Results: The median time to delivery was 9.45 hours in cervical ripening balloon group and 13.2 hours in the oxytocin group in multiparous women. The differences were statistically significant ($p<0.001$). The median time until delivery was 11.48 hours in cervical ripening balloon group and 13.46 hours in the oxytocin group; the differences were statistically significant ($p<0.001$). Cesarean delivery ratios were similar in both groups ($p=0.431$).

Conclusion: The results of the present study are promising for balloon use, especially in multiparous women. It is beneficial to support these data with wide ranging population-based studies. (J Turk Ger Gynecol Assoc 2019; 20: 231-5)

Keywords: Double-balloon catheter, labor induction, unfavorable cervix

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Introduction

The mechanisms of birth and the initiating factors of labor are still obscure. The main goal of obstetrics is the health of both the mother and the baby. The management of the labor process is an important part of obstetric practice. Although labor initiates spontaneously in a vast majority of pregnancies, induction of labor (IOL) may be needed in a wide variety of conditions including post-term pregnancies, preterm premature membrane rupture, preeclampsia, eclampsia, hemolysis, elevated liver enzyme levels, and low platelet levels-HELLP syndrome, fetal demise, maternal diabetes mellitus, fetal distress, maternal cholestasis, and chorioamnionitis. In such situations, an unfavorable uterine cervix is one of the major obstacles to successful IOL (1).

The uterine cervix retains its physical integrity by remaining firm during pregnancy until the beginning of labor. Just before labor, the cervix softens and becomes more distensible; this process is called cervical ripening. However, if induction is indicated before onset of spontaneous labor, cervical conditions come to the fore for a successful delivery. The Bishop scoring system is commonly used for evaluation of the cervix (2,3). In general, when the Bishop score is less than six, the success rate of IOL is poor (3-5). Unfortunately, nearly 50% of women with an indication of IOL have an unfavorable cervix with a Bishop score of less than six (2).

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Oxytocin is one of the commonly preferred pharmacologic methods used for inducing labor (6). However, systemic adverse effects including tachycardia, flushing, and uterine tachysystole are the disadvantages experienced during its infusion. The primary effect of oxytocin is on myometrial contractility and the secondary effect is on cervical ripening in interactions with steroid hormones, prostaglandins (PGs), and probably nitrite oxide (NO) (7,8).

Alternatively, a cervical ripening balloon is used for IOL. Such balloons may be speculated to cause fewer systemic adverse effects because of their local and limited effect and because they are mechanical in nature (9). On the other hand, high cost and application difficulties are some of the disadvantages experienced when these devices are preferred for IOL. The proposed mechanism of the cervical ripening balloon during labor induction is apparently mechanical, but interestingly, cervical ripening is also achieved pharmacologically via the Ferguson reflex. This is a neuroendocrine reflex and has as its afferent limb the sensory nerves from the vagina and cervix (Franken Hauser nerves), the ascending somatosensory pathways in the spinal cord (the anterolateral columns) and an incompletely described projection through the brainstem and medial forebrain bundle that ultimately reaches the hypothalamic magnocellular nuclei. The efferent limb of the reflex is the blood-borne carriage from the paraventricular nucleus and supraoptic nucleus of oxytocin. Oxytocin secretion is a consequence of this neuroendocrine reflex induced by tactile stimulation of the reproductive tract (9).

Although both procedures are implemented in different ways, both oxytocin and cervical ripening balloons have similar mechanisms in terms of their molecular action (8,9). Hypothetically, a cervical ripening balloon might have lesser adverse effects during IOL as compared with oxytocin because of having a closer physiologic mechanism to oxytocin secretion (10,11). In this study, we aimed to compare the effectiveness and safety of oxytocin (Synpitan®) and cervical ripening balloons (Cook®) for IOL in unfavorable cervices.

Material and Methods

This prospective randomized study was conducted at a tertiary care hospital. Ethics committee approval was obtained (approval number 154-4958/2009). After giving informed consent, patients were assigned to one of the induction methods according to a computer-generated randomized list. A total of 80 pregnant women with singleton pregnancies between 37 and 41 gestational weeks and intact membranes with unfavorable cervices were randomized and assigned to either the cervical ripening balloon group (n=40) or the oxytocin infusion (n=40) group. A visual analogue scale (VAS) was used to determine pain perception in both groups. All participants rated the pain using the VAS during the labor process (in the VAS scale, pain is rated from zero to ten in which zero indicates no pain and ten the worst possible pain).

The presence of vaginal bleeding, pregnancies from in vitro fertilization, non-cephalic presentation, multi-fetal pregnancies, prior uterine or cesarean surgery, an estimated fetal weight over 4500 grams, and obstetric complications (hypertensive disorders, post-term pregnancies or fetal growth disorders) were the exclusion criteria for labor induction.

Oxytocin infusion

Administration started with ten units of oxytocin per 1000 mL 0.9% saline with a rate of 10 mU/min adjusted by infusion pump. The infusion rate was increased by 1 mU/min every 15 minutes, until three strong uterine contractions were achieved in a one-minute period (200-250 Montevideo units). The maximum dosage was set as 60 mU/minutes. All cases were monitored for fetal heart rate and uterine contractions until delivery.

Cervical ripening balloon insertion

All patients were placed in the lithotomy position. The balloon was inserted using ring forceps passing through the internal cervical ostium and into the extra-amniotic space. The uterine part of the balloon was inflated with 40 mL saline and pulled back to the level of the internal ostium and the vaginal part of the balloon was inflated with 20 mL saline. The position of the balloon was checked. Once the correct position was established, both balloons were inflated with up to 80 mL. All patients were monitored by means of fetal heart rate and uterine contractions until delivery.

The groups were compared with respect to the interval between the time of induction and delivery, the amnion membrane rupture time, pain perception during labor, the amount of hemorrhage after delivery, the route of delivery, and the first and fifth minute APGAR scores. The primary outcomes were the induction to delivery interval and the cesarean ratios. Secondary outcomes included the effect of parity on the time to the onset of labor, in addition to obstetric and perinatal outcomes.

Statistical analysis

After performing normalization tests on parametric data, Student's *t*-test was used for data with normal distribution. The Mann-Whitney U test was used for data with non-normal distribution. Non-parametric values were analyzed using the chi-square test. IBM statistics SPSS 20.0 software was used for statistical analysis. P values <0.005 were considered statistically significant.

Results

The study was conducted at a tertiary care hospital in the department of obstetrics and gynecology. A total of 80 pregnant women aged between 22 and 29 years were enrolled. The mean ages were 24.75±3.57 years in the oxytocin group and 25.13±3.84 years in the balloon group. The demographic parameters of both groups were similar (Table 1). The cesarean ratio in the oxytocin group was 20% (eight out of 40 women) and in the balloon group it was 27.5% (11 out of 40 women). The cesarean ratios were similar between the groups (p=0.431) (Table 1). The mean time until delivery was 13.46 hours in the oxytocin group and 11.48 hours in the cervical ripening balloon group; the differences were statistically significant (p<0.001). In the multiparous women, the median time until delivery was 13.2 hours in the oxytocin group and 9.45 hours in the cervical ripening balloon group; the differences were statistically significant (p<0.001) (Table 2, 3). The estimated fetal weight and APGAR scores of the newborns were similar in both groups. The mean hemoglobin changes from the pre-partum to post-partum period were 1.7 g/dL; the hemoglobin changes were not statistically significant (p=0.884). Pain perception scores were higher in the oxytocin group and this change was statistically significant (p<0.001). Neonatal intensive care hospitalization was needed for 10% (four out of 40 babies) in the oxytocin group and 2.5% (one out of 40 babies) in the balloon group. The difference was not statistically significant (p=0.359). The mean time to amnion membrane rupture was 10.9 hours in the oxytocin group and 9.6 hours in the balloon group. This difference was statistically significant (p=0.019) (Table 4).

Table 1. Patient characteristics descriptive statistics were performed. The mean and median values are demonstrated. Standard deviation is marked with ± for the mean values. Minimum and maximum values were demonstrated for the median values with parenthesis

	Induction protocol		p value
	Balloon group	Oxytocin group	
Patients (n)	40	40	-
Age	25.13±3.84	24.75±3.57	0.879
BMI (kg/m^2)	28.07±3.25	27.94±4.37	0.751
Gravida	2 (1-4)	2 (1-4)	0.996
Parity	1 (0-3)	1 (0-3)	0.876
Gestational weeks	40.1±1.1	39.3±1.4	0.780
Vaginal delivery	32 (80%)	29 (72.5%)	0.481
Cesarean delivery	8 (20%)	11 (27.5)	
BMI: Body mass index			

Discussion

In this study, we compared the effects of oxytocin and cervical ripening balloons in term pregnancies with unfavorable cervices. The most commonly preferred induction agent is intravenous oxytocin infusion. Oxytocin is known as the hormone that starts uterine contractions. However, according to recent research, the effect of oxytocin for parturition is not limited to the initiation of uterine contractions, it is also effective in cervical ripening (8). The proposed mechanism of oxytocin for cervical ripening is the secretion of local NO and PGs (especially PG F2α) through oxytocin receptor action located at the amnion membrane and placental decidua (8). Similarly, cervical ripening balloons initiate secretion of oxytocin, NO and PGs (especially PG F2α) via the Ferguson reflex (9). At the molecular level, both induction methods may have similar mechanisms in IOL.

When comparing the two methods in respect to labor time, a shorter time interval from induction to delivery was observed in

Table 2. Mean time interval to delivery. Subgroup analyses were performed to observe time interval of latent phase and active phase. The results were statistically significant

	Induction of labor protocol		p value
	Balloon group	Oxytocin group	
Time interval to 6 cm dilatation	8.57±2.14	9.91±2.31	0.009*
Time interval from 6 cm dilatation to delivery	2.45±0.71	3.8±0.84	0.005*
Time interval to delivery	11.48±2.48	13.46±2.74	0.001*
*Statistically significant			

Table 3. Time interval to delivery according to parity

	Induction of labor protocol			
	Balloon group		Oxytocin group	
Parity	**Primiparous**	**Multiparous**	**Primiparous**	**Multiparous**
Time interval to delivery	12.4±1.6[a]	9.77±1.57[b]	12.4±1.59[a]	13.26±2.98[b]
[a]Comparing time interval to delivery in primiparous women (p=0.884); [b]Comparing time interval to delivery in multiparous women (p<0.001)				

Table 4. Statistical analysis was established using Students-t test. The mean and median values are demonstrated. Standard deviation is marked with ± for the mean values. Minimum and maximum values are demonstrated for the median values with parenthesis

	Induction protocol		p value
	Balloon group	Oxytocin group	
Bishop score	2 (1-3)	2 (1-3)	0.428
Apgar 1	8 (7-9)	8 (7-9)	0.464
Apgar 5	9 (8-10)	9 (8-10)	0.049
Fetal weight	3365±421	3317±417	0.667
Neonatal ICU* admission	1 (2.5%)	4 (10%)	0.359
Postpartum hemoglobin change	1.76±0.39	1.77±0.40	0.884
Time interval to amniotic membrane rupture	9.61±2.45	10.89±2.51	0.019**
Pain perception (VAS)	6.7±0.51	8.7±0.54	<0.001
Maternal/fetal infection	0	0	-

*Intensive care unit; **Statistically significant; ICU: Intensive care unit; VAS: Visual analogue scale

women induced with balloon catheters. In multiparous women induced with double-balloon catheters, the time interval to delivery was observed to be markedly shorter than in that experienced by the oxytocin group. A relatively high positioning of the fetal head in multiparous women may place insufficient pressure on the uterine cervix in the oxytocin group. The imitating effect of the fetal head provided by the balloon catheter may be the cause of this difference. In studies comparing cervical ripening balloons with dinoprostone (known as a good cervical ripening agent), the mean time interval from induction to delivery was found to be similar between groups (12-15). Pressure on the uterine cervix may be the major factor providing similar cervical changes with dinoprostone. Interestingly, in our study, the time interval to delivery in primiparous women was similar between both groups. Engagement of the fetal head with the pelvis may the determining factor affecting cervical ripening and the time interval to delivery. Some studies have argued that cervical ripening balloons could impede fetal head engagement with the birth canal and risk cord prolapse (16,17). Other recent studies found that cervical ripening balloons function like a pillow for the fetal head, owing to the ellipsoid and cylindrical nature of the balloon (18,19). In accordance with this idea, caesarean ratios were detected as being similar in both groups.

In a review published in 2008, increased maternal and fetal infection rates were reported as the result of balloon catheter use in labor induction (20). On the other hand, more recent studies found no risk of increased infection due to balloon catheter use (21,22). The results of the present study support these latter studies.

Perception of pain is one of the troublesome adverse effects of catheter insertion. Contrary to our expectations, pain perception in the balloon group was lower as compared with the oxytocin group. There can be several factors affecting this result; perhaps the lack of a need for traction with double-balloon catheter use or the physiologic secretion of oxytocin ensured tolerable pain during labor. However, as the balloon application is a newly preferred method for inducing labor, more frequent patient contact could be achieved as a result of the desire to manage possible problems that might hinder the induction process. This increased attention can cause bias in patients such that they felt more comfortable and safer as compared with those in the oxytocin group.

In the present study, our priority was to compare easily obtained, preserved, applied, and cost-effective methods for the IOL in unfavorable cervices. Dinoprostone could be a good alternative for the study design, as it involves simplicity in application, but its high cost and the difficulties involved in its preservation ruled out its use in this study. However, misoprostol is a good alternative for the study design because it provides cost effectiveness, ease of application, and preservation conditions. Unfortunately, misoprostol is not licensed for use in labor induction in our country. Another limitation of the study was our relative inexperience with the balloon in comparison with oxytocin for labor induction.

In summary, the argument regarding labor induction in unfavorable cervices is still continuing. The aim of the this study was to compare our routine labor induction procedure (oxytocin) with the new cervical ripening balloon catheters in women with unfavorable cervices. Similar cesarean ratios were observed in both groups. The balloon catheters provided a shorter time interval to delivery and lower pain perception during labor, especially in multiparous women. Post-partum hemorrhage, APGAR scores, and fetal and maternal outcomes were similar in both groups.

Oxytocin is still a safe, effective, easily applicable, easily preserved, and cost-effective method for labor induction. However, the results of our study are promising for balloon use, especially in multiparous women. The main problems with cervical ripening balloons are the involved cost and application difficulties. By manufacturing reusable catheters, the cost problem can be solved; application difficulties can be resolved by increasing application frequency. The latter situation is bound up with cost effectiveness. Once these disadvantages are resolved, cervical ripening balloons may become a good alternative to oxytocin for inducing labor, especially in multiparous women. It would be beneficial to support these data with large-scale population-based studies.

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Oocyte donors' awareness on donation procedure and risks: A call for developing guidelines for health tourism in oocyte donation programmes

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Abstract

Objective: In the recent years, oocyte donation programmes have widely spread worldwide becoming the drive of health tourism. In some countries, donation programmes are tightly regulated, whereas in others, the guidelines or regulations are not well defined. To evaluate donors' awareness of the donation programmes and the ethical consequences in enrolling these programmes.

Material and Methods: A detailed questionnaire-based survey was conducted to evaluate the donors' main drive to get involved in the donation programme and the donor's knowledge and awareness of risk factors.

Results: The majority of the donors (70%) were undergoing donation programmes for financial gains through compensation. The donors were especially not aware of the long-term medical risks and the possibility of identity exposure through genetic screening.

Conclusion: The main duty of health professionals is to counsel donors about the basic procedures and any possible problems they may face during the donation programmes. Reimbursement of oocyte donors is a slippery slope in oocyte donation programmes. High compensation may make women think that donation is a profession without considering possible risks. Furthermore, with the wider use of direct-to-consumer genetic testing, and genetic anonymity may be at risk, thus the donors have to be counselled properly. Therefore, in this era of health tourism, it is crucial to set up well-defined counselling bodies in all oocyte donation centres and enable donors to make an informed choice in becoming oocyte donors. (J Turk Ger Gynecol Assoc 2019; 20: 236-42)

Keywords: Oocyte donation, donor programme, donors, ethics

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Introduction

Third-party reproduction has become one of the widely used fertility treatments that involve use of gametes or embryos. With the improvements in oocyte cryopreservation techniques, a new era of health tourism has been initiated. The first oocyte donation was performed in 1983 in Austria and since then it has become a part of routine assisted reproductive technology (ART) treatments (1). Thousands of oocyte donations have been applied throughout the world resulting in thousands of births (2). The main drive of oocyte donations is the inability of females to get pregnant using their own gametes due to poor oocyte quality after several failed in vitro fertilization (IVF) attempts or low/absent ovarian reserve because of advanced maternal age or premature ovarian failure. Oocyte donation can also be offered to woman with a heritable genetic disease to prevent the transmission of the disorder to the next generation, though preimplantation genetic diagnosis is usually preferred with no history of infertility. Least commonly, oocyte donations can be offered to same-sex male couples in adjunct to surrogacy.

Reproductive cells, especially oocyte cells, are supplied by a limited number of donors, similar to other organ and

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tissue donations. Controlled ovarian hyperstimulation (COH) protocols have to be applied to all patients undergoing ART treatments. These protocols have been long revised and although more studies are being performed to enhance them, they are very standardised. However, there is always a worry that higher doses of drugs may be used to obtain more oocytes from the donors because the number of patients seeking donor oocytes is always higher than the number of donors. The short- and long-term medical risks of COH have been investigated with a limited number of studies. These studies have suggested that there is an increased risk of early menopause and ovarian cancers (3). Furthermore, donors may undergo multiple COH cycles, especially in countries where there are no regulations.

The oocyte donation process is considered to be a slippery slope because it does not benefit the donor directly (4-7). The majority of oocyte banks provide financial compensation that raises substantial ethical concerns on the quality of informed consent (8) with the exploitation of oocyte donors (9-11). There has always been considerable concern on the undue inducement that affects the judgement of the women's ability to rationalise and evaluate the burdens and risks of participating in the oocyte donation programme (12). There have been long-running discussions regarding females with low socioeconomic status being exploited by donation programmes (13). One of the other concerning areas in the donation programmes is the sufficiency of counselling provided to the donors.

In addition to these objective and subjective matters of oocyte donations, concerns of elimination of anonymity have arisen due to the direct to consumer (DTC) genetic testing. Throughout the world, oocyte banks have been established with stored donor oocytes of mostly anonymous donations (2). In recent years, more and more people are taking DTC genetic testing without even consulting a physician. Mostly, individuals are taking these tests to uncover their ancestry or to learn about their possible health issues (14). However, a number of cases have been reported where the child is seeking the biologic parent using the results of DTC genetic testing (1,15). Furthermore, we are entering into the era of personalised medicine, where genomic databases storing patients' information are being formed to provide better individualised medical care. A person's genome can be sequenced even before they are born. It is still not exactly clear as to whom this data will be available and under what circumstances. However, it is highly unrealistic to expect genomic anonymity in this genomic era.

