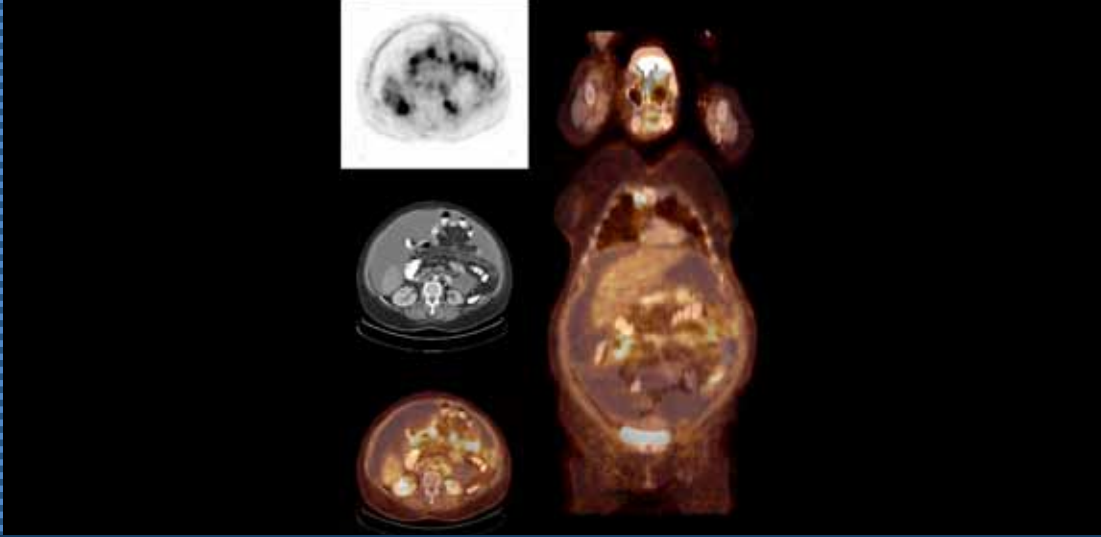




TURKISH-GERMAN GYNECOLOGICAL EDUCATION and RESEARCH FOUNDATION

*Journal of the*  
**Turkish-German**  
*Gynecological Association*



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# Journal of the Turkish-German Gynecological Association

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Journal of the Turkish-German Gynecological Association is an official journal of the Turkish-German Gynecological Education and Research Foundation, Turkish-German Gynecological Association and the Turkish Society of Reproductive Medicine and is published quarterly on March, June, September and November.

The target audience of Journal of the Turkish-German Gynecological Association includes gynaecologists and primary care physicians interested in gynecology practice. It publishes original work on all aspects of gynecology. The aim of Journal of the Turkish-German Gynecological Association is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor and case presentations are also published.

It is an independent peer-reviewed international journal printed in English language. Manuscripts are refereed in accordance with "double-blind peer reviewed" process for both referees and authors.

Papers written in English language are particularly supported and encouraged.

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## Editorial

Dear Colleagues,

Our journal - JTGGA - is at your disposal again with a new issue full of high quality content. I would like to extend my sincere thanks to the scientists submitting their high quality scientific manuscripts to our journal.

In this issue, you will find an important research that presents normative values of intracranial translucency in the first trimester screening of normal singleton pregnancies of the Turkish population. As you all know, clomiphene citrate has some negative effects on the endometrium. On this matter, another interesting research published is the one that determines the effect of vaginally administered local estrogen on endometrial thickness and pregnancy rates in clomiphene citrate stimulated cycles. An exciting study from Georgia aimed to determine the prevalence of dysmenorrhea in female adolescents living in Tbilisi, Georgia; searching possible risk factors and establishing an association, if any, with nutrition and sleep hygiene. An attractive article about metabolic syndrome, trying to identify biochemical factors that serve as predictors for the metabolic syndrome in patients with polycystic ovary syndrome, and investigating the value of adipocytokines in the prediction of metabolic syndrome is also in this issue. Besides these research articles, you will find interesting case reports and a quiz in this issue.

I would like to remind my young colleagues at the beginning of their academic career that our journal is indexed by many internationally accepted databases such as SIIC, Tübitak/Ulakbim Turkish Medical Index, Turkish Citation Index, EBSCO host, SCOPUS, Excerpta Medica (EMBASE), DOAJ database, Gale/Cengage Learning, ProQuest, CINAHL and Index Copernicus. Our young colleagues can also contact the editorial team directly in order to be a reviewer and become actively involved in the evaluation process of our journal.

Besides these scientific studies, I want to report proudly that our 4th Social Responsibility Project - "Just For Me" was held in Rize on June 6-7, 2012 with the support and active participation of distinguished foundation member professors all over the country. Financed solely by our foundation, the project was held in four steps. The first step was the public awareness meetings for the community about general women's health, gynecologic cancers, pregnancy process and contraception. The conferences for the health professionals such as doctors, nurses and midwives working in the area followed these meetings. Simultaneously, a colposcopy course was held for the first time in Rize by some renowned gynecologic oncology professors in our group. Our colleagues, working in difficult conditions, enjoyed meeting and sharing ideas with experienced scientists and motivated themselves for serving the public. The third step of our project was breast cancer screening at the specially equipped mobile screening vehicle provided by the Cancer Department of the Ministry of Health. The professors have been involved in breast ultrasonography, breast examination and mammography. Two women were diagnosed with cervical cancer and their treatment was planned by the experts. Even this result proved to us that we are successful in this project. Finally, we have visited the Rize State Hospital and donated a NST device for the use of our colleagues. We felt the satisfaction of being involved in a social responsibility project to do something useful for our country. I would like to thank specially the director of this project Assoc. Prof. Batuhan Özmen, all TAJEV members and SERENAS team involved in the project.

I sincerely hope that the third issue of this year will be a valuable contribution for our dear colleagues and Gynecology and Obstetrics community.

Best regards,

**Prof. Dr. Cihat Ünlü**  
**Editor in Chief of the JTGGA**  
**President of TAJEV**



# The nomogram of intracranial translucency in the first trimester in singletons

## Tekil gebeliklerdeki birinci trimester fetal intrakraniyel translüsenssi nomogramı

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### Abstract

**Objective:** Measurement of intracranial translucency (IT), which is a recent earlier recognizable sonographic marker, has been suggested for detection of spina bifida. In this prospective study we aimed to determine normative values of IT in the population of Turkish singleton pregnant women during the first trimester of pregnancy.

**Material and Methods:** Between January 2011 and July 2011, all consecutive singleton pregnant women were screened for intracranial translucency. Pregnancies were followed until birth. Pregnancies with any detected/suspicious anatomical or genetic fetal anomalies, biochemical abnormalities, increased nuchal translucency measurements, pregnancies of artificial reproduction techniques and anomaly detected at birth were set to be excluded from the data analyses. Mean±standard deviation, median and percentile values of intracranial translucency were calculated. Linear regression analyses were done between the length of intracranial translucency and gestational week and CRL.

**Results:** Data analyses included 201 fetuses. Median maternal age was 31 years. Median CRL was 62 mm. Median gestational week was 12.57 weeks. Median IT value for the first trimester was 1.7 mm (range 1.00-2.40). Calculated formulae are; IT (mm)=[0.237xGestational Week]-1.27, R<sup>2</sup>=0.302; p<0.001 and IT (mm)=[0.020xCRL (mm)]+0.44, R<sup>2</sup>=0.381; p<0.001. IT measurements increased with increasing CRL and advancing gestational weeks. During follow up none of the fetuses was found to have any anatomical abnormality or anomaly.

**Conclusion:** This study presents normative values of intracranial translucency in the first trimester screening of normal singleton pregnancies of the Turkish population. In accordance with previous reports, intracranial translucency increases linearly with advancing CRL. (J Turkish-German Gynecol Assoc 2012; 13: 153-6)

**Key words:** Screening, spina bifida, intracranial translucency, pregnancy, ultrasonography

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### Özet

**Amaç:** Yeni erken saptanabilen sonografik bir marker olan intrakraniyel translüsenssi ölçümü sipina bifida varlığının saptanması için önerilmektedir. Prospektif olan çalışmamızda Türk popülasyonundaki tekil gebeliklerde birinci trimester fetal intrakraniyel translüsenssi (IT) ölçümlerinin normal dağılımının saptanması amaçlandı.

**Gereç ve Yöntemler:** Ocak 2011 ve Temmuz 2011 tarihleri arasında tüm ardışık tekil gebelerde intrakraniyel translüsenssi ölçümleri bakıldı. Gebeler doğuma kadar takip edildi. Şüpheli veya gösterilen anatomik/genetik fetal anomalileri, biyokimyasal değer anormallığı, artmış nokal kalınlığı yada doğumda anomali saptanan fetüsler ve yardımcı üreme teknikleri uygulanmış fetüsler değerlendirme dışında tutuldu. İntrakraniyel translüsenssi için ortalama±standart deviasyon, medyan ve persentil değerleri hesaplandı. Lineer regresyon analizleri ile intrakraniyel translüsenssi ve gebelik haftası ve CRL arasındaki ilişki saptandı.

**Bulgular:** Veri analizlerine 201 fetus dahil edildi. Medyan anne yaşı 31 yıl, medyan CRL 62 mm ve medyan gebelik haftası 12.57 hafta saptandı. Birinci trimester için medyan IT değeri 1.7 mm (aralık 1.00-2.40) bulundu. Hesaplanan formüller; IT (mm)=[0.237xGebelik Haftası]-1.27, R<sup>2</sup>=0.302; p<0.001 ve IT (mm)=[0.020xCRL (mm)]+0.44, R<sup>2</sup>=0.381; p<0.001. IT ölçümleri artan CRL ve ilerleyen gebelik haftası ile birlikte artıyor olarak saptandı. Takip esnasında hiçbir fetüste anomali saptanmadı.

**Sonuç:** Mevcut çalışmada Türk popülasyonundaki tekil gebeliklerde birinci trimester fetal intrakraniyel translüsenssi normal dağılımları gösterilmektedir. Literatür ile uyumlu olarak, intrakraniyel translüsenssi ilerleyen gebelik haftası ve CRL ile lineer olarak artmaktadır.

(J Turkish-German Gynecol Assoc 2012; 13: 153-6)

**Anahtar kelimeler:** Tarama, spina bifida, intrakraniyel translüsenssi, hamilelik, ultrason

**Geliş Tarihi:** 27 Şubat 2012

**Kabul Tarihi:** 09 Nisan 2012

### Introduction

The detection of fetal abnormalities as well as its timing is of utmost importance to pregnant women. Ultrasonography has been proved to be the ideal and preferable non-invasive technique for the detection of fetal abnormalities. However, it necessitates repeatable and easily recognizable markers of

fetal abnormalities. NT, which is an important sample to these markers, identifies fetuses with trisomy 21 or other major aneuploidies, skeletal dysplasia and cardiac defects in the first trimester. Another such important earlier recognizable sonographic marker which has been suggested for detection of spina bifida, is an intracranial translucency (IT) (1-3). It is actually the fourth cerebral ventricle, seen as anechoic

behind the brain stem in the mid-sagittal plane of the fetal face, used for NT and nasal bone in the first trimester. Its absence or occlusion can be a sign of open spina bifida (1-3).

In this prospective study we aimed to determine normative values of IT in the population of Turkish singleton pregnant women, during the first trimester of pregnancy.

## Material and Methods

Ethics committee approval was obtained for this prospective study. Between January 2011 and July 2011, all consecutive singleton pregnant women were screened for intracranial translucency as a part of first trimester fetal screening. Written informed consents were obtained for the study as a part of consent of first trimester screening. Ultrasonographic fetal screenings were performed by one of two sonographers using one of two ultrasound machines (Voluson 730 Expert (USA)).

In the second trimester the pregnant women were screened for the "lemon" or "banana" sign for spina bifida as well as any other anatomical abnormalities. Pregnancies were followed up to birth. Pregnancies with any detected/suspicious anatomical or genetic fetal anomalies, biochemical abnormalities, increased nuchal translucency measurements, pregnancies of artificial reproduction techniques and anomaly detected at birth were excluded from the data analyses. Ultrasonographic evaluation and measurement of intracranial translucency had been performed in accordance with that previously stated in the literature (1-3). To measure IT, the exact mid-sagittal plane of the fetal face is obtained and the image is magnified to include the fetal head and upper thorax. The exact mid-sagittal plane of the fetal face is defined by the echogenic tip of the nose and rectangular shape of the palate anteriorly, the translucent thalamus in the center and the nuchal membrane posteriorly. In this plane, the fluid is identified within the third ventricle between the right and left thalami and the aqueduct of Sylvius between the cerebral peduncles, although the thalami and peduncles themselves are not visible. The two lines that define the IT are the posterior border of the brain stem anteriorly and the choroid plexus of the fourth ventricle posteriorly. At 11-13 weeks the brain stem appears hypoechoic whereas the IT is anechoic. IT has a slightly curved appearance and the widest anteroposterior diameter is in the middle part of the fourth ventricle. It is recommended to select the translucency in the widest diameter placing the calipers on the anterior and posterior echogenic borders (1-3) (Example in Figure 1).

Mean±standard deviation, median and percentile values of the intracranial translucency were calculated. The linear regression analyses were done between the length of intracranial translucency and gestational week and CRL. Statistical analyses were done with SPSS ver. 14.0.

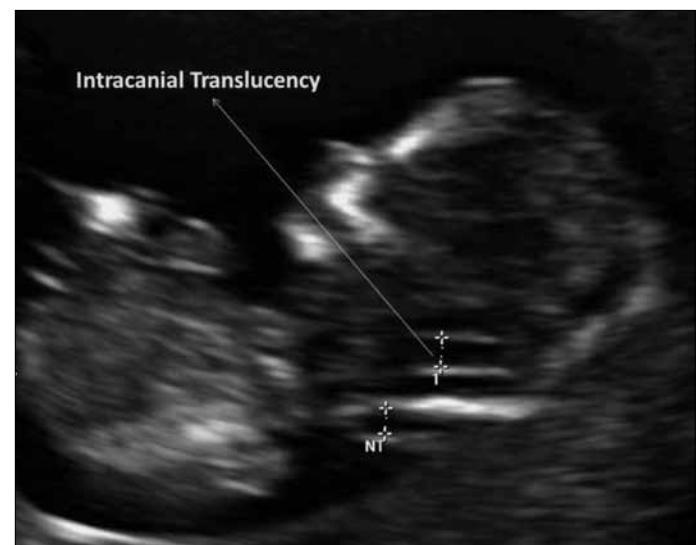
## Results

During the study period, 206 pregnant women were screened for intracranial translucency and followed up. In five of them, intracranial translucency could not be measured in the first trimester but their follow-ups were normal and no anomaly

was detected. Data analyses were done for the remaining 201 fetuses.

Median maternal age was 31 years (range 20-48). Median CRL was 62 mm (range 45-84). Median gestational week was 12.57 weeks (range 11<sup>+0</sup>-13<sup>+6</sup>). Median IT value for the first trimester was 1.7 mm (range 1.00-2.40), reference percentile ranges are shown in Table 1. The measurement values of IT according to the CRL ranges are shown in Table 2. IT measurements increased with increasing CRL range ( $p<0.001$ ).

The equations of linear regression analyses of intracranial translucency measurements and gestational week and CRL are as follows;  $(IT \text{ (mm)})=[0.237 \times \text{Gestational Week}]-1.27$ ,  $R^2=0.302$ ;  $p<0.001$  and  $(IT \text{ (mm)})=[0.020 \times \text{CRL (mm)}]+0.44$ ,  $R^2=0.381$ ;  $p<0.001$ ). The graphs of the linear regression analyses are



**Figure 1.** For the measurement of intracranial translucency, the exact mid-sagittal plane of the fetal face should be obtained and the image is magnified to include only the fetal head and upper thorax. The exact mid-sagittal plane of the fetal face has been defined by the echogenic tip of the nose and rectangular shape of the palate anteriorly, the translucent thalamus in the center and the nuchal membrane posteriorly

**Table 1.** Percentile values of IT

	Percentiles				
	5	25	50	75	95
IT (mm)	1.30	1.50	1.70	1.90	2.20
N.S.: Not significant, Mean Data±Standard Deviation, <sup>a</sup> : Median Value					

**Table 2.** The values of IT according to CRL ranges

CRL Ranges	Number	Mean	Std. Deviation	Median
45-54 mm	38	1.46	.22	1.40
55-64 mm	82	1.65	.19	1.70
65-74 mm	68	1.84	.24	1.85
75-84 mm	13	2.03	.25	2.00
Total	201	1.70	.27	1.70

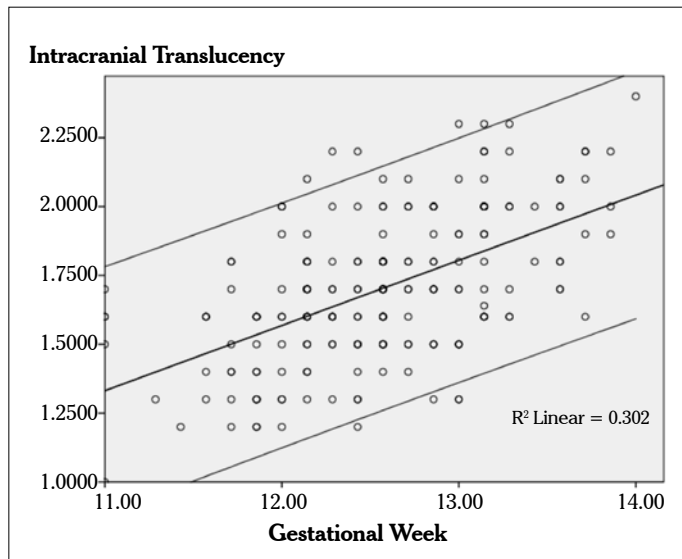
seen in Figure 2 and Figure 3. IT measurements increased with increasing CRL and advancing gestational weeks. During follow up, none of the 201 fetuses was found to have any anatomical abnormality or anomaly.

**Discussion**

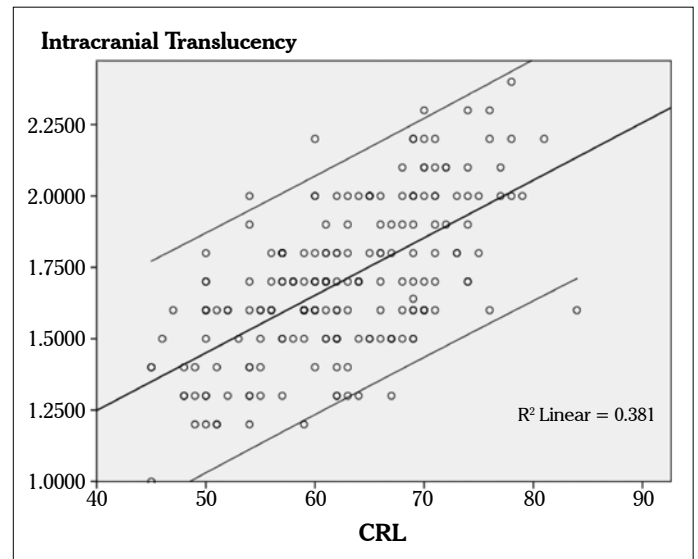
With increasing knowledge and experience in fetal ultrasonography in the past decades, new repeatable and easily recognizable markers for fetal abnormalities have been suggested. Being one of above mentioned markers, absence of intracranial translucency has been suggested to be related to spina bifida, which can be scanned earlier in the first trimester compared to the “lemon” or “banana” sign in the second trimester screening (1-4). Although intracranial translucency

measurement is mentioned as reproducible (5, 6), it has been shown that specific training increased visualization ratios of intracranial translucency (5). In this study, the retrospective series detection rate was 56-58% versus 91-92% in prospective series, indicating that experience as well as stored planes suitable for NT measurement may not always be suitable for IT visualization (5). In our study, experienced sonographers could not see intracranial translucency in five fetuses during the study period and they had no anomaly at birth. The prevalence of neural tube defects is approximately 1/1000 and our study population was lower, therefore absence of anomaly in our series might be due to this.

Although the absence of intracranial translucency has been suggested to be related to spina bifida; the intracranial translucency was reported to be normal in a case of 13 week fetus



**Figure 2.** IT measurements according to gestational week (Lines indicating 95<sup>th</sup>, 50<sup>th</sup>, 5<sup>th</sup> percentiles above to bottom respectively)



**Figure 3.** IT measurements according to CRL (Lines indicating 95<sup>th</sup>, 50<sup>th</sup>, 5<sup>th</sup> percentiles above to bottom respectively)

**Table 3.** Different studies indicating intracranial translucency measurements in the first trimester of pregnancy

Type of Study / Center	Number of fetuses	Median Age	Median CRL	Median Gestational Week	Formula of intracranial translucency	IT at 45 mm CRL*	IT at 84 mm CRL*
Retrospective Multicenter Europe (1)	200	NA	65 mm (Range 45-84)	12 (range 11 <sup>+0</sup> -13 <sup>+6</sup> )	NA	1.5**	2.5**
Prospective China (6)	111	31 years (range 20-44)	64.6 mm (range 48.5-82.4)	12 <sup>+4</sup> (range 11 <sup>+5</sup> to 13 <sup>+4</sup> )	(0.004×CRL) +1,63 (R <sup>2</sup> =0.006)	1.8	2.0
Prospective Greece (9)	611	NA	NA	NA	(0.016×CRL) +0.803 (R <sup>2</sup> =0.17)	1.5	2.1
Prospective Turkey (Present Study)	201	31 years (range 20-48)	62 mm (range 45-84)	12.57 weeks (range 11 <sup>+0</sup> -13 <sup>+6</sup> )	(0.020×CRL) +0.44 (R <sup>2</sup> =0.381)	1.3	2.1

\*: Calculated from given formula, \*\*: Given in the original article, NA: Not Applicable

with spina bifida (1, 7). However, in a case series report, it has been noted that in some fetuses, fluid can be observed in the posterior brain and misdiagnosed as a 'normal IT' (3). In these cases with some fluid visualized, the typical posterior border of the IT was not identified and it was stressed that there was a thickened brainstem with an increased brainstem diameter to brainstem-occipital bone distance (BS/BSOB) (3). Also, in another retrospective study, a previously reported high degree of accuracy for detection of open spina bifida by assessment of IT at the 11-13-week scan was not confirmed (8). Rather, presence was accepted to be useful to exclude spina bifida (8). Also, fetal position was held responsible for non-visibility of intracranial translucency. The prone position was found to be related to a high ratio of non-visibility compared to the supine position (9).

The values related to various previous studies indicating intracranial translucency measurements, including the ones above mentioned, are shown in Table 3.

## Conclusion

This study presents normative values of intracranial translucency in the first trimester screening of normal singleton pregnancies of the Turkish population. In accordance with previous reports, intracranial translucency increases linearly with advancing CRL.

## Conflict of interest

No conflict of interest was declared by the authors.