Although in some countries the donation programmes are tightly regulated; in some, there are no strict guidelines or regulations (1). This introduces many issues in such countries where oocyte donations become the drive of health tourism. A scarce number of studies have investigated this; however, many factors, such as the demographics, education level, and socioeconomic status, may alter the reasons of the involvement in oocyte donation programmes.

The aim of this study was to evaluate the ethical aspects of oocyte donations, the counselling services provided to donors, and donors' awareness of the consequences in undergoing donation programmes. More specifically; we intended to determine the counselling quality before the treatment, and the depth of the information provided by health specialists. We aimed to investigate the donors' knowledge regarding the fate of the donated oocytes, the short- and long-term medical risks, and the ethical implications of the donation. Furthermore, we investigated the reasons for the donors to get involved in the oocyte donation programmes.

Material and Methods

Ethical approval

A total of 50 donors volunteered for the study. Ethical approval was granted by the Near East University Ethics Committee (Project number: YDU/2018/58-604) prior to commencement of the study and informed consent was obtained from all participants. A questionnaire-based survey was used as an evaluation method. A thorough literature review was performed to prepare the questionnaire using a Likert scale (Appendix 1). The questions were mainly focused on the evaluation of the reasons for involvement in the donation programme, donor's awareness, and knowledge of risk factors and genetic screening.

Study population

The participation was anonymous and voluntary. The women included in this study were recruited as oocyte donors in a private IVF clinic as part of a donation programme. They had to meet specific requirements to become a donor and these requirements also determined their suitability to be included in this study. Donors had to be aged between 18 and 32 years and they had to be screened negative for sexually transmitted infections including human immunodeficiency virus, cytomegalovirus, and hepatitis B and C. They had to have a normal physical and gynaecologic examination and no familial history of congenital malformations or hereditary diseases. A good physical and mental health were also required to be an eligible donor. The potential donors meeting these requirements were assessed by the gynaecologist and counselled by the same gynaecologist and/or IVF nurse.

Subjective and objective questionnaires

The questionnaire was presented to the participants after they had been through the oocyte collection procedure and were fully recovered from anaesthesia. The survey was performed

by an experienced nurse and/or an experienced embryologist. The demographic characteristics were reported for all the donors. These included age, level of education, marital status, reasons of involvement in the donation programme, and socioeconomic status. Subjective questionnaires were designed to interrogate the perceived understanding of the oocyte donations. These involved questions to rate how well the donors understood the oocyte donation process. The objective questionnaires involved interrogating the objective understanding, including the understanding of matters, such as "There is a risk that I can become pregnant naturally if I engage in a sexual relationship during the donation process" or "I can change my mind about donating my oocytes". Open-ended questions were also recorded to specify the motivation of the involvement in the donation programme and to identify what had driven them to donate their oocytes. The answers were mainly grouped as, financial gain, helping couples who cannot get pregnant using the female partner's oocytes, and other reasons, such as knowing the person who needed the oocytes.

Statistical analysis

Fisher's exact test was performed and a two-tailed p value of less than 0.05 was considered to be statistically significant.

Results

In this study, a total of 50 donors volunteered to perform the survey investigating the main drive in the involvement of oocyte donation programmes and the awareness of the procedure and risks. Our cohort of oocyte donors indicated that the main reason for donating their oocytes was due to financial reasons. Seventy percent (n=35) of the donors underwent this programme to benefit from financial gains, 22% (n=11) stated that they had always wanted to help someone going through infertility problems and were donating mainly for altruistic reasons. The remaining 8% (n=4) donated oocytes for other reasons or did not want to disclose their particular reason (Figure 1).

These donors were further questioned regarding the number donation programmes they were involved in. The majority of the donors (80%, n=40) had donated their oocytes multiple times. Overall, 12.5% (n=5) did not want to reveal the number of donations they had previously been through. Sixty-eight percent (n=38) of the donors had undergone an average of 4.76 previous oocyte donations ranging from 2 to 9 COH cycles. Twenty-one percent (n=7) of the donors further specified that they had donated oocytes in different ART centres.

The final part of this study investigated the knowledge of donors on: (i) the procedure before the initiation of donation, (ii) the procedure during/after donation, (iii) the fate of oocytes after donation, (iv) the short-term medical risks of donation, (v) the long-term medical risks of donation, and (vi) the ethical implications of donation, such as the possibility of identity disclosure through genetic testing. Overall, only 38% (n=19) of donors were fully aware of all the procedures, the fate of the oocytes, and the medical risks. Of these well-informed donors, only 4% (n=2) were first-time donors (Figure 2). More than half of the donors with previous experience in donations (57.5%, n=23) were not fully aware of what the procedures were. The average rate of knowledge among donors with previous donation history was 88% (n=35), where the average rate of knowledge of first-time donors was 71% (n=7). The least informed donor was a first-time donor with a knowledge rate of 29%. Donors were relatively well informed about the fate of the oocytes before and during the donation procedure. Overall, the donors were least informed about the long-term medical risks, in which 52% (n=26) stated that they had not been informed about these risks at all. Furthermore, they were not well informed about the short-term medical risks such as risks associated with anaesthesia, infection/bleeding after

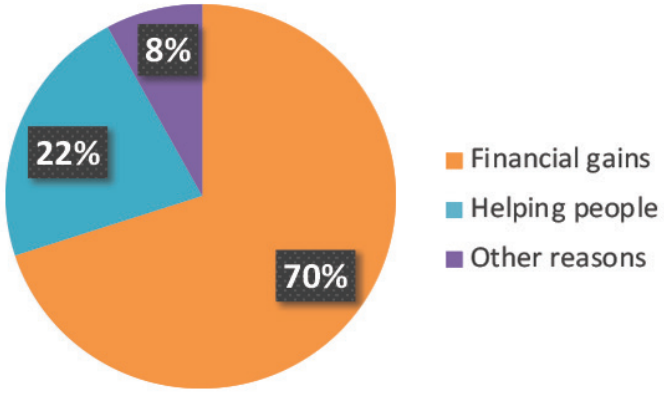

Figure 1. Pie chart representing the main reasons for donation in our cohort of oocyte donors

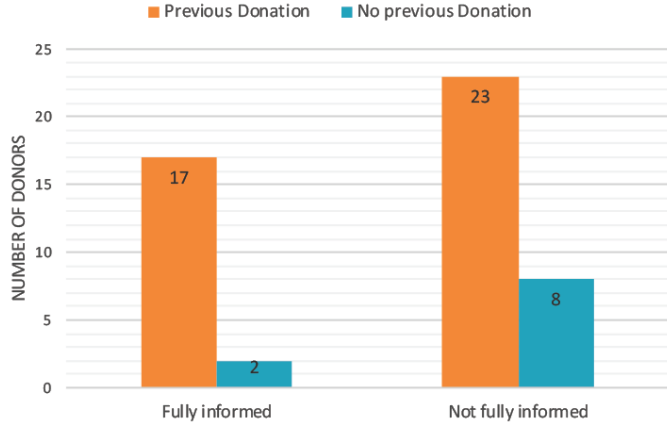

Figure 2. Bar chart representing counselling provided to the donors about the procedures and risks of donations

oocyte collection, and bruising from injections/withdrawal of blood. Twenty-four percent (n=12) of the donors also stated that they were not informed about the possibility of identification after genetic testing. All donors were aware of the risk of ovarian hyperstimulation syndrome (OHSS); however, only four percent were aware of the risk of pregnancy during treatment cycle in case of unprotected sexual intercourse.

To investigate the knowledge of the donors with previous donations, 2×2 contingency tables were formed. The association between previous donation and the awareness was statistically significant ($p<0.0001$) for the first four categories; (i) the procedure before initiation of donation, (ii) the procedure during/after donation, (iii) the fate of oocytes after donation, (iv) the short-term medical risks of donation. The association between previous donation and awareness of the possibility of identity disclosure through genetic testing was also statistically significant ($p<0.05$). However, donors with a previous donation history were equally as unaware as first-time donors about the long-term medical risks of donation ($p>0.05$, Figure 3).

Discussion

Ethical and psychological aspects of oocyte donation programmes present distinct challenges in reproductive medicine. Donating oocytes is risky in terms of the actual medical procedure of obtaining oocytes and in terms of the short- and long-term health risks that the procedure and the use of (repeated) supra-physiologic hormones might have on the donors. Moreover, donors are often faced with the ethical dilemma of how their reproductive cells might be used and will usually have no hereditary autonomy after the donation procedure. Depending on the demographics of women participating in donation programmes, the reasons of becoming a donor show variability. Although some donors volunteer to become a donor with no reimbursement, some receive different amounts of compensation introducing issues

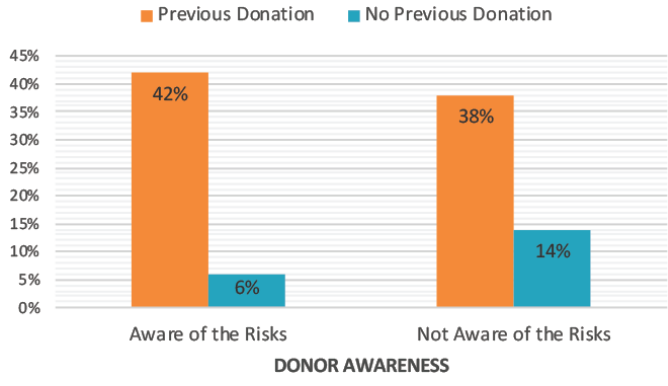

Figure 3. Bar chart representing the awareness of donors about the procedures and risks of donations

on donor exploitation. The aim of this study was to investigate the drive of becoming oocyte donors and to assess how well these donors were informed about the stages of the procedure and the associated risks.

One of the most important aspects of donation programmes is sufficient counselling. The results of this study showed that the counselling on the most basic procedure, possible short- and long-term medical risks were not reasonable in North Cyprus. This finding is particularly worrying because it implies that oocyte donors do not give true informed consent. This was in agreement with previously published studies, in which only 34% of the donors were aware of the COH procedure, only 20% were aware of the risk of bleeding or infection, and only 15% of ovarian torsion or damage (14). Therefore, it is crucial to develop counselling services to improve the subjective and objective understanding for the oocyte donors. Although counselling methods show variations in different ART clinics, they have the responsibility to ensure that donors are well informed about the procedures and the risks. One-on-one discussion was suggested to be the only intervention to improve donor perception on the subject (16). Therefore, it is a possibility that clinics may adapt their counselling programme with a one-on-one discussion. Another important part of counselling is to cover the concept of anonymity. Both the parents using the donated oocytes and the donors have to be fully informed that the DNA obtained from the children reveals information on the biological parents. The anonymity in the genomic era where DTC genetic testing is becoming more and more available will be eliminated rapidly. The challenges faced due to compensation combined with limited information given might make donating oocytes seem a risk-free, comfortable way of earning money.

One of the greatest societal and ethical concerns of oocyte donations is the amount of reimbursement to avoid donor exploitation. In most countries, there are strict criteria, governmental laws, and regulations for the performance of any kind of reproductive donation. A recent study conducted in the Netherlands reported that a typical donor was very well-educated, feminine, and a fellow who has agreed to make donations for altruistic reasons (13). None of the women who participated in the study reported that the earnings from the financial direction motivated them and did not show them as the reasons for the donation (13). Another multi-centre study reported that the majority of donors undergo the donation programme for altruistic reasons; however, the sociodemographics and motivation of donors vary hugely depending on the country of donation (9). On the contrary, financial motivation, especially as the compensation increases, has been reported to be the main drive of oocyte donors in the United States of America (USA) (9). Similarly, in this study, 70%

of the participants clearly stated that financial gains were their main reason for going through the donation programme.

One of the main reasons of the inconsistency of these motivations may be due to the differences in the socioeconomic status of the local donors in North Cyprus compared with other European countries where similar studies were conducted. This introduces critical ethical and psychological issues in countries like North Cyprus, which is considered to be a third world country. The greatest difficulties in such countries are the lack of robust, local, governmental regulations and/or enforcement of existing regulations on ART centres carrying out oocyte donation procedures. Therefore, especially not having a set limit on the reimbursement makes it very tempting for potential donors. On the other hand, even though reimbursement is controversial, it has been proposed that it is not realistic for women to go through the donation programme just based on altruistic basis (9,17). Even women with altruistic motivation, inconveniences such as transport to the ART clinic and taking time off work, should have a small financial compensation to encourage them to go through the donation process (9). Previously published studies reported that these donors with altruistic motivation were usually married and well-educated (17-20), whereas donors who go through the donation programme due to financial gains have variable demographics and tend to be single and younger (21-23). The mean age of the oocyte donors in this study was lower than reported in other studies (19,23-25), and they were mostly single students. Therefore, the demographics of the donors in North Cyprus are different compared with other parts of Europe.

Due to the differences of laws and regulations in each country, the results of these kinds of studies vary significantly (26). Throughout Europe and the USA, the legislation on anonymity or reimbursement vary reflecting the different drives of involvement in the oocyte donation programmes. There is a need of more studies to investigate the motivation and counselling in different countries to obtain a better insight to avoid donor exploitation. Our results showed that the donors were being informed selectively about certain risks and they gained knowledge with multiple donation cycles by experience. All donors were informed about OHSS because this is a very serious and life-threatening condition. However, fewer donors knew about the risk of (multiple/ectopic) pregnancy following unprotected sexual intercourse during the treatment cycle. Both of these are very serious conditions with short-term effects and the clinic would be in huge distress in the event that they happened. However, information provided about long-term medical risks, which might frighten the potential donor, were not provided sufficiently. Even though donors with a previous donation history were generally more informed, they were equally as uninformed as a first-time donor about the possible long-term consequences of donation. Therefore, a standardised counselling protocol is missing. Furthermore, there is a critical risk of donor exploitation, especially in third world countries where donors tend to have lower socioeconomic status and lower self-esteem. Therefore, it is advised to set up large-scale longitudinal studies to establish sufficient counselling services and set an amount for compensation for each country without the risk of donor exploitation.

Ethics Committee Approval: *Ethical approval is granted by Near East University Institutional Review Board, YDU/2018/59-605.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - P.T.; Design - P.T.; Supervision - P.T.; Materials - O.A.; Data Collection and/or Processing - P.T., O.A.; Analysis and/or Interpretation - P.T., O.A.; Writer - O.A., P.T.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

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Questionnaire for Oocyte Donors Appendix

1. Why would you like to be an egg donor?

a. I think it would be a rewarding experience for me
b. I always wanted to help someone with infertility difficulties
c. I think the process is exciting
d. I need the money
e. Other

2. Is this your first time as a donor?

a. Yes
b. No

If no, how many donation cycles were you involved in?
Was it in a different clinic?

.....
.....

3. Were you informed that you will be treated with fertility drugs to assist ovulation?

a. Yes
b. No

4. Were you informed that you will be monitored with ultrasound equipment?

a. Yes
b. No

5. Were you informed that you will have eggs removed surgically either by laparoscopic or ultrasound direct follicle aspiration?

a. Yes
b. No

6. Were you informed that the assisted reproductive technology medical staff will attempt to fertilize some or all of the donated eggs with sperm collected from the husband or with donated sperm?

a. Yes
b. No

7. Were you informed that the embryos produced will be transferred to the uterus of the female recipient?

a. Yes
b. No

8. Were you informed that you relinquish all claims to the eggs and any child that results from the use of eggs donated and from the moment of retrieval of the eggs, the eggs belong to the recipient and that the recipient has the sole and exclusive right to determine any medical procedures and treatment regarding the eggs?

a. Yes
b. No

9. Were you informed that physical examination will be conducted, including taking blood and other body fluids, as well as a test for exposure to the HIV (AIDS) virus, drug screening, genetic testing and psychologic screening for the purpose of giving the assisted reproductive technology medical staff sufficient information to determine whether you are an acceptable egg donor?

a. Yes
b. No

10. Your personal information is definitely not shared with the patients. Were you informed that there is a possibility that a genetic test may identify you as the biological mother of any children born as a result of donation?

a. Yes

b. No

Were you informed the risks of egg donations that may include:

11. Overstimulation of ovaries, which could result in a feeling of bloating or abdominal discomfort?

a. Yes

b. No

12. Risks associated with general anaesthesia if used in connection with egg retrieval?

a. Yes

b. No

13. Discomfort, infection and bleeding from laparoscopic or vaginal ultrasound recovery of eggs?

a. Yes

b. No

14. Pregnancy or multiple pregnancies resulting from having vaginal intercourse during the cycle if adequate contraception is not used?

a. Yes

b. No

15. Bruising from injections and withdrawal of blood?

a. Yes

b. No

16. There may be certain long-term risks associated with the use of fertility drugs. These risks include ovarian cyst formation or rupture, ovarian over-stimulation, possible increased risk for ovarian cancer?

a. Yes

b. No

The effect of cadaveric hands-on training model on surgical skills and confidence for transobturator tape surgery

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Abstract

Objective: To demonstrate the role of cadaveric hands-on training model on surgical skills and confidence levels of surgeons during transobturator tape (TOT) surgery.

Material and Methods: A pre-test and post-test evaluation to measure skills during the practice of the steps of TOT surgery was performed on a total of 15 postgraduate urologists and gynecologists during a urogynecologic cadaveric dissection course. The course was shaped with regard to theoretical lessons, full pelvic cadaveric dissection and TOT surgery on cadavers.

Results: Good handling of the TOT needle, identifying the right place for groin incision, adequate size of groin incision, identifying the right place for incision at the anterior vagina, dissection of bladder pillars from the vagina, identifying the right place at the vaginal foramina for TOT needle exit, and good placement of mesh were reviewed. The post-test scores were statistically significant for all parameters and also for self confidence level ($p<0.001$).

Conclusion: Cadaveric workshops are important landmarks of surgical education to improve surgical skills, and gain experience and confidence. (J Turk Ger Gynecol Assoc 2019; 20: 243-6)

Keywords: Cadaveric, pelvis, dissection, education, transobturator

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Introduction

Many residents and young surgeons attend to cadaveric dissection courses, which are in association with surgical anatomy and procedures, because detailed anatomic knowledge is essential for accurate practice in surgical procedures (1,2). Despite learning how to perform surgical procedures, the management of complications and related close anatomic landmarks are lacking in residency. However, it is not unusual to see cadaveric workshops as an integrated part of residency curricula in some gynaecology and obstetrics centres from the first year in residency (3,4).

Postgraduate courses highly focus on improving anatomic knowledge in surgical procedures, and physicians need a good knowledge of anatomy to manage patients intra-operatively and post-operatively. Cadaveric workshops have a beneficial status on the learning points of surgical procedures without any stress of the operation room (5).

Stress urinary incontinence (SUI) is highly common among pre and postmenopausal women, the prevalence is between 4% and 35% (6), and there are many abdominal and vaginal procedures to treat this problem. In general, abdominal procedures such as Burch's surgery require opening of the

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Retzius space, which is rich in vascular venous networks and complications may increase morbidity (7). However, a mid-urethral tension-free sling, transobturator tape (TOT) reduces surgical complications (8) and provides adequate support to the urethra in the event of increased abdominal pressure to prevent SUI.

This study aimed to analyse the effect of a cadaveric dissection course on the surgical competence of participants for TOT procedure with a pre/post-test.

Material and Methods

In total, 15 postgraduate physicians, urologists and gynaecologists, attended this urogynecologic cadaveric dissection course to gain adequate knowledge in pelvic reconstructive surgery and urogynecology. The faculty consists two urologists, one gynaecologist, and one anatomist. After theoretical lessons, a detailed pelvic dissection was performed with two fresh-frozen female cadavers and afterwards a pre/post-test, which measured 8 parameters. The points scale was from 0 (no success) to 5 (very good) and 3 physicians evaluated all participants. First, participants tried to perform a TOT procedure, then mentors showed the tips and tricks of the TOT procedure with step-by-step anatomic landmarks, the participants repeated the procedure and were evaluated for each described intervention (flowchart). One of the check points after the test the measurement of the confidence level of the participants for TOT procedures.

Statistical analysis

Statistical analyses were performed using the SPSS software version 21. Visual and analytical methods were used to investigate whether the variables were normally distributed. Median and minimum-maximum values were used to present non-normally distributed variables. The Wilcoxon test was used to compare the change in scores between the pre-test and post-test analysis. A p value of less than 0.05 was considered to show a statistically significant result.

Surgical technique

A 16-F Foley catheter was placed in the bladder, clamped, and tracked to localise the position of the urethra neck. Then, the clitoris was palpated and the parallel location at the genitofemoral fold was used for the groin incision, 0.5-1 cm in length. A mid-urethral vaginal incision 1-1.5 cm in length was performed and, with sharp and blunt dissections, the pubo-cervico-vaginal fascia was opened cranio-laterally until the superposed obturator foramina. After the bladder was removed from the operation field, a TOT needle was inserted from the groin incision (out-in technique) and two clicks was felt while getting into the lateral field of vagina. The pathway of the needle after the skin is the obturator externus muscle, obturator membrane, obturator internus muscle, endopelvic fascia, and vaginal incision. Afterwards, the needle was directed to the vaginal incision to exit. This intervention was performed bilaterally; the tape was laid in front of the urethra with a distance of a Metzenbaum scissor.

Results

Parameters that were identified to measure the ability for TOT procedure were: good handling of the TOT needle, identifying the right place for the groin incision, adequate size of groin incision, identifying the right place for incision at the anterior vagina, dissection of the bladder pillars from the vagina, identifying the right place at the vaginal foramina for TOT needle exit, and good placement of mesh. At the end, the participants self-evaluated their confidence level as per their situation before the demonstration by mentors and after the course. All parameters were detected as statistically significant ($p<0.05$) (Table 1).

Table 1. Pre/post-test median scores with minimum and maximum values

Parameters	Pre-test median score	Pre-test minimum-maximum values	Post-test median score	Post-test minimum-maximum values	p
Good handling of TOT needle	3	1-5	5	4-5	<0.001
Identifying the right place for groin incision	3	1-5	5	5-5	<0.001
Adequate size of groin incision	4	1-5	5	5-5	0.001
Identifying the right place for incision at the anterior vagina	3	1-5	5	5-5	<0.001
Dissection of bladder pillars from the vagina	2	1-5	4	4-5	<0.001
Identifying the right place at the vaginal foramina for TOT needle exit	2	1-5	5	4-5	<0.001
Good placement of mesh	3	1-5	5	4-5	<0.001
TOT procedure confidence level	3	2-5	5	4-5	<0.001

TOT: Transobturator tape

Discussion

TOT procedures are very commonly performed by gynaecologists and urologists to increase the quality of life of women due to SUI. It has quite low rates of complications and discomfort after surgery with a desirable improvement in symptoms (9,10). Despite the low complication rates, mesh erosion, sexual discomfort, urinary infection, post-surgery voiding dysfunction, and bladder injury are some complications that may occur after TOT procedures (11,12). Many surgeons need to gain practice and experience in this surgical procedure to feel confident in the operation room and perform better surgeries with increased patient satisfaction.

This study has an important corner, which is in conjunction with urogynecologic practice and anatomic cadaveric studies. It showed that proper surgical education at the cadaveric workshops improved the surgical skills for all steps of a procedure, with increased confidence levels for the operation room.

Surgical training needs a good knowledge of anatomy and clinical consideration for better surgery. In general, there are no formal post-graduate training centres in surgery. However, surgical anatomy education programs should be based on the need of physicians and course directors should plan the flow-chart of the training module according to deficits in surgical anatomy. Additionally, observation and one-to one dissection with mentors, and performing procedures under their guidance increases the basic and advanced anatomic knowledge of participants with clinical concordance (13).

A literature search revealed many anatomic cadaveric studies in the field of urogynecology. Smajda et al. (14) explored the pertinent anatomy during the blind pass of the needle for translevator posterior intravaginal slingplasty. Hubka et al. (15) assessed the tape position in the foramen obturatorum during transvaginal tape (TVT) ABBREVO technique and found no relation of TVT with the obturator nerve. Cadaveric studies were also used in the improvement of anatomic terminology, and the relationship between the external anal sphincter and the bulbocavernous muscle has been investigated previously (16). The functional role of anatomic structures, proximity of anatomic landmarks to the surgical field, and management of probable complications have also been discussed (17-19). Moreover, cadaveric dissection studies were compared with anatomic imaging and histologic studies of the human body to improve the detailed knowledge of anatomic structures, so far that will be used in the surgical technique.

This urogynecologic cadaveric dissection course aimed to make surgical procedures easier with cadaveric dissections. Detailed pelvic anatomy with self-practice of participants aimed to increase the topographic view of pelvic and nearby anatomic landmarks during pelvic and urogynecologic surgeries. For surgical approaches, many physicians find it beneficial to attend cadaveric workshops to improve surgical skills in an atmosphere of less stress. This course was planned to be held 3 times per year with 15 participants to improve skills in pelvic reconstructive and urogynecologic surgery.

All the steps of TOT procedure were analysed by mentors and mistakes in the practice of surgery were solved with self-practice. During these steps, close anatomic landmarks were discussed and identified with dissection of the pelvis, perineum, and obturator space. The axis of the trocar needle must be accurate and that needs a good level of experience because the path of the trocar needle is blind and it passes many tissues and fascia around the vessel and nerve structures. Otherwise, the obturator artery, obturator vein or obturator nerve be injured on the lateral pelvic wall, additionally the bladder may also be damaged. Movement of TOT needles were analysed and viewed from the abdominal incision to check probable complications by all participants; the proximity of obturator nerve and obturator foramina, the position of bladder, the location of vagina and endopelvic fascia were evaluated during vaginal dissection and TOT needle insertion (Figure 1). At the end of the course, all participants gained statistically significant surgical skills in performing TOT procedures and had increased surgical confidence levels.

All anatomic steps during TOT surgery (20), place of groin incision, place of vaginal incision, dissection of bladder pillars, TOT needle insertion, path of needle, and placement of mesh

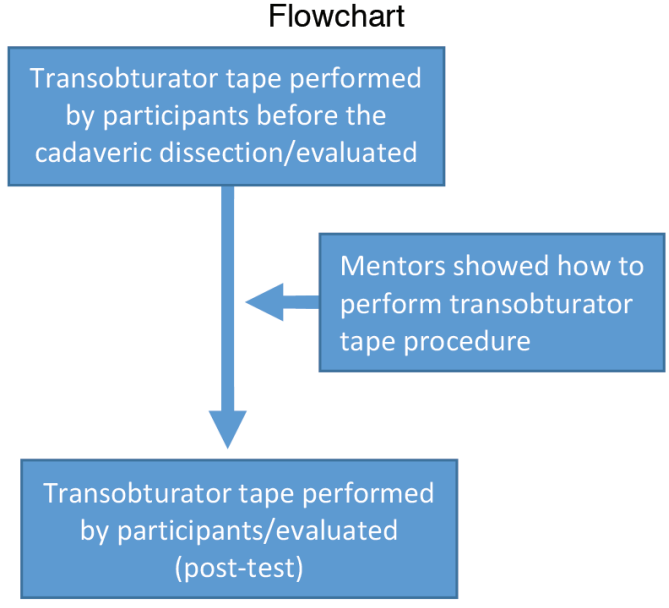

Figure 1. Right pelvic side wall, dissection of obturator space and close anatomic landmarks (star indicates the superposed location where the transobturator tape needle passes)

were found statistically significant. This infrastructure showed the importance of learning anatomic landmarks for a definitive practice of TOT surgery. Cadaveric courses increase the anatomic knowledge and technical skills, which yields a more confident state for surgeons.

As a conclusion, cadaveric workshops improve basic and advanced anatomic knowledge during the steps of transobturator tape procedure with improved skills and increased confidence status.

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The mammalian target of rapamycin protein expression in human granulosa cell tumors

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Abstract

Objective: To investigate the role of mammalian target of rapamycin (mTOR) in human granulosa cell ovarian tumors and the therapeutic effect of rapamycin in COV434 mitotic granulosa cell lines.

Material and Methods: A retrospective evaluation of the medical records and pathologic sections of patients with granulosa cell ovarian carcinoma was performed. mTOR and p-mTOR expression was immunohistochemically investigated. A *COV434* cell culture were treated with 0.5, 1, 2, and 5 μM rapamycin. Real-time growth curve analysis via xCELLigence system and apoptotic cell analysis via YO-PRO™-1 Iodide were performed to assess the therapeutic effect of rapamycin on cancer cells.

Results: A total of twenty patients were evaluated. mTOR staining was detected in 18 (90%) patients. Mild, moderate, intense, and very intense staining was observed in three (15%), eight (40%), six (30%), and one (5%) sample, respectively. The mean mTOR staining ratio was 59±41%. P-mTOR staining was observed in two (10%) patients. One (5%) patient had 5% staining, and one (5%) patient had 100% staining for p-mTOR. Both of the latter patients had very intense staining. Rapamycin caused a dose-dependent growth arrest and induced apoptosis in *COV434* mitotic granulosa cells. The real-time growth curves of the cells treated with these drugs were distinguished by a marked reduced slope after exposure for several hours, indicating a rapid onset of apoptosis. Live/dead cell analysis with YO-PRO-1 staining showed that rapamycin induced apoptosis in 24% of the cells when used at 1 μM concentration, whereas the rate increased to 61% and 72% when the cells were treated with 2 μM and 5 μM rapamycin, respectively.

Conclusion: mTOR expression is observed in various degrees in 90%, and p-mTOR expression is observed in only 10% of patients with granulosa cell ovarian carcinoma. Rapamycin caused a dose-dependent growth arrest and apoptosis in *COV434* mitotic granulosa cells. (J Turk Ger Gynecol Assoc 2019; 20: 247-54)

Keywords: Granulosa cell ovarian tumor, mTOR, rapamycin, ovarian cancer

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Introduction

Granulosa cell ovarian tumors constitute approximately 5% of all ovarian cancers. Contrary to the well-investigated epithelial ovarian tumors, there is very little known about the molecular and genetic changes in granulosa cell tumors. The studies are more or less focused on pathways playing a role in normal granulosa cell proliferation. The most important of these pathways is the follicle-stimulating hormone (FSH) pathway. Recently, a somatic missense mutation was revealed in the Forkhead box L2 *(FOXL2)* gene in 97% of adult-type granulosa cell tumors. Granulosa cell tumors are treated with surgery, adjuvant treatments (radiation or conventional chemotherapy), and hormone therapies (gonadotropin-releasing hormone

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antagonists, tamoxifen, and aromatase inhibitors). In metastatic or recurrent disease after primary surgical resection, adjuvant treatment is considered an option; however, the efficacy of systemic chemotherapy remains controversial. Based on our experiences on epithelial ovarian tumors, our first-line chemotherapy is platin-based chemotherapy. Agents such as doxorubicin, cyclophosphamide, vinblastin, bleomycin, and etoposide were combined with cisplatin, and the response rates were detected to be between 60-83% (1). With a better understanding of molecular and genetic features of granulosa cell ovarian tumors, treatment options will certainly increase.

mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase and is part of the phosphatidyl inositole-3-kinase (PI3K)/AKT signal pathway. It plays a critical role in cellular development, metabolism, and the cell cycle of cancer cells (2). Disturbances of the PI3K-dependent signalling pathway may lead to a variety of tumors including ovarian, endometrial, and cervical cancer. In clinical studies, first-line mTOR inhibitors were shown to have a promising clinical efficacy in ovarian and endometrial cancer (3). However, on a molecular level, the sensitivity or resistance rates of these agents are still unknown. At this point, we see that studies on the potential overactivity of mTOR pathway in granulosa cell ovarian tumors are scarce. In our study, we investigated the expression of mTOR and phospho-mTOR in granulosa cell ovarian tumor sections in order to better understand the molecular genetic features of granulosa cell ovarian tumors and determine whether mTOR inhibitors could be used in treatment. Moreover, we evaluated the possible therapeutic effects of rapamycin in *COV434* mitotic granulosa cell lines.

Material and Methods

The medical records and pathologic sections of patients who underwent surgery İstanbul University, Cerrahpaşa School of Medicine, Gynecologic Oncology Clinic between 1999 and 2011 and diagnosed as having granulosa cell ovarian tumor were evaluated retrospectively.

Our study was approved by İstanbul University, Cerrahpaşa School of Medicine, Medical Ethics Committee (Date: September 6th, 2011, No. 32476), and was supported by the İstanbul University, Scientific Research Fund of İstanbul University (Project number: 17384).

In our search of medical records and pathology archives, we detected paraffin sections of 20 patients with granulosa cell ovarian tumors.

Pathological evaluation

Serial sections of 3 microns were cut from paraffin blocks using a Thermo Scientific® microtome (MI, USA). The sections were incubated and dried at 80 °C for 20 minutes. Immunohistochemical staining was performed using streptavidine biotine with a fully automated Ventana Benchmark Ultra (Arizona, USA) immunohistochemistry staining instrument. Deparaffinization of the sections was completed by incubation at 72 °C for 8 minutes followed by washing with Ez Prep (Ventana, USA). In the next step, sections were incubated with CC1 (Ventana, USA) at 95 °C for 8 minutes for the antigen retrieval process. Sections were incubated with primary antibody mTOR (7C10) Rabbit mAb (1:50, Cell Signalling Technology) at 24 °C for 48 minutes. Phospho-mTOR (Ser2448) (49F9) Rabbit mAb (IHC Specific) (Cell Signaling Technology, USA). Normal antibody Diluent (Scytek, USA) was used for antibody dilution. After treatment with primary antibodies, sections were treated with Blocker A and B (Ventana, USA) for 4 minutes each. Sections were incubated with biotinated secondary antibodies (IView DAB Delection Kit, Ventana, USA) and streptoavidine conjugated horseradish peroxidase (IView DAB Delecton Kit, Ventana, USA) for 8 minutes.

Sections were incubated with diaminobenzidine (DAB, IView DAB Delection Kit, Ventana, USA) and mordant application was performed by Copper (DAB, IView DAB Delection Kit, Ventana, USA). In all washing steps, Reaction Buffer (Ventana, USA) was used. Negative staining was performed using hematoxylin II (Ventana, USA) for 12 minutes. Sections were washed in tap water followed by alcohol baths. Xylene was used to clear the sections followed by covering with Consul-Mount (Thermo Scientific, UK) coverslip medium.

Mammarian ductal carcinoma in situ sections were used for positive mTOR. Normal mammarian gland sections were used for positive P-mTOR.

Finally, sections were evaluated under a light microscope (Olympus BX 50, Olympus Corporation, Japan). M-TOR and P-mTOR expression were evaluated semiquantitatively; the staining extent was defined as the percentage of staining, and the staining intensity was defined as absent, weak, moderate, strong, and very strong. The scoring scales are represented in Figure 1A-F (percentage) and Figure 2A-D (intensity).

Cell culture

A human immortalized granulosa cell line (COV434) was maintained in Dulbecco's modified Eagle's medium: F12 supplemented with 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin *Amphotericin* B Solution (Gibco, 15240-062) at 37 °C with 5% CO_2. The cells were routinely harvested using trypsinization with 0.25% trypsin–EDTA, and counted using a hemocytometer and 0.4% trypan blue.