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# The effect of administering vaginal estrogen to clomiphene citrate stimulated cycles on endometrial thickness and pregnancy rates in unexplained infertility

*Açıklanamayan infertilite tedavisinde klomifen sitrat ile uyarılan sıkluslara vajinal östrojen eklenmesinin endometrial kalınlık ve gebelik oranlarına etkisi*

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## Abstract

**Objective:** Clomiphene citrate (CC) has some negative effects on the endometrium. We aimed to determine the effect of vaginally administered local estrogen (LE) on endometrial thickness (ET) and pregnancy rates in CC stimulated cycles.

**Material and Methods:** This was a prospective randomized crossover study that took place in a university hospital. The patients had received CC due to unexplained infertility (UI). Two different treatment protocols were given sequentially as, either CC+LE or CC alone. Each protocol was planned for two cycles and there was a one-month wash-out period between protocols. The effects of LE on the 3<sup>rd</sup> and 9<sup>th</sup> day, the ovulation day (OD) and 7<sup>th</sup> postovulation day (POD7) were investigated

**Results:** A total of 6 pregnancies were achieved with 3 patients from each protocol. The ET was 7.6±1.4 mm and 8.3±2.1 mm respectively and significantly different in the CC group and the CC+E2 groups on the OD (p=.039), while these values were 9.7±2.3 mm and 10.9±3.0 mm respectively and significantly different on the POD7 (p=.007). There was no significant difference between the groups for arterial PI values on the OD and POD7. The frequency of thin endometrium (<6 mm) was 15.2% and 12.2% respectively in the CC group and the CC+E2 group on the OD (p=.628) and 5.1% and 1.2% respectively on the POD7 (p=.182).

**Conclusion:** Adding vaginal LE to CC stimulated cycles led to a significant increase in ET on the OD and POD7. However, this difference in thickness was not reflected in the pregnancy rates in this study.

(J Turkish-German Gynecol Assoc 2012; 13: 157-61)

**Key words:** Infertility, clomiphene citrate, estrogen, endometrial thickness, pregnancy

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## Özet

**Amaç:** Klomifen sitrat (CC)'in endometrium üzerinde bazı olumsuz etkileri vardır. CC ile uyarılmış sıkluslara vajinal yoldan uygulanan lokal östrojen (LE)'nin endometrial kalınlık (ET) ve gebelik oranları üzerine etkisini belirlemeyi amaçladık.

**Gereç ve Yöntemler:** Üniversite hastanesinde gerçekleştirilen prospektif randomize çapraz karşılaştırmalı bir çalışmadır. Hastalar açıklanamayan infertilite (UI) nedeniyle CC aldılar. İki farklı tedavi protokolü ardışık olarak ya CC+LE ya da tek başına CC verildi. Her protokol iki siklus olarak planlandı ve protokoller arasında bir ay kadar yıkama için bekleme süresi bırakıldı. LE'nin etkileri 3. gün, 9. gün, yumurtlama günü (OD) ve yumurtlama sonrası 7. gün (POD7) incelendi.

**Bulgular:** Her protokolda 3'er olmak üzere toplam 6 gebelik elde edildi. CC grubu ve CC+E2 grubunda ET sırasıyla 7.6±1.4 mm ve 8.3±2.1 mm OD anlamlı farklı (p=.039) iken, bu değerler POD7 sırasıyla 9.7±2.3 mm ve 10.9±3.0 mm yine anlamlı farklı idi (p=.007). OD ve POD7 arteriyel PI değerleri için gruplar arasında anlamlı fark yoktu. İnce endometrium (<6 mm) sıklığı CC grubu ve CC+E2 gruplarında sırasıyla OD %15.2 ve %12.2 (p=.628) ve POD7 %5.1 ve %1.2 (p=.182) idi.

**Sonuç:** CC ile uyarılan sıkluslara vajinal yolla LE eklenmesi OD ve POD7'de ET için anlamlı artış sağlamıştır. Ancak ET artışı bu çalışmada gebelik oranlarına yansımamıştır.

(J Turkish-German Gynecol Assoc 2012; 13: 157-61)

**Anahtar kelimeler:** Infertilite, klomifen sitrat, östrojen, endometrium kalınlığı, gebelik

**Geliş Tarihi:** 11 Şubat 2012

**Kabul Tarihi:** 12 Mayıs 2012

## Introduction

Clomiphene citrate (CC) accelerates the development of an ovum suitable for fertilization with its systemic effect on the hypothalamus-ovary axis, but can also have no effect or a detrimental effect on the endometrium the fertilized ovum will attach to (1, 2). The endometrial proliferation can sometimes even be less than the ideal thickness (3). It therefore prevents

endometrial development and can cause a problem regarding implantation in some cases. A thin endometrium leads to a risk of inability to achieve pregnancy even if ovulation and fertilization take place, as the endometrium is not thick enough for the blastocyst to be implanted (4). Although ovulation is found in 80% of women with a cumulative effect in 6-8 months, the pregnancy rate can still be very low in these women (1, 2). The possible causes are the anti-estrogenic effects of CC at the

endometrium and cervical mucus level (2, 5). The endometrial effect is without doubt one of the most important handicaps in infertility treatment. The pregnancy rate can be very low, especially if the endometrial thickness (ET) is <6-8 mm (1, 3, 6). The systemic administration of estrogen has been used to overcome the ET lowering effect of CC. A major limitation of oral estrogen therapy is undoubtedly the first-pass effect in the gastrointestinal system that leads to metabolism of a major portion of estrogen before reaching the target tissue. Additionally, higher serum estrogen levels can interfere with the anti-estrogenic effect of CC that leads to superovulation. We hypothesized that vaginal administration of estrogen can protect the estrogen from liver metabolism before reaching the endometrium, thus obtaining a favorable ET for achieving better pregnancy rates. As far as we are aware, it is yet unknown whether vaginally added local estrogen (LE) can be effective in decreasing the unfavorable effect of CC on endometrium and occurrence of pregnancy. The aim of this study was to evaluate the effect of vaginally administered estrogen on the ET and uterine blood flow and therefore on pregnancy rates in women who had undergone ovulation induction (OI) with CC.

## Material and Methods

### Patients

Before entering the study, all patients received a complete infertility workup and were diagnosed as having unexplained infertility (UI) for at least one year. Unexplained infertility was defined as normal findings in the infertility evaluation. A total of 67 UI patients were admitted to Atatürk University Medical Faculty Department of Obstetrics and Gynecology between July 1, 2008 and July 1, 2009 and were scheduled to receive OI with CC. The patients were provided oral and/or written information and Ethic Committee permission was also obtained. Patients aged 18 to 35 who had received a diagnosis of UI with a 3<sup>rd</sup> day FSH value <10 mIU/ml and estrogen (E2) value >40 pg/ml, with bilateral tubal patency found on hysterosalpingography or laparoscopy, were included in the study. Those with a major medical problem, patients who had past ovarian or adnexial surgery, where a male factor that could cause infertility was present, patients with an endocrine disease such as hyperprolactinemia or abnormalities of their thyroid function test results, those who had used a hormonal drug in the last 6 months, patients with a body mass index >30 kg/m<sup>2</sup> and those with an organic pelvic disease were excluded from the study.

### Treatment protocols

We had two treatment protocols as the CC+E2 group and the CC alone group. Ovulation induction was administered to both groups for 5 days with CC 50 mg b.i.d oral tablets (Klomen® tablet, Koçak Farma, Turkey) on the 4<sup>th</sup> to 8<sup>th</sup> days of the cycle for a total of 100 mg daily in patients where no pathology was found on the 3<sup>rd</sup> day (D3) basal vaginal ultrasonography (USG). Estrogen (E2) administration was with 25 microgram vaginal tablets (Vagifem® tablet, Novo Nordisk, Denmark) for 15 days starting on the 4<sup>th</sup> day of the cycle in the CC+E2 group. Ovulation was triggered by 10 000 IU hCG when the mean diam-

eter of a leading follicle was ≥18 mm. Follicle sizes were the average of two dimensions at transvaginal USG. Ovulation day was determined by ultrasonographical examination.

The study was planned to have a crossover design and closed envelope randomization was performed. The assignment was not known to the treating physician or the patient until consent was obtained. The waiting duration (wash-out period) for changing from one treatment method to the other was about one month. Each protocol was planned for two cycles and there was a one-month wash-out period between protocols. USG was performed following spontaneous bleeding on the D3, 9<sup>th</sup> day (D9), the ovulation day (OD) and the 7<sup>th</sup> post-ovulation day (POD7) in all patients. Ovulation was confirmed by a serum level of progesterone exceeding 5 ng/ml on POD7. The ET, subendometrial blood flow (SEBF), uterine artery blood flow (UABF) and follicular development were measured in both ovaries. The systemic E2, FSH, LH and progesterone levels were also studied on the same days. The presence of pregnancy was tested with the beta-hCG level (>100 mIU/ml) and then evaluated with USG during follow-up.

### Statistical analyses

The SPSS 15.0 software was used for statistical analysis. Parametric data were expressed as mean and standard deviation. Differences between groups were compared by Student's *t* test for independent samples for continuous values and  $\chi^2$  test or Fisher's exact test for categorical variables. Variance analysis was used to compare means between the groups. Results with a *p* value less than 0.05 were considered to be statistically significant.

## Results

A total of 64 of the 67 UI patients who were scheduled to undergo OI with CC and were invited to the study agreed to participate. Patients who did not come regularly for follow-up (*n*=5), developed ovarian cysts during follow-up (*n*=2), or developed additional medical problems (*n*=1) were excluded for a total of 8 exclusions. The study has two protocols and each protocol was planned for two cycles in crossover design, but it was not possible for each patient to administer two cycles as planned. Ultrasonographical measurements could be obtained at 161 cycles where ovulation was achieved and were analyzed. CC had been used in 79 cycles (49.07%) and CC + E2 in 82 cycles (50.93%). The mean patient age was 26.8±6.2 and the mean infertility duration 34.2 months.

The ET values in the CC group and CC+E2 group of the patients was 7.6±1.4 mm and 8.3±2.1 mm on OD (*p*=0.039) and 9.7±2.3 mm and 10.9±3.0 mm on POD7 (*p*=0.007), respectively (Table 1). We found that ET increased in a significant manner on the OD and POD7 day with LE. However, this increase did not lead to a change in pregnancy rates. Evaluating the patients' pregnancy status showed 3 pregnancies in 79 cycles in the CC group and 3 pregnancies in 82 cycles in the CC+E2 group for a total of 6 pregnancies out of a total of 56 (10.7%). There was no significant difference between groups regarding pregnancy rates.

**Table 1. Endometrial thickness (mm)**

	CC group	CC + E2 group	p
3 <sup>rd</sup> day	3.9±1.0	3.7±1.1	0.472
9 <sup>th</sup> day	6.3±1.2	6.1±1.2	0.285
Ovulation day	7.6±1.4	8.3±2.1	0.039
7 <sup>th</sup> post-ovulation day	9.7±2.3	10.9±3.0	0.007

**Table 2. Endometrial thickness (mm) on ovulation day and 7<sup>th</sup> post-ovulation day**

Treatment Type		Ovulation day			7 <sup>th</sup> post-ovulation day		
		<6	6-7.9	≥8	<6	6-7.9	≥8
CC	n	12	28	39	4	13	62
	%	15.2	35.4	49.4	5.1	16.5	78.5
CC+E2	n	10	26	46	1	14	67
	%	12.2	31.7	56.1	1.2	17.1	81.7
P		.628	.673	.019	.182	.684	.002
n: number							

**Table 3. Uterine artery PI value on ovulation day and 7<sup>th</sup> post-ovulation day**

Treatment Type	Ovulation day		7 <sup>th</sup> post-ovulation day	
	Mean	Std. Deviation	Mean	Std. Deviation
CC	2.8071	.78394	2.1875	.87092
CC+E2	2.6115	1.32222	2.3333	1.05363
P	.616		.649	

The ET of the patients who achieved or did not achieve pregnancy was 8.1±1.5 and 7.9±1.8 mm on the OD and 10.0±2.1 mm and 10.3±2.7 mm on the POD7 respectively. There was no significant difference on the OD or the POD7 between the ET of patients who achieved and did not achieve pregnancy.

The ET was evaluated on the OD and the POD7 in a total of 161 cycles and classified as 8 mm and over, 6 to 7.9 mm, and less than 6 mm. When divided into treatment groups, the ET values in the CC group and CC+E2 group were presented OD in Table 2 and POD7 in Table 3. There was no difference between the CC and CC+E2 groups for OD and POD7 when the ET was categorized as 8 mm or more, 6 to 7.9 mm and 6 mm or less.

The systemic E2, FSH, LH and progesterone levels of all patients in the CC group and CC+E2 group were measured. There was no significant difference between the groups for the systemic hormone levels on D3, D9, OD and POD7. The UABF and SEBF pulsatility index (PI) was measured in all patients on the OD and POD7. There was no significant difference between the groups for arterial PI values on those days.

## Discussion

We tried to determine whether it was possible to administer LE daily for fifteen days, starting from the 4<sup>th</sup> day of the cycle in unexplained infertile patients using CC to prevent the possible detrimental effects of CC on the endometrium. The effect of vaginally administered estrogen on endometrial thickness in

patients receiving clomiphene induction showed a significant increase on ovulation day and 7<sup>th</sup> postovulation day.

The endometrium makes uterine attachment possible and can be evaluated directly with an endometrial biopsy but this method is invasive. Non-invasive methods have been investigated and various ultrasonographical parameters such as ET and uterine vascularization have been developed. Healthy blood perfusion of the uterus and ovaries may affect the success of assisted reproductive treatment (6-9). We therefore used the ET measurement to evaluate the endometrium and a Doppler investigation to determine whether the treatments we administered had led to a change in blood flow.

Estrogen may be used to prevent endometrial thinning but systemic estrogen (SE) administration might decrease the effect of CC by competitive inhibition. SE might also not reach a concentration high enough to thicken the endometrium. We believed these handicaps could be overcome by administering local (vaginal) E2. This study was mainly planned to determine whether it was possible to attain a relatively high local concentration with intravaginal E2 usage and therefore prevent the negative effects of CC on the endometrium, also preventing the systemic effects, and whether this could contribute to achieving implantation and pregnancy. In the end, as a result of the study, the ET increased significantly on OD and POD7 with CC and LE compared to CC alone. However, this difference in thickness did not lead to a difference in pregnancy rates.

The fact that pregnancy rates were found to be higher in the untreated group in a study where one group received CC for infertility and the control group received no treatment was attributed to the anti-estrogenic activities of CC in estrogen-sensitive organs (10). The ET has been shown to remain thinner than natural cycles when CC was administered and this effect has been interpreted as the change created by CC in the estrogen receptor kinetics of the endometrium (11, 12). However, there is also a study stating that even if the endometrium stays thin, this does not lead to a significant change in pregnancy rates with ET values for the 6 mm or less, 6-8 mm and 8 mm or more groups on the ovulation day at 17.1%, 19.1% and 21.5% respectively (13). These unwanted physiological effects of CC continue to be scientific areas of interest. Some studies report that CC has a negative effect on uterine perfusion and the implantation rate. The ET as measured by USG has been shown not to increase in CC-induced cycles as much as in spontaneous or gonadotropin-induced cycles, and that even endometrial cell damage could develop (14, 15).

It is obvious that CC has multifactorial negative effects on reproductive end organs. We may postulate that this adverse effect on end organs may be minimized by LE without making any difference in the ovulation-inducing systemic effects. Other methods have been tried to eliminate these adverse effects that include low-dose CC use, starting CC intake on different days, starting at the early follicular phase, adding various agents, and various results have been reported (16-18).

The implantation and pregnancy rates have been found to be significantly higher compared to placebo in groups receiving E2 in studies on the effect of SE on infertile patients undergoing in vitro fertilization (19). Patients undergoing induction with CC were found to demonstrate a beneficial effect of SE on ET but effects that were not significant on the endometrial blood flow and the number of prevovulatory follicles (20). Fanchin et al. (21) have compared the effect of LE and SE in infertile patients receiving no other treatment and found that the LE group had better ET and SEBF. This is the first study aiming to minimize the adverse effects of CC on end organs where LE was added to CC. A rest period of one month was used when changing from one treatment method to the other due to the cumulative effect of CC (22).

There may be various reasons for the lack of a difference in pregnancy rates in this study. The ET for all patients together was 6 mm or less in 13.7% on the ovulation day and 3.1% on the POD7. Kolibianakis et al. (13) have measured an endometrium thinner than 6 mm in 20.8%, 6-8 mm in 40.4%, and 8 mm or more in 38.6% of their cases, but did not specify the POD7 measurements. The small percentage of patients with very thin endometrium compared to the general population in our study may be a reason for the lack of a difference in pregnancy rates between the treatment groups. Palomba et al. (23) have reported that the ET in the periovulatory and luteal phases decreased significantly, compared to the first cycle, on the 4<sup>th</sup> cycle. An increasing number of cycles during treatment increases the degree the endometrium is affected and the thinning continues. Thickness is also not the only important factor for implantation and Hosie et al. (15) have shown by

electron microscopic investigation of the endometrium that areas with marked cellular damage and lesions consisting of irregularly shaped cells are present on the endometrial surface and affect implantation.

Unfer et al. (19) have studied administering oral estrogen for 10 days in addition to CC and found no significant change in the FSH, LH and estrogen levels, while there was a significant increase in ET in the estrogen group. Another study on the association between ET and implantation measured the mean ET in those that did and did not achieve pregnancy as  $10.2 \pm 2.6$  mm and  $8.6 \pm 3.4$  mm respectively with pregnancy rates 13.3% in those with values under 7.5 mm and no pregnancies in those with values under 5 mm (3).

Two studies on ET and continuing pregnancy compared infertile patients with ET measurements of less and more than 9 mm and found a significantly higher rate of continuing pregnancy in those with an ET more than 9 mm (24). Another study on the relationship between pregnancy and ET in patients who had undergone intrauterine insemination found a significant difference in ET between those who were pregnant ( $10.1 \pm 3.0$  mm) and not pregnant ( $7.7 \pm 3.5$  mm) (25). El-Toukhy et al. (26) have reported that both very thin (<7 mm) and very thick (>14 mm) endometrium have an adverse effect on implantation, with the highest pregnancy rates being seen in patients with an ET between 9 and 14 mm.

Uterine vascularization and blood flow have been suggested as other possible noninvasive measures that determine uterine receptivity. Despite the anti-estrogenic effects of CC and its adverse effects, especially on ET and endometrial maturation, no significant difference has been found between natural cycles and those under CC regarding endometrial and sub-endometrial flow (11). We also did not find any significant difference between the treatment protocols. Unfer et al. (19) have similarly not observed a difference regarding Doppler flow parameters between the groups receiving and those not receiving E2.

## Conclusion

We found that the ET results on OD and POD7 in patients receiving clomiphene induction showed a significant increase with LE addition. It may be beneficial in selected cases, but this increase was not reflected in the pregnancy rates in the current study. Larger series are required to demonstrate the effect of adding LE to CC stimulated cycles on pregnancy rates.

## Conflict of interest

No conflict of interest was declared by the authors.

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# Primary dysmenorrhea: prevalence in adolescent population of Tbilisi, Georgia and risk factors

## *Primer dismenore: Tiflis, Gürcistan'ın adolesan popülasyonunda prevalans ve risk faktörleri*

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### Abstract

**Objective:** The study aimed to determine the prevalence of dysmenorrhea in female adolescents living in Tbilisi, Georgia; find possible risk factors and establish an association, if any, with nutrition and sleep hygiene.

**Material and Methods:** A cross-sectional study was used. A retrospective case control study was used to identify risk factors. Participants: A total of 2561 women consented to participate in the research. 431 participants were included in the case-control study. Interventions: Detailed questionnaire included: reproductive history, demographic features, menstrual pattern, severity of dysmenorrhea and associated symptoms; information about nutrition and sleep hygiene.

**Results:** The prevalence of dysmenorrhea was 52.07%. Due to pain, 69.78% reported frequent school absenteeism. The risk of dysmenorrhea in students who had a family history of dysmenorrhea was approximately 6 times higher than in students with no prior history. The prevalence of dysmenorrhea was significantly higher among smokers compared with non-smokers 3.99% vs. 0.68% ( $p=.05$  OR:6.102). Those women reporting an increased intake of sugar reported a marked increase of dysmenorrhea compared to women reporting no daily sugar intake (55.61% vs. 44.39%,  $p=.0023$  LR:0.0002). However, alcohol, family atmosphere and nationality showed no correlation with dysmenorrhea. Our study revealed two most important risk factors of dysmenorrhea: meal skipping 59.78% vs. 27.03%,  $p=.00000$  LR: 0.00000 OR:4.014 and sleep hygiene-receiving less sleep 38.77% vs. 19.59%,  $p=0.000055$  LR: 0.000036 OR:2.598.

**Conclusion:** Primary dysmenorrhea is a common problem in the adolescent population of Tbilisi Georgia. It adversely affects their educational performance. Meal skipping and sleep quantity are associated with dysmenorrhea and may cause other reproductive dysfunctions. (J Turkish-German Gynecol Assoc 2012; 13: 162-8)

**Key words:** Primary dysmenorrhea, adolescent, nutrition, sleep, lifestyle

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### Özet

**Amaç:** Çalışma Tiflis, Gürcistan'da yaşayan adolesan kızlarda dismenore prevalansını belirlemeyi, olası risk faktörlerini bulmayı ve eğer varsa beslenme ve uyku hijyeni ile ilişki kurmayı amaçlamıştır.

**Gereç ve Yöntemler:** Kesitsel çalışma yapıldı. Risk faktörlerini tanımlamak için retrospektif olgu kontrol çalışması kullanıldı. Katılımcılar: toplam 2561 kadın araştırmaya katılmak için olur verdi. Olgu-kontrol çalışmasına 431 katılımcı dahil oldu. Girişim: Ayrıntılı anket şunları içermekteydi: reproduktif öykü, demografik özellikler, menstrual patern, dismenorenin şiddeti ve ilişkili semptomlar; beslenme ve uyku hijyeni hakkında bilgiler.

**Bulgular:** Dismenore prevalansı %52.07 idi. %69.78'i ağrı nedeniyle sık olarak okula gidemediğini bildirdi. Ailede dismenore öyküsü olan öğrencilerde dismenore riski, öyküsü olmayan öğrencilerden yaklaşık 6 kez daha yüksekti. Dismenore prevalansı sigara içenlerde sigara içmeyenlerle kıyaslandığında anlamlı olarak daha yüksekti, %3.99'a karşılık %0.68 ( $p=.05$  OR: 6.102). Artmış şeker alımı bildiren kadınlar, günlük şeker alımı olmadığını bildiren kadınlara kıyasla belirgin dismenore artışı rapor etti (%55.61'e karşılık %44.39,  $p=0.0023$ , LR:0.0002). Buna karşın, alkol, aile çevresi ve uyruk dismenore ile bir korelasyon göstermedi. Çalışmamız dismenorenin en önemli iki risk faktörünü ortaya çıkardı: öğün atlama %59.78'e karşılık %27.03,  $p=0.00000$ , LR:0.00000, OR:4.014 ve uyku hijyeni-az uyumak %38.77'ye karşılık %19.59,  $p=0.000055$ , LR: 0.000036, OR:2.598.