Real-time growth curve analysis via xCELLigence system

An xCELLigence System (ACEA Biosciences Inc. San Diego, CA, USA) was used according to manufacturer's instructions. In brief,

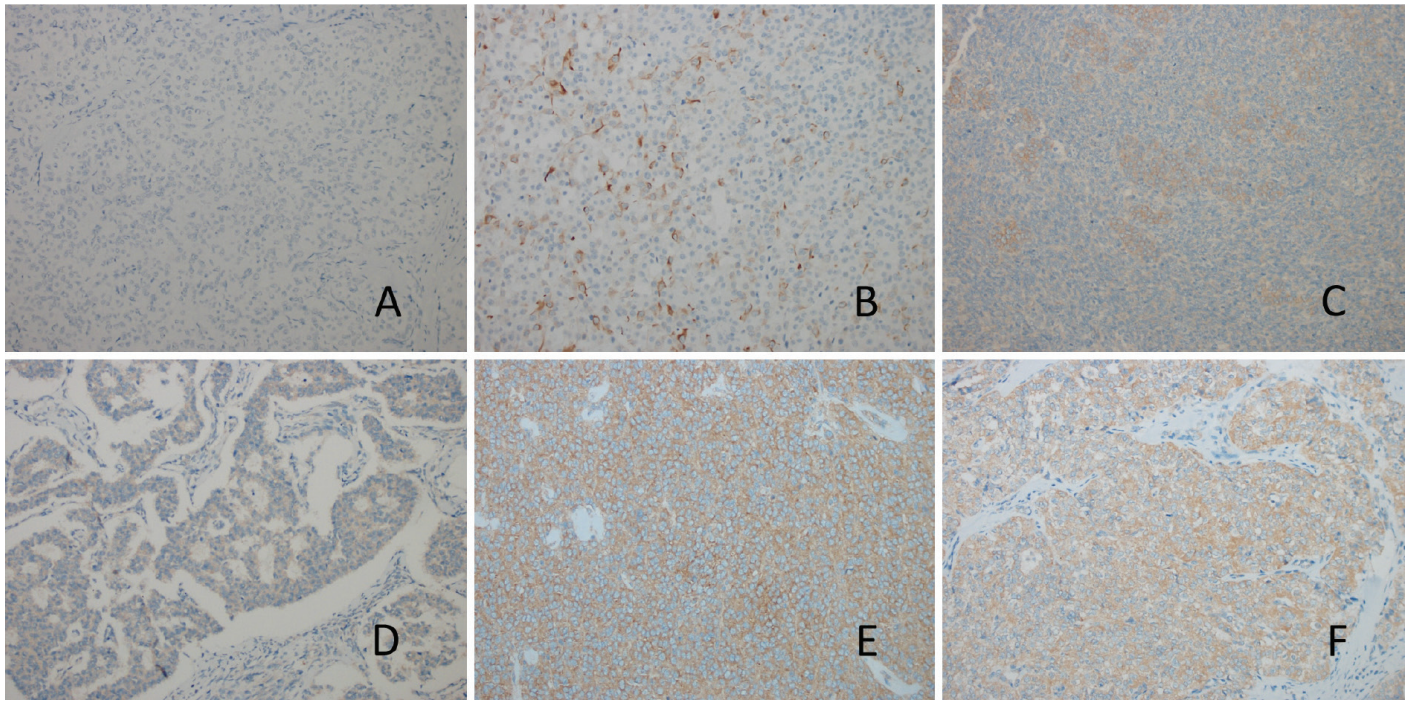

Figure 1A-F. Scoring scales representation as percentage. mTOR and p-mTOR expression were evaluated semiquantitatively; the "staining extent" was defined as the percentage of staining. (A) No staining (×200); (B) 5% staining (×200); (C) 30% staining (×200); (D) 50% staining (×200); (E) 90% staining (×200); (F) 100% staining (×200)
mTOR: Mammalian target of rapamycin

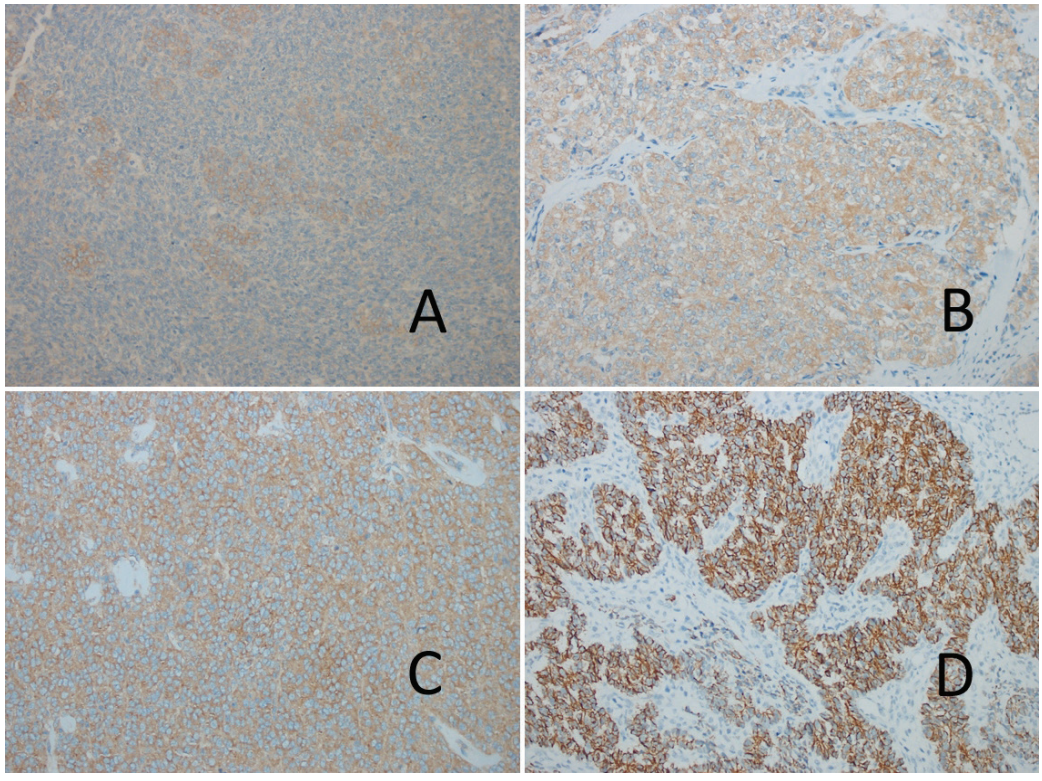

Figure 2A-D. Scoring scales representation as intensity. The "staining intensity" was defined as "absent, weak, moderate, strong, and very strong". (A) (+) staining; (B) (++) staining; (C) (+++) staining; (D) (++++) staining

100 μL of culture media was added to the each well, incubated at room temperature for 15 minutes, and the background impedance was measured. The trypsinized COV434 cells were centrifuged, resuspended in complete media, and seeded in a 96-well E-Plate at the density of 10.000 cells per well in a final volume of 200 μL. The cells were incubated at 37 °C with 5% CO_2, and continuously monitored on the real-time cell analysis (RTCA) system at 30 minute intervals. When they reached the log growth phase, they were treated with 0.5, 1, 2, and 5 μM concentrations of rapamycine. The effects of rapamycin on viability and proliferation of COV434 cells were monitored on the RTCA system for up to 200 h. The results are expressed as normalized cell index (CI), which was derived from the ratio of CIs before and after the addition of the compounds. Recording and normalization of CI were performed using the RTCA Software 1.2.

Apoptotic cell analysis via YO-PRO™-1 iodide

YO-PRO-1 is a carbocyanine nucleic acid stain used in identification apoptotic cells. Apoptotic cells become permeant to YO-PRO-1, whereas live cells are not stained with YO-PRO-1. Culture media of both the control and rapamycin-treated cells were aspirated and replaced with YO-PRO-1 containing culture media (1 μM). Hoechst 33342 was used as a counterstain. After 10 minutes of incubation at 37 °C with 5% CO_2, they were observed under appropriate channels using an IF microscope (Olympus IX71, Japan).

Statistical evaluation

Parametric variables are expressed as mean ± standard deviation, and non-parametric variables are expressed as median, minimum and maximum. Student's t-test and analysis of variance were used for the comparison of parametric variables, and the chi-square test was used to compare nonparametric variables. Pearson's correlation test was used for the evaluation of possible correlations between parametric variables, and Spearman's correlation test was used for the evaluation of possible correlations between non-parametric variables. The Statistical Package for the Social Sciences (11.0, Chicago, IL, USA) was used for statistical evaluations. $P<0.05$ was accepted as significant.

Results

A total of 20 patients with granulosa cell ovarian tumor were evaluated. The mean age was 46.05±11.5 (minimum: 26, maximum: 71). At the time of diagnosis, eleven (55%) patients were premenopausal and nine (45%) were post-menopausal. Eleven (55%) patients had stage 1a, five (25%) had stage 1c, one (5%) had stage 3b, and three (15%) had stage 3c disease. All patients had adult-type granulosa cell tumors. The mean tumor size was 92 mm ± 50 mm (minimum: 15 mm, maximum: 190 mm).

Necrosis was present in seven (35%) patients. The mitotic number was 1-4 in seven patients, 4-8 in eight patients, and more than 8 in three patients. Nuclear atypia was absent in one (5%) patient. Eight (40%) had mild, five (25%) had moderate, and two (10%) had severe atypia.

mTOR staining was not seen in two (10%) patients. Three (15%) patients had mild staining, eight (40%) patients had moderate, six (30%) had strong, and one (5%) patient had very strong staining. Mean mTOR staining percentage was 59±41 (minimum: 0, maximum: 100). mTOR statining features were given in Figure 3A-C.

There was no correlation between age and tumor size, mTOR staining percentage or intensity, p-mTOR staining percentage and staining. There was a positive correlation between mTOR staining percentage and staining intensity ($p<0.001$, $r=0.819$) (Table 1).

P-mTOR staining was not observed in 18 (90%) patients. One patient had 5% and the other one had 100% staining. Staining intensity was very strong in both patients. P-mTOR staining features are presented in Figure 4A, B.

The patient with 5% p-mTOR staining was a 26-year-old woman with stage 3c disease, 5 cm tumor size, widespread tumor implants, 19 mitoses in 10 high-power field (HPF), and high-

Table 1. Correlations between age, stage, tumor size, presence of atypia and necrosis, and mTOR staining percentage and intensity

	mTOR staining percentage		mTOR staining intensity	
	p	r	p	r
Age	0.750[a]	-0.076[a]	0.584[a]	-0.0130[a]
Stage	0.128[a]	0.352[a]	0.109[b]	0.370[b]
Tumor size	0.296[a]	-0.253[a]	0.094[a]	-0.395[a]
Presence of atypia	0.868[a]	0.043[a]	0.443[b]	0.199[b]
Presence of necrosis	0.550[a]	0.146[a]	0.367[b]	0.219[b]

[a]Pearson correlation test; $p<0.05$ is significant; [b]Spearman correlation test; $p<0.05$ is significant; r: Correlation coefficent; mTOR: Mammalian target of rapamycin

grade atypia. The patient with 100% p-mTOR staining was a 42-year-old woman with stage 1a disease, 12 cm tumor size, 2/10 HPF mitoses, and low-grade atypia. The low number of p-mTOR-positive cases rendered the statistical evaluation impossible.

The growth curve characteristics of cells treated with different doses of rapamycin were analyzed to observe cell proliferation/apoptosis rate. Compared with the untreated control group, rapamycin caused dose-dependent growth arrest and triggered apoptosis in *COV434* mitotic granulosa cells. The real-time growth curves of the cells treated with these drugs were distinguished by a marked descendent curve after exposure for several hours, indicating a rapid onset of apoptosis (Figure 5A, B).

To further validate the findings obtained from the xCELLigence system and confirm apoptotic death after exposure to rapamycin, live/dead cell analysis with YO-PRO-1 staining was perfomed.

Overall, rapamycin induced apoptosis in 24% of the cells when used at 1 μM concentration, whereas the rate increased to 61% and 72% when the cells were treated with at 2 μM and 5 μM concentration, respectively (Figure 6A, B).

Discussion

mTOR is abundant in cytoplasm, especially in the perinuclear area in normal granulosa cells (4). The kinase active serine 24-48 phosphorylated form of mTOR, in other words the active form, p-mTOR, is generally increased during the M phase of the cell cycle. P-mTOR is observed near mitotic spindles and around contractile circle during cytokinesis. Inhibition of mTOR by rapamycin causes a dose-dependent decrease in granulosa cell proliferation and follicular development *in vitro*. However, in the presence of rapamycin, follicles do not undergo atresia in cell cultures. Yaba et al. (4) performed a study on rat ovaries and detected that mTOR expression was increased in cytoplasm compared with nuclei; they also showed that inhibition of mTOR in primary granulosa cell culture was associated with cell death in G2/M stages of cell cycle. Contrarily, granulosa cells survived in the presence of rapamycin, although tissue size was decreased. Therefore, rapamycin does not seem to stimulate folicle atresia directly, but it regulates follicular growth by acting as a check point.

Yu et al. (5) evaluated mTOR expression in mouse ovaries, primary mouse granulosa cells, and spontaneously immortalized rat granulosa cell lines (SIGC). mTOR expression was best seen

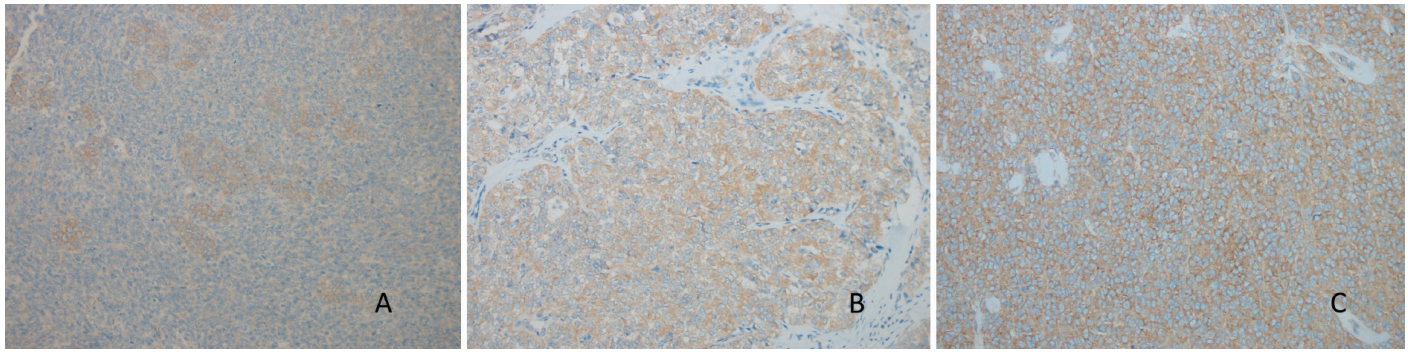

Figure 3A-C. (A) 30% (+) mTOR staining granulosa cell ovarian tumor (×200); (B) 100% (++) mTOR staining granulosa cell ovarian tumor (×200); (C) 90% (+++) mTOR staining granulosa cell ovarian tumor (×200)

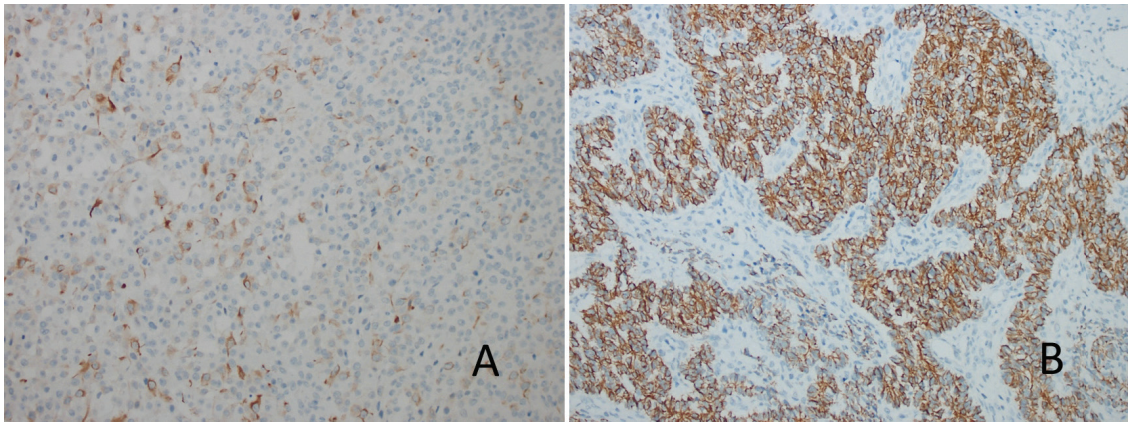

Figure 4A, B. (A) 5% (++++) f-mTOR staining granulosa cell ovarian tumor (×200); (B) 100% (++++) mTOR staining granulosa cell ovarian tumor (×200)

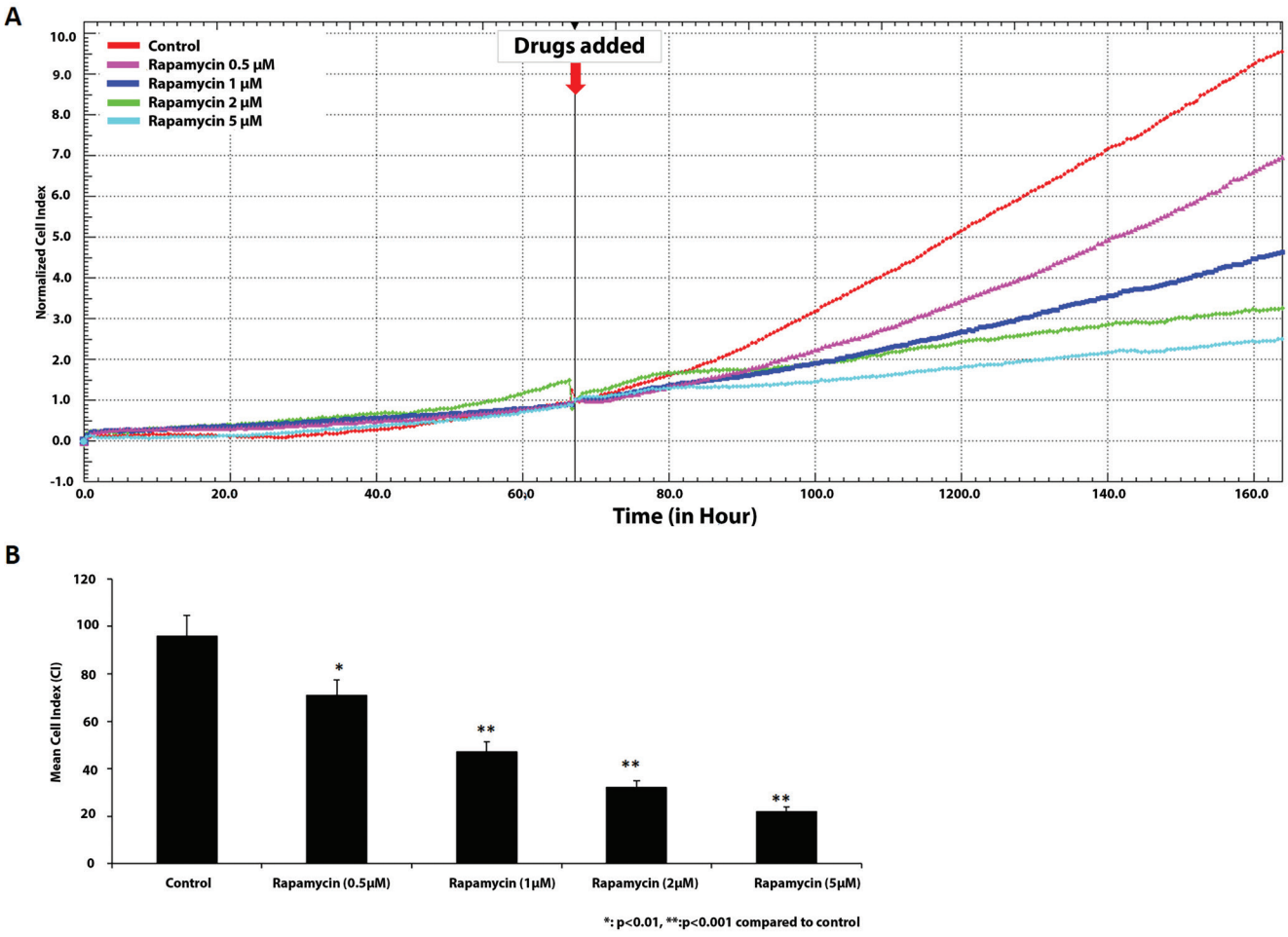

Figure 5A, B. The real-time growth curves of the cells treated with these drugs were distinguished by a marked descendent curve after exposure for several hours, indicating a rapid decrease of the cell proliferation. (A) The real-time growth curves of the cells treated with rapamycin. (B) Mean cell index according to the various rapamycin doses

in M phase in primary mouse granulosa cells and SIGC groups. In mouse granulosa cells, p-mTOR was detected to be increased in G2/M phase. In a recent study by Rico et al. (6), mTOR inhibition was shown to slow tumor development in a transgenic mouse model. In our study, mTOR staining was not seen in two (10%) patients, whereas three (15%) patients had mild, eight (40%) patients had moderate, six (30%) had strong, and one (5%) patient had very strong staining. P-mTOR staining was not observed in 18 (90%) patients. Detecting the stage of the cell cycle is possible in cell cultures but not in paraffinized sections. We cannot know for sure which stage of the cell cycle we observe when we stain sections for mTOR and p-mTOR. Therefore, this may be regarded as an inevitable confounding factor.