**Sonuç:** Primer dismenore Tiflis Gürcistan'ın adolesan popülasyonunda yaygın bir problemdir. Eğitim performanslarını olumsuz olarak etkilemektedir. Öğün atlama ve uyku miktarı dismenore ile ilişkilidir ve diğer reproduktif işlev bozukluğuna neden olabilir.

(J Turkish-German Gynecol Assoc 2012; 13: 162-8)

**Anahtar kelimeler:** Primer dismenore, adolesan, beslenme, uyku, yaşam tarzı

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### Introduction

Except for the newborn and early infant years, no period of the human life span encompasses more dramatic changes than does adolescence. Providing optimal health care to individuals of this age group requires an in depth understanding of the biological, cognitive, and sociocultural changes that

occur, their interrelatedness and their potential impact on an adolescent's health (1). Primary dysmenorrhea is by far the most common gynecological problem in this age group (2). Studies conducted on female adolescents report the prevalence range of primary dysmenorrhea from 20% to 90% (3). Morbidity due to dysmenorrhea represents a substantial public health burden. It is one of the leading causes of absen-

teism from school and work and is responsible for significant loss of earnings and diminished quality of life (4-8). Despite its high prevalence and associated negative effects, many women do not seek medical care for this condition (9).

In primary dysmenorrhea, no pelvic pathological feature is identified; an inflammatory response, mediated by prostaglandins and leukotrienes causes lower abdominal cramps and systemic symptoms. The following risk factors have been associated with more severe episodes of dysmenorrhea: earlier age at menarche; long menstrual periods; heavy menstrual flow; smoking and positive family history, obesity and alcohol consumption (10-13). Physical activity and the duration of the menstrual cycle do not appear to be associated with increased menstrual pain (11). There is a lack of data of an association between primary dysmenorrhea and nutritional deficiency despite the fact that adolescence is a peak time for eating disorders (14). Adolescents require increased nutrients to sustain the rapid growth of the brain, bones, body tissues, and sexual maturation. Significant nutritional deficiencies in adolescence can lead to decreased adult height, osteoporosis, and delayed sexual maturity (15). To meet the energy requirements of adolescents, a variety of food sources is recommended, with 60% of calories from carbohydrates, 10% to 20% from protein, and less than 30% from fat (15). The role of diet in the pathogenesis of dysmenorrhea is discussed in the literature and results are diverse. There are no clear recommendations on dietary habits, but there are clear points. The effects of dieting may bring women to the attention of the gynecologist and may be responsible for symptoms that may not seem readily related to dieting. Dietary behaviors and associated chronic disease risk factors established during adolescence have been shown to track into adulthood, suggesting that adolescence is a key time to address nutritional issues such as meal skipping (16-18). In Georgia there is lack of information about the nutritional status of adolescents and its relationship to reproductive health. There is existing evidence that adequate nutrition plays a key role in achieving normal adult size and reproductive capacity, which increases our interest in this issue.

To our knowledge, a review of studies focusing on sleep and primary dysmenorrhea in adolescents is at present absent. There are several converging reasons to focus on sleep regulation in relation to primary dysmenorrhea and adolescent development:

- i. Sleep appears to be particularly important during periods of brain maturation.
- ii. There are substantial biological and psychosocial changes in sleep and circadian regulation which exist across pubertal development.
- iii. Increasing evidence exists that many adolescents frequently obtain insufficient sleep.
- iv. There is mounting evidence that sleep deprivation has its greatest negative effects on the control of behavior, emotion, and attention, which is a regulatory interface critical in the development of social and academic competence, and psychiatric disorders (19).

The outcomes of inadequate or dysfunctional sleep are well documented. Sleep affects immune, cardiovascular, and cerebrovascular function and can have an effect on menstrual disorder.

A six-year longitudinal study among adolescents given a ten-hour sleep opportunity suggested that adolescents needed nine hours of sleep on average (20, 21). Healthy young adults, who were deprived of sleep for forty hours, showed increased circulating pro- and anti-inflammatory factors (22). The association of sleep deprivation, pain, and inflammatory processes is seen in a variety of medical disorders and conditions (23).

## Material and Methods

The study was conducted with two-stage cluster sampling. The primary unit of random selection was the capital of Georgia, Tbilisi. Selection of clusters used probability proportional to size (PPS) sampling, based on statistic data collected in 2008. With cluster sampling, the schools and universities to be included were first selected. Enrollment data in these schools was obtained from the Ministry of Education. The second stage consisted of systematic equal probability sampling. Students were randomly selected from within the selected schools and they were eligible to participate in the survey. Informed consent was obtained from respondents before collecting data. A specially designed form providing information about the study was given to respondents. Since this study involved disclosure of intimate knowledge, participants were assured of confidentiality and anonymity. Parents' consent letters were also given to the students explaining what the study was all about and reassuring them that the information obtained would be strictly anonymous and confidential.

A letter was previously sent to heads of all selected schools for their consent to undertake the survey. The purpose and details of the survey were discussed with the school authorities.

The teachers and school personnel were not present during administration of the questionnaire to encourage the students to provide their own answers without influence. Out of 2890 selected, 2561 agreed to participate. They answered survey questions in the classroom, and all of them underwent a physical exam. Vital Signs: Blood pressure, heart rate, respiration rate and temperature were checked. Anthropometry: Body mass index (BMI) was calculated and pelvic ultrasound was conducted in all students. Based on including and excluding criteria, 431 students were included in the case control study. The medical school "Aieti" ethics committee approved the study protocol. Students were informed of their right to withdraw from the study at any time.

*The eligibility criteria included the following:*

- Age 14-20 with clinical signs of dysmenorrhea
- Nulliparity
- Written or oral informed consent.

*Exclusion criteria included:*

- Acute or chronic pelvic pathology that can be a cause of secondary dysmenorrhea.
- Physical illness affecting eating behavior or causing pain
- Any history of mental illness or a structural abnormality that could account for pain or sleep
- Planning pregnancy
- Taking any kind of psychotropic drugs
- Refusal to participate

Information about the girls' reproductive history was gathered. Ultrasound was conducted again in all participants to rule out pelvic pathology. The questionnaire included data regarding demographic features, menstrual pattern (menarche age, cycle length, menstrual flow length, and menstrual blood quantity) use of contraception, severity of dysmenorrhea and associated symptoms, the body area, frequency, intensity (if pain was experienced during the last three cycles), number of years of painful menstruation, duration of pain, region of pain, presence of other complaints accompanying dysmenorrhea and impact of menstrual pain on daily activities. In addition, the questionnaire addressed information about menstrual abnormalities in close relatives, extra genital pathologies and treatments used. The questions were also related to the impact of dysmenorrhea on school performance and attendance.

A retrospective case control study was used to identify risk factors. In the focus group were included adolescents suffering from dysmenorrhea with no underlying pelvic pathology. In the control group: healthy adolescents, with regular menstrual cycles, no physiological or somatic complaints during menstruation.

Questions concerning nutrition were straightforward. Students were asked to circle the number of meal intakes throughout the 24 hours. The U.S. Department of Health and Human Services recommends at least six servings of grains and at least five servings of fruits and vegetables per day. So, twice or less meal intake was considered as infrequent food intake.

Gibney and Wolever (24), classifies an eating episode as an event that provides at least 50 kcal, and, as generally one snack does not provide the above mentioned amount, we did not regard it as "meal" intake. We considered that meal skipping can be used as a screening tool to identify adolescents of unhealthy nutrition, since meal skipping is associated with numerous health-compromising eating behaviors and less adequate dietary intakes. Excessive carbohydrate and sugar intake was determined based on individual self-report of excessive consumption of bread, table sugar, soda, chocolate, ice cream and sweets in their daily diet (25). Nine possible responses ranging from "never" to "six or more times a day" were recorded. Four and more was considered as excessive. Some other questions included: Which meals do you regularly eat? When do you snack? What are your favorite snack foods? Do you think you have healthy eating habits? We consider that this questionnaire is reliable, for rapid assessment of eating habits. However, we are aware that it is hardly ever possible to assess the absolute validity of dietary questions in population-based surveys (26).

There is evidence supporting the validity of self report sleep habits surveys, when the goal is to describe group-level sleep patterns of large samples of adolescents (27, 28).

Questions were: What time do you go to bed on school days? What time do you wake up? On school days when do you go to bed? How long does it take you to fall asleep? How many times a week are you awake the whole night? Based on answers, we calculated hours of sleep received in the participant 2890 women aged 14-20 years who were randomly selected. A total of 2561 women consented to participate in the research.

431 participants were in the case-control study. Participants were divided in two groups. One group comprised 148 healthy women with no dysmenorrhea and with regular ovulatory cycles, the second group consisted of 276 women suffering from painful menstruation, and 7 participants were excluded due to genital malformation.

The survey data were analyzed using the SPSS version 13.0.

## Results

The prevalence of dysmenorrhea was 52.07%. In the adolescent population of Tbilisi. Georgia Case group- #1 adolescents with dysmenorrhea the mean age of the girls was  $16.03 \pm 1.39$  years, age of menarche  $12.58 \pm .2$ , menstrual flow duration  $4.92 \pm 1.36$  days. Control group-#2, healthy adolescents without dysmenorrhea: the mean age was  $15.55 \pm 0.87$  years, age of menarche  $12.74 \pm 1.06$  years, menstrual flow duration  $4.53 \pm 1.24$  days. BMI of participant was  $23 \pm 1$ .

Out of women from group having dysmenorrhea  $\text{kg/m}^2$ , 63.7% started experiencing pain from 12-15 years, 36.3% reported late development of the disease from 15 to 19 years, 16.88% of adolescent associated onset of painful cycles with psychological stress, 13.42% -with physical work, and 11.26% reported a stressful event before the start of painful cycles. Other factors reported were: allergic reactions in 7.79%, frequent viral or bacterial infection in 9.92%, and start of sexual activity in 1.3%. Analysis showed that the occurrence of dysmenorrhea differed significantly among students in high school, compared with university, with a higher prevalence in school compared with universities, which can be due to age (87.68% vs 12.32%  $p < 0.001$ , LR 0.0007).

Most patients, 34.42%, reported a duration of pain of one day and more: 27.90% one hour and more. 23.19% less than an hour, 10.51%, one day with intervals, -1.81%. a week: The majority of women, 43.84%, reported localization of pain in the lower part of the abdomen and 5.94% in the lumbar region, or both 27.17% (Figure 1).

Pain associated with menstruation is also accompanied by various other symptoms. Associated physiological symptoms are presented in Figure 2. Due to pain and the presented symptoms, 69.78% reported frequent school absenteeism.

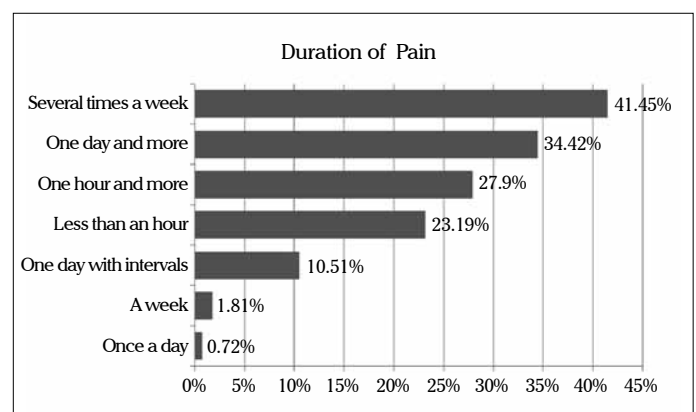
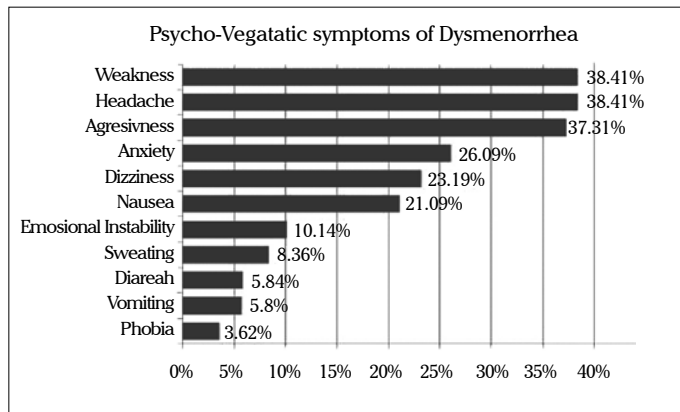


Figure 1. Pain duration reported

**Table 1. Gynecological family history and dysmenorrhea**

		Dysmenorrhea		P (Asymp. Sig.)		Odds
		Dysmenorrhea	Healthy	Pearson Chi-Square	Likelihood ratio	Ratio
Menstrual cycle irregularity	yes	23.19%	15.54%	0.063055	0.058665	1.641
	no	76.81%	84.46%			
Dysmenorrhea	yes	30.43%	6.76%	0.000000	0.000000	6.038
	no	69.57%	93.24%			
Amenorrhea	yes	5.43%	2.03%	0.097123	0.078711	2.778
	no	94.57%	97.97%			
Premenstrual syndrome	yes	5.80%	2.03%	0.073679	0.057007	2.974
	no	94.20%	97.97%			
Pelvic inflammatory disease	yes	6.52%	2.70%	0.091020	0.075388	2.512
	no	93.48%	97.30%			



**Figure 2. Psycho-vegetatic symptoms associated with dysmenorrhea**

The risk of dysmenorrhea in students who had a family history of dysmenorrhea was approximately 6 times higher than in students with no prior history. Questions included mother and anyone in the family separately. Although in Georgia there is high degree of attachment and close family relationship, the most positive answers were checked with the mother. Other gynecological diseases in the mother did not influence the prevalence of dysmenorrhea (Table 1).

The prevalence of dysmenorrhea was significantly higher among smokers compared with non-smokers 3.99% vs. 0.68% p.0.05 OR 6.102. d, Our results revealed a significant difference between high and low intakes of sugar products and frequency of dysmenorrhea. Those women reporting an increased intake of sugar reported a marked increase of dysmenorrhea compared to women reporting no daily sugar intake, 55.61% vs. 44.39% p<0.001 LR 0.0002. However, alcohol, family atmosphere and nationality showed no correlation with dysmenorrhea. Our study revealed the two most important risk factors of dysmenorrhea to be meal skipping- inadequate nutrition 59.78% vs 27.03% p<0.001 LR 0.00000 OR 4.014 and sleep hygiene-receiving less sleep 38.77% vs. 19.59% p .0.001 LR 0.000036 OR 2.598. All studied risk factors are presented in Table 2.

We also studied the association of dysmenorrhea with extra-genital pathology. No association was observed between reported diabetes, thyroid diseases, gastro-intestinal, vascular, or renal diseases. Allergy reaction in patients with dysmenorrhea was more frequent, 14.23% compared with the healthy group 4.73% Pearson Chi-Square 0.003 Likelihood Ratio 0.001494 Odds Ratio 3.343.

On the question of what relieved pain, half of the women, 50.72%, used medication for the management of their pain, 17.69% used rest, napping as pain relief, 13.08% used trying to concentrate on other tasks, 14.23% preferred a horizontal position, 4.23% did not specify. Medications used for dysmenorrhea were: NSAID's 9.82%, other pain relievers, analgesics 71.78%, spasmolytics 6.13%, combined oral contraceptives 9.82%.

**Discussion**

It is estimated that the prevalence of dysmenorrhea varies from 20% to 95% (29-31). The results of this study confirm that dysmenorrhea is common in young women, as 52.0% of our sample experienced dysmenorrhea. We consider that variation of the prevalence may be due to different diagnostic tools or different attitudes of different cultures towards menstruation. Geographic location cannot be ignored as in Turkey, on the border of Georgia, the prevalence of dysmenorrhea was quite close 55.5% (32). According to another study, prevalence in northern Turkey was also 53.6% (33). We want to mention that this is the first and only study in our country examining the prevalence of dysmenorrhea, but in Turkey in other studies the prevalence of painful menstruation was low. Polat reported 45.3% (34) Cakir reported 31.2% (35).

The highest number of women having dysmenorrhea, 57%, was observed at the age of 14. As the mean age of menarche in our sample was 12.58±1.2, dysmenorrhea occurred soon after the onset of menstruation. We found no significant difference in the mean age of menarche between the two groups. Our data differs from many other studies where age of menarche is an important factor (36-39). The same results were observed by Pawlowski,

**Table 2. Risk factors of dysmenorrhea**

		Dysmenorrhea		P (Asymp. Sig.)		Odds
		Yes	No	Pearson Chi-Square	Likelihood ratio	Ratio
Education	High school college	87.68%	97.97%	0.000344	0.000071	6.790
		12.32%	2.03%			
Living conditions	Satisfactory non satisfactory	93.12%	95.95%	0.238306	0.224929	0.572
		6.88%	4.05%			
Family income	High income medium income poverty refugee	9.42%	19.59%	0.012283	0.014966	
		85.51%	75.68%			
		4.35%	2.70%			
		0.72%	2.03%			
Family atmosphere	Harmonic conflictive	83.27%	89.19%	0.100820	0.093935	0.603
		16.73%	10.81%			
Alcohol use	Yes No	4.35%	3.38%	0.627653	0.623123	1.300
		95.65%	96.62%			
Tobacco use	Yes No	3.99%	0.68%	0.050112	0.028883	6.102
		96.01%	99.32%			
Drug use	Yes No	1.45%				
		98.55%	100%			
Sleep hygiene	Yes No	38.77%	19.59%	0.000055	0.000036	2.598
		61.23%	80.41%			
Meal skipping	Yes No	59.78%	27.03%	0.000000	0.000000	4.014
		0.22%	72.97%			
School absenteeism	Yes No	56.88%	45.95%	0.031456	0.031468	1.552
		43.12%	54.05%			

who did not find any difference in the ages of menarche between dysmenorrheic and non-dysmenorrheic women (40).

Again, our research pointed out that primary dysmenorrhea causes recurrent absenteeism from school, or other activities. Despite great advances in the study of the pathogenesis and treatment options, the fact that students tended to be absent from school, unable to focus on their courses, and distracted from lectures due to dysmenorrheal symptoms, is stable. As Ondervan in 1998 indicated that dysmenorrhea is responsible for school absenteeism, later other studies from 2000- through 2007 again confirm female adolescents absenteeism was common due to excessive pain (36, 41-44).

Although a high percentage of women suffer with pain each month, which leads to school absenteeism, our study highlighted that most women do not seek medical advice. It is known that non-steroidal anti-inflammatory drugs are highly effective, but most participants are unaware of the effectiveness of the treatment. Only 9.82% used NSAID's. In our country university entrance exams are conducted on a pre-determined day, so it is important to be aware of treatment options, if menstruation coincides with examinations. There is need to educate girls and their parents regarding effective dysmenorrhea treatment,

emphasizing NSAID use, appropriate dosing, including prophylactic administration and dosing frequency.

Our results demonstrated that the prevalence of dysmenorrhea was dependent on family income. Similarly, Widholm and Kantero noted a higher prevalence of dysmenorrhea among higher income groups (45). However, some researchers have indicated that economic status is not consistently associated with dysmenorrhea, suggesting that further research is necessary to clarify this factor (46).

Our research indicated that, while no particular gynecologic disease in the mother is a predictor of dysmenorrhea, family history of dysmenorrhea seems to be an important risk factor for women with dysmenorrhea. The results are consistent with previous studies (32, 44, 47).

Our study identifies tobacco to be an important risk factor. Previous studies have found an association between current cigarette smoking and the prevalence of dysmenorrhea (48, 49). We agree that cigarette smoking may increase the duration of dysmenorrhea, presumably because of nicotine-induced vasoconstriction (50).

Our study demonstrated that meal skipping significantly increases the prevalence of dysmenorrhea. To our knowledge, this is

the first research that considered meal skipping as a risk factor, although there are several studies suggesting that nutrition during adolescence affects reproductive function in young women and dysmenorrhea as well. A low-fat vegetarian diet was associated with a decrease in duration and intensity of dysmenorrhea in young adult women (7). Dietary supplementation with omega-3 fatty acids had a beneficial effect on the symptoms of dysmenorrhea in adolescents in one study (29).

Nonetheless, it has been demonstrated that menstrual regularity can be influenced by diet (51). Specific dietary nutrients may have direct effects or exert their effects by altering the status of circulating sex steroids. So, as inadequate nutrition is a cause of low energy availability and can alter hormonal status, we consider that meal skipping causes menstrual disorders.

Our study revealed that sugar consumption was associated with the prevalence of dysmenorrhea. We found only one study that observed same results. A possible explanation that Nebahat Ozerdogan provided was that sugar interferes with the absorption and metabolism of some vitamins and minerals, thus causing nutritional imbalances, which in turn can cause difficulty in muscle functioning and lead to muscle spasms (32). We consider excessive sugar can alter circulating steroid quantity, but further research is necessary to prove this hypothesis.

Excessive sleepiness among children and adolescents has become a major international health concern. There are several reports of poor sleep hygiene and sleep disorders in pediatric groups (52).

Our study also indicated that a high percent of the Georgian adolescent population report late sleeping habits and getting inadequate sleep. The data is consistent with large-scale community surveys, and between 14 and 33% of adolescents report a subjective need for more sleep (53-57).

Our study once again demonstrated that, despite cultural differences, adolescents' worldwide sleep is significantly less than the recommended 9-10 h. Although our study has limitations, findings strongly point out that self-reported shortened total sleep time, is negatively associated with primary dysmenorrhea. We consider that the effect of sleep disorder on dysmenorrhea is due to the fact that the sleep/wake rhythm seems to be influenced by the estrogen and progesterone receptors and vice versa. It well known that sleep deprivation compromises cognitive, emotional, neurologic, metabolic, and immune functions and thereby can have a great effect on reproductive health (58).

We also found that frequent overnight wakefulness can affect dysmenorrhea. The same results were shown by a survey of 2264 participants, that women working a shift schedule, found an increase in dysmenorrhea and menstrual irregularity (27).

Sleep deprivation has an impact on dysmenorrhea and reproductive health, but a more detailed assessment is necessary to specify this effect. Our study is a step forward to emphasize that, despite its significance and frequency, sleep disturbance is an area of adolescent health that is almost entirely unaddressed.

## Conclusion

Primary dysmenorrhea is a common problem in the adolescent population of Tbilisi, Georgia. It adversely affects their educa-

tional performance. As most students are unaware of effective treatment, we suggest improving female adolescents' self-care behavior towards dysmenorrhea through enhanced health education in schools. The findings of this study can serve as a guide to healthcare providers who want to design an effective systematic menstrual health education program for female adolescents. Meal skipping is associated with dysmenorrhea and may cause other reproductive dysfunctions. Meal skipping and excessive sugar consumption can be used as a screening tool to identify adolescents in need of nutrition education. Future management of dysmenorrheal pain may be considered a promising nutritional therapy for the relief of pain and symptoms associated with dysmenorrhea. However, large, prospective, and controlled studies will be necessary to establish the findings of this study. At this point professionals should educate teens on the adverse effects associated with meal skipping, including, excessive snacking of unhealthy foods. The results of this study suggest that sleep quality and sleep hygiene has a great effect on reproductive health quality. Disturbed sleep can be both a cause and a result of ill health and, if recognized, can improve adolescent development and reproductive health. Sleep deprivation is associated with a variety of adverse consequences, which are often not appreciated by patients or clinicians. In summary, dysmenorrhea trials are especially important because this condition is undertreated and leads to high morbidity in adolescence.