Although the role of mTOR and p-mTOR in the proliferation of granulosa cell and in vitro SIGC line is well-established, no rigorously validated immunohistochemical study or targeted therapy on human granulosa cell tumors has been reported to date.

The K-RAS oncogene is found in 48% of borderline and serous ovarian tumors (7,8). Stable transfection with H-RAS and other oncogenes may be used to immortalize granulosa cells (9). However, because RAS mutations have never been reported in ovarian granulosa cell tumors, the immortalized granulosa cell ovarian tumors may not represent the real human granulosa cell tumor proliferation mechanism. mTOR and p-mTOR are known to play a role in normal granulosa cell development and proliferation, but this pathway is not the only one that maintains protein expression. During cancer development, pathways other than mTOR could be activated.

Abnormal hyperstimulation of the pathways and oncogenic signalling are not the only pathologic mechanisms in cancer cell survival. Follicular growth and differentiation include complex mechanisms from the primordial stage until full establishment of the corpus luteum; less than 0.1% of follicles succeed. Female fertility depends on a delicate balance between survival signals for maturing follicle cells and death

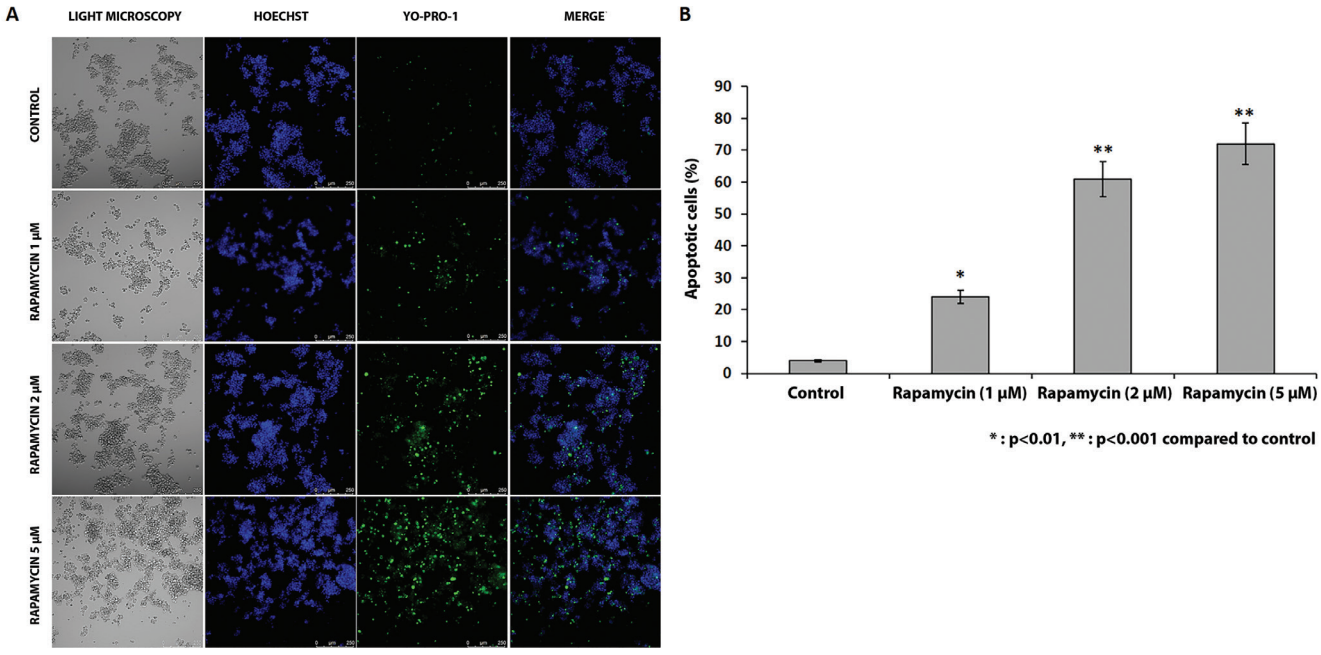

Figure 6A, B. Live/dead cell analysis with YO-PRO-1 staining was carried out to confirm the apoptotic death after exposure to rapamycin. Overall, rapamycin induced apoptosis in 24% of the cells when used at 1 µM concentration, whereas the rate increased to 61% and 72% when the cells were treated with at 2 µM and 5 µM concentration, respectively. (A) Live/dead cell analysis with YO-PRO-1 staining (B) Rate of apoptotic cells according to the various rapamycin doses

signals, leading the other to undergo atresia. For this reason, a disturbance in the apoptosis process may cause granulosa cell tumor development (1).

In cellular and molecular levels, ovarian cancer is known to be heterogenic. Altomare et al. (10) suggested that the PI3K pathway, which also includes mTOR, was active in 70% of all ovarian cancer types and its up-regulation was the main interfering factor in drug resistance. However, in this pathway, there are negative and positive feedback loops and alternative escape mechanisms interacting with the other pathways (11). For example, inhibition of mTORC1 by rapamycin leads to a short-term increase in mTORC2, which eventually increases the hyperstimulation of AKT. Hyperstimulation of AKT opposes the suppressive effect of mTOR inhibition. Besides, inhibition of mTORC1 leads to a loss of effective feedback of p70 and IRS-1 on each other. In addition to the PI3K and AKT pathways that are activated by FSH, there is an alternative MEK/ERK pathway activated by tyrosine kinases. There are cross-interactions between the above-mentioned pathways. Although normal granulosa cell proliferation depends on FSH signalling, proliferation may be accomplished without FSH in carcinoma cells.

In order to evaluate the mTOR pathway *in vitro*, the growth curve characteristics of cells treated with different doses of rapamycin were analyzed to observe mitosis/apoptosis rate. Compared with the untreated control group, rapamycin caused a dose-dependent growth arrest and apoptosis in COV434 mitotic granulosa cells. The real-time growth curves of the cells treated with these drugs were distinguished by a marked descendent curve after exposure for several hours, indicating a rapid onset of apoptosis.

To further validate these findings obtained using the xCELLigence system and confirm the apoptotic death after exposure to rapamycin, live/dead cell analysis with YO-PRO-1 staining was performed.

Overall, rapamycin induced apoptosis in 24% of cells when used at 1 µM concentration, whereas the rate increased to 61% and 72% when the cells were treated with at 2 µM and 5 µM concentration, respectively. These findings show that rapamycin may be a therapeutic option in vivo; however, further studies are needed to assess this hypothesis.

The main limitation of our study is the limited number of patients. Studies with a greater number of patients are needed to confirm the results of our study.

In conclusion, mTOR expression is observed in various degrees in 90% of patients with granulosa cell ovarian carcinoma, and p-mTOR expression is observed in only 10%. Rapamycin caused a dose-dependent growth arrest and induced apoptosis in COV434 mitotic granulosa cells, which was confirmed with live/dead cell analysis through YO-PRO-1 staining. There is a strong need for studies on expression of mTOR and p-mTOR in human ovarian granulosa cell tumor cultures.

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Polycystic ovarian syndrome: Environmental/ occupational, lifestyle factors; an overview

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Abstract

Polycystic ovary syndrome (PCOS) is a multifaceted disease of women with infertility that has diverse etiologic factors. Some women may have only a few PCOS-linked symptoms or mild symptoms, whereas others will have severe or all PCOS-linked symptoms. Therefore, PCOS symptoms can differ among women. PCOS is a state of hormonal imbalance, excess terminal hair (hirsutism), hair loss (alopecia), menstruation impairments, metabolic disorders, and cystic appearance on the ovaries. The cysts hamper ovulation, thus reducing the ability of women to become pregnant and result in infertility. The available data suggest that PCOS might originate in utero and the phenotypic appearance of PCOS symptoms may be developed in later life, which could be linked with host factors (endogenous) and exogenous factors like lifestyle, and dietary, environmental or occupational factors. Based upon the available information, it can be postulated that prenatal exposure to excessive androgens might be responsible for androgenization of the fetus, which in turn may alter the program of differentiating target tissues and the phenotypic characteristics of PCOS can be persuaded by exposure of female offspring to various endogenous and exogenous factors at later life. Genetic/ host and environmental/lifestyle factors might be related to the pathophysiology of PCOS after prenatal exposure to androgen. Additional studies are necessary to understand the exact mechanism responsible for the manifestation of PCOS because it is a very important issue in female reproduction. (J Turk Ger Gynecol Assoc 2019; 20: 255-63)

Keywords: Polycystic ovary syndrome, environmental, occupational, hyperandrogenism, anovulation, genetic factors, androgenization, infertility

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Introduction

Polycystic ovary syndrome (PCOS) is a problem of teenage girls or women in which their hormonal levels are impaired. It can cause problems in the menstruation cycle and difficulty in conceiving. In this disease, many small cysts develop in the ovaries of women, hence its name. PCOS is globally considered to be the foremost reason for infertility in women. PCOS is a multifaceted disease with various etiologic factors, which might be related with the disease or exaggerate the problem or induces PCOS phenotypic characteristics in women in adulthood. Owing to anovulation, it is the main cause of infertility and most common endocrine disorder of women. It affects the lives of women from *in utero* life to till death.

PCOS is also linked with several health hazards, which in turn elevate morbidity, impair quality of life, and increase mortality rate (1). The prevalence of PCOS differs because several diverse criteria are used for the diagnosis of PCOS by different investigators and diverse norms for diagnosis are also suggested by various organizations. It is an endocrine syndrome with menstruation irregularities, hyperandrogenism, and polycystic ovaries of women (2). Based upon the criteria of diagnosis for PCOS by the European Society for Human Reproduction & Embryology/American Society for Reproductive Medicine, the prevalence of PCOS is about 15-20% (2). Later, a meta-analysis was conducted by including studies that were published from 2006 to 2011 from Iran. The prevalence rate based on the criteria of the National Institute of Child Health and Human Disease

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of the United States was 6.8%, 19.5% based on Rotterdam norms, and 4.4% based on ultrasound criteria (3). About 5 to 10% of reproductive-aged women of Indian sub-continent are reported to be affected with PCOS (4).

Polycystic ovaries, chronic anovulation, and hyperandrogenism are the distinctive characteristics of PCOS and with the existence of insulin resistance, hyperinsulinemia, hypertension, abdominal obesity, and dyslipidemia are responsible for long-term serious outcomes such as endometrial hyperplasia, type 2 diabetes mellitus, and coronary artery disease (4). They also mentioned that it is only the interaction of environmental factors (obesity) with genetic factors that result in the appearance of PCOS, even though a woman may be genetically susceptible for the development of PCOS (4).

Many women may have PCOS illnesses and live with it without being diagnosed clinically. March et al. (5) drew attention to this important issue of PCOS diagnosis in the community and mentioned that about 68% of women with PCOS had not been tested for PCOS in the past. This showed the seriousness of the diagnostic problem of PCOS in the community. Further, the appearance of PCOS may also affect the mental health of the subjects, which might also be related to the psychiatric problems among such patients due to the difficulty in conceiving. The prevalence of depression and anxiety was 27.5% and 13.3%, respectively, in subjects with PCOS as compared with 3.0% and 2.0% among control subjects (6). Further, about 65-70% of women with PCOS had compensatory hyperinsulinemia and insulin resistance (7). In addition, an inherent ovarian defect (possibly genetically) existed in PCOS women, which makes the ovary vulnerable to insulin stimulation of androgen production. However, limited data/evidence also suggests hyperinsulinemia might stimulate androgen production in ovaries (8). Later, Baptiste et al. (9) described several steps for the appearance of PCOS: (a) enzymatic default in the ovarian and/or adrenal steroidogenesis; (b) variation in the gonadotropin-releasing hormone that encourages luteal hormone secretion; or (c) amendments in insulin actions leading to insulin resistance with compensatory hyperinsulinemia. Some women with the characteristic of PCOS do not show insulin resistance, this advocates the hypothesis of a genetic predisposition to PCOS. This would be exhibited by the progression of insulin resistance and compensatory hyperinsulinemia in the majority of women with PCOS, but not all women with PCOS (9).

In addition to the endogenous or host factors, it is also recognized that some factors such as chemical, physical, dietary, lifestyle, occupational, and environmental factors are accountable for hostile consequences on human male and female reproduction and they might also affect pregnancy and its outcome. Several reports and reviews on the role of occupational and environmental aspects on different aspects of human reproduction (10-14) and controlled experimental studies on certain environmental and lifestyle factors on reproductive health have been published (15-17) from this laboratory.

The hazard factors of PCOS comprised menstrual cycle impairment [odds ratio (OR)=5.8], bad mood (OR=2.8), family history of diabetes (OR=7.0), infertility in the family (OR=11.9), mother menstrual irregularity (OR=2.5) and physical exercise deficiency (OR=1.8) (18). The existing data on various environmental factors suggest their potential contribution in the etiology, prevalence, and modulation of the syndrome. There is evidence that advocates that environmental factors might play a significant role in deteriorating reproductive health and some environmental factors are vital behind the deterioration, including environmental toxins, diet and nutrition, socioeconomic status, and geography (19). Nevertheless, research/data on these environmental factors with reference to the causation of PCOS are limited or inconsistent and further well-planned studies are needed.

This overview is furnished based on available information on the role of occupational, environmental, and lifestyle factors in PCOS. The information was collected through searching various websites such as Google, PubMed, Medline, Toxline, and other websites and consulting related books. This overview is separated into various segments and the first section deals with the existing information on the recognized host/genetic factors associated with PCOS. The second and third sections deal with occupational/chemical exposures and lifestyle factors that might be associated with PCOS. In addition, some light is also shed on oxidative stress in the occurrence of PCOS. The majority of on-hand reviews on PCOS are available on the host/genetic factors rather than the role of both occupational/environmental toxicants exposure and lifestyle influences and PCOS.

In this review, importance has been given to parental environmental exposure and lifestyles factors in PCOS, covering mainly human studies. The possible etiologic factors related to the progression/development of PCOS are depicted in Figure 1.

Results and Discussion

The exact mechanism for the manifestation of PCOS is not yet completely understood. The syndrome appears to involve genetic, environmental, dietary, metabolic components. The origin of PCOS starts from early life in the mother's womb, extending throughout the lifecycle, and environmental insults and lifestyle issues may affect vulnerable women, leading to the occurrence of phenotypic characteristics of PCOS. Diet seems to be one of the foremost environmental determinants for the occurrence of PCOS. Hormone levels are imbalanced among women with PCOS. Generally, women with PCOS have

elevated male hormone (androgens) and lower levels of the female hormone (estrogen). High androgen concentrations can also have a significant impact on female reproductive development and function.

PCOS seems to be one of the ancient ailments that continued through human evolution (20). A report indicated that the anti-mullerian hormone (AMH) level was 2-3 three times higher in women with PCOS as compared with normal levels (10±2.2 ng/mL), and this high AMH level is a good indicator of infertility and PCOS (21). Further, women with PCOS are stated to have significantly higher levels of serum AMH as compared with controls, and the occurrence of negative and positive correlations with other hormonal parameters showed involvement of AMH, at least in part, in the manifestation and development of PCOS (22). The data available on serum levels of AMH in subjects with PCOS suggest its use as a diagnostic biomarker and it can serve as a reliable tool to describing the severity of the disorder, monitoring, and forecasting a prognosis of the diseases.

Recently it has been stated that AMH is raised and strongly associated with several reproductive, metabolic, and endocrine impairments in subjects with PCOS. AMH also has an inhibitory function in follicular growth and recruitment. The follicle-stimulating hormone (FSH)-induced aromatase production due to the preventive action of AMH probably contributes to hyperandrogenism, which further increases the insulin resistance in women with PCOS. In addition, elevation in serum AMH levels is extrapolative of poor treatment response in women with PCOS i.e. loss of weight, induction of ovulation, and laparoscopic ovarian drilling, whereas improvement in several other clinical parameters after treatment was related to declining serum AMH levels. This advocates a significant role of this hormone in the pathophysiology of PCOS (23).

The pregnancy-related difficulties were reported to be more frequent in women with PCOS. Various etiologic factors involved in PCOS and allied co-morbidities may also be connected to compromised pregnancy and/or its outcomes. A possible relationship between genetic, environmental, clinical and biochemical, and dietary factors is involved in the occurrence of this complex syndrome, with pregnancy complications and its outcome (24). In this overview, more emphasis has been given on lifestyle, occupational, and environmental issues in PCOS. However, some information on other factors is also incorporated to understand the overall possible causative factors/mechanism connected with PCOS.

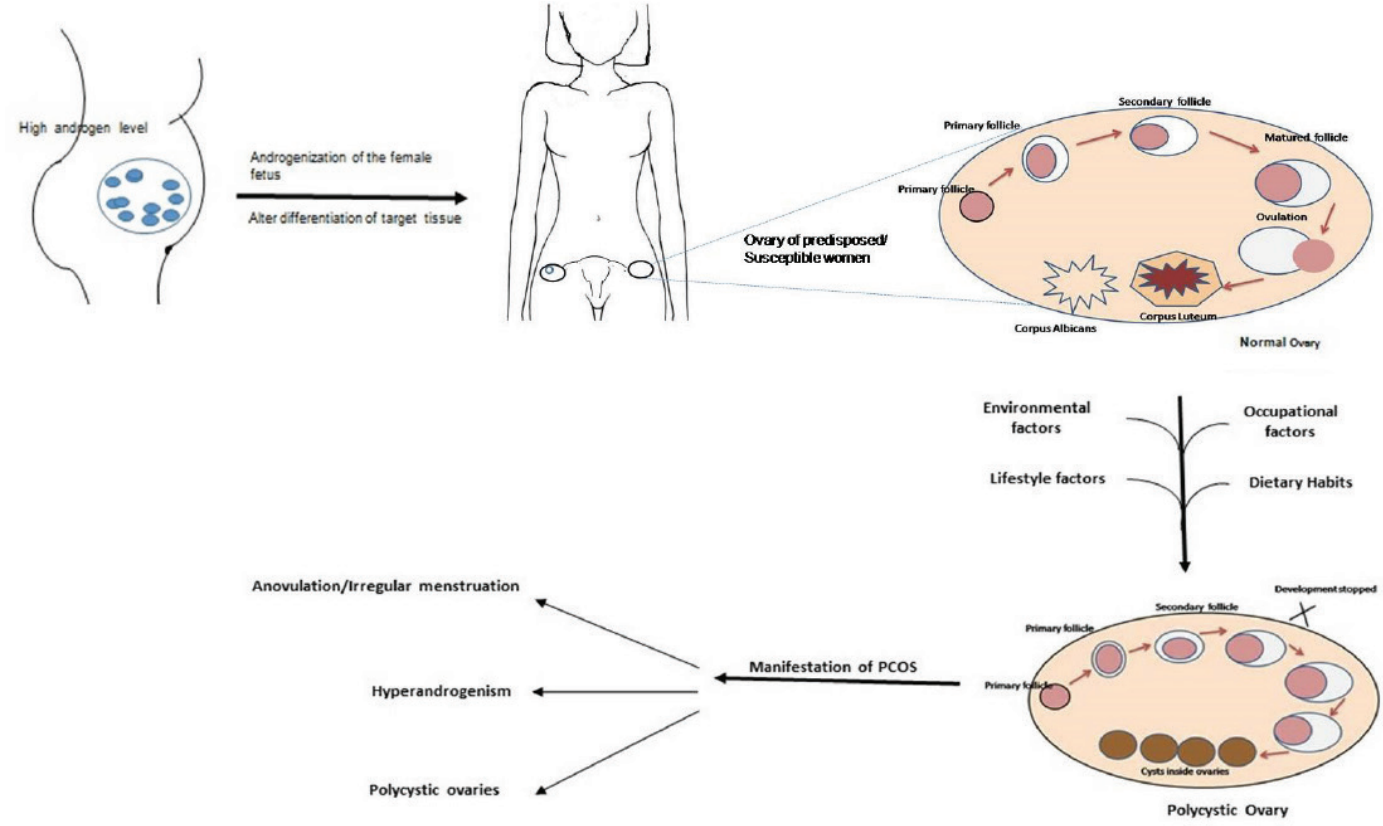

Figure 1. Possible factors of occurrence of PCOS
PCOS: Polycystic ovary syndrome

Endogenous/host/genetic factors

Several genetic/host factors might be related to the development/occurrence of PCOS. The genetics behind the appearance of PCOS are yet not fully understood, but ample evidence has been provided of their role in PCOS. Further, a considerably higher number of women in the PCOS group was reported to have a family history of diabetes and some women in this group also had a self-history of diabetes, whereas no women were diabetics in the controls (25). The presence of type 2 diabetes is noticeably higher in middle-aged women with PCOS, which suggests that body mass index, glucose, and sex hormone-binding globulin levels are connected to the risk stratification of PCOS (26).