## Conflict of interest

No conflict of interest was declared by the authors.

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# Endometrial spiral artery Doppler parameters in unexplained infertility patients: is endometrial perfusion an important factor in the etiopathogenesis?

## *Açıklanamayan infertilite olgularında endometrial spiral arter Doppler parametreleri: endometrial perfüzyon etyopatogenezde önemli bir faktör mü?*

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### Abstract

**Objective:** Uterine perfusion, particularly the endometrial blood flow, may have an important role in endometrial receptivity. In order to assess the contribution of sub endometrial blood flow in the etiopathogenesis of unexplained infertility mid luteal- peri-implantation period spiral artery transvaginal color Doppler parameters were measured and compared with fertile controls.

**Material and Methods:** Forty-two consecutive patients admitted to Izmir Katip Celebi University Atatürk Training and Research Hospital, Department of Obstetric and Gynecology with the diagnosis of unexplained infertility after standard diagnostic work up constituted the study group and they were compared with a fertile control group admitted to hospital with non specific gynecological complaints or for check-up in the same period. Mid luteal transvaginal color Doppler ultrasonography was applied to each patient by the same radiologist who was blind to the diagnosis of the particular patient and, RI (resistance index) and PI (pulsatility index) values were calculated.

**Results:** There were no significant differences between the two groups, in respect to age, body mass index, basal hormonal and mid luteal progesterone levels ( $p>0.05$ ). For the fertile control group, mid luteal-peri-implantation phase endometrial spiral artery mean RI values were calculated as  $0.48\pm 0.08$  SD and mean PI values as  $0.65\pm 0.18$  SD. For the study group, mean RI values were calculated as  $0.54\pm 0.07$  SD, PI values were calculated as  $0.80\pm 0.16$  SD. The differences for RI ( $p=0.009$ ) and PI ( $p=0.004$ ) were statistically significant.

**Conclusion:** According to Doppler parameters, unexplained infertility patients have high impedance blood flow in spiral arteries which means that peri-implantation blood flow in these patient is lower than fertile controls. These findings suggest that endometrial perfusion may have an important contribution to etiopathogenesis of unexplained infertility. (J Turkish-German Gynecol Assoc 2012; 13: 169-71)

**Key words:** Unexplained infertility, endometrial blood flow, endometrial receptivity, transvaginal ultrasonography, colored Doppler

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### Özet

**Amaç:** Endometrial kan akımının, endometrial implantasyonda önemli rol oynadığı düşünülmektedir. Biz çalışmamızda sub-endometrial kan akımının açıklanamayan infertilitedeki önemini belirleyebilmek için mid-luteal- periimplantasyon dönemindeki kan akımını TVUSG doppler ile ölçerek fertil kontrol grubu ile karşılaştırdık.

**Gereç ve Yöntemler:** Çalışmamıza İzmir Katip Çelebi Üniversitesi Atatürk Eğitim ve Araştırma Hastanesi, Kadın Hastalıkları ve Doğum Kliniği'ne infertilite nedeni ile başvurmuş ve yapılan tetkikler sonucunda açıklanamayan infertilite olarak tanı almış 42 hasta ile fertilitte dışındaki jinekolojik şikayetler ile başvurmuş fertil hastaların mid-luteal dönemindeki endometrial kan akımı değerlendirildi. Ölçümler; rezistans indeksi ve pulsatilite indeksi, hastaların hangi gruba ait olduğunu bilmeyen tek radyolog tarafından yapıldı.

**Bulgular:** Yaş, boy, kilo oranı, bazal hormonal durum ve mid-luteal progesteron düzeyleri açısından iki grup arasında fark yoktu ( $p>0.05$ ) Fertil kontrol grubunun mid-luteal-peri-implantasyon fazındaki spiral arter ortalama RI değeri  $0.48\pm 0.08$  SS, PI değeri ise  $0.65\pm 0.18$  SD olarak ölçüldü. Çalışma grubu için ise RI değeri  $0.54\pm 0.07$  SS, PI değeri  $0.80\pm 0.16$  SS olarak ölçüldü. Ölçümler istatistiksel olarak değerlendirildiğinde anlamlı olarak bulundu (RI  $p:0.009$ , PI  $p:0.004$ ).

**Sonuç:** Doppler parametrelerinin değerlendirilmesi ile, açıklanamayan infertilite grubundaki hastaların, endometrial spiral arter dirençleri kontrol grubundan daha yüksek olarak bulundu. Sonuç olarak açıklanamayan infertilite grubundaki hastalarda, periimplantasyon dönemindeki subendometrial kanlanma kontrol grubuna göre daha az olarak ölçülmüştür ve bu sonucun infertilite etyopatogenezinde önemli rol oynadığı düşünülmüştür.

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**Anahtar kelimeler:** Açıklanamayan infertilite, endometrial kan akımı, endometrial implantasyon, transvajinal ultrasonografi, renkli Doppler

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## Introduction

Unexplained infertility is one of the most common diagnoses in a fertility clinic with the prevalence of 15-30% (1, 2). In fertile women uterine spiral artery perfusion has been found to improve during the luteal phase, which coincides with the implantation window (3). Endometrial receptivity is regulated by many factors including uterine perfusion (4, 5). Several studies have shown that uterine receptivity is decreased when the uterine artery impedance has been increased during the mid luteal phase (2, 6). Abnormal uterine perfusion may be a contributing factor to etiopathology of infertility especially in couples with unexplained infertility.

Transvaginal Doppler pulsed ultrasound is an important tool for examining the female reproductive system and is a non-invasive method to assess the uterine perfusion (7, 8). The aim of this study is to evaluate the mid-luteal phase endometrial spiral artery blood flow in unexplained infertility patients and compare those parameters with fertile controls in order to reveal the possible role of the uterine perfusion in the infertility etiopathogenesis.

## Material and Methods

The study group is composed of consequent 42 unexplained infertility patients admitted to Izmir Katip Celebi University Atatürk Training and Research Hospital, Department at Obstetric and Gynaecology between August 2010 and August 2011. Values of the study groups were compared with 20 volunteers admitted during the same time frame mostly for Pap smear controls. Patients in the control group had normal physical and pelvic examinations with normal menstrual cycles and had given a live birth during the year before the admission and did not have intrauterine device or use oral contraceptives. They were below 40 years of age, did not smoke and did not have any systematic or clinical disease. In the study group, infertility was defined as unexplained if a comprehensive infertility evaluation including transvaginal ultrasonography, mid-luteal progesterone value, histerosalpinghography, detailed semen analysis, failed to reveal any apparent cause.

In this study we measured mid-luteal phase spiral artery parameters by using transvaginal color Doppler ultrasonography (TVCDUSG) in 42 unexplained infertile and 20 fertile patients. TVCDUSG has been performed by the same radiologist, who was blinded to the groups.

The ultrasonographic examination was done on the 21<sup>st</sup> menstrual day by using GE logic P6 (GE Healthcare, Buckinghamshare) ultrasound machine with a 6-10MHz endovaginal transducer. After placing the transducer in to the vagina, transverse and sagittal images at the uterus and ovaries were obtained. Color Doppler examination of the endometrium was performed with a 1.1 kHz pulse repetition frequency (PRF) to evaluate a minimum flow velocity at 5 cm/sec. in the spiral arteries. Triplex mode examination included gray scale image combined with color frame and a flow spectrum on the spectral wave, resistance (RI) and pulsatility indexes (PI) were measured automatically by using the software program in the equipment. SPSS 16.0 for Windows was used for the statistical analysis 95% confidence interval was considered. Continuous variables were analyzed with sample t-test and  $p < 0.05$  was considered statistically significant.

## Results

No significant difference was reported in serum mid-luteal progesterone, basal FSH levels between two groups and similarly no significant difference was observed in terms of demographic characteristics like age and BMI ( $p > 0.05$ ). Endometrial spiral arteries RI and PI values were found to be  $0.48 \pm 0.08$  and  $0.65 \pm 0.18$  in midluteal peri-implatation period of the fertile control group. This values in study group were detected as RI  $0.54 \pm 0.07$ , PI  $0.80 \pm 0.16$ . The difference in both indexes are statistically significant between two groups (RI  $p < 0.009$ , PI  $p < 0.004$ ).

## Discussion

It has been previously demonstrated that impedance of uterine and spiral arteries blood flow change periodically during the normal ovulatory menstrual cycle (1, 2, 8). It is interesting that the lowest impedance at spiral artery blood flow has been detected just at the time at mid-luteal phase, during which endometrium has been transformed from proliferative phase to secretory phase (2, 8, 9). At this specific time period blood supply of uterus is rich and implantation is most likely to occur (2, 3, 6, 10). In addition to this it was reported that impedance of spiral artery blood flow in women with unexplained infertility was significantly higher than that of the fertile counterparts (1, 7). Similarly Steer et al. (7) suggested that decreased uterine perfusion might be a cause of unexplained infertility (7).

**Table 1. The mean values of age, BMI, progesterone, FSH and endometrial artery impedance in unexplained infertility group and fertile control group**

	Unexplained (n=42) Mean±SD	Control (n=20) Mean±SD	P
Age	26.69±4.4	28.25±3.71	0.176
BMI (kg/m <sup>2</sup> )	25.41±3.31	25.55±2.61	0.874
Resistance index	0.54±0.07	0.48±0.08	0.009
Pulsatility index	0.80±0.16	0.65±0.18	0.004
Progesteron ng/mL	10.86±6.56	10.84±5.12	0.992
FSH (basal) mIU/mL	7.6±1.41	7.58±1.2	0.941

BMI: Body Mass Index, FSH: Follicule Stimulating Hormone, SD: Standard Deviation

We therefore hypothesized that impaired blood flow could be an important contributing factor to infertility in women with no other relevant cause was present.

There are various methods to assess the endometrial receptivity including endometrial biopsy and immunohistochemical analysis. The introduction of pulsed Doppler ultrasonography has provided a non-invasive mode of evaluation of uterine impedance and reveal physiological data, rather than anatomical information alone.

There was significant correlation of uterine PI and biochemical markers of endometrial receptivity including endometrial histological dating (11, 12). The measurement of impedance to uterine blood flow in IVF cycles has provided an indirect measure of endometrial receptivity (9, 11, 13). In studies where endometrial and subendometrial blood flow were analysed by color or power Doppler ultrasonography absence of blood flow signal has been found to be associated with significantly low pregnancy rate or absence of pregnancy in IVF cycles (13, 14). Battaglia et al. (9) reported the highest pregnancy rate in the group with lower resistance to blood flow in the uterine spiral arteries. This finding revealing the decrease in peripheral impedance in the uterine vasculature reflected by a low uterine artery PI was considered to be a consequence of increased blood flow and a sign of high tissue perfusion, and this might be an important prerequisite for successful invitro fertilization and embryo transfer cycle. In this study the best uterine receptivity was achieved in lower resistance group and no pregnancy were detected when  $PI > 3.0$ . Similarly Steer et al. (11) reported that 35% of women who failed to conceive in on IVF programme had a mean uterine artery PI value  $> 3.0$ . Therefore, they suggested that embryo cryopreservation in those patients with a uterine artery  $PI > 2.99$  for transfer in subsequent cycles should be considered.

In other studies it was also found that there was significantly lower impedance at uterine artery blood flow in the conception group than in non-conception group in IVF-ET program. When spiral artery  $RI > 0.72$ ,  $PI > 1.6$  were present or there was absence of vascular signal in spiral artery, endometrium was considered to be non receptive, endometrial condition was suboptimal and implantation was unlikely to occur, so the patient was at high risk of ART treatment failure (11, 15).

In our study we observed a significantly lower impedance in uterine vasculature infertile control group in comparison to unexplained infertility group. Despite the similar demographic parameters and luteal phase progesterone profile spiral artery PI and RI values in unexplained infertility group were significantly higher than fertile control group. These findings are in accordance with the ones in literature. Pulsed Doppler ultrasonography is technically difficult especially with tiny vessels like subendometrial signals and spiral arteries and operator dependant. This may be a limitation to the wide spread use of this technique. In our study all of the measurements have been performed by the same author and the operator was blinded to the groups an this may be considered as a positive effect on objective evaluation. In conclusion our results can be considered as a sign for a significant endometrial contribution as derivation of blood flow impedance to unexplained group. Mid-luteal or peri-implantation period Doppler measurements should be considered in infertility patients and if impedance to uterine vasculature is found to be high these patients may

become candidates for therapies increasing the luteal blood low like aspirin and omega 3 in unexplained infertility and IVF patients (16, 17). Embryo freezing and subsequent hormonal preparation of endometrium can be considered.

#### Conflict of interest

No conflict of interest was declared by the authors.

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# Maternal plasma prolidase, matrix metalloproteinases 1 and 13, and oxidative stress levels in pregnancies complicated by preterm premature rupture of the membranes and chorioamnionitis

*Preterm erken membran rüptürü ve koryoamnionitle komplike gebeliklerde maternal plazma prolidaz, matriks metalloproteinaz 1 ve 13, ve oksidatif stres seviyeleri*

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## Abstract

**Objective:** This study aimed to investigate the role of various biochemical markers in preterm premature rupture of membranes (PPROM) and in prediction of chorioamnionitis in patients with PPRM.

**Material and Methods:** This case-control study included a total of 100 pregnant women at 26-34 gestational weeks. Of these women, 50 were healthy and 50 had PPRM. The biochemical markers in the maternal plasma including prolidase, matrix metalloproteinase (MMP) 1 and 13, total oxidative status (TOS), total antioxidant capacity (TAC), glutathione peroxidase (GPx), catalase (CAT), paraoxonase-1 (PON-1), tumor necrosis factor alpha (TNF- $\alpha$ ), and high sensitive C-reactive protein (hs-CRP) were assayed. These levels were compared between the PPRM and control groups and between women with or without chorioamnionitis in the PPRM group.

**Results:** Compared to the control group, the levels of prolidase, MMP-13, and TOS were significantly higher (p values <0.001, 0.020, and 0.035, respectively) and those of TAC and PON-1 were significantly lower in the maternal plasma of the PPRM group (p values=0.012 and <0.001, respectively). The plasma prolidase and TOS levels were significantly higher (p values=0.033 and 0.005, respectively) and the plasma TAC and PON-1 levels were significantly lower in women with chorioamnionitis as compared with the corresponding values in women without chorioamnionitis in the PPRM group (p values =0.041 and 0.048, respectively). The multivariate logistic regression analysis observed that prolidase, TAC, and PON-1 were important markers for the presence of PPRM and prolidase and TOS were important markers for predicting chorioamnionitis.

**Conclusion:** This study suggested that maternal plasma prolidase, TAC, and PON-1 may be useful for the diagnosis of PPRM, and prolidase and TOS may be used to predict chorioamnionitis in patients with PPRM. (J Turkish-German Gynecol Assoc 2012; 13: 172-7)

**Key words:** Preterm premature rupture of membranes, maternal plasma, prolidase, matrix metalloproteinase, oxidative stress

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## Özet

**Amaç:** Bu çalışmanın amacı çeşitli biokimyasal belirteçlerin preterm erken membran rüptürü (PEMR) ve PEMR olan hastalarda koryoamnioniti predikte etmedeki rollerini araştırmaktır.

**Gereç ve Yöntemler:** Bu vaka-kontrol çalışma, 26 ile 34 gebelik haftaları arasında 100 gebe kadını kapsadı. Bu gebe kadınların 50'si sağlıklı, 50'si PEMR'ne sahipti. Maternal plazmada, biokimyasal belirteçler olarak, prolidaz, matriks metalloproteinaz 1 ve 13, total oksidatif status (TOS), total antioksidan kapasite (TAC), glutatyon peroksidaz (GPx), katalaz (CAT) ve paraoksonaz-1 (PON-1), tümör nekrozis faktör alfa (TNF- $\alpha$ ) ve yüksek sensitif C-reaktif protein (hs-CRP) test edildi. Biokimyasal belirteçlerin plazma düzeyleri, hem PEMR ve kontrol grubu arasında, hem de PEMR grubunda koryoamnionit gelişen ve gelişmeyen hastalar arasında karşılaştırıldı.

**Bulgular:** Kontrol grubuyla karşılaştırıldığında, PEMR olan hastalarda maternal plazma prolidaz, MMP-13 ve TOS düzeyleri anlamlı yüksek (p değeri; <0.001, 0.020, 0.035); TAC ve PON-1 düzeyleri anlamlı düşüktü (p değeri; 0.012, <0.001). PEMR grubunda, koryoamnionit gelişen kadınlarda, koryoamnionit gelişmeyen kadınlardaki değerlerle karşılaştırıldığında plazma prolidaz ve TOS düzeyleri anlamlı yüksek (p değeri; 0.033, 0.005); TAC ve PON-1 anlamlı düşüktü (p değeri; 0.041, 0.048). Multivaryans lojistik regresyon analiz ile prolidaz, TAC ve PON-1'in PEMR'nün varlığı için; prolidaz ve TOS'un koryoamnioniti predikte etmek için önemli belirteçler oldukları bulundu.

**Sonuç:** Bu çalışma maternal plazma prolidaz, TAC ve PON-1'in PEMR'nün tanısında faydalı olabileceğini; prolidaz ve TOS'un PEMR olan hastalarda koryoamnionitin öngörülebilmesinde kullanılabileceğini gösterdi. (J Turkish-German Gynecol Assoc 2012; 13: 172-7)

**Anahtar kelimeler:** Preterm erken membran rüptürü, maternal plazma, prolidaz, matriks metalloproteinaz, oksidatif stres

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## Introduction

Premature rupture of membranes (PROM) is defined as the rupture of fetal membranes any time before the start of labor. If this rupture occurs before the thirty-seventh week of gestation, it is known as preterm premature rupture of membranes (PPROM). PPRM occurs in approximately 1% of all pregnancies and in 30% of all preterm deliveries (1). Lower socioeconomic level, lower maternal mass body index (BMI), smoking, history of preterm birth, urinary and genital tract infections, increase in membrane tensile strength due to excessive uterine distension as in polyhydramnios, vaginal bleeding at any stage of pregnancy, amniocentesis, and cerclage have been considered to be risk factors for PPRM (2). Although impaired collagen metabolism, increased oxidative stress, and choriodecidual inflammation or infections are suspected to be involved in its etiopathogenesis, its etiology has not yet been fully elucidated (3-5). PPRM can be diagnosed by the presence of amniotic fluid leakage from the external cervical ostium observed during sterile speculum examination. Although some pregnant women may complain of symptoms suggestive of PPRM, the examination findings might not support this diagnosis. In such cases, establishment of diagnosis can be challenging. In the treatment of PPRM, if there are no findings of labor, fetal distress, and chorioamnionitis, expectant management is preferred for the maturation of fetal lungs (6). Chorioamnionitis is an important infection, increasing both maternal and neonatal morbidity and mortality (7, 8). Therefore, it is important to detect subclinical intrauterine infection before the development of clinical chorioamnionitis. Although culture of fluid obtained by amniocentesis is a safe method for diagnosing intrauterine infection, due to its invasive nature several studies have aimed to develop noninvasive diagnostic tests (9).

In this study, our aim was to determine the importance of plasma markers in the prediction of PPRM and chorioamnionitis in pregnant women. To this end, we measured the prolidase, matrix metalloproteinase 1 and 13 (MMP1 and MMP13, respectively), total oxidative status (TOS), total antioxidant capacity (TAC), glutathione peroxidase (GPx), catalase (CAT), paraoxonase 1 (PON 1), tumor necrosis factor alpha (TNF- $\alpha$ ), and high sensitive C-reactive protein (hs-CRP) in the plasma of healthy pregnant women and pregnant women with PPRM.

## Material and Methods

This prospective case-controlled study was conducted at the Dicle University Faculty of Medicine, Department of Gynecology and Obstetrics between January and December 2011. The study protocol was approved by Ethics Committee of our university. Informed written consents were obtained from all the pregnant women participating in the study.

This study included a total of 100 pregnant women at 26-34 weeks of gestation, of whom 50 were healthy (control group) and 50 had PPRM (PPROM group). Women with preeclampsia, placenta previa, ablatio placentae, polyhydramnios, multiple pregnancies, major fetal anomaly, diabetes mellitus, chronic systemic disease, and suspected PPRM were excluded from

the study. PPRM was diagnosed by observing the accumulation of amniotic fluid in the posterior fornix during sterile speculum examination as well as by a positive nitrazine test. Routine laboratory examination for all patients included complete urinalysis, routine whole blood count, biochemical tests, as well as obstetric ultrasonography (US) examinations to determine the gestational age, major anomalies, amniotic fluid index, and number of fetuses. In the PPRM group, high-sensitive C-reactive protein (hs-CRP), cervicovaginal cultures and postpartum placental histopathological examination were performed.

The expectant management was applied to pregnant women in the PPRM group. All the women were given prophylactic antibiotic therapy (with ampicillin) as medical intervention. Also, standard antenatal corticosteroid therapy (with betamethasone) was administered for accelerating fetal lung maturation in pregnant women with PPRM before 32 weeks of gestation (1). The pregnancy was terminated with the development of fetal distress, active labor, and clinical chorioamnionitis.

The patients in the PPRM group were closely followed up for clinical infection indices such as fever, heart rate, vaginal discharge, uterine sensitivity, white blood cell count, and hs-CRP levels. Chorioamnionitis was clinically diagnosed by 2 or more positive results among the following tests: uterine sensitivity and irritability, foul-smelling and purulent discharge, leukocytosis ( $>15000$  cells/mm<sup>3</sup>), and maternal fever of  $\geq 38^{\circ}\text{C}$  during the hospitalization (10). Histologically, the diagnosis of chorioamnionitis was established by findings of acute inflammatory changes in the chorionic placenta and its membranes. However, funisitis was defined as detection of neutrophilic infiltration into the umbilical vessel wall and Wharton gel (11). In the PPRM group, participants with or without clinical and/or histopathological chorioamnionitis were defined as chorioamnionitis (+) and (-), respectively.

Participants in the control group were pregnant women at 26-34 weeks of gestation who delivered at term and did not show any emergent complications of pregnancy during routine prenatal controls.

## Sampling

We collected one blood sample from each patient on hospital admission prior to administration of steroids and antibiotics in the PPRM group and on routine pregnancy follow-up in the control group at the same time of gestation. Blood samples drawn from the antecubital vein were centrifuged at 3000 rpm for 10 minutes, and plasma portions of the samples were stored at  $-80^{\circ}\text{C}$  until laboratory analysis.

## Biochemical analyses

Enzymatic activity of prolidase, TAC, and TOS were analyzed as described previously (12). CAT and PON-1 activities were measured using the method of Aebi et al. (13) and spectrophotometrically by the modified Eckerson method, respectively (14). Enzyme-linked immunosorbent assay (ELISA) kits were used to measure the MMP1, MMP13 (Raybiotech Inc. USA), TNF- $\alpha$ , and hs-CRP levels (Diasource Immunoassays S.A., Belgium). GPx activity was measured according to the method described by Kayabasi et al. (15).

For the quantitative analysis of whole blood count and of routine biochemical parameters, an Abbott Cell Dyn 3700 Hematology Analyzer (Abbott Diagnostics, IL, USA), and a Roche/Hitachi P 800 module autoanalyzer (Roche Diagnostics, IN, USA), respectively, were used.

### Statistical Analysis

Data were analyzed using the Microsoft Statistical Package for Social Sciences (SPSS) version 18.0 for Windows. The sample size was calculated with Cohen's power analysis method using effect size  $d=0.8$ , 80% power,  $\alpha=0.05$ , and considering the results of previous studies (16).

The normality of distribution of data was tested using the Kolmogorov-Smirnov statistical method. Mann-Whitney *U* and Student's *t* tests were used for continuous variables, and the chi-square, Pearson's chi-square, and Fisher's exact tests were used for categorical variables. Logistic regression analyses were carried out to assess the correlations between these parameters and detect PPRM and chorioamnionitis in cases with PPRM.  $P < 0.05$  was considered statistically significant.

### Results

The study included 50 pregnant women with PPRM and 50 healthy pregnant women (control group). There were no signif-

icant differences in the demographic parameters between both the groups (Table 1). In the PPRM group, all pregnant women received prophylactic antibiotic therapy. For those with age of gestation of  $<32$  weeks, corticosteroid therapy was instituted to support fetal lung maturation. At the time of hospital admission, the mean time after membrane rupture was  $1.93 \pm 0.37$  days. The mean time until the delivery after membrane rupture was  $6.75 \pm 2.9$  days.

Cervicovaginal cultures were positive in 10 of the 50 (20%) women, with *Candida albicans* in 6, Enterobacteriaceae in 3, and group B streptococci in 3. In the PPRM group, clinical chorioamnionitis developed in 7 (14%) women. Indices of clinical chorioamnionitis, including increased CRP levels ( $n=7$ ; 14%), leukocytosis ( $n=12$ ; 24%), fever  $\geq 38^\circ\text{C}$  ( $n=7$ ; 14%), foul-smelling vaginal discharge ( $n=7$ ; 14%), and uterine sensitivity ( $n=3$ ; 6%), were detected. Further, 13 (26%) of the 50 pregnant women exhibited histological evidence of chorioamnionitis.

The maternal plasma levels of prolidase, MMP-1, MMP-13, TOS, TAC, PON-1, CAT, GPx, TNF- $\alpha$ , and hs-CRP in both the groups are presented in Table 2. As compared with the control group, the prolidase, MMP-13, and TOS levels were significantly higher in the PPRM group, while the TAC and PON-1 levels were found significantly lower. The MMP-1, GPx, CAT, TNF- $\alpha$ , and hs-CRP levels did not differ significantly between groups (Table 2). Multivariate logistic regression analyses of statistically signifi-

**Table 1. Demographic characteristics**

	PPROM group N (50)	Control group N (50)	P value
Maternal age (year)	28.9 $\pm$ 4.80	29.2 $\pm$ 6.80	0.852
Parity	2.18 $\pm$ 1.99	2.120 $\pm$ 1.95	0.850
Abortion	0.65 $\pm$ 1.29	0.37 $\pm$ 0.89	0.254
Gestational age (week in first evaluation)	30.75 $\pm$ 3.89	31.10 $\pm$ 3.94	0.684
History of PPRM	5 (10)	3 (6)	0.715
BMI (kg/m <sup>2</sup> )	27.5 $\pm$ 1.68	27.8 $\pm$ 1.65	0.527

Data has been given as mean $\pm$ standard deviation (SD) or n (%). BMI: Body mass index, PPRM: preterm premature rupture of membrane

**Table 2. The maternal plasma levels of parameters**

Parameters	PPROM group N (50)	Control group N (50)	P value
Prolidase (U/L)	793.2 $\pm$ 141.6	525.2 $\pm$ 107.1	<0.001
MMP 1 (pg/mL)	244.6 $\pm$ 65.59	220.8 $\pm$ 48.26	0.060
MMP 13 (pg/mL)	380.8 $\pm$ 122.1	325.6 $\pm$ 89.82	0.020
TOS ( $\mu\text{mol H}_2\text{O}_2$ Equiv./L)	12.87 $\pm$ 3.43	11.55 $\pm$ 2.03	0.035
TAC (mmol Trolox Equiv./L)	0.93 $\pm$ 0.42	1.17 $\pm$ 0.41	0.012
GPx (U/mL)	1.73 $\pm$ 1.34	2.14 $\pm$ 1.57	0.201
CAT (U/L)	599.5 $\pm$ 166.3	646.8 $\pm$ 150.7	0.18
PON 1 (U/L)	61.26 $\pm$ 14.05	98.84 $\pm$ 36.41	<0.001
TNF- $\alpha$ (pg/mL)	14.37 $\pm$ 2.71	14.88 $\pm$ 5.08	0.571
hs-CRP (mg/dL)	0.78 $\pm$ 0.52	0.68 $\pm$ 0.35	0.328

Data has been given as mean $\pm$ standard deviation (SD). PPRM: preterm premature rupture of membrane, MMP 1: matrix metalloproteinase 1, MMP 13: matrix metalloproteinase 13, TOS: total oxidative status, TAC: total antioxidant capacity, GPx: glutathione peroxidase, CAT: catalase, PON 1: paraoxonase 1, TNF- $\alpha$ : tumor necrosis factor alpha, hs-CRP: high sensitive C-reactive protein

cant parameters revealed that the prolidase, TAC, and PON-1 levels were important predictors of PPRM (Table 3).

Comparison of the biochemical parameters of the women in the PPRM group with negative and positive cervicovaginal cultures revealed no significant differences. In the PPRM group, the prolidase and TOS levels were significantly higher while the TAC and PON-1 levels were significantly lower in women with chorioamnionitis when compared with the corresponding levels in women without signs of chorioamnionitis (Table 4). Furthermore, multivariate logistic regression analyses for these parameters were performed, and it was found that the plasma prolidase and TOS levels were important predictive markers of chorioamnionitis (Table 5).

## Discussion

PPROM is an important obstetric problem whose etiopathogenesis has been suggested to involve factors such as impaired collagen metabolism, increased oxidative stress, choriodecidual inflammation, and/or infection. Fetal membranes are comprised of the amnion and chorion, which are both strong and flexible. These membranes consist of collagens and noncollagenous materials. Prolidase and MMPs are enzymes that play important roles in the metabolism of collagen. Also, PPRM has been suggested to be related to increased oxidative stress and infection and/or inflammation at the amniochorionic site. Therefore, we investigated the levels of the above-mentioned

**Table 3. The multivariate logistic regression analysis of parameters related to PPRM**

Parameters	Beta-coefficient	P value	Odds ratio (95% CI)
Prolidase	0.016	<0.001	1.016 (1.007-1.026)
MMP 13	0.007	0.163	1.007 (0.997-1.017)
TOS	0.136	0.461	1.146 (0.798-1.646)
TAC	-3.091	0.016	0.045 (0.004-0.568)
PON 1	-0.067	0.006	0.935 (0.891-0.981)

CI: confidence interval, PPRM: preterm premature rupture of membrane, MMP 13: matrix metalloproteinase 13, TOS: total oxidative status, TAC: total antioxidant capacity, PON 1: paraoxonase 1

**Table 4. The maternal plasma levels of parameters in pregnant women with and without chorioamnionitis in the PPRM group**

Parameters	Chorioamnionitis (+) (N:13)	Chorioamnionitis (-) (N:37)	P value
Prolidase (U/L)	854.7±87.09	754.6±151.4	0.033
MMP 1 (pg/mL)	261.8±42.74	235.7±73.84	0.158
MMP 13 (pg/mL)	387.2±138.8	377.5±114.9	0.970
TOS (μmol H <sub>2</sub> O <sub>2</sub> Equiv./L)	14.73±3.79	11.90±2.83	0.005
TAC (mmolTrolox Equiv./L)	0.75±0.31	1.03±0.45	0.041
GPx (U/mL)	1.67±1.16	1.76±1.44	0.862
CAT (U/L)	552.6±177.5	623.8±157.9	0.220
PON 1 (U/L)	54.26±13.56	64.89±13.09	0.048
TNF- α (pg/mL)	14.82±3.31	14.13±2.37	0.665
hs-CRP (mg/dL)	0.89±0.57	0.72±0.48	0.393

Data has been given as mean±standard deviation (SD). PPRM: preterm premature rupture of membrane, MMP 1: matrix metalloproteinase 1, MMP 13: matrix metalloproteinase 13, TOS: total oxidative status; TAC: total antioxidant capacity, GPx: glutathion peroxidase, CAT: catalase, PON 1: paraoxonase 1, TNF-α: tumor necrosis factor alpha, hs-CRP: high sensitive C-reactive protein

**Table 5. The multivariate logistic regression analysis of parameters related to chorioamnionitis**

Parameters	Beta-coefficient	P value	Odds ratio (95% CI)
Prolidase	0.007	0.049	1.007 (1.002-1.013)
TOS	0.285	0.021	1.329 (1.044-1.693)
TAC	-2.017	0.064	0.133 (0.016-1.127)
PON 1	-0.072	0.084	0.931 (0.858-1.010)

CI: confidence interval, PPRM: preterm premature rupture of membrane, MMP 13: matrix metalloproteinase 13, TOS: total oxidative status, TAC: total antioxidant capacity, PON 1: paraoxonase 1

enzymes, oxidative stress, and inflammation in women with PPROM and in women who developed chorioamnionitis during the clinic follow-up in the PPROM group, assuming that increased or decreased levels of the above parameters would aid in the diagnosis of PPROM as well as in the prediction of chorioamnionitis. In this study, the levels of prolylase, MMP-1, and MMP-13, which play important roles in collagen metabolism; TOS, TAC, PON-1, CAT, and GPx as oxidative stress markers; and TNF- $\alpha$  and hs-CRP as inflammatory markers were analyzed in the maternal plasma of pregnant women with PPROM and in healthy pregnant women (controls). In patients with PPROM, while the levels of prolylase, MMP-13 and TOS were significantly higher, the TAC and PON-1 levels were lower as compared to the control group. While significantly increased levels of prolylase and TOS were found, the TAC and PON-1 levels were significantly decreased in patients with PPROM who subsequently developed chorioamnionitis.

Prolidase is a cytoplasmic enzyme that degrades imidopeptides containing C-terminal proline and hydroxyproline. Prolidase activity plays an important role in the production of collagen and other proteins with a proline content, and it is an indicator of increased collagen turnover (17, 18). Prolidase activity has been found to increase in diseases associated with collagen metabolism disorders as pathogenetic processes (19-21). Moreover, collagen metabolism disorder has been proposed as an etiopathogenetic factor for PPROM. Therefore, we investigated the prolylase activity in the plasma samples of pregnant women with PPROM. Prolidase activity was significantly increased in the PPROM group, and it was significantly higher in women with chorioamnionitis than in women without this condition. Moreover, multivariate regression analyses revealed that prolylase was a significant predictive marker for both PPROM and chorioamnionitis.

The amniochorionic extracellular matrix consists of intense connective tissue and is considerably resistant to proteases. Their physiological degradation requires involvement of specific proteases, namely, matrix metalloproteinases (MMPs). MMPs are released in an inactive form and inhibited by tissue-specific inhibitors of MMPs. Their activation has been demonstrated to be an important marker in PPROM (22-24). MMP-1, or collagenase I, and MMP-13, or collagenase III, are 2 members of the proteinase family found in the fetal membrane and amniotic fluid (24). In the present study, the relationship of these 2 enzymes with PPROM was investigated. Although higher plasma MMP-13 activities were detected in cases with PPROM, multivariate regression analysis revealed that the diagnostic value of MMP-13 activity was insignificant for PPROM. The MMP-1 levels did not differ significantly between the two groups.

Oxidative stress (OS) is defined as an imbalance between the production of reactive oxygen species and the protective capacity of antioxidants. Pregnancy is a state causing oxidative stress due to increased metabolic activity and decreased antioxidant capacity (25). The oxidative stress induced in the intrauterine compartment reflects in the maternal circulation (20). In many studies, significant increase in the oxidative stress and decrease in antioxidant capacity have been reported in conditions with adverse outcomes in pregnancy, such as preeclampsia, fetal

growth restriction, and preterm birth, when compared with the levels in normal pregnancies (26, 27). Few studies have investigated the association between PPROM and oxidative stress. In our study, we investigated the antioxidant markers CAT, PON-1, GPx, and TAC and the oxidative marker TOS. In the PPROM group, as well as in the subset of this group who developed chorioamnionitis, the TOS levels were higher and the TAC and PON-1 levels were lower as compared to the control and chorioamnionitis (-) subset, respectively. There was no significant difference in the CAT and GPx level between the groups. The TAC and PON-1 levels were found to be significant predictive markers for PPROM, whereas the TOS level was revealed to be significant as a predictive marker of chorioamnionitis.

In pregnancy, the production of anti-inflammatory and proinflammatory cytokines are balanced. Anti-inflammatory cytokines are dominant during the early stages of pregnancy, and the level of proinflammatory cytokines increases as the pregnancy progresses and during its advanced stages. Even labor is said to be induced as a result of an inflammatory process. Increase in the proinflammatory cytokines has been reported in the etiology of PPROM (28). Increased levels of IL-6 and IL-8 in patients with PPROM have further supported these findings (9, 29). In our study, comparison of the TNF- $\alpha$  and hs-CRP levels in both the groups as well as in the chorioamnionitis (+) and (-) groups revealed no significant differences.

One of the theories in the pathogenesis of PPROM is intrauterine infection (1). Intrauterine infection can be effectively diagnosed by assessments such as gram stain, culture, and by evaluation of glucose and interleukin-6 levels of amniotic fluid obtained by amniocentesis (30). The studies demonstrated that the prophylactic antimicrobial therapy has beneficial effects, including prolongation of pregnancy and prevention of chorioamnionitis and neonatal sepsis. The studies were performed to investigate prolonged pregnancy with prophylactic antimicrobial therapy, considering infection as the pathogenesis of PPROM (31, 32). These studies reported that antimicrobial treatment significantly delayed delivery in pregnant women with PPROM. Since amniocentesis is an invasive method, no amniocentesis was performed in this study; however, all pregnant women with PPROM received prophylactic antimicrobial therapy with ampicillin. Clinical chorioamnionitis developed in 7 (14%) women, and the mean time until delivery was found to be  $6.75 \pm 2.9$  days.

## Conclusion

We determined that the maternal plasma levels of prolylase, TAC, and PON-1 are significant predictive factors for PPROM and that the prolylase and TOS levels are a significant predictive factor for chorioamnionitis. Further study is required to determine whether or not these markers can be used in non-invasive tests as predictors for both PPROM and subsequent chorioamnionitis.

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**Conflict of interest**

No conflict of interest was declared by the authors.

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# Prediction of metabolic syndrome in women with polycystic ovary syndrome

## *Polikistik over sendromlu kadınlarda metabolik sendromun öngörüsü*

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### Abstract

**Objective:** To identify biochemical factors that serve as predictors for the metabolic syndrome (MetS) in patients with polycystic ovary syndrome (PCOS) and to investigate the value of adipocytokines in the prediction of metabolic syndrome.

**Material and Methods:** A total of 91 pre-menopausal women with PCOS diagnosed according to the Rotterdam consensus criteria were recruited as study subjects. Waist circumference, blood pressure, body mass index (BMI), fasting glucose, serum lipids, insulin, FSH, LH, E<sub>2</sub>, total testosterone, homeostatic model assessment–insulin resistance (HOMA-IR), serum leptin and adiponectin levels were evaluated for all patients.

**Results:** Of the 91 women with PCOS, 15 patients met the criteria for MetS. Body weight, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, fasting glucose, total cholesterol, triglyceride, and VLDL concentrations were significantly higher and HDL was significantly lower in women with PCOS+MetS compared with those with PCOS only. However, the level of LDL, FSH, LH, E<sub>2</sub> and total testosterone was not significantly different between these two groups. Women with PCOS+MetS had significantly higher levels of leptin and HOMA-IR, and significantly lower levels of adiponectin compared to the women with PCOS only. In the multiple logistic regression model, the association between HOMA-IR and leptin, and MetS remained statistically significant (p=0.001 and 0.018), while the association between adiponectin and MetS was no longer statistically significant.

**Conclusion:** Aside from the biochemical markers such as glucose, cholesterol and triglyceride, adipose tissue factors and insulin resistance are valuable parameters in the prediction of MetS in patients with PCOS. (J Turkish-German Gynecol Assoc 2012; 13: 178-83)

**Key words:** Metabolic syndrome, polycystic ovary syndrome, leptin, adiponectin, HOMA-IR

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### Özet

**Amaç:** Polikistik over sendromu (PKOS) tanısı almış hastalarda metabolik sendromun (MetS) öngörüsünde kullanılabilecek biyokimyasal faktörleri tanımlamak ve adipositokinlerin metabolik sendromun öngörüsündeki yerini araştırmak.

**Gereç ve Yöntemler:** Rotterdam kriterlerine göre PKOS tanısı almış olan 91 premenapozal kadın çalışma grubunu oluşturdu. Tüm hastaların bel çevresi, kan basıncı, vücut kitle indeksi (VKİ), açlık kan şekeri, serum lipid düzeyleri, insülin, FSH, LH, E<sub>2</sub>, total testosteron, HOMA-IR (homeostatic model assessment–insulin direnci), serum leptin ve adiponektin seviyeleri değerlendirildi.

**Bulgular:** PKOS tanısı almış 91 kadından 15'i MetS kriterlerini taşıyordu. PKOS+MetS tanılı kadınlarda sadece PKOS tanılı kadınlara göre vücut ağırlığı, VKİ, bel çevresi, sistolik kan basıncı, diastolik kan basıncı, açlık kan şekeri, total kolesterol, trigliserid ve VLDL düzeyleri anlamlı olarak yüksekken, HDL seviyesi anlamlı olarak düşük bulundu. Ancak, bu iki grup arasında LDL, FSH, LH, E<sub>2</sub> ve total testosteron seviyeleri açısından istatistiksel olarak anlamlı bir fark yoktu. PKOS+MetS tanılı kadınlarda diğer kadınlara göre leptin ve HOMA-IR düzeyleri anlamlı olarak yüksek, adiponektin seviyesi ise anlamlı olarak düşüktü. Çoklu lojistik regresyon analizinde HOMA-IR ve leptin ile MetS arasındaki ilişki istatistiksel olarak anlamlı kalırken (p=0.001 ve 0.018), adiponektin ile MetS arasındaki ilişki istatistiksel anlamını yitirdi.

**Sonuç:** Kan şekeri, kolesterol ve trigliserid gibi biyokimyasal belirteçlerin dışında, yağ dokusu faktörleri ve insülin direnci de PKOS hastalarında MetS öngörüsü açısından değerlidir.

(J Turkish-German Gynecol Assoc 2012; 13: 178-83)

**Anahtar kelimeler:** Metabolik sendrom, polikistik over sendromu, leptin, adiponektin, HOMA-IR

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### Introduction

Polycystic ovary syndrome (PCOS) is a frequent endocrine disorder affecting 6-12% of reproductive-age women (1). Oligo-amenorrhea, chronic anovulation, hyperandrogenism, hirsutism, and infertility are some of the well-defined clinical

manifestations of PCOS. It is also considered as a metabolic disorder, since the components of metabolic syndrome (MetS), namely obesity, glucose intolerance, atherogenic dyslipidemia, and hypertension, are the common features of this syndrome. Although MetS and PCOS have overlapping features, and both cause an increased risk of cardiovascular

disease, the pathophysiology that may link these two remains unclear. Possible theories regarding the association between MetS and PCOS include: (1) insulin resistance underlies the pathogenesis of both MetS and PCOS, (2) obesity and related adipose tissue factors (adipocytokins) independent of insulin resistance are the major pathogenic contributors to both conditions.

With the identification of adipose tissue as the source of leptin in 1994, adipose tissue transformed from a passive organ storing energy to an active endocrine organ which produces a variety of locally and systemically functioning bioactive molecules. The adipocyte-derived 16 kDa hormone leptin is a critical mediator of energy balance that relays information regarding the depletion or accumulation of fat stores to the brain. Effects on immune system, reproduction, angiogenesis and blood pressure are some of the widely varying peripheral and central actions of leptin (2). Both the granulosa and theca cells of the human ovarian follicle have leptin receptors and it has been shown that granulosa cells can secrete leptin, which indicates a direct paracrine role for leptin at the ovarian follicular level (3). Moreover, elevated leptin level is a common feature in obese subjects, and it has been suggested that it could represent an additional factor involved in the development of insulin resistance and in the impairment of ovarian function, particularly in women with PCOS (4).

Another molecule secreted by adipose tissue, adiponectin, is a 224-amino acid adipose specific protein, which was first identified in 1995. It has a molecular weight of 30 kDa, and is also referred to as Adipocyte Complement-Related Protein 30 kDa (ACRP30). Adiponectin suppresses almost all processes involved in atherosclerotic vascular changes including the expression of adhesion molecules in vascular endothelial cells, the proliferation of vascular smooth muscle cells and the formation of foam cells *in vitro*, and it exhibits an anti-atherosclerotic activity *in vivo* (5). Adiponectin is the only known protein that is produced in adipose tissue and it decreases in obesity (6). It is demonstrated that adiponectin has insulin-sensitizing activity, and the high level of plasma adiponectin is a negative risk factor for type 2 diabetes in diabetes-prone people (7). It has been shown to be correlated negatively with triglyceride level, fasting plasma glucose level, plasma glucose level two hours after a meal and fasting insulin concentration, and correlated positively with the high-density lipoprotein level (7, 8). Recent studies have also shown that adiponectin is related to endothelium-dependent vasodilatation and its plasma concentrations are low in subjects with essential hypertension (9).

In the present study, we aimed to find the predictive value of adiponectin and leptin in the assessment of MetS and to evaluate their relationship with severity of insulin resistance and hyperandrogenemia in patients with PCOS. We also aimed to find biochemical predictors of MetS in women with PCOS.