Based upon animal studies and reinforced by clinical studies, it is reported that PCOS has its origin in fetal life, and exposure to excess androgens from the time of the growth of the ovaries in fetal life to the commencement of puberty leads to distinctive features of PCOS, along with irregularities in luteinizing hormone secretion and insulin resistance (27). PCOS arises from ancestral gene variants, which are an ancient syndrome. Such ancient genes were likely to be transmitted trans-generationally through offspring conceived amongst fertile carrier males and sub-fertile affected females (20). PCOS is a heterogeneous syndrome and commonly determined by the implication of two vital factors i.e. hyperandrogenism and insulin resistance (28). Alterations in genes that control the ovarian steroidogenesis are possibly the foremost contributing factors of hyperandrogenism. Insulin resistance may be due to different gene variations such as insulin receptor substrate-1 and 2, calpain-10, and peroxisome proliferator-activated receptor (28).

In most subjects, PCOS seems to be determined by the association of gene polymorphisms common in the general population, but gene polymorphisms alone are incapable of determining phenotypic consequences. The heterogeneity of the ailment can be explained by numerous combinations of multiple gene polymorphisms and environmental factors (28). Based upon numerous available studies, a sturdy genetic element is evident for the etiology of PCOS. Keeping in view the vast genetic and phenotypic heterogeneity of PCOS and inadequate large cohort studies to identify precise causative genes, only a few definite outcomes have been provided that concluded the heterogeneity of the disease and inadequate sample sizes complicated the identification of exact genes responsible for PCOS (29). Heritable predispositions have been stated in the occurrence of PCOS, and a genome-wide association study (GWAS) regarding PCOS showed evidence for the genetic mechanisms in the pathophysiology of PCOS. They suggested that studies using innovative techniques such as next-generation sequencing would be beneficial to understand more about underlying variants for PCOS (30). Further, PCOS is reported to be linked with oligomenorrhea, hirsutism, hyperandrogenism, insulin resistance, obesity, and risk of type 2 diabetes mellitus by ~ 7-folds (31).

Most women with PCOS (both obese and lean) have insulin resistance. The mini-satellites of the insulin gene, specifically class III alleles and III/III genotypes, are connected to the risk of type 2 diabetes and determine the predisposition to anovulatory PCOS. In addition, the appearance of estrogen receptor and 5-alpha-reductase gene (*SRD5A1-2* genes) activity in granulosa and theca cells indicated a significant variation in the expression of estrogen receptor (ER) alpha and ER beta in PCOS that may be linked with anomalous follicular development (31). The higher frequency of individuals with PCOS and the extensive range of phenotypic appearances of the disease can be elucidated by the interaction of several main genes with environmental factors (32). However, some confirmation of familial segregation and clustering of the ailment in the first-degree relatives of women with PCOS has been demonstrated with no pattern of inheritance. The existing genetic studies put forward a strong familial element and PCOS is considered a polygenic trait, which might be a consequence of the interaction of vulnerable and defensive genomic variants and environmental aspects during pre or postnatal life (32). Based upon all these studies, it can be inferred that PCOS has a sturdy genetic element in the occurrence of this syndrome along with other factors.

Duniaf and Thomas (33) mentioned that family history showed a genetic vulnerability of PCOS. Women with PCOS with insulin resistance have an ~ 50% chance of their sister having polycystic ovaries; hyperandrogenemia and high low-density lipoprotein are consistent with genetic traits. Family-based studies on linkage and association of factors have implicated numerous genes in the causation of PCOS (33). Further, it is apparent that genome-wide association studies have evolved powerful means for studying the genetic architecture of human disease (34). PCOS reduces fertility without changing in prevalence and it was considered as an evolutionary paradox (35). Overall, 17 single nucleotide polymorphisms (SNPs) were identified by GWAS studies, which were related to PCOS, with different allele frequencies, ethnicity-related, in 11 susceptible loci. The authors further examined phenotype-genotype correlations of PCOS *in silico* and suggested that PCOS was a genetic gradient that resulted from genetic drift due to the consequences of a series of events that took place in early human migrations (35). A few GWAS studies were published on PCOS from diverse areas and different ethnic groups of the world e.g. European countries, China, Korea. The GWAS study on PCOS was conducted among Han Chinese and acknowledged robust evidence of an association between

PCOS and three loci: 2p16.3; 2p21 and 9q33.3. These results offer a new understanding of the pathogenesis of PCOS (36).

Lee et al. (37) recognized a novel locus with genome-wide implication and seven moderately linked loci in Korean women with PCOS. The strongest relationship was found on chromosome 8q24.2, and other association signals were situated at 4q35.2, 16p13.3, 4p12, 3q26.33, 9q21.32, 11p13, and 1p22. The strongest signal was situated upstream of KHDRBS3, which is linked with telomerase activity, and that might be resulted to PCOS and associated phenotypes (37). Later, common genetically susceptible loci were also reported in European lineage women and three loci were reported to have genome-wide implication in a case-control meta-analysis i.e. two novel loci mapping chr 8p23.1 and chr 11p14.1, and also the chr 9q22.32 locus found earlier in Chinese women with PCOS. PCOS diagnosis and LH levels were strongly linked with the chr 11p14.1 SNP, rs11031006, in the region of the *FSH B polypeptide* gene (38). The genetic risk calculated by GWAS studies was significantly connected with PCOS and associated clinical features (39). There is a report that indicated that androgen metabolism was deteriorated in women with PCOS, thus the *CYP19* gene, which is associated in this pathway, can be a novel gene for investigation (40). Studies also revealed an association between an SNP of the *CYP19* gene in hyperandrogenism and PCOS in some ethnic groups. The studies further stated that among Iranian women, variants of SNP rs.2414096 in CYP19 could be responsible for the occurrence of PCOS (40). All these studies clearly suggest the role of genetic factors in the appearance of PCOS.

Environmental/Occupational factors

Exposure to some of lifestyle, occupational, environmental factors may enhance the elevation in the occurrence of PCOS or exaggerate the incidence of PCOS and/or phenotypic signs of PCOS, but the cause-effect relationship of these aspects with PCOS is still lacking or inadequate. There are also inadequate or inconsistent studies on exposure to environmental/occupational/lifestyles factors with regards to PCOS. Further, women are exposed to several chemicals during their day-to-day activities without their knowledge and some of these chemicals may have estrogenic or anti-estrogenic, androgenic or anti-androgenic properties. These chemicals act at a very low dose and are known as endocrine disruptors (EDs). EDs might act through membrane-bound estrogen-receptors; estrogen-related receptors; nuclear receptors; interaction with targets in the cytosol and variations in endogenous hormones metabolism; cross-talk between genomic and non-genomic pathways, interfering with feedback regulation; cross-talk with estrogen receptors after binding on other receptors and alterations in neuroendocrine cells; and DNA methylation or histone alterations (41). There is a report that indicated that experimental exposure to industrial endocrine disruptive chemicals contributed to the worsening of normal reproductive function and metabolic regulation, perhaps contributing to the growth of or enhancing PCOS-resembling clinical ailments. Industrial chemicals may also contribute to the causative role of a hostile environment to unveil PCOS characteristics in genetically susceptible individuals or further deteriorate the hormonal steadiness and fertility status of women with PCOS (42).

In addition, hormonal activity is affected due to exposure to chemicals in the womb, which may exaggerate the development of PCOS. The elevated concentrations of bisphenol-A (BPA) in women with PCOS and a noteworthy positive relationship between androgens and BPA suggests a probable role of this endocrine disruptor in the causation of PCOS (43). Further, elevated serum BPA levels were found in teenage girls with PCOS, independent of obesity, more than in the controls. BPA levels were also evidently associated with androgen concentration, leading to the inference that BPA might have a considerable role in the occurrence of PCOS in teenage girls (44). Exposure to EDs that mimic natural hormones during prenatal development might contribute to deviating the fetal programming of target tissues, which may be associated with PCOS and may have several potentially adverse trans-generational health effects (45). Chronic or acute exposure to advanced glycation end-products and EDs during various stages of the life cycle may result in the interruption of hormonal homeostasis, which is linked to the deterioration of reproductive functions. They may also interfere with metabolic changes such as insulin resistance, obesity, and compensatory hyperinsulinemia, which can contribute to PCOS consequences such as cardiovascular disease and type 2 diabetes (45). However, phthalic acid esters, BPA and octylphenol, do not induce an apparent effect on the manifestation of PCOS or contribute to insulin resistance, but octylphenol may play a considerable role in insulin resistance in subjects with PCOS (46).

Vagi et al. (47) also conveyed that subjects with PCOS might have different environmental contaminant profiles from controls, and reported that women with PCOS had higher serum concentrations of perfluoro octanoate and perfluoro octane sulfonate, and lower concentrations of mono-n-butyl phthalate and mono-benzyl phthalate in urine. They also mentioned that more studies are required to confirm these findings. Environmental factors are likely to be related with the occurrence of PCOS. Women with PCOS were found to be consuming plastic-packaged food, eating fruit with pericarp, pesticide exposure, living close to a garbage heap, working at an acid plant, taking Chinese medicines, smoking, and ingesting alcohol more commonly than controls. Eating plastic-packaged

food, eating fruit with pericarp, and alcohol consumption were independent hazards for the manifestation of PCOS (48). The authors further reported that relationships of these factors with PCOS should be confirmed by conducting additional studies.

Earlier it was also reported that environmental issues linked to PCOS were occupation, education, disposable plastic cups for drinking, indoor decoration, and cooking oil fumes, and all were significantly connected to PCOS (49). It is recognized that PCOS has the characteristic of endocrine disturbances, thus EDCs might be one of the underlying causes of PCOS. Based upon experimental studies, it was stated that BPA exposure in the perinatal stage, often at doses comparable to human exposure, interrupted ovarian and reproductive function. BPA seems to have obesogenic qualities, affecting standard metabolic function and the body becomes prone to being overweight. Cross-sectional studies suggested that PCOS women had higher BPA levels with respect to women with good reproductive health. They also suggested that additional investigations are required to extrapolate the mechanisms, wherein EDs might be linked with PCOS and critical time periods of EDCs exposure, which might have a trans-generational effect (50).

Recently, the relationship between PCOS and the anogenital distance (AGD) was explored by different investigators, which is a biomarker of androgen exposure in fetal development, and observed that subjects with PCOS exhibited higher AGD as compared with controls. This suggests that PCOS has an intrauterine origin, and fetal hormonal environment may be responsible for the advancement of PCOS in later life (51). It is acknowledged through animal studies that, prenatal exposure to high testosterone induces PCOS-like phenotypes, even though the etiology of PCOS is unfamiliar. Further, infant girls born to women with PCOS have longer AGD, which implies excess prenatal testosterone exposure with respect to girls born to women without PCOS (52). The prevailing information on prenatal exposure to three important categories of EDCs i.e. phthalates, BPA, androgenic EDCs, and the occurrence of PCOS and/or PCOS-linked anomalies were described thus: (1) maternal BPA exposure modifies sexual maturation and postnatal development in rodents; (2) gestational exposure to dibutyl phthalate and di-(2-ethylhexyl) phthalate induces polycystic ovaries and PCOS like hormonal profile; and 3) androgenic EDCs such as 3,4,4'-trichlorocarbanilide and nicotine, generate fetal hyperandrogenic environment. EDC exposure during prenatal growth may be accountable for altering fetal programming (53).

Lifestyles and dietary factors

Lifestyles and dietary factors may indirectly contribute to the occurrence of PCOS because exposure to these factors has been linked with the appearance of PCOS in girls who are susceptible to PCOS. PCOS is a common ailment, and women with PCOS have reproductive, metabolic, and psychological consequences. Weight gain and obesity deteriorate the characteristic features of PCOS, while weight decline, diminishes the characteristic features of PCOS (54). The excess weight loss through lifestyle alteration leads to menstrual regulation and regulates reproductive outcomes in women with PCOS. The available data support that a moderate diet with carbohydrates, poly and mono-unsaturated fats, and a high content of fiber with lean protein sources, are beneficial to overall health parameters in women with PCOS. Further, the incorporation of exercise in daily life showed a positive effect on the clinical representations of PCOS (55). Therefore, management of PCOS must include better lifestyle approaches with regard to proper diet, exercise, optimization of body weight, and improving insulin sensitivity, to target PCOS-related health apprehensions.

Recently, lifestyle (diet and exercise) intervention was shown to recover levels of FSH, sex hormone binding globulin, androstenedione, total testosterone, free androgen index, and Ferriman-Gallwey scores in women with PCOS (56). Losing body weight and exercise are important factors reported to improve the condition of menstrual impairment, and infertility was noticed in obese women with PCOS (57). The lifestyle variation program with importance on behavioral management, dietary, and workout interventions has been described as being successful in lowering the risk of diabetes and metabolic syndrome in the general population and accomplishing an improvement in fertility outcomes in patients with PCOS (58). These data clearly exhibited the positive role of adopting a healthy lifestyle for managing PCOS to some extent.

Trace and heavy metals in women with PCOS

Some metals in trace quantities are essential for various physiologic functions in the human body. Hence these are called essential trace metals. Essential trace and heavy metals were studied in human subjects with PCOS and serum copper (Cu), and zinc (Zn) concentrations were found to be significantly higher, whereas manganese (Mn) and lead (Pb) values were lower in subjects with PCOS. These findings should be explored further to find new insights into metals and PCOS (59). Further, no considerable differences in the median levels of barium, lead, cadmium, chromium, arsenic, strontium, gallium, and vanadium were reported amongst subjects with PCOS and controls. In contrast, serum nickel and copper concentrations were significantly higher, and Zn was significantly lower in subjects with PCOS compared with controls. Thus, metals such as copper and nickel might be implicated in the causation of PCOS and linked with impairment of reproductive hormone levels (60).

The relationship between hormonal impairments and the alterations of trace element (manganese), macro elements (magnesium and calcium), heavy metals such as cadmium, and lead in both obese and non-obese subjects with PCOS was studied and significantly higher blood Pb and Cd levels were found in subjects with PCOS (non-obese and obese) as compared with control subjects, and significantly low levels of magnesium, calcium, and Mn were recorded in subjects with PCOS (61). The serum FSH level was significantly lower in obese subjects with PCOS compared with control (obese and non-obese) subjects. A positive association was observed among serum testosterone and Cd levels in obese women with PCOS. This study established that elevated blood Pb and Cd concentrations and lower levels of serum calcium, magnesium, and Mn were observed in PCOS subjects as compared with controls (61). Later, Taher and Mhaibes (62) reported that serum copper and nickel levels were considerably elevated in subjects with PCOS, whereas the concentration of serum zinc was declined in subjects with PCOS (obese and non-obese) in comparison with controls (obese and non-obese). Further, Sedighi et al. (63) compared the lifestyle of women with PCOS and reported a noteworthy association between the manifestation of PCOS and improper diet, low physical activity, but no association with unhealthy behaviors. The data on lifestyle, diet, and some metals suggest that these factors may also have some role in the occurrence of PCOS phenotypic symptoms and weight management, healthy lifestyle, and regular exercise might be beneficial in the reduction of PCOS linked features in young adult girls.

Oxidative stress and PCOS

In addition to androgenization of female fetus, genetics, host, dietary and other environmental, lifestyle factors, oxidative stress might also be related in the occurrence of PCOS. Oxidative stress is a phenomenon of disproportion between the excessive generation of free radicals and the balancing system of antioxidant status in the body to detoxify these extra free radicals efficiently. Based upon a review on oxidative stress indicators in women with PCOS, it was mentioned that circulating indicators of OS were unbalanced in women with PCOS independent of excess weight, suggesting that OS might play a significant role in the occurrence of PCOS (64). An increased level of ROS and myeloperoxidase in subjects with insulin resistance and PCOS were also reported. Further, inflammation in subjects with PCOS brings about leukocyte-endothelium interactions and a concurrent elevation of tumor necrosis factor-α, interleukin-6, leukocytes, and adhesion molecules i.e. E-selectin, ICAM-1, and VCAM-1, and these situations are heightened by the existence of insulin resistance (65). Further, OS is a significant factor of the cardio-metabolic risk found in women with PCOS and adjusting oxidative stress with supplementation of antioxidants along with measuring antioxidant status could have a valuable effect on OS-induced hyperandrogenism and insulin resistance found in non-obese women with PCOS (66).

Earlier, González et al. (67) reported that ROS production in response to hyperglycemia from mononuclear cells was elevated in subjects with PCOS, which are independent of obesity. The resultant OS might subscribe to a proinflammatory state, which encourages hyperandrogenism and insulin resistance in women with PCOS. In addition to hormonal imbalances, defects in insulin signaling and dysfunction of adipose tissue, oxidative stress has been robustly implicated in the etiology of occurrence of the PCOS. Oxidative stress, with other etiologic factors of PCOS and involvement of environmental factors, leads to a hostile redox status that stigmatizes the normal progression of PCOS (68). The strong association of insulin resistance with OS at the visceral adipose tissue level was also observed indicating local OS and defects of insulin signaling in adipose tissue may play a vital role in the causation of PCOS (69) along with other genetic, host, and dietary or environmental factors.