## Material and Methods

### Subjects, definitions and study design

This was a cross-sectional study conducted in the Gynecology Department of Kanuni Sultan Suleyman Research and Teaching

Hospital between April 2008 and June 2010. A total of 91 Turkish pre-menopausal women with PCOS were recruited as study subjects. The diagnosis of PCOS was based on the Rotterdam PCOS consensus (Rott-PCOS) criteria (10). According to these criteria, PCOS was diagnosed if at least two of the following criteria were present: oligo/amenorrhea, clinical or biochemical hyperandrogenism and PCO on ultrasonography. Clinical hyperandrogenism was defined as the presence of hirsutism (Ferriman-Galwey score >8) and/or acne. Biochemical hyperandrogenism was defined as elevated total testosterone. PCO was defined as the presence of at least one ovary with 12 or more follicles measuring 2-9 mm in diameter (11). Other etiologies that could mimic PCOS, such as Cushing syndrome, thyroid dysfunction, hyperprolactinemia, adrenal hyperplasia or androgen producing neoplasm were excluded. The use of medications known or suspected to affect reproductive or metabolic function was prohibited within 60 days prior to study entry. This study was approved by the institutional review boards of our center and all subjects provided written informed consent.

MetS was defined according to NCEP ATP III guidelines (12). MetS was diagnosed if at least three of the following five features were present: (i) central obesity with waist circumference >88 cm, (ii) elevated serum triglyceride >150mg/dL, (iii) reduced serum high-density lipoprotein (HDL)-cholesterol <50 mg/dL or the use of lipid lowering medication, (iv) elevated systolic and/or diastolic blood pressure >130/85 mmHg or the use of antihypertensive medication, (v) impaired fasting glucose >100mg/dL.

Insulin resistance (IR) was assessed using the homeostatic model assessment (HOMA-IR: fasting insulin ( $\mu$ U/mL) x fasting glucose (mg/dL)/405) (13).

### Physical examination, ultrasound and laboratory analyses

A standard questionnaire was used to document personal, medical, and drug history, regularity and length of menstrual cycles, ovulation status, symptoms of hirsutism and acne. Signs of androgen excess were noted during the physical examination. Body weight (kg), body height (m), and waist circumference (WC) (cm) were measured. Waist circumference was taken as the narrowest measurement between the top of the iliac crest and the lower rib margin. Sitting systolic and diastolic blood pressures (SBP-DBP) were measured after a 5-min rest using a standard sphygmomanometer. Hirsutism was established by using the Ferriman-Gallwey score. Transvaginal ultrasonography was systematically performed by the same investigator (E.A.) on a Logiq 9, using the 4 MHz transvaginal probe. Overnight fasting blood specimens were obtained in all women for measurement of fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), adiponectin, and leptin. Serum LH, FSH, and total testosterone concentrations were measured on the third day of either spontaneous or progesterone-induced menstruation.

After clot formation, blood samples were centrifuged at 4°C for 15 minutes at 2000×g. Serum fasting blood glucose, lipid levels, thyroid function tests, fertility hormones and insulin levels were

determined on the same day. Aliquots of serum samples were stored at  $-80^{\circ}\text{C}$  until leptin and adiponectin assaying. Glucose, total cholesterol, triglycerides, HDL and LDL cholesterol, free T4, free T3, TSH, FSH, LH, estradiol, progesterone, prolactin, and testosterone levels were analyzed with Architect c16200 Integrated System (Abbott Diagnostics Europe, Wiesbaden, Germany). Serum insulin levels were determined by Siemens Immulite 1000 immunoassay system (Siemens Healthcare Diagnostics, USA) Serum adiponectin levels were determined using AssayMax Human adiponectin ELISA Kit (Acrp30) (catalog EA2500-1, Lot 7250521), purchased from Assay Pro (USA) following the manufacturer's instructions. AssayMax Human adiponectin ELISA Kit employs the quantitative sandwich enzyme immunoassay technique. Intra-assay and inter-assay coefficients of variation were 4.2% and 7.3% respectively. Adiponectin levels were expressed as  $\mu\text{g}/\text{mL}$ . Serum Leptin levels were measured using DRG Leptin (Sandwich) ELISA kit (EIA-2395) purchased from DRG Instruments (GmbH, Germany) following the manufacturer's recommendations. Intra-assay and inter-assay coefficients of variation were 5.9% and 8.6% respectively.

#### Statistical analysis

In this study, the data were analyzed using NCSS 2007. Continuous variables were presented as mean $\pm$ SD and ana-

lysed using independent sample t-test. Categorical variables were presented as  $\chi^2$  test. Cut off for HOMA-IR, leptin and adiponectin variables was determined and areas under ROC curves were calculated and compared. For determining factors that were effective in the differential diagnosis of metabolic syndrome, logistic regression analysis was performed. Statistical significance was considered present if the P-value was  $<0.05$ , 95%CI.

#### Results

Table 1 shows the comparison of clinical and biochemical characteristics of women with PCOS only and women with PCOS + MetS. Of the 91 women, 15 patients met the criteria for MetS. Women in these two groups were similar in age and height. As expected, compared with the women who did not meet criteria for the MetS, those with the MetS had a significantly higher body weight, BMI, waist circumference, systolic blood pressure, and diastolic blood pressure. In addition, fasting glucose, total cholesterol, triglyceride, and VLDL concentrations were significantly higher in those with PCOS+MetS. Also, HDL was significantly lower in the PCOS+MetS group. However, the difference in LDL was not significant between these two groups, nor were the levels of FSH, LH,  $\text{E}_2$  and total testosterone.

**Table 1. Comparison of clinical and biochemical characteristics of women with PCOS only and women with PCOS+MetS**

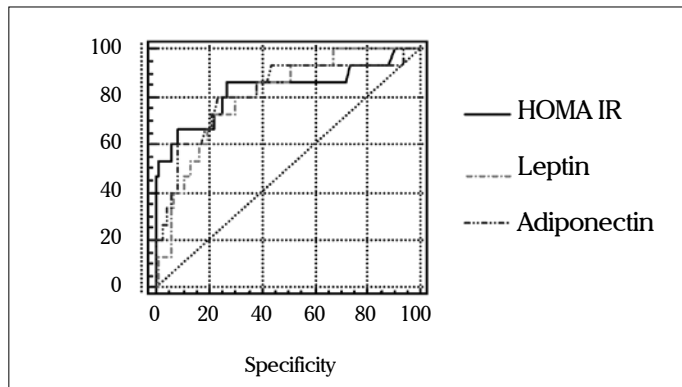
	PCOS only (n:76)	PCOS+MetS (n:15)	P
Age (year)	24.11 $\pm$ 4.73	25.93 $\pm$ 4.95	NS
Height (m)	1.6 $\pm$ 0.06	1.58 $\pm$ 0.07	NS
Weight (kg)	63.72 $\pm$ 12.84	78.87 $\pm$ 11.15	0.0001
BMI (kg/m <sup>2</sup> )	24.8 $\pm$ 5.01	31.47 $\pm$ 4.1	0.0001
WC (cm)	82.46 $\pm$ 9.45	94.6 $\pm$ 6.89	0.0001
SBP (mmHg)	110.13 $\pm$ 14.28	124.67 $\pm$ 13.02	0.0001
DBP (mmHg)	68.42 $\pm$ 8.8	76.67 $\pm$ 10.47	0.002
Glucose (mg/dL)	87.5 $\pm$ 13.57	109.33 $\pm$ 15.37	0.0001
Total cholesterol (mg/dL)	160.86 $\pm$ 39.63	186.53 $\pm$ 24.97	0.018
HDL-C (mg/dL)	42.33 $\pm$ 9.2	37.27 $\pm$ 7.69	0.049
Triglyceride (mg/dL)	94.51 $\pm$ 49.01	151.07 $\pm$ 52.64	0.0001
VLDL-C (mg/dL)	18.9 $\pm$ 9.8	30.21 $\pm$ 10.53	0.0001
LDL-C (mg/dL)	99.7 $\pm$ 36.86	119.09 $\pm$ 20.37	NS
FSH (mIU/mL)	5.18 $\pm$ 1.71	4.78 $\pm$ 1.96	NS
LH (mIU/mL)	8.46 $\pm$ 4.65	6.64 $\pm$ 4.9	NS
$\text{E}_2$ (pg/mL)	70.34 $\pm$ 62.66	51.8 $\pm$ 35.41	NS
Total testosterone (pg/mL)	0.87 $\pm$ 0.35	0.71 $\pm$ 0.33	NS
HOMA- IR	1.95 $\pm$ 1.72	8.81 $\pm$ 7.59	0.0001
Leptin (ng/mL)	30.35 $\pm$ 11.6	44.37 $\pm$ 14.34	0.0001
Adiponectin ( $\mu\text{g}/\text{mL}$ )	9.76 $\pm$ 4.57	6.15 $\pm$ 3.54	0.005

MetS, metabolic syndrome; NS, not significant; BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, LDL-C: low-density lipoprotein cholesterol, VLDL-C: very low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol, HOMA-IR: the homeostatic model assessment of insulin resistance

Moreover, the women with PCOS+MetS had significantly higher levels of leptin and HOMA-IR (both  $p=0.0001$ ), and significantly lower levels of adiponectin compared with the women with PCOS only ( $p=0.005$ ).

We constructed receiver operating characteristic (ROC) curves (Figure 1) and calculated the area under the curve (AUC) value as 0.833 for HOMA-IR, 0.805 for leptin, and 0.819 for adiponectin. Then we made pairwise comparison of ROC curves and we found statistically no difference between the AUC values of these parameters. For predicting metabolic syndrome, cut-off points for HOMA-IR, leptin and adiponectin were determined. When HOMA-IR was  $\geq 2.51$  sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratio were 86.67%, 73.68%, 39.4%, 96.6%, and 3.29%, respectively. When leptin level was  $\geq 35.7$  the corresponding statistics were 73.33%, 77.63%, 39.30%, 93.70% and 3.28%, and when adiponectin level was  $\leq 6.45$  they were 80%, 76.32%, 40%, 95.1% and 3.38%, respectively (Table 2).

In the multiple logistic regression model, the association between HOMA-IR and leptin, and MetS remained statistically significant ( $p=0.001$  and  $0.018$ ), while the association between adiponectin and MetS was no longer statistically significant (Table 3).



**Figure 1.** ROC curves for selected variables and the presence of metabolic syndrome

**Discussion**

The prevalence of MetS has been shown to range between 8.2% and 14.3% according to the criteria used in women with PCOS, as opposed to 2.7% and 6.6% in the control group (14). The most likely pathogenic link between PCOS and MetS is insulin resistance and compensatory hyperinsulinism. In one study, the prevalence of insulin resistance was reported to be 32% among women with PCOS (15). Although hyperinsulinemic euglycemic clamp technique is the gold standard for measuring insulin sensitivity, it is expensive, time consuming, and invasive (16). The homeostatic model assessment of IR (HOMA-IR) is a simple and noninvasive method for estimating insulin sensitivity from the steady glucose and insulin concentrations measured under fasting conditions. It generally correlates well with clamp techniques. In this study we used HOMA-IR as a surrogate marker of insulin resistance, and we postulated that women with concomitant PCOS and MetS would have more insulin resistance than women with PCOS only. We found HOMA-IR values of these two groups  $8.81 \pm 7.59$  and  $1.95 \pm 1.72$ , respectively ( $p=0.0001$ ). This finding is in agreement with other studies who have found that all surrogate markers of reduced insulin sensitivity are more obvious in women with concomitant PCOS and MetS than women without MetS (17-19).

Hyperandrogenism has been shown to be a risk factor for insulin resistance in PCOS cases (15). However, in our study women with PCOS only and women with PCOS+MetS did not show any significant difference in terms of total testosterone. While this finding is consistent with some studies in the literature (18, 19), it is in contrast with others (20). It has been also shown that hyperinsulinemia and lipid abnormalities remained unchanged despite the use of GnRH to suppress androgens in hirsute hyperandrogenic women (21). As a consequence of these findings, it can be suggested that hyperandrogenism by itself may not contribute to the development of MetS in women with PCOS.

**Table 2.** Diagnostic performance of HOMA-IR, leptin and adiponectin in the differentiation of metabolic syndrome

	Cut Off	Sensitivity	Specificity	PPV	NPV	+LR	-LR
HOMA IR	>2.51	86.67	73.68	39.4	96.6	3.29	0.18
Leptin	>35.7	73.33	77.63	39.3	93.7	3.28	0.34
Adiponectin	<6.45	80.00	76.32	40.0	95.1	3.38	0.26

PPV: Positive predictive value, NPV: Negative predictive value, LR: Likelihood ratio

**Table 3.** Multivariate logistic regression analysis

	B	S.E.	p	95.0% C.I. for EXP(B)		
				Exp (B)	Lower	Upper
HOMA-IR	0.657	0.17	0.001	1.77	1.26	2.48
Leptin	0.069	0.07	0.018	1.07	1.01	1.13
Adiponectin	-0.32	0.18	0.076	0.73	0.51	1.03
Constant	-3.77	2.62	0.004			

Although current theories focus on insulin resistance as the prime factor linking visceral obesity with adverse metabolic changes, studies suggest that the pathophysiology of MetS cannot be explained by insulin resistance alone. Beyond glucose homeostasis; dyslipidemia, blood pressure, and many other pathophysiologic features have been characterized in individuals with MetS. Also, adipose tissue factors (adipocytokins) play an important role in the pathogenesis of this syndrome. Previous studies showed that adiponectin was closely associated with visceral fat accumulation and insulin resistance, and the low levels of adiponectin were linked to the components of MetS (7, 8, 22). It has been also reported that adiponectin level might be useful for the diagnosis of MetS in obese Japanese children (23). According to the effects of excess adiposity and IR on adiponectin levels, a marked decrease of adiponectin levels in women with PCOS can be assumed. This assumption has been confirmed by the findings of numerous studies (24, 25). As mentioned above, both MetS and PCOS are related with low adiponectin levels and it is expected that women with concomitant PCOS and MetS would have lower adiponectin levels compared to women with PCOS only. In this study, similar to previous studies, adiponectin levels were detected lower in PCOS patients with MetS.

Clearly, serum leptin concentrations rise in proportion to the body adiposity. Therefore, obese individuals with MetS generally have higher circulating leptin concentrations. However, obese individuals seem to be resistant to the hypothalamic effects of leptin, and the catabolic pathways designed to reduce appetite and increase energy expenditure are not activated, therefore, excess body weight is maintained. In animal models of pharmacologically induced PCOS (rats with dihydrotestosterone-induced PCOS) real-time reverse-transcriptase PCR detected that the level of leptin expression in visceral fat of rats with PCOS was higher than that of controls (26). Several reports show that women with PCOS have higher circulating concentrations of leptin as compared with controls (27, 28). The role of hyperleptinemia in women with PCOS is not clear yet, but there is a possibility that women with PCOS are leptin resistant. In this study leptin levels were significantly elevated in women with concomitant MetS and PCOS. It is probably the result of the concomitant presence of two leptin resistant conditions, PCOS and MetS, in the same group of patients.

Screening all women with PCOS for the presence of MetS is expensive. For this reason, screening should be limited to only those women particularly at risk for MetS. At the Rotterdam consensus meeting, it was mentioned that obese PCOS patients should be screened for MetS, but they did not give specific criteria or validity for screening these women. In the literature, there are a few studies that were performed to find the methods of the prediction of MetS in patients with PCOS. Goverde et al. (29) found that a combination of waist circumference and free androgen index (FAI) offers the best selection criterion for the presence of either MetS or IR. Another study suggested use of the triglyceride/HDL-cholesterol ratio (TG/HDL-C ratio) >3.2 as a criterion to screen for MetS in hyperandrogenic women (AUC 0.941, sensitivity 90.9%, specificity 87.5%) (19).

In this study, HOMA-IR, adiponectin and leptin were found to be suitable for the differential diagnosis of MetS and cut off points

were defined in a cohort of women diagnosed with PCOS. However, this study was not without limitations. HOMA-IR may have underestimated the true prevalence of IR. Even though the gold standard for the diagnosis of IR is euglycemic hyperinsulinemic clamp, we opted to use HOMA-IR, because euglycemic hyperinsulinemic clamp technique is too expensive and complicated to use in clinical practice and large-scale studies.

## Conclusion

In the management of PCOS it is essential to differentiate the women particularly at risk for MetS. To our knowledge this is the first study that investigate the value of adipocytokines in the prediction of MetS in patients with PCOS. We have found that adiponectin, leptin and HOMA-IR levels are significantly different between women with PCOS only and women with PCOS+MetS, as well as the other biochemical markers, such as glucose, cholesterol and triglycerides. However, these markers are very expensive and impractical for clinical use. Therefore, more studies are required in order to find the optimum biochemical or clinical markers that predict the presence of MetS in patients with PCOS.

## Conflict of interest

No conflict of interest was declared by the authors.

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# Perinatal outcomes and anomalies associated with fetal right aortic arch

## *Fetal sağ aortik ark: perinatal sonuçları ve ilgili anomaliler*

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### Abstract

**Objective:** To evaluate the prenatal findings, associated anomalies, and prognosis of right aortic arch (RAA) anomalies.

**Material and Methods:** All cases referred for detailed ultrasonography and fetal echocardiography between October 2006 and July 2009 were systematically examined for aortic arch anomalies and associated cardiac and extracardiac anomalies. Prenatal findings of all cases with aortic arch anomalies and intracardiac and extracardiac findings were prospectively registered in an electronic database that included fetal echocardiography. Outcomes of the cases were collected postnatally from the patients' obstetricians, neonatal unit archives, and pediatric cardiologists.

**Results:** We detected 12 cases of RAA (0.37%; n=12/3200). Mean gestational age at diagnosis was 24 weeks (range, 21–33 weeks). Of the 12 cases of RAA, five (41.7%) had a major cardiac defect, including tetralogy of Fallot (n= 3), atrioventricular septal defect (n=1), and ventricular septal defect (n=1). An extracardiac anomaly was observed in three cases (25%). The fetal karyotype was trisomy 21 in one case with increased nuchal translucency (6.6 mm). Microdeletion 22q11 analyses performed in three cases were normal. The postnatal courses of the cases with isolated RAA were uneventful. Two cases associated with major cardiac and extracardiac anomalies were lost in the early neonatal period. The case of trisomy 21 was terminated. The other four cases of RAA with an associated cardiac anomaly are currently in follow up.

**Conclusion:** Aortic arch anomalies, particularly RAA, can be diagnosed by fetal echocardiography. The prognosis for isolated RAA is relatively good compared with that for RAA with associated anomalies. (J Turkish-German Gynecol Assoc 2012; 13: 184-6)

**Key words:** Right aortic arch, aortic arch anomaly, fetal echocardiography, fetus, prenatal diagnosis

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### Özet

**Amaç:** Prenatal dönemde, sağ aortik ark tanısı alan hastaların prognozu ve diğer anomalilerle birlikteliğini değerlendirmek.

**Gereç ve Yöntemler:** Kasım 2006 ile Temmuz 2009 yılları arasında ünitemize obstetrik ultrasonografi ve fetal ekokardiyografi yapılması için refere edilen vakalar sağ aortik ark ve ilgili diğer kalp ve kalp dışı anomalilerin varlığı için sistematik olarak muayene edildi. Prenatal dönemde, sağ aortik ark tanısı ve diğer kalp ve/veya kalp dışı anomali tanısı alan hastalar prospektif olarak elektronik ortama kaydedildi. Postnatal dönemdeki sonuçlar hastanın kadın doğum hekiminden, neonatal ünite dosyalarından ve pediatrik kardiyoloji hekiminden alındı.

**Bulgular:** Çalışma süresi boyunca on iki hastaya sağ aortik ark tanısı konuldu (%0.37; n:12/3200). Tanı ortalama olarak 24'üncü gebelik haftasında kondu (ortalama: 21-33 hafta). Aortik ark anomali tanısı konan 12 hastanın beş (%41.7) tanesinde major kardiyak anomali saptandı. Bu anomaliler; fallot tetralojisi (TOF, n:3), atriyoventriküler septal defekt (AVSD, n:1) ve ventriküler septal defekt (VSD, n:1) idi. Çalışma grubumuzdaki üç hastada (%25) kalp dışı anomali varlığı tespit edildi. Ense saydamlığı (NT: 6.6 mm) artmış olan bir hastanın karyotip analizinde Trizomi 21 tespit edildi. Microdelesyon 22q11 analizi yapılan üç hastanın sonuçları normal olarak bulundu. Major kardiyak anomali ve kalp dışı anomali olan iki hasta erken neonatal dönemde ex oldu. Trizomi 21 tanısı konulan hastanın gebeliği sonlandırıldı. Diğer sağ aortik ark ve ilgili kalp anomali tanısı alan dört hastanın takipleri halen sürmektedir.

**Sonuç:** Aortik ark anomalilerinin, özellikle sağ aortik ark anomalisinin tanısı fetal ekokardiyografi ile konulabilir. İzole sağ aortik ark anomalilerin prognozu, sağ aortik ark ve eşlik eden diğer anomaliler varlığı ile karşılaştırıldığında nispeten daha iyidir.

(J Turkish-German Gynecol Assoc 2012; 13: 184-6)

**Anahtar kelimeler:** Sağ aortik ark, aortik ark anomali, fetal ekokardiyografi, fetus, prenatal tanı

**Geliş Tarihi:** 19 Mart 2012

**Kabul Tarihi:** 01 Haziran 2012

## Introduction

Right aortic arch (RAA) is a relatively rare anomaly. The true incidence is unknown, but it is estimated to be 1 in 1000 in low-risk populations (1). RAA may occur in isolation or in association with cardiac and extracardiac anomalies. Prenatal diagnosis of RAA anomalies has not been a focus of attention until recently. So far, a few case series and some case reports have been published, but information regarding outcomes and associated anomalies is still limited. In this study, we report the outcomes and associated anomalies in cases of RAA detected in the fetus (2-8).

## Material and Methods

All cases referred for detailed ultrasonography (US) and fetal echocardiography between October 2006 and July 2009 were systematically examined with a US 4-8 MHz curved array probe (GE Voluson 730, Vienna, Austria), and all cardiac and extracardiac anomalies were noted prospectively. Fetal echocardiography was carried out using the segmental approach with standardised anatomical planes and the three-vessels-trachea view as an integral part of all examinations. RAA was diagnosed when an aortic arch was present on the right side of the trachea or on the right side with a U-shaped confluence (Figure 1, 2). Other aortic arch anomalies associated with cardiac and extracardiac anomalies were also identified. Fetal karyotype and 22q11 microdeletion analyses were performed in three cases. Prenatal findings of all cases were registered in an electronic prenatal database that included fetal echocardiography. Outcomes of cases were collected postnatally from the patients' obstetricians, neonatal units, and pediatric cardiologists. Prenatal diagnoses of RAA and associated anomalies were confirmed by pathological examination or postnatal echocardiography.