The program of differentiating target tissues to the occurrence of PCOS phenotypic characteristic later in life might result from the prenatal androgenization of the female fetus due to both genetic and environmental factors and their interaction (70). The data available from both experimental and clinical studies suggest that maternal hyperandrogenism is a causative factor of PCOS, and variations in the gestational endocrine environment due to hyperandrogenism in women with PCOS in pregnancy, may play a vital part in the vertical transmission of PCOS. The scarcity of data in humans at early gestational stages has been emphasized, and the importance of experimental data to understand the cellular and molecular mechanisms involved in the programming of adult diseases. The two-hit hypothesis was proposed for the appearance of PCOS i.e. perinatal organizational and postnatal activation events (71).

Based upon available clinical and experimental data, one can infer that PCOS is a consequence of androgenization as well as alteration of the programming of target tissue differentiation during fetal development, metabolic disorders, exposure to EDs during pre and postnatal development, along with lifestyle and dietary factors in later life are associated with development of PCOS phenotypic symptoms; this also supports the two-hit hypothesis i.e. prenatal organizational and post-natal activation as reported earlier (71). The management of PCOS can be achieved through better lifestyles such as appropriate diet, exercise, optimization of body weight, and improving insulin sensitivity to control this syndrome.

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Oral care in pregnancy

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Abstract

Pregnant women are susceptible to a wide range of oral health conditions that could be harmful to their own health and the future of their baby. There are many myths about the safety of dental care during pregnancy. As a result, pregnant women receive less dental care than when they are not pregnant. In our review, we tried to emphasize the importance and safety of routine dental care for pregnant women. (J Turk Ger Gynecol Assoc 2019; 20: 264-8)

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Introduction

To sustain oral and dental health for a life time, effective and adequate care is essential. In women, dental care is much more important during pregnancy, breastfeeding, and menopausal periods. Pregnancy is not a disease state but instead it is a sign of being healthy. A healthy person is not expected to lose their teeth without any reason. The same rules are valid for pregnant women. If they take some simple precautions they will not have any loss of teeth or other dental problems. Nevertheless, mothers are known to face tooth decay and gingival problems during pregnancy. Due to bad oral health in pregnancy, pregnant women can experience premature delivery, low birth weight baby, pre-eclampsia, gingival tissue ulcerations, pregnancy granuloma, gingivitis, pregnancy tumors (epulis gravidarum), loose teeth, mouth dryness, and dental erosions. The changing hormone levels in pregnancy directly affect gum problems, and indirectly, tooth decay (1-6).

Changes seen in the gums

Pregnant women undergo hormonal balance changes during pregnancy. Many tissues undergo certain changes because the placenta produces higher levels of estrogen and progesterone during pregnancy. In this period, excessive sensitivity to irritations occurs in the gingiva. In pregnancy, gingivitis or epulis gravidarum, commonly known as pregnancy tumors, can be seen very often. Pregnancy gingivitis usually starts at the second month of gestation and reaches the highest level at the eighth month, and heals spontaneously after birth. They originate from pyogenic granuloma and disappear after 1-2 months. Surgical removal is recommended if they do not vanish spontaneously. Surgical excision can be performed by conventional methods or laser. Laser treatment may give more comfortable results to the patient because pyogenic granulomas have tendency to bleed. In fact, if they do not disturb the patient and if they do not bleed excessively, there is no need for treatment during pregnancy.

Silk et al. (5) reported that gingivitis was found in 40% of pregnancies (3-7). However, it is argued that healthy gingiva is unaffected by pregnancy, which is only a reaction caused by increased plaque and gingivitis (8). The gum papillae may appear red, like a swollen strawberry. Fissures can occur on the edges of the gingiva and on the papillae. Bleeding, and even pain can be reported. In the ones that are in hyperplastic character gingiva is dull, light pink, and the surface is rough and dry. Sometimes all gums can be dark red in color. When the growth of the gingiva is localized to one area, then pregnancy tumors may be seen. Generally,

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the underlying reason is an irritant. These gingival changes are named as: Gingivitis simplex, gingivitis ulcerosa, gingivitis hypertrophicans, and pregnancy tumor. The cause of these changes has been shown to be the rising level of progesterone in the blood stream, which increases vascular permeability. Another cause of this phenomenon is thought to be the low levels of vitamin C (9). Giving vitamin C, Ca, P, and Fl is thought to be beneficial (10,11). Mothers who have attachment loss have a higher risk of giving birth to low birth weight babies (small for gestational age) when compared with mothers with healthy periodontiums (12-16). Periodontal diseases are related to many systemic diseases, including gestational complications.

Changes that occur in the teeth

It is generally known that tooth decay increases during pregnancy. The teeth are painful and tooth losses can be seen. There is no scientific basis for the belief that fetal need for calcium required for intrauterine growth is obtained from the mother's teeth and that every pregnancy has tooth loss. This phenomenon can be explained by dentists as follows: nausea and vomiting are seen in 70% of pregnancies. Vomiting can affect oral hygiene negatively or may cause erosion on the maternal enamel layer. During pregnancy, a decrease in Ca concentration occurs. However, in the amount of ionized Ca, there is no difference compared with pre-pregnancy levels, although bone turnover is doubled during pregnancy. Increasing oral hygiene habits during pregnancy will help to prevent this problem.

The deterioration of oral and dental health during pregnancy depends on the following factors:

* During the first months of pregnancy some mothers may have extreme interest in some foods, especially carbohydrates, and tooth brushing can be neglected after they eat these kinds of food.

* Pregnant women bleed more readily due to the effect of pregnancy hormones (estrogen, progesterone), and may consequently avoid brushing their teeth. As a result, bacterial plaque increases. Therefore, in pregnancy, the mouth needs more care.

* Vomiting, especially during the first months of pregnancy, increases the acidic environment in the mouth. After vomiting, in the first few months, the mother may not pay enough attention to oral care. If the teeth are not brushed sufficiently, an acidic environment will form in the mouth.

* Saliva flow decreases. For these reasons, the formation of caries increases during this period.

* Mothers can neglect their own oral and dental health care while they are dealing with the health of the baby, which in turn causes a deterioration of oral health.

For these reasons, it is necessary to pay more attention to dental health care during this period (1-9).

The importance of nutrition on dental health during pregnancy

Adequate intake of energy and nutrients constitutes positive health effects, and inadequate intake affects mother and baby health negatively. During this period, it is necessary for the mother to take 1200-1500 mg daily calcium for herself and her babies bones to be healthy. During pregnancy, mothers should meet their calcium requirements by taking calcium-rich foods such as milk and dairy products and green, leafy vegetables. With a good diet and adequate oral health care, there will be no different tooth problems during pregnancy. Nutrition during pregnancy is very important for general health and oral health for both the mother and the baby. Baby's tooth development during pregnancy starts at the 5^{th} and 6^{th} weeks. The purpose of the nutrients taken in pregnancy is to balance the body's nutritional requirements and to provide the necessary energy and nutrients for normal growth of the fetus. It has been shown that Ca and Mg values are increased in the molar teeth due to pregnancy, whereas there is no change in Zn value. It is thought that the increasing values of placental lactogen and insulin-like growth factor-1 during pregnancy is responsible for the increase in Ca (17).

Dietry guidance during the whole pregnancy period in terms of oral and dental health;

• Fruits, vegetables, cereal, milk, dairy products, meat, fish and eggs that are rich for A, C, D vitamins, calcium and phosphorus must be taken in a balanced diet.

• Sugar should be avoided as much as possible, especially between meals.

• Dried fruit and toffees should be avoided.

• Nutrition during this period affects the health of the mother, as well as the baby, that is going to be born. The effect of vitamins A and D on enamel formation is known. There is no clear evidence that prenatal fluoride use can prevent decay (18).

Nicotine and alcohol consumption during pregnancy

Smoking negatively affects oral health, especially the gums. Periodontal inflammation and destruction increases in patients who smoke. With the increasing number of filiform and fungiform papillae on the surface of the tongue, a so-called "smooth tongue" can be seen and therefore oral hygiene becomes difficult to control. Due to the anemic effect of smoking, wounds in the mouth may recover slowly and spontaneous bleeding can be seen (16). The oral health of the mother changes, which also affects the baby indirectly. There are around 4000 different chemicals in cigarette smoke. The

acids, aldehydes, ketones, cyanide, and carbon monoxide that are among these, have direct toxic effects. Carbon monoxide, which is present in 4% of cigarette smoke, is known to prevent transport of oxygen by binding to hemoglobin in red blood cells. The hemoglobin oxygen transport capacity of smokers is reduced by 2.5% to 15%. As a result, the oxygenation of the fetus decreases, like the organs of the mother. The possibility of abortion or stillbirth increases among smokers. Also, when the baby is born alive, the birth weight is less than normal. Early or late neonatal deaths are seen much more frequently among babies of mothers who smoke (19,20).

Overuse of alcohol consumption is teratogenic in babies and can cause fetal alcohol syndrome. Epithelial growth factor receptors are responsible for dental proliferation and differentiation. Changes in these receptors due to alcohol consumption can lead to dental anomalies. Encountered findings when pregnant rats were given alcohol at certain doses, small teeth, structural deterioration of the enamel, and delayed tooth rupture were seen in young rats. It can also cause hepatic and oral pathologies in the mother and can indirectly affect the baby's condition (3,21-23).

Oral care recommendations during pregnancy

The combination of personal and professional treatment during pregnancy is very important, it plays a major role in improving oral health. Zanata et al. (24) found a correlation between preventive maintenance procedures performed during pregnancy and plaque accumulation and caries prevalence (25).

* Daily oral and dental care should be continued non-stop.

* A full oral examination must be done before gestation to achieve optimal oral hygiene and gain the habit of maintaining it because there is a direct relationship between hormonal changes during pregnancy and plaque accumulation and gingival diseases. The hormone increase during pregnancy makes the mouth mucosa more sensitive to external factors, especially against bacterial plaques.

* Effective dental care should be obtained by using toothbrushes and dental floss at least twice a day.

* Gargling with mouthwashes or warm salty water must be performed. Warm salty water relaxes gums and reduces gum sensitivity.

Treatments that can be performed during pregnancy

Many dentists think that if there is approval from the doctor of the pregnant woman they can perform uncomplicated treatments. However, most procedures to be performed in dentistry are important in the first three months and the last three months, in terms of the stresses to which the mother and the baby will be exposed. Effective dental treatment in the first trimester should be avoided. This period is a very sensitive period because it is the stage of organogenesis. Unnecessary interventions can lead to abortions. However, in cases when there is pain or if no intervention will cause more harm, the teeth must be urgently treated. Under these circumstances, tooth extraction and canal treatment can be performed. The second trimester is the most appropriate period for making many treatments, for those that if postponed until the end of pregnancy would be dangerous, such as tooth extraction, filling, and canal treatment. In the third trimester, it is not easy for the mother to take the necessary positions for the dental treatment, and may become disturbed. The baby has grown considerably in the womb and the delivery is close. It should also be remembered that if a pregnant women, in the last trimester, sits for too long in the dental chair, it may cause vena cava inferior syndrome (supine hypotensive syndrome). In this situation, turning the mother to the left side in a semi-sloping manner will help to relieve the venous circulation (1,26). Just as in the first trimester, the intervention of the dentist is not recommended except for emergency treatments.

Although some pregnant women hesitate to receive antenatal oral care, recent publications indicated that many dental treatments can be performed safely during pregnancy, such as extractions, local anesthetic, root canal treatment, scaling, and root planning (4,5).

In emergency cases such as tooth and gingival inflammation, existing infections can affect the baby’s health much more adversely than the adverse effects of dental treatment. Therefore, dental treatment must be provided according to the advice of an obstetrician.

In order to decide on the procedures to be performed, the amount of ionized radiation in a single radiograph that is taken can be reduced with lead gowns, fast films, well-calibrated instruments and collimator, which will not cause damage to the fetus. The National Radiation Protection Committee reported that the cumulative amount of radiation should not exceed 0.20 Gy, higher doses may cause microcephaly and mental retardation (27-30). Nitrous oxide used for anesthesia is known to cause abortion and congenital anomalies in pregnancy (27,31). The manufacturer’s recommendations must be taken into account in the use of local anesthetics during pregnancy for tooth extraction or any intervention. If there is no special warning, there is no inconvenience. Local anesthetics such as lidocaine and prilocain can be safely used during pregnancy in the context of the Food and Drug Administration (FDA) recommendation (1,30).

Although the mercury gases released during dental treatment are unlikely to produce teratogenic effects, they should be avoided so that the patient or workers do not inhale intensified

mercury gases. In addition, it has been suggested that during the pre-pregnancy period ≥1 μg/day of mercury exposure is associated with attention-deficit/hyperactivity disorder in infants (32,33).

The ideal number of dental checks in the 1st trimester is two, and one in the second and third trimester. After a good evaluation at the first check, it should be checked whether oral hygiene is provided in the 2nd trimester and the planned treatment should be performed in this period (e.g. tooth extraction, filling).

When medication is necessary, penicillin, erythromycin, cephalosporins are safe antibiotics to use during pregnancy. However, tetracycline (coloring in teeth), vancomycin (ototoxic/nephrotoxic), streptomycin (ototoxic) have adverse effects and are inappropriate to use during pregnancy. In addition, according to ADA, ciprofloxacin, benzodiazepines, and barbiturates should be avoided absolutely. Prenatal vitamin supplement is recommended.

Pain originating from the teeth can be a reason for contractions to start, by putting the patient under stress. Therefore, it is recommended to prescribe pain relievers with consultation. Narcotic analgesics can depress the central nervous system and non-steroidal anti-inflammatory drugs can cause patent ductus arteriosus, a such their use should be avoided during pregnancy. Acetaminophen can be preferred throughout pregnancy. Pain relief medications that can be used in pregnancy, in line with FDA recommendations, are given in Table 1 (32,33).

Table 1. The first letter in the B/D or C/D listing indicates analgesic availability for the first two trimesters, second the third trimester

Pain killer type	FDA category for use in pregnancy	Usage in pregnancy
Acetaminophen	B	Can be used
ASA	C/D	Can not be used in third trimester
Diflunisal	C/D	Can not be used in third trimester
Flurbiprofen	B/D	Can not be used in third trimester
Ibuprofen	B/D	Can not be used in third trimester
Ketorolac	B/D	Can not be used in third trimester
Ketoprofen	B/D	Can not be used in third trimester
Naproxen	B/D	Can not be used in third trimester
Codeine	C	Can be used low dose, short term
Oxycodone	B	Can be used low dose, short term
Hydromorphone	B	Can be used low dose, short term
Meperidine	B	Can be used low dose, short term
Pentazocine	B	Can be used low dose, short term
FDA: Food and Drug Administration; ASA: Acetyl salicylic acid		

Conclusion

During pregnancy, oral and dental care requires special attention. Oral health is a part of general health, and it is of even greater importance during this period because it concerns both the mother and the fetus.

It should also be kept in mind that neglecting oral and dental health during pregnancy does not only cause problems such as tooth decay and tooth loss, but may also lead to problems such as premature birth, low birth weight infant, and pre-eclampsia. Pregnancy is a period in which the mother must obey certain rules in order to protect her health and her baby's' health. In this period, mothers can protect their oral health by taking the necessary precautions and then they can prevent dental problems that may be irreversible.

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What is your diagnosis?

Mrs X, a 26-year-old primigravida woman presented for a morphology scan at 20 weeks of gestation. She had been married for 7 months and had conceived following one cycle of ovulation induction with clomiphene citrate. She was diagnosed as having gestational diabetes earlier in pregnancy and was started on a diabetic diet following which her sugars were controlled. She had an ultrasound scan at 6 weeks, which showed a single live intrauterine pregnancy corresponding to the gestational age. She had no history of intake of any teratogenic drugs.

A morphology scan was suggestive of a monochorionic monoamniotic twin gestation. One of the twins was well formed and had normal anatomy and biometry corresponding to 20 weeks. Attached to the upper abdomen and thorax of the normal twin, was a significantly underdeveloped 'co-twin' that had only a trunk, both lower limbs, and rudimentary upper limbs (Figure 1).

The scan findings were discussed in details with the parents. Risks of surgical separation of conjoined twins, the chances of survival of the normal twin and the need for lower segment cesarean section as a mode of delivery in case the pregnancy was allowed to continue, were discussed. The parents opted for termination of pregnancy.

Autopsy findings revealed partial conjoined twins with a male autosite weighing 340 grams. The weight of the parasite could not be determined separately. The parasite co-twin was attached to the autosite at the level of the epigastrium.

The autosite showed a patent anus and no significant gross abnormality. The parasite had malformed limb buds, intestinal atresia, and a poorly developed spine (Figure 2a, b). Microscopic cut sections of umbilical vessels showed four arteries and a single umbilical vein.