## Results

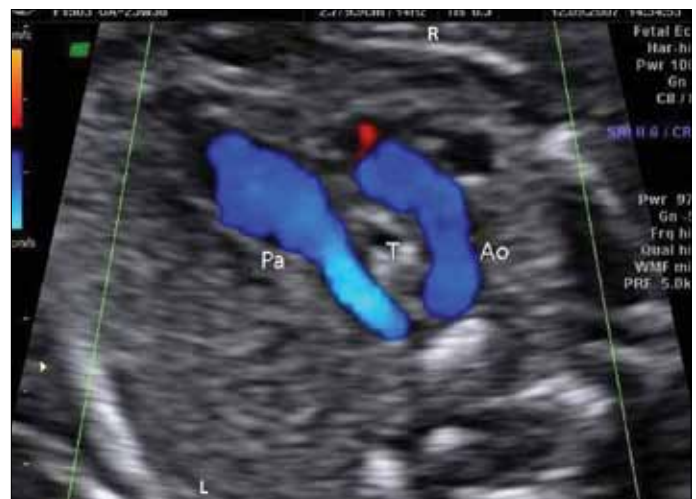
We detected 12 cases with RAA (0.37%; n=12/3200) (Table 1). Mean gestational age at diagnosis was 24 weeks (range, 21-33 weeks). Of the 12 cases of RAA, five (41.7%) were complicated with other major cardiac defects, including tetralogy of Fallot (TOF, n=3), atrioventricular septal defect (AVSD, n=1), and ventricular septal defect (VSD, n=1). Extracardiac findings were observed in three cases (25%). The fetal karyotype was trisomy 21 in one case with increased nuchal translucency (6.6 mm). Microdeletion 22q11 analyses were performed in three cases, and results were normal. The postnatal courses of the isolated RAA cases were uneventful. Two cases associated with major cardiac and extracardiac anomalies were lost during the early neonatal period. The case of trisomy 21 was terminated. The other four cases of RAA with associated cardiac anomalies are currently in follow-up.

## Discussion

Among the various anomalies of the aortic arch involving the vessel course and/or its branching pattern, those most

commonly seen include RAA with aberrant left subclavian or innominate arteries, RAA with mirror-image branching, double aortic arch, circumflex retroesophageal aortic arch, or left aortic arch with an aberrant right subclavian artery (1). The frequency of RAA among adults is approximately 0.1%, and a similar incidence was reported in a prenatal low-risk cohort (2, 3). Many undiagnosed cases are detected when RAA is investigated retrospectively.

RAA detected in the fetus is frequently associated with other cardiac and extracardiac malformations. The risk of concomitant congenital heart disease is >90% with the mirror-image branching type of RAA and only 10% with RAA and an aberrant left subclavian artery (3). Berg et al. (3) reported 71 fetuses with RAA, and additional cardiac abnormalities were noted in 22 (30%) of these cases. The most common cardiac lesions in that study were TOF, pulmonary atresia with VSD, and a common arterial trunk. Zidere et al. (4) reported 50 (66%) fetuses with other cardiac anomalies, the most common being TOF with pulmonary atresia. In our series, RAA was found in association



**Figure 1. Fetal right aortic arch**

Ao: aorta, Pa: pulmonary artery, T: trachea



**Figure 2. Fetal right aortic arch with tetralogy of Fallot**

Ao: aorta, Pa: pulmonary artery, T: trachea

**Table 1. Intracardiac, extracardiac anomalies and outcome in the cases of right aortic arch (n:12)**

No.	GA at diagnosis	Cardiac anomaly	Extracardiac anomaly	Outcome
1	23	-	-	Alive
2	22	-	-	Alive
3	21	-	-	Alive
4	24	-	-	Alive
5	23	-	-	Alive
6	33	-	Trachea and anal atresia, polyhydramnios	NND
7	24	VSD	Pelvic kidney	Alive
8	23	TOF	-	Alive
9	24	TOF	-	Alive
10	28	AVSD	-	Alive
11	30	TOF with absent pulmonary valve	-	NND
12	22	-	NT: 6.6 mm, Trisomy 21	Termination

GA: gestational age, week, RAA: right aortic arch, TOF: tetralogy of Fallot, VSD: ventricular septal defect, AVSD: atrioventricular septal defect, NT: nuchal translucency, NND: neonatal death

with additional intracardiac malformations in five (41.7%) cases (TOF, n=3; AVSD, n=1; VSD, n=1). Furthermore, RAA was associated with an extracardiac anomaly in three fetuses (25%). In a study by Berg et al., (3) this finding was observed in 12% of the entire series. These findings suggest that a meticulous inspection of fetal cardiac and extracardiac anatomy, including the brachiocephalic branching pattern, should be performed in prenatally detected cases and that cytogenetic testing for 22q11 deletions should be considered carefully.

A right-sided aortic arch may be asymptomatic. Sometimes, affected infants and children can present at any time with symptoms of airway obstruction, usually stridor. Symptoms in infancy are related to congenital heart anomalies or to compression of mediastinal structures such as the trachea or the esophagus.

### Conclusion

RAA can be prenatally diagnosed by fetal echocardiography and may be associated with cardiac anomalies. Once RAA is diagnosed, cardiac and extracardiac anomalies, fetal karyotype, and 22q11 deletion should be suspected. The prognosis depends on whether RAA is associated with cardiac and extracardiac anomalies. The prognosis is relatively good for isolated RAA.

### Conflict of interest

No conflict of interest was declared by the authors.

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# Combined lower segment cesarean section and cholecystectomy in single sitting-our initial experience

## *Tek oturumda kombine alt segment sezaryen ameliyatı ve kolesistektomi-başlangıç deneyimimiz*

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### Abstract

**Objective:** To study feasibility and results of cholecystectomy at the time of cesarean section.

**Material and Methods:** Thirty-two patients were subjected to cholecystectomy at cesarean section. Most of them were diagnosed with cholelithiasis at or before the first antenatal scan. Cholecystectomy was performed by subcostal mini-laparotomy, after assessing the anatomy via the cesarean wound.

**Results:** Cholecystectomy was combined with lower segment cesarean section in all the patients. Under general anaesthesia, surgeries were performed with an mean duration of 90 minutes. Difficult anatomy at calots was found in 3 patients, who required extension of subcostal incision by 3-4 cm. One woman required blood transfusion during operation. There were no other intraoperative or postoperative complications. No extra antibiotics or analgesics doses were needed. Patients were discharged on 5<sup>th</sup>-7<sup>th</sup> postoperative day.

**Conclusion:** Combined cesarean section and cholecystectomy avoids rehospitalisation for separate cholecystectomy. With an additional small subcostal incision, single anaesthesia, and single hospital stay, the combined procedure confers valuable advantages for both patient and hospital in time, cost, and convenience, including avoiding the separation of mother from newborn entailed by reoperation. It also prevents the possibility of developing acute cholecystitis while the patient is waiting for cholecystectomy. Our results indicate that the combination approach is safe, effective, and well accepted.

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**Key words:** Lower segment cesarean section (LSCS), cholecystectomy, combined approach, gall bladder disease, pregnancy

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### Özet

**Amaç:** Sezaryen ameliyatı sırasında kolesistektominin uygulanabilirliğini ve sonuçlarını incelemek.

**Gereç ve Yöntemler:** Otuz iki hastada sezaryen ameliyatı sırasında kolesistektomi yapıldı. Hastaların çoğu doğum öncesi ilk tarama sırasında veya öncesinde kolelithiazis tanısı almıştı. Kolesistektomi sezaryen yarısı aracılığıyla anatominin değerlendirilmesinden sonra kosta altı mini-laparotomi ile gerçekleştirildi.

**Bulgular:** Hastaların tamamında kolesistektomi alt segment sezaryen ameliyatı ile kombine edildi. Ameliyatlar, genel anestezi altında, ortalama 90 dakikalık süre içinde yapıldı. Üç hastada "calot"larda zor anatomi saptandı ve kosta altı kesinin 3-4 cm uzatılması gerekti. Bir kadında ameliyat sırasında kan transfüzyonu gerekti. Bunlar dışında ameliyat sırasında veya sonrasında komplikasyon gözlenmedi. İlave antibiyotik veya analjezik dozları gerekmedi. Hastalar ameliyat sonrası 5-7. günde taburcu edildi.

**Sonuç:** Kombine sezaryen ameliyatı ve kolesistektomi, ayrıca kolesistektomi için yeniden hastaneye yatışı önler. İlave küçük bir kosta altı kesisi, tek bir anestezi ve hastaneye bir kez yatış ile kombine işlem; hem hasta hem de hastane için zaman, maliyet ve rahatlık bakımından değerli avantajlar sağlar, anne ve yenidoğan tekrar bir ameliyat sonucu ayrı kalmamış olur. Ayrıca hastanın kolesistektomi için beklerken akut kolesistit geçirme olasılığını da önler. Sonuçlarımız kombine yaklaşımın güvenli, etkili ve iyi kabul gördüğünü göstermektedir.

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**Anahtar kelimeler:** Alt segment sezaryen ameliyatı (LSCS), kolesistektomi, kombine yaklaşım, safra kesesi hastalığı, gebelik

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### Introduction

Lower segment cesarean section (LSCS) is one of the most common operative procedures in women of reproductive age. Gallstones are three times more common in women than men and cholecystectomy is the most common major operation worldwide. While 2-4% of pregnant patients are found to have gallstones by obstetric ultrasound, symptomatic

cholelithiasis and cholecystitis during pregnancy occur in only five to 10 of every 10.000 births. Most patients are effectively managed with conservative, nonoperative therapy. In some patients, however, surgery is required for refractory symptoms or complications (1). The incidental finding of gallstones has increased considerably as so many patients undergo ultrasound imaging of abdomen for a variety of condition (2). It has been shown that cholecystectomy for gallstones dur-

ing laparotomy for unrelated condition may sometimes be appropriate because such patients are at greater risk of developing symptoms (3). Many women undergoing gynaecological surgery ask for cholecystectomy to avoid future hospitalization and another operation. One appropriate approach could be to perform combined cesarean section and cholecystectomy in one sitting. A number of procedures have been done at the time of cesarean section, including gynaecological procedures, hernia repair, appendectomy and cholecystectomy (4-7). The combination of cholecystectomy with gynaecologic surgery or cesarean section is virtually undocumented outside of a case report (8, 9). This study evaluates the feasibility and results of cholecystectomy at the time of cesarean section in peripheral hospitals where facilities for laparoscopic surgery are lacking.

## Material and Methods

This study was done in the rural hospitals in two districts of Kashmir from June 2007 till Nov 2011. A total of 2210 women were registered for antenatal care. Sixty five patients (2.94%) were found to have gall bladder disease, either at or before the first antenatal scan, of which 35 women were scheduled for cholecystectomy at the time of cesarean section. Patients who did not agree to a combined procedure or had associated cardiovascular or pulmonary illnesses, acute cholecystitis in third trimester, gall bladder mass and symptoms or investigations suggestive of common bile duct stones were excluded from the study. In our study group, three patients were operated for their gall stone disease in the second trimester of their pregnancy and were also excluded from the study. The remaining 32 patients were either managed conservatively for their symptomatic gallbladder disease or were asymptomatic during their pregnancy. Indications for cesarean section were either cephalo pelvic disproportion (CPD), previous cesarean section, transverse lie, twin pregnancy or placenta praevia. Cholecystectomy was indicated for gall stone disease in the majority of the patients.

Written informed consent was obtained for combined procedures at admission. All patients received prophylactic intravenous antibiotics. Lower segment cesarean section (LSCS) was done first making a Pfannenstiel incision. Upper abdominal anatomy was assessed via the cesarean wound after the uterus was closed. Lax abdominal wall was easily retracted allowing assessment of the upper abdomen. The cesarean wound was closed and followed by a Minilap-cholecystectomy, making a 5-cm subcostal incision. In case of difficulty, the incision was extended. The common bile duct was not explored in any patient. Closed suction drain was placed in the hepato-renal pouch in selected patients. All the patients were encouraged to be ambulatory a day after the operation.

Data recorded included age, parity, associated illnesses, biliary symptoms, laboratory and radiological investigations, operative procedures, operative findings, intraoperative complications, the time taken for cholecystectomy after completion of cesarean section, postoperative complications, length of hospital stay from the day of operation, mortality and pathological findings of gall bladder.

## Results

The ages of women ranged between 22-40 years. All except three were multigravida. Out of 35 patients planned for cholecystectomy at cesarean section, gall stones with or without sludge was seen in 32 (91.4%) patients, gall bladder polyps in two (5.7%) and cholesterosis in one (2.8%) of the patient. Patients with gall stone disease had a history of biliary symptoms like episodic upper abdominal pain and/or dyspepsia in 18(56.2%), acute cholecystitis in early second trimester in 3 (9.3%), while eleven (34.3%) women had silent gallstones. All the patients with GB polyp or cholesterosis were asymptomatic. Three patients, who were excluded from the study, were operated in their second trimester for their gallstone disease at a referral centre, as one patient developed empyema of the gall bladder and the remaining two had frequent admissions for their recurrent intractable biliary colic. Other patients with symptomatic gallstones, including those with acute cholecystitis, were managed conservatively during their pregnancy. The indications for LSCS were CPD in 19, previous LSCS in 10, and transverse lie, twin pregnancy and placenta praevia in one each of the patients.

Two patients were operated at 37-38 weeks of pregnancy because of early onset of labour, while the remaining 30 were operated at full term. Under general anaesthesia, lower segment cesarean section (LSCS) was first done using the Pfannenstiel incision. Anatomy in the upper abdomen was assessed via the cesarean wound after closing the uterus. Three (9.3%) patients were found to have unfavourable anatomy including dense adhesions, GB lump or contracted intrahepatic gall bladder. Cholecystectomy was completed in all the patients. A 5-cm subcostal incision was used in all the patients. The incision was extended by 3-4 cm in the women with unfavourable anatomy in the right upper abdomen.

During surgery, adhesions of various intensity were found in 7 patients (21.8%), distended gallbladder in 5 (15.6%), inflamed gallbladder with oedema of wall in 4 (12.5%), and mucocele in 1 (3.1%) patient. Eight women (25%) had a contracted thick walled gall bladder suggestive of chronic cholecystitis. None of the patients had empyema of gallbladder, pericholecystic oedema, pericholecystic abscess or common bile duct stones. All the patients had gallstones with or without biliary sludge. Anatomy in the calots triangle was distorted, requiring extension of subcostal incision and cholecystectomy by fundus first method in three (9.3%) patients. Short cystic duct was encountered in 2 (6.2%) cases, while significant bleeding from the liver bed occurred in 1 (3.1%).

Surgeries were done within a mean operating time of 90 minutes. The mean extra time taken after LSCS for completion of cholecystectomy was 25 minutes (20-35 m). Six women also had bilateral tubal ligation done in the same sitting. Closed suction drain was placed in the hepato-renal pouch in 12 (37.4%) patients. There were no intraoperative or postoperative complications except for one women who required blood transfusion during the operation. There were no deaths in our series. No extra antibiotics or analgesic doses were needed. Patients were discharged on the 5<sup>th</sup>-7<sup>th</sup> postoperative day. Histopathology of

the gall bladder specimen showed chronic cholecystitis in 14, acute inflammation in three, benign polyps in three, cholesterosis in one and a normal gallbladder in 11 specimens.

## Discussion

The most common causes of gall bladder disease in pregnancy are gall stones and biliary sludge. The incidence of gall bladder disease in pregnancy is approximately 0.05%-0.3%, and asymptomatic gall stones occur in 3.5%-10% of all pregnancies. However the need for cholecystectomy occurs in 1 in 1.600 to 1 in 10.000 pregnancies (10-12). Most of the patients with symptomatic gall bladder disease in pregnancy are effectively managed conservatively, and cholecystectomy is performed selectively during the postpartum period (10). Some women require surgery and/or endoscopic retrograde cholangiopancreatography (ERCP) during pregnancy, for refractory symptoms or complications (1, 13).

Although gallstone disease in pregnancy is uncommon, the potential maternal and fetal morbidity from both the disease and its surgical therapy are significant. Pregnant patients who develop symptomatic gallstone disease have a high rate of recurrent symptoms (14). After open cholecystectomy, the rate of preterm labour is about 7% overall and 40% in the third trimester (15). The rate of spontaneous abortion is 0-18%, and the rate of preterm delivery is 0-22%, depending on the severity of the underlying disease and gestational age (12).

Faced with a pregnant patient with symptomatic gallstone disease, the clinician must decide between operative or non-operative management. This decision must balance the operative risks against those of the disease itself. The main operative risks include fetal teratogenicity and spontaneous abortion for patients treated early in pregnancy and preterm labour or delivery in those treated in the third trimester. With nonoperative management, the main concern relates to the severity of nausea and/or pain and the potential development of complications of gallstones, including acute cholecystitis, obstructive jaundice, and pancreatitis (14). In this series, 21 out of 32 patients managed non-operatively had documented recurrent symptoms prior to delivery. Three patients who were excluded from the study developed complications prior to delivery and were operated at a referral centre.

If surgery is considered in the pregnant patient, the options include either an open or a laparoscopic approach. Any abdominal operation during pregnancy may adversely affect the fetus and/or mother by several mechanisms. These include direct uterine trauma, altered uteroplacental blood flow, anaesthetic teratogenic effects and altered homeostasis in fetus and mother respectively, increased risk of thromboembolic disease, effects of postoperative medications and increased risk of incisional hernias (16). Laparoscopic surgery has potential advantages compared to open abdominal surgery. These include reduced exposure of the uterus to trauma and air, more rapid maternal recovery and mobilization, decreased maternal dependence on postoperative pain medications, improved operative exposure in some conditions, and decreased risk of incisional hernias (14).

In an era when cost containment in surgery has become increasingly important, a new approach has been combined procedures in laparoscopic surgery as well as open general and gynaecological surgery (17-19). Since we did not have facilities for laparoscopic procedures in all the peripheral hospitals, most of the patients were planned for open cholecystectomy at the time of cesarean section. In our series, ability to perform effectively combined LSCS and minilap-cholecystectomy in selected patients with minimum complications has established the safety of this procedure. There is a paucity of studies on this subject but all previous studies have shown that in selected patients this combined approach can be considered by both the gynaecologists and general surgeons (8, 9).

Studies have also shown that cholecystectomy does not increase the morbidity and mortality rates of concomitant gastric, colonic, hepatic, pancreatic and urological surgeries (20). A healthy young patient with no co morbid conditions and uncomplicated cesarean section is a good candidate. Obese patients with co-morbid medical conditions, acute cholecystitis in the third trimester, associated CBD stones, symptoms or investigations suggestive of common bile duct stones and those encountering complications of LSCS would be better served by delayed cholecystectomy. The combined procedure was completed in all the patients in our series.

Fourteen patients in our study were asymptomatic, which included 11 patients with silent stones, two with gall bladder polyp, and one with cholesterosis. Although the latter two are definite indications for cholecystectomy, there is no consensus on management of silent stones. The development of symptoms in silent stones is 2% per year and morbidity and mortality is approximately equal to those with cholecystectomy (21). So, in patients with silent stones receiving general anaesthesia for other reasons than gall bladder disease, cholecystectomy can be done to avoid complications (4).

The risks of combined surgery are that of longer anaesthesia and operation time, complications of two incisions, increased blood loss and the presence of two visceral peritoneal defects (22). However in our study, it was only 20 - 35 extra minutes for completion of cholecystectomy, and only one patient who had significant bleeding from the liver bed required blood transfusion during surgery. The duration of hospital stay was 5-7 days. No extra antibiotics or analgesia was required. Combined procedure in selected patients, apart from having many advantages, is a cost effective method of treatment, as most of the patients receiving treatment at the rural hospitals are poor.

Combined cesarean section and cholecystectomy avoids rehospitalisation for separate cholecystectomy. With an additional small subcostal incision, single anaesthesia, and single hospital stay, the combined procedure confers valuable advantages for both patient and hospital in time, cost, and convenience, including avoiding the separation of mother from newborn entailed by reoperation. It also prevents the possibility of developing acute cholecystitis while the patient is waiting for cholecystectomy. Our results indicate that the combination approach is safe, effective, and well accepted.

## Conflict of interest

No conflict of interest was declared by the authors.

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# Research on embryos in Turkey with ethical and legal aspects

## *Etik ve yasal açıdan Türkiye’de embryo üzerinde arařtırmalar*

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### *Abstract*

Technically, the term embryo refers to the products of conception after implantation into the wall of the womb, usually nearly two weeks after fertilization, up until the eighth week. Embryos contain stem cells which, according to scientists, could be used to cure a wide range of conditions. Stem cells can be coaxed into growing cells of any other type, which makes them potentially very useful indeed. However, removing stem cells from an embryo will kill the embryo, which some people object to. From the mid 1970s, IVF was being developed and research was carried out on the spare embryos produced. This research helped to improve IVF techniques, as well as to better understand the earliest stages of human development. Research also shed light on a variety of inheritable disorders. In Turkish Law, assisted reproduction treatment (ART) services are regulated with the Regulation of Assisted Reproductive Treatment Centers Act (RAPTCA) The Regulation was issued in 1987, but it has been amended several times since. Also, article 90 of the Turkish Penal Code covers some aspects of research on embryos. At the same time, the Biomedicine Convention (Oviedo Convention), signed by Turkey and which entered into force in 2003, has binding regulations about this issue. Different legal regulations and some ethical guidelines are in conflict with each other, creating much confusion for the researchers. In this paper these conflicts are discussed, giving some practical proposals.

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### *Özet*

Embryo terimi fertilizasyondan itibaren sekizinci haftaya kadar olan gebelik materyalini tarif etmek için kullanılır. Embryo, çok geniş ve farklı yelpazedeki patolojileri tedavi etmek için kullanılacak kök hücrelere sahiptir. Kök hücreler köken aldıkları hücre tipinden farklı yapıdaki dokuları oluşturmak üzere yönlendirilebilirler ki bu özellikleri nedeniyle kullanışlı olma potansiyelleri artmaktadır. Ancak kök hücrelerin embryodan uzaklaştırılması embryonun ölümüne neden olacaktır ki bu durum birçok kişinin karşı çıktığı noktadır. 1970’lerin ortalarından itibaren uygulanan in vitro fertilizasyon (IVF) işlemleri sırasında elde edilen fazla embryoların araştırma amacıyla kullanılması gündemdedir. Bu arařtırmalar hem IVF tekniklerinin gelişmesine hem de insan gelişiminin ilk evrelerinin daha iyi anlaşılmasına yardımcı olmuştur. Bu arařtırmalar aynı zamanda birçok kalıtsal hastalığın aydınlatılmasına da yardımcı olmuştur. Türkiye’de üremeye yardımcı tedaviler Üremeye Yardımcı Tedavi Merkezleri Yönetmeliği ile düzenlenmektedir. Bu yönetmelik 1987 yılında yürürlüğe girmiş olup ardından bir dizi değişiklikler geçirmiştir. Ayrıca Türk Ceza Kanunu’nun 90. maddesi embryo üzerinde yapılacak arařtırmalarla ilgili bazı hususları kapsamaktadır. Ayrıca Türkiye’nin 2003 yılında imzaladığı Avrupa Biyotıp Sözleşme (Oviedo Konvansiyonu) konuyla ilgili bağlayıcı hükümleri vardır. Türkiye’deki yasal düzenlemeler arařtırmacılar açısından karışıklığa yol açacak nitelikte ve netlikten yoksun gözükmektedir, konuya özel olarak belirlenmiş spesifik bir yasal düzenlemenin bulunmayışı büyük eksiklik yaratmaktadır. Bu makalede konunun yasal ve etik yönleri bazı öneriler doğrultusunda tartışılmıştır. (J Turkish-German Gynecol Assoc 2012; 13: 191-5)

**Anahtar kelimeler:** Embryo, araştırma, genetik, hukuk, etik

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### **Introduction**

Ethical and legal debates on embryo research have again become an important item in our agenda when European Court of Human Rights (ECHR) General Assembly passed judgment on the application of a British lady in 2007. This late legal debate requires long attention from those who are engaged in medicine and legal sciences. We can make a short summary of the event as follows:

A British woman has to undergo major surgery because of her health problems. She was engaged at that time. The

woman’s ovaries are to be taken with this operation, and there would be no chance of her having a child after the operation. Therefore, sperm and eggs are taken from the couple prior to the surgery, eggs are fertilized artificially and embryos are stored frozen. After the medical intervention, the engagement is broken due to a dispute between the couple. Her ex-fiancee stated that he did not want to have a child with the woman and demanded the termination of the frozen embryos.