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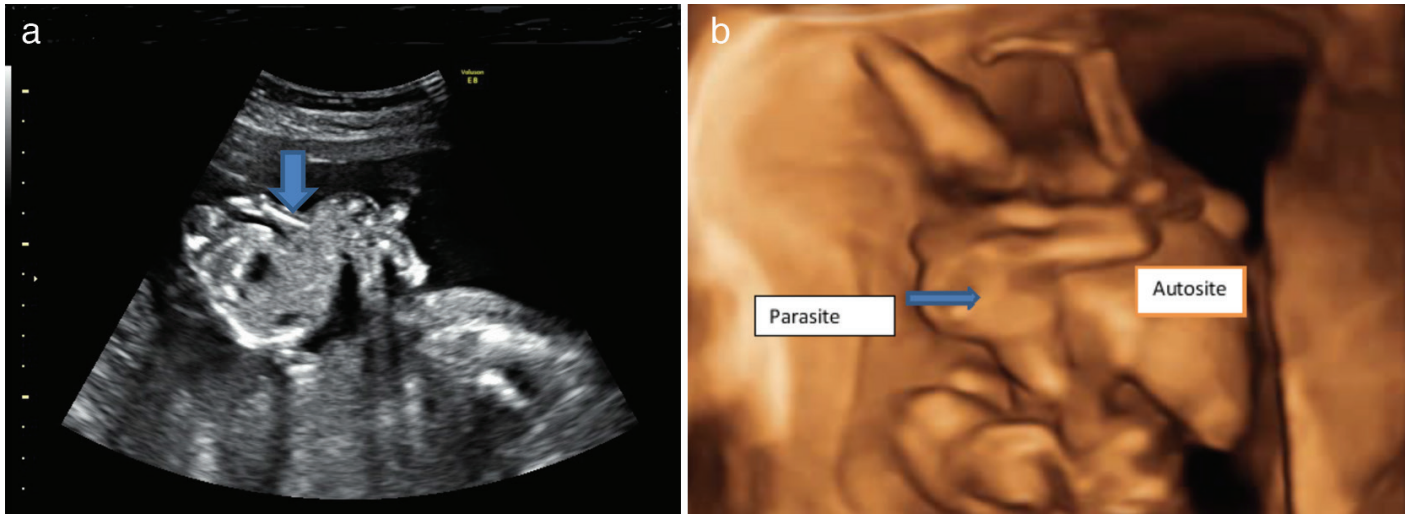

Figure 1. a) 2D ultrasound image showing transverse axial view of the upper abdomen of auto site with the stomach bubble. The arrow shows the origin of heteropagus with trunk, two lower limbs and malformed upper limbs, from the epigastric region of the autosite, b) 3D image of the epigastric heteropagus twin, parasitic twin arising from epigastric region of the autosite

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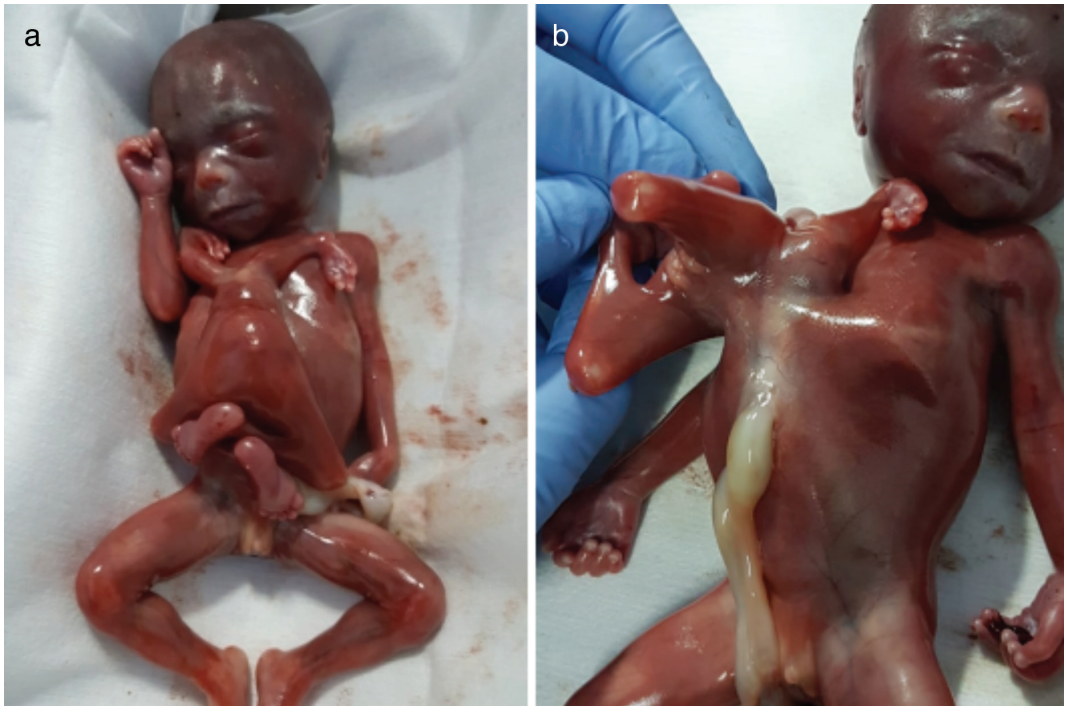

Figure 2. a) Gross specimen showing normal appearing male twin, arising from the epigastric region of which is the parasitic co-twin. b) Soft tissue pedicle connecting the parasite to the autosite and its relation to cord insertion on the autosite. Parasitic co-twin was also male with no skeletal muscle in the lower limbs

Answer

Epigastric heteropagus conjoined twins are very rare. They are also known as asymmetrical or parasitic conjoined twins, and are a rare complication of monozygotic twins (1). Their prevalence is 1 in 1-2 million births (2). The well-formed twin is known as an autosite, whereas its under developed counterpart is considered as a parasite because it is dependent on the former for its growth. The term 'heteropagus' was coined for the first time by Potter and Craig (3).

Asymmetrical conjoined twins are 20 times less common than symmetrical twins (4). Heteropagus twins are predominantly (78%) males, whereas symmetrical conjoined twins are mostly (70%) females. In our case also, both abortuses were males suggesting monozygotic twinning (5). Symmetrical conjoined twins share bowel and other organs, whereas asymmetrical twins do not share organs (6).

The pathophysiology of the heteropagus twinning has been explained in three theories.

The 'fission' theory suggests incomplete separation of the embryo (7), and the 'fusion' theory proposes coalition of two originally distinct parts (7). The third theory postulates that it occurs due to vascular compromise in utero, leading to death and partial resorption of one of the twins (7).

Most of the cases described in the literature were diagnosed postnatally (8). Few, however, were diagnosed prenatally like ours (9). The significance of the absence or presence of sharing of organs between the parasite and the autosite is that surgical separation is less complicated compared with symmetrical conjoined twins. There is still no consensus about the mainstay of therapy because the management of such cases is solely based upon case reports. When diagnosed early in pregnancy, termination may be a viable option especially in the developing world where surgery required for twin separation may not be widely accessible.

Surgical separation and closure of the incision may be attempted after babies are born. Wound breakdown is a dreaded complication following surgical separation (10). Long-term complications include hernia and teratoma at the incision site (11). Long-term follow-up of these babies following separation is very limited. One baby who was followed up to 52 months following surgical separation had normal growth and development (12).

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Tips and tricks for laparoscopic interval transabdominal cervical cerclage; a simplified technique

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Abstract

With the advance of laparoscopic surgery, several minimally invasive cervical cerclage techniques have been described and the outcomes of those have been promising. With this video article, we describe a simplified technique for laparoscopic interval transabdominal cervical cerclage. The suture material is a standard non-absorbable, braided polyester Mersilene tape, which is also used for transvaginal cerclage. The straightened needle is passed medial to the uterine vessels and lateral to the cervico-isthmic junction in anteroposterior direction on both sides, and pulled out above the uterosacral ligament. The knot is tied posteriorly, just above the uterosacral plate. The advantages of straightened needles are easy insertion into the abdominal cavity through the 5-mm ports, and more accurate direction of the suture in anteroposterior direction. In addition, posterior knots can be removed via colpotomy in the event of pregnancy failure in the second trimester, and this allows vaginal delivery. (J Turk Ger Gynecol Assoc 2019; 20: 272-4)

Keywords: Cervical cerclage, interval, laparoscopy, technique

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Introduction

The two main indications for trans-abdominal cervical cerclage are grossly damaged cervical tissue due to previous surgeries or absence of vaginal portion of cervix, and previously failed elective vaginal cerclage (1). With the advance of laparoscopic surgery, several minimally invasive techniques have been described and the outcomes of those have been promising (2-4). With this video article, we describe a simplified technique that might reduce the risk of complications such as uterine artery or lower urinary tract injuries (Video 1).

Technique

Under general anaesthesia, the patient was positioned in a low dorsal lithotomy position in booted support stirrups. A urethral catheter was inserted prior to surgery. A uterine manipulated is placed into the endo-cervical canal to move the uterus during surgery and avoid obstruction of the cervical canal. The suture material is a standard non-absorbable, braided polyester Mersilene tape, which is also used for trans-vaginal cervical cerclage (Ethicon US, LCC, USA). First, the utero-vesical peritoneal fold is incised at the cervico-isthmic level and in order to identify the uterine vessels, the incision is extended laterally on both sides. Generally, the bladder is not reflected downwards. However, previous caesarean section or other anterior uterine surgeries that result in adhesions may necessitate dissection and bladder reflection. The straightened needle is passed medial to the uterine vessels and lateral to the cervico-isthmic junction in an anteroposterior direction with a right angle to the cervix (Figure 1), and pulled out from the posterior surface of broad ligament, 1 cm above the uterosacral ligament. Then, the same procedure is repeated on the left side. The knot is tied on the posterior surface of cervico-isthmic junction, just above the uterosacral plate (Figure 2).

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The Mersilene tape is carefully laid flat on the anterior surface of the cervix (Figure 3). The ends of the tape are cut at least 1 cm beyond the knot after tying. It is not essential to close the peritoneum on the anterior surface over the tape. After achieving haemostasis, the bladder catheter is removed if there is no contra-indication and the patient is discharged on postoperative day 0/1.

Although the suture may be inserted in either direction, we believe in that placing the suture from anterior to posterior has the advantages of better visualisation, and reduced risk of bowel injury and bladder erosions. In addition, posterior knots can be removed via colpotomy in the event of pregnancy failure in the second trimester and this allows vaginal delivery. Fibrosis can occur around and within the braided fibres of the Mersilene tape and make removal more difficult. However, a posterior knot can make it easy to remove when necessary.

The procedure can be simplified further by straightening the needles before insertion to the abdominal cavity. The two important advantages of straightened needles are easy insertion into the abdominal cavity through the 5 mm ports, and more accurate direction of the suture from the anterior to posterior direction at the cervico-isthmic level. An anterior knot may be beneficial to avoid adhesions in the Douglas pouch, and can also be easily removed in laparoscopy. However, it has the disadvantage of increased risk of bladder erosion.

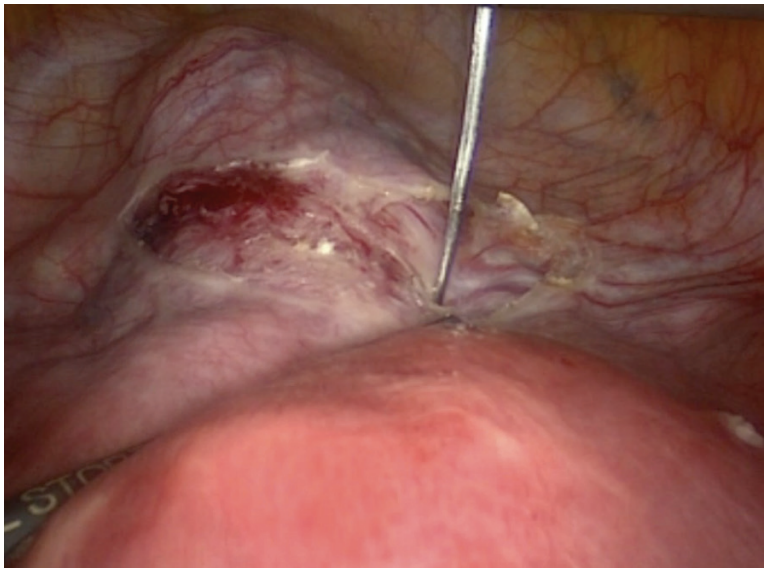

Figure 1. The needle is passed between the uterine vessels and cervico-isthmic junction with a right angle

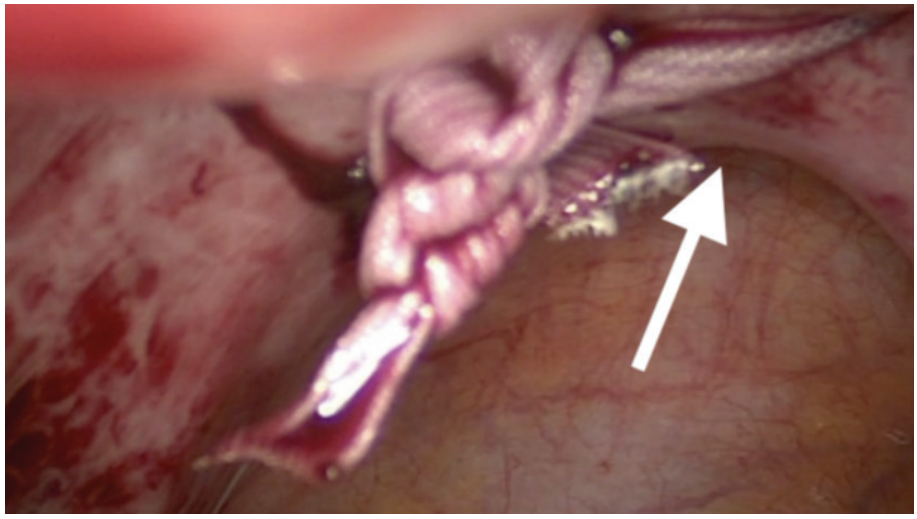

Figure 2. Knot-tied posteriorly, just above the uterosacral plate

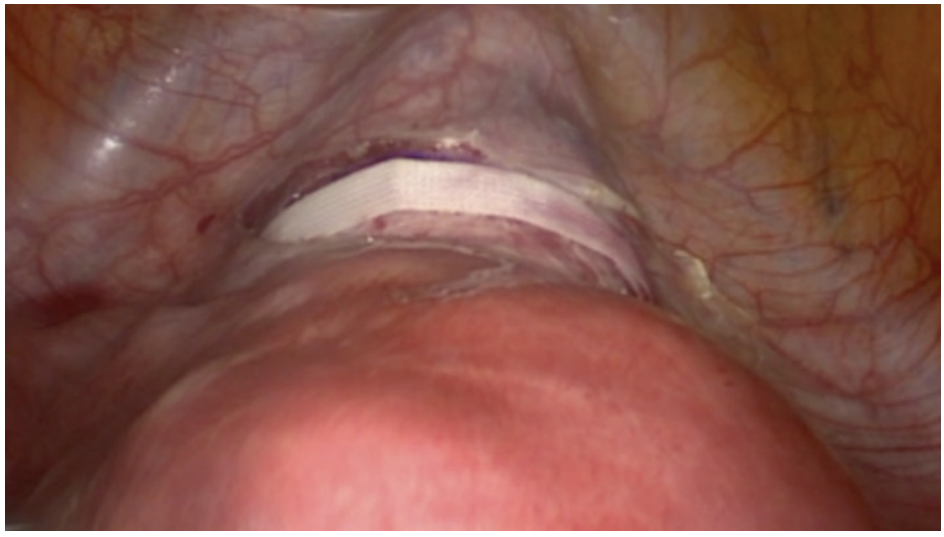

Figure 3. The tape is laid flat on the anterior surface of the uterus

***Editor-in-Chief's note**: Yavuz Emre Şükür is the member of the Editorial Board of Journal of the Turkish-German Gynecological Association. However, he did not take place at any stage on the editorial decision of the manuscript.*

Video 1. A simplified technique for laparoscopic interval transabdominal cervical cerclage
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Özel A, Alıcı Davutoğlu E, Erenel H, Karslı MF, Korkmaz SÖ, Madazlı R. Outcome after prenatal diagnosis of fetal urinary tract abnormalities: A tertiary center experience. J Turk Ger Gynecol Assoc 2018; 19: 206-9.

Table 2 of the article given above has been corrected as following:

Table 2. Postnatal persistency and surgery rates in the low and high-risk groups

	Low-risk n, %	High-risk n, %	p
Postnatal persistency			
Yes	15 (65.2)	12 (100)	0.02
No	8 (34.8)	0 (0)	
Postnatal need for surgery			
Yes	2 (8.7)	7 (58.3)	<0.002
No	21 (91.3)	5 (41.7)	

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Acknowledgements for the Year 2019
(Reviewers contributed at the review process in 2019)

On behalf of the office staff and the Editorial Board of the *Journal of The Turkish German Gynecological Association*, we would like to thank to all of our reviewers of the past year for their outstanding contributions. Their thorough reviews and expertise enable our journal to improve its scientific quality. We certainly look forward to their ongoing support, suggestions and recommendations as to how to continue to advance the overall quality of the *Journal of The Turkish German Gynecological Association*.

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Yanping Kuang
Yasemin Sert
Yavuz Tokgöz
Yusuf Üstün
Zahra Motagi

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CONGRESS CALENDER

INTERNATIONAL MEETINGS

(for detailed International Meeting please go website:
http://www.medical.theconferencewebsite.com/conferences/obstetrics-and-gynaecology)

January 9-11, 2020	**Obstetrics, Gynecology, Perinatal Medicine, Neonatology and The Law 36th Annual Conference, Waimea, HI, United States**
January 9-11, 2020	**13th Joint Conference of the UK Fertility Societies: The Association of Clinical Embryologists, British Fertility Society and The Society for Reproduction & Fertility, Edinburgh, United Kingdom**
February 3-8, 2020	**Society for Maternal-Fetal Medicine's 40th Annual Pregnancy Meeting, Texas, United States**
March 4-7, 2020	**International Society of Gynecological Endocrinology 19th World Congress, Florence, Italy**
March 5-8, 2020	**International Society for the Study of Womens Sexual Health Annual Meeting Orlando, United States**
March 11-14, 2020	**Society for Reproductive Investigation (SRI) 67th Annual Scientific Meeting, Vancouver, Canada**
March 25-28, 2020	**Royal Congressof Obstetricians and Gynecologists World Congress, Muscat, Oman**
March 28-31, 2020	**Society of Gynecologic Oncology (SGO) Annual Meeting, Toronto, Canada**
April 3-5, 2020	**16th ISUOG International Symposium, Cairo, Egypt**
April 30-May 3, 2020	**17th World Congress on Menopause, Melbourne, Australia**
May 8-11, 2020	**14th World Congress on Endometriosis (WCE), Shanghai, China**
June 10-13, 2020	**XXVII European Congress of Perinatal Medicine (ECPM), Lisbon, Portugal**
July 5-8, 2020	**European Society of Human Reproduction and Embryology (ESHRE) 36th Annual Meeting, Copenhag, Denmark**
September 11-13, 2020	**International Gynecologic Cancer Society (IGCS) 2020 Meeting, Rome, Italy**
October 1-4, 2020	**IFCPC – 2020 – 17th World Congress for Cervical Pathology and Colposcopy, Hyderabad, India**
October 11-14, 2020	**ESGE 29th Annual Congress, Lisbon, Portugal**
October 17-21, 2020	**American Society for Reproductive Medicine (ASRM) 76th Annual Meeting, Portland, United States**
October 17-21, 2020	**30th World Congress on Ultrasound in Obstetrics and Gynecology, Glasgow, United Kingdom**
November 15-19, 2020	**49th American Association of Gynecologic Laparoscopists (AAGL) Global Congress on Minimally Invasive Gynecologic Surgery (MIGS), Colorado, United States**
November 19-21, 2020	**World Congress on Controversies in Obstetrics Gynecology & Infertility (COGI), Berlin, Germany**

CONGRESS CALENDER

NATIONAL MEETINGS

(for detailed International Meeting please go website:
http://www.kongre2019.com)

December 5-8, 2019	**İstanbul Üniversitesi 9. Kadın Doğum Günleri, İstanbul, Turkey**
February 19-22, 2020	**3. Minimal İnvaziv Jinekolojik Cerrahi Kongresi, İstanbul, Turkey**
February 20-23, 2020	**4. Uluslararası Gebelik Doğum Lohusalık Kongresi ve 2. Ulusal Riskli Bebek Kongresi, Bolu, Turkey**
March 6-8, 2020	**8. Acıbadem Kadın Doğum Günleri, İstanbul, Turkey**
March 19-21, 2020	**8. Uluslararası Fetal Hayattan Çocukluğa İlk 1000 Günde Anne ve Çocuk Beslenmesi Kongresi, Ankara, Turkey**
April 18-22, 2020	**18. Ulusal Jinekoloji ve Obstetrik Kongresi-TJOD, Antalya, Turkey**
June 10-13, 2020	**9. Ulusal Jinekolojik Endoskopi Kongresi ve 3. Genç Endoskopistler Sempozyumu, İstanbul, Turkey**
November 4-8, 2020	**9. Üreme Sağlığı ve İnfertilite Kongresi – TSRM, Antalya, Turkey**

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