Since there is no chance for her to have a child because her ovaries are removed and trusting only to the frozen embryos,

the woman applies to the sperm bank to get pregnant with frozen embryos. With her ex-fiancée's objection, the issue becomes a judiciary issue. Consuming domestic remedies and stated unjust in every judicial body in the UK, she carries the issue to the ECHR. ECHR Court No. 5 judges that the man is equally entitled to the embryos and rejects her request. As last resort she applies to the Grand Chamber of the ECHR. In 2009, the General Assembly of the Grand Chamber decides that both parties have unique rights of ownership on sperm/ovaries and the woman can not be impregnated via In vitro fertilization without the consent of her ex-fiancee. While her ex-fiancee is waiting for the legal process of termination of the embryos in a short time, he is shocked by the news that she was in fact pregnant, taking the embryo from the bank by imitating his signature and via fake documentation. Whether or not the statutory period of time for embryo termination is passed, since a medical necessity, or a legal requirement, and most importantly the consent of the woman is not present, abortion of the embryo/fetus was not an option from that point on, and despite his objections, the ex-fiancee is to become a father (1).

Both this case and some other cases which have been brought in front of the ECHR in the last three years have caused a big ethical, medical and legal debate around European Council Convention of Biomedicine provisions.

When we look at the legal approach regarding in vitro fertilization in Turkey, we see that the in-vitro fertilization method is accepted as a method of treatment and not a research method, and social insurance institutions are able to cover the treatment expenses (Council of State Department 5, 1998/3529 Acts in 20.09.2001 and 2001/3138 Acts). Before these laws, Turkish laws had judged with artificial reasons that in-vitro fertilization was not a method of treatment and its medical expenses could not be paid for by the state as health care, even though the regulation concerning this issue had clearly stated otherwise. Likewise, the Religious Affairs Department has defined IVF as "a procedure which hurts feelings of humanity" and "adultery" in its decision of the High Council of Religious Affairs in 2002 (1).

### Contributions From Turkey

However; to give way to scientific research and to prevent abuses, various studies within the framework of human rights, human dignity and freedom of science and art are currently being conducted in European countries. These studies aim to make the human dignity concept tangible and functional, pursue the freedom of science and try to prevent abuse of medical research projects, particularly subjects like embryo research, in-vitro fertilization, stem cells and drug trials. For example; ZiF in Bielefeld, Germany (Cross-Cultural Research Center) has launched the "Human Design, and Human Dignity" project 2 years earlier in this context. The project employs dozens of expert scientists working on different branches such as law, ethics, religion and social studies, who work seamlessly in this regard to achieve tangible outcomes. One of the major sub-topics of this project, which began on 2009 and is to be published as a book in 2010, is embryonic research (2).

With contributions from Prof. Dr. Yener Ünver and Dr. Altan Heper from Turkey, the project aims to eliminate the com-

mon dilemmas between law and science, to achieve common, secure, open and fundamental human design criteria suitable for the 21<sup>st</sup> century and to create theories regarding basic human rights. One of the most controversial issues in this subject is whether or not the embryo should benefit from the human dignity concept and rights, how to maintain its legal protection regarding the human dignity concept, while technically not being human itself (2).

## Discussion of Current Legal Legislations About the Issue

### The Biomedicine Convention (Oviedo Convention)

The Biomedicine Convention which is promulgated by the Council of Europe and accepted by Turkey in 2003, includes arrangements on this issue in its articles numbered 2, 11, 13, 15, 16, 17 and in particular 18 and 23. Article 18/1 of this convention regulates the necessity of protection for the embryo on the basis of national regulation allowing IVF research. Article 18/2 of the convention, on the other hand, prohibits the creation of human embryos only for research purposes. This issue should be considered along with the ban on cloning stated in the European Declaration of Fundamental Rights. Several European countries such as Germany, have not yet acknowledged this agreement with a concern of prohibition of cloning for stem cell research and planning to do so after an additional protocol is maintained or the main agreement text is modified regarding this issue. These countries have the widely held opinion that the Convention, in its current form, seriously limits the scientific research projects contrary to the dominant purpose of these studies. Additional protocol to the Convention prohibits human cloning and states (in the introduction section) that cloning is a misuse of medicine technique and an abuse of human dignity (3).

Turkey's only objection to the Convention was in terms of Organ-Tissue Transplantation Act (OTTA). This incorrect drawback has not yet been removed since 2003 and OTTA is yet to be changed to compensate for the contradiction between the Convention. Turkey's objection to the Convention is based on OTTA article no. 5 which bans inter-family organ and tissue transplantation on minors (younger than 18 years old) according to Turkish legislation (4).

### Turkish Penal Code

Turkish Constitution article 90/5 dictates that "No appeal to the Constitutional Court can be made with regard to international agreements, on the ground that they are unconstitutional." This Convention is subject to that article.

Turkish Constitution article 17, entitled "Personal Inviolability, Material and Spiritual Entity of the individual" dictates in 2. that "The physical integrity of the individual shall not be violated except under medical necessity and in cases prescribed by law; he shall not be subject to scientific or medical experiments without his consent." According to article 27, entitled "Freedom of Science and Arts", paragraphs 1 and 2; "Everyone has the right to study and teach freely, explain, and disseminate science and arts and to carry out research in these fields." and "The right to disseminate shall not be exercised for the purpose of changing the provisions of Articles 1, 2 and 3 of this

Constitution.” In other words, the right to exercise material sciences shall not be interpreted as the right to ignore the individual’s rights to live, protection and development of tangible and intangible assets (4).

Article 90 of Turkish Penal Code states that any person who carries out a scientific experiment on a human being shall be sentenced to a penalty of imprisonment for a term of one to three years. However, these regulations do not cover research studies on embryos. Currently, there is no direct legal regulation that prevents embryo research. There is only the ODNK law to apply to embryo plantation situations, since an embryo consists of tissues and tissue transplantation is-incorrectly-regulated in the ODNK law. So in case of embryo transplantation, those who participated shall be subject to relevant provisions in the Turkish Penal Code article 15 which regulates criminal penalties regarding tissue transplantation (5).

#### **Regulation of Assisted Reproductive Treatment Centers Act (RAPTCA)**

There is no unique regulation or act regarding In Vitro Fertilization (IVF) in Turkey. This area is regulated via the Regulation of Assisted Reproductive Treatment Centers Act.

A short time ago (2009) a new Regulation on Clinical Research made by the Ministry of Health was put into effect. While article 13 of the Turkish Constitution clearly states that “regulations regarding fundamental rights shall only be achieved through laws and no other legal norms”, with above statute the Ministry of Health effectively limited the freedom of research and science. For example the Turkish Penal Code article 90 regulates that, in certain conditions, experimentation on humans can be done. However, the above guideline completely prohibits any research on children, pregnant women or people who are not able to give written consent, even though this is not written in the law (Article 5). The exception to this rule is that: “if it provides a direct benefit in terms of people treated and if there is written permission from the Ministry of Health, it is possible to conduct tests and trials”. This arrangement cannot be done via guidelines and should be arranged via law. This is a requirement of both relevant articles of Biomedicine Convention and articles 13 and 90/5 of the Turkish Constitution.

According to this regulation, including revision text dated March 6, 2010; uncontrolled ovarian hyperstimulation by physicians or other individuals, opening special clinics for in-vitro fertilization (IVF) and micro-injection (ICSI) practices or assigning a part/area of current clinics for these purposes are prohibited. These types of activities shall be immediately stopped by the governor’s office and the republican prosecutor’s office shall be informed of the involved parties concerning judicial process. This provision does not have any importance in terms of penal law; and these kindsof medical interventions, despite the provisions of this regulation, shall not constitute a crime (6).

According to article 18/12 of RAPTCA; in the case of obtaining more embryos from the candidates, embryos are stored frozen with the consent of both spouses. Should the storage period exceed one year, spouses should re-apply every year stating their continuous demand for preservation of the embryo, with written and signed declarations. In cases of mutual request

from the spouses, death of one of the spouses, divorce or end of the fixed period of preservation, the embryos shall be destroyed/terminated after being recorded by a commission established by the directorate. Should there be an electronic record system set forth by the Ministry of Health, information regarding the embryos shall be recorded in that system.

According to article 18, paragraph 13 of RAPTCA, the samples aforementioned in the 11. and 12. paragraphs of the same article shall be stored no more than five years. A storage period of more than five years is subject to the permission of the Ministry of Health. Counting and evaluation of the stored samples shall be carried out by means of the commission which is to be established within the relative directorate. Should samples which have not been recorded in time are detected, administrative sanction in the form of supervision shall be carried out. Since these actions do not constitute a crime, they do not have a criminal law penalty and are subject to administrative sanctions (7).

Legal arrangements should be made in order to prevent illegal reproductive tourism from Turkey to Greece, Belgium, Rhodes Island and Northern Cyprus. Illegal ova/embryo transplantation should be recognized as a crime regulated by law by these arrangements. The lack of these regulations, along with many dangers and risks, causes these operations to be carried out in above-mentioned countries, crimes mentioned in Turkish Penal Law articles 91-93 to be committed, organ-trafficking and young girls to sell their ovaries for money. When these actions, committed in foreign countries, constitute a criminal offence, their prosecution in Turkey is not possible according to the Turkish Penal Code article 11. These actions should be regulated so that they constitute a criminal offence even when they are committed in a foreign country, the need for realization of the action as a crime by the relevant foreign country should not be a necessity and it should be ensured that these crimes can be prosecuted also in Turkey (8).

If heterologous artificial fertilization is not legally regulated and sperm banks, and more importantly sperm and ova donations are not subject to coding and categorizing, both medically and legally unfavorable situations are sure to arise, including unintended inter-sibling marriages in future years. Neither medicine nor law can be said to aim at these consequences (9, 10).

#### **Discussion**

Today, often one or two fetal/fetuses are selected in multiple pregnancies and the remainder are removed by curettage after the statutory 10 week period, although there is no medical necessity. The parent’s consent/request only, does not make this process legal for both parents and the medical staff, the process still remains illegal and if proven, those involved are still subject to a legal penalty regarding the abortion law stated in the Turkish Penal Code article 99.

On the other hand, application for embryo reduction is carried out mostly via the abdominal route and on weeks 11-12 of the pregnancy. Since in this case this operation cannot be regulated within the framework of “abortion crime” (Turkish Penal Code Articles 99-100), this subject should be re-regulated

in the Turkish Penal Law and Law on Population Planning. Also the future planned Embryo Protection Act should include provisions regarding this issue.

A similar medical practice is the determination of the sex of the fetus via intervention by medical personnel. These practices, the use of which the Biomedicine Convention has permitted-only-on genetic diseases and prohibits their usage on all other cases, are still not in current legislation in Turkey, so these actions go unpunished since they are not recognized as types of crimes (3, 8).

Although the above-mentioned Regulation of 2009 prohibits pharmaceutical research on pregnant, nursing and puerperant women; because it is not possible to recognize a practice as a criminal act by Regulations (not laws) and also because bans on scientific research (which is a human right) are unimportant when it comes to Turkish Constitution article 13, these regulations are basically meaningless (11).

Turkish legislation in this area lacks important and overdue regulations to be filled. These legal arrangements that should be carried out are essentially a part of international law and approved conventions. In this context, a special law for the protection of the embryo should be regulated, the Stem Cell Act should be legalised, an urgent law regulating Protection of Personal Data, Data (DNA) Bank, Biobanking Act should be passed and a law concerning Biobanks should be constituted (7).

Over-production of embryos should be recognized as a crime and should be punishable. All the same, unlawful destruction of the embryos, embryonic research against the law (experiments and trials) must be punishable and embryo storage, transportation and disposal should be controlled by strict regulations. Just like blood transfusions, tissue transplantation must be arranged separately from organ and tissue transplantation laws, regulated by its own law (12).

Naturally produced embryos are under the protection of the Turkish Penal Code articles 99 and 100. The regulations of aforementioned articles in TPC apply to embryos after their placement into the uterus, but there are no provisions about pre-placement. Legal regulations on this subject are needed. Abuse of in-vitro fertilization methods and human embryo should be a crime; embryo/fetal gender determination interventions should be illegal on the norms of penal law unless there is a medical necessity; the law should clearly state the permitted and forbidden forms of using reproductive cells for the purpose of conception after death; a sperm bank should be established and regulations which block scientific research should not be given a place in the law. A discrimination ban law should be established regarding disabled fetal-infants. Again, the "surrogate mother/womb renting" issue should be clearly organized by law concerning the impact on the Turkish Civil Code and penal law fields. Creation of human-animal embryo hybrids and medical interventions using a different genetic mix of cells to create embryos should be recognized as a crime and should be prohibited.

Again, if spouses demand their frozen sperm/ova from the institution in order to use it for artificial insemination in a different public or private institution, they are often denied the request or given the sperm/ova without due diligence. Even if these

stored sperm or fertilized/unfertilized eggs are given to spouses on demand; this extra action is recognized both as physical violence and sexual assault in legal scope because it is illegal to obtain and/or freeze the ova without the consent of spouses. Also storage of the ova is in violation of the regulation article 17/5. These actions require administrative sanction regulated in the aforementioned regulation article 18 paragraph 1. However, there is no regulation regarding the penal code.

RAPTCA article number 17/1 dictates that "Possession, usage, transfer and sale of embryos, usage and application of embryos obtained from a candidate on different candidates or application of embryos obtained from a non-candidate on a candidate are strictly prohibited". The Council of Europe Convention on Biomedicine article number 21 dictates that "The human body and its parts shall not, as such, give rise to financial gain." Article 22 of the Convention regulates that "When, in the course of an intervention, any part of a human body is removed, it may be stored and used for a purpose other than that for which it was removed, only if this is done in conformity with appropriate information and consent procedures".

However there is no penal code regulation or legal arrangement for medical inventions done in hospital-clinics not due to the Ministry of Health and no administrative penalty applies (13).

According to the article text, "No more than three embryos shall be transferred in medical centers which employ assisted reproductive techniques. In cases where the transfer of more than three embryos are required, such as the age factor, quality of embryos and similar medical necessities, the doctor shall document the medical rationale." In contrast, there is comparative law protection of the embryo, which will be used, and in particular the prohibition of unlawful destruction (required) number of banning the production of more embryos, and in particular there is a regulation that punishes the criminal law norms. On the contrary, RAPTCA clearly provides an opportunity for the production of more (than required) embryos and does not regulate their necessity of termination after and this is not acceptable by law.

RAPTCA article number 17/5 states that, with the consent of both spouses, freezing and storing of the embryos are possible and these embryos can be used by the same candidate within the period specified by the registration center (this period could not exceed 5 years). However, regulation article 17/5 lacks some information like embryo conditions and who to be responsible for the embryos when the medical center is shut down or relocated to another city, whether embryos are to be transferred to another facility or to be terminated, the legal responsibility for transferee medical clinics and centers, how the procedure is to be carried out when the patient requests his/her embryos for transfer to another facility. These should be regulated along with embryo storage provisions (14).

The Turkish Penal Code article 99 paragraph 6 dictates that "Where a woman is pregnant due to an offence that she was a victim of, no penalty shall be imposed upon any person who terminates such pregnancy, where the term of pregnancy is not more than twenty weeks and there is consent from the woman. " However, both the termination of the embryos and producing more than the required amount should be regulated

and recognized as a criminal act and the Turkish Penal Code article 99 paragraph 6 should be reorganized in order to correct the mistakes and fill in the lacking provisions. Therefore, the Turkish Penal Code article 99 paragraph 6, which dictates: "Where a woman is pregnant due to an offence of which she was a victim, no penalty shall be imposed upon any person who terminates such pregnancy, where the term of pregnancy is not more than twenty weeks and there is consent from the woman." should be revised (4).

## Conclusion

The concept of Human dignity should be widened to allow understanding and protection of the life of the fetus. Since the aforementioned article requires only the consent of the mother and does not require any medical necessity and employs no control mechanism, it allows abuse from the institution and provides opportunity for the violation of human dignity. Worse yet, there is no criminal or penal responsibility if medical personnel, health care providers, including the mother, abuse this system. The fetal body/placenta etc. from abortion is subject to be used for the pharmaceutical industry and this violates the norms which prohibit using human body parts for financial gain. Current lack of legal regulations also give way to frauds carried out against the law by hiding behind the Turkish Penal Code articles 91 and 93, which regulate organ and tissue transplantation laws and this allows people who discriminate between male-female children to terminate undesirable gender fetuses, for example female fetuses can be put to death when spouses do not want a girl baby (4).

The revision of article 99 of the Turkish Penal Code should include the following: Pregnancy duration must not be less than 20 weeks. The fact that the cause of pregnancy was really a criminal act must be determined "not only" by the mother's statement and by other legal mechanisms. Whether or not there is a reason preventing anti-abortion measures must be checked during the normal abortion period (not exceeding 10 weeks) and these and following interventions on the fetus must be regulated by law (4).

Finally, the Turkish Penal Code article 92, which regulates organ trafficking states "A penalty may be reduced or not imposed at all, after considering the social and economic conditions of the person selling his own organs or tissue." This statement is against the law and is often being abused. How satisfactory is an arrangement that penalizes a doctor, coordinator or the receiver of the organ (who possibly saves his/her own life by doing so) and not giving a normal penalty to countrymen who sell their organs for money, or reducing their penalty?

The goal must be the protection of human dignity and the paving of the way for scientific research at the same time. Unrestricted, excessive prohibitions are part of the problem not the solution.

Surely there is an important issue which should be taken into consideration here. The first article of the Turkish Constitution regulates that human dignity is inviolable, but it is not enough for human dignity to be protected. Currently, the human dignity concept is vague, there is no clear definition of human dignity which is satisfactory for everyone.

## Conflict of interest

No conflict of interest was declared by the authors.

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# Evaluation of ovarian reserve in infertile patients

## *İnfertil hastalarda over rezervinin değerlendirilmesi*

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### Abstract

Diminished ovarian reserve is a more common occurrence as more women postpone childbearing in modern societies due to social and demographic trends. Diminished ovarian reserve is one of the primary reasons for poor ART outcome. Due to high costs, side effects and heavy burden on patients on ART treatments, patient selection and counseling for prognosis is an important aspect before starting ART. Proper prediction of ovarian reserve before initiation of the treatment can decrease cycle cancellations, help clinicians to establish alternative treatment options (i.e. oocyte donation) for poor prognosis patients. However, indicators of ovarian reserve are not fully successful in predicting the outcome of the treatment. In this review, our aim was to discuss the efficacy of ovarian reserve tests on predicting poor ovarian response and treatment outcome in ART patients.

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**Key words:** ART, ovarian reserve, poor ovarian response, cycle cancellation, treatment

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### Özet

Günümüzde modern toplumlarda kadınların evliliklerini çeşitli nedenlerle geciktirmesi nedeniyle düşük over rezervi ile daha sık karşılaşılmaktadır. ART tedavisi sırasında düşük over rezervi başarıyı azaltan başlıca nedenlerden birisidir. Tedavilerin maliyeti, yan etkileri, hasta açısından zorlukları göz önüne alındığında uygun hasta seçimi ve uygun prognoz belirlenmesinin oldukça önemli olduğu görülmektedir. Tedavi öncesi ovaryen yanıtın öngörülmesi ile yetersiz ovaryen yanıt nedeniyle siklus iptalleri azaltılabilmektedir; aynı zamanda hekime uygun tedavi stratejileri geliştirme ve kötü prognozlu hastaları alternatif yöntemlere (oosit donasyonu) yöneltmeyi sağlayabilmektedir. Günümüzde bu amaçla kullanılan birçok belirteç mevcuttur. Ancak bu belirteçler tedaviye verilen yanıt hakkında fikir verse de hiç birisi tek başına tedavi başarısını göstermede tam güvenilir değildir. Bu derlemede ART tedavisi alan hastalarda kötü ovaryen yanıtı ve başarıyı ön görmede kullanılan over rezerv testlerinin etkinliği tartışılmaktadır. (J Turkish-German Gynecol Assoc 2012; 13: 196-203)

**Anahtar kelimeler:** ART, over rezervi, kötü ovaryen yanıt, siklus iptali, tedavi

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### Introduction

In modern societies, an increasing proportion of women delay marriage for various reasons and attempt to have their first pregnancies at a more advanced age (1). However, their chance of becoming pregnant begins to decline in this period of reproduction. The incidence of subfertility gradually increases with female partner age as 6% at age 20 to 24, 9% at age 25 to 29, 15% at age 30 to 34, 30% at age 35 to 39 and 64% at age 40 to 44 (2). According to data derived from natural populations without contraception, fecundity is halved at 35 years as compared to 25 years of age; thus, 35 years of age in women is considered to be the age when the decline in fertility becomes more pronounced (3, 4).

Other factors associated with impaired fertility may augment the effect of aging on fertility in women. These are increased incidence of conditions such as endometriosis, myomas and pelvic inflammatory disease with age, as well as decreased frequency of coitus and increased incidence of male infertility in older couples. To control the effect of the male factor and coital frequency on impaired fertility, data from donor

insemination studies can be used. In a donor study, female partners of men with azoospermia who were supposed to be comparable in reproductive health to fertile women have been inseminated with normal donor sperms and a cumulative pregnancy rate of 74% for one year was noted in women under 31 years of age as compared to 54% in women over 35 years of age (4). Data from another insemination program revealed a 3.5% decrease per year in the probability of having a healthy child in women after the age of 30 (5).

### Physiology of Reproductive Aging

The probable theoretical causes of decline in reproductive potential in women beginning at the third decade of life may be classified as:

- i. Diminished ovarian reserve
  - a. Quantitative decrease in oocytes
  - b. Qualitative changes in oocytes
- ii. Diminished uterine receptivity for implantation

The diminished ovarian reserve, either by decreased quantity and/or quality of the resting follicle pool, might decrease fertility after age 30. There is enough evidence for both situations.

The primordial follicle count, which is about 20 millions at the 20<sup>th</sup> week of intrauterine life, starts to decrease with the process of apoptosis (6-8). The primordial follicles left are about 1 million at birth and 300 thousands at puberty. At a mean age of 37-38 years only about 25 thousands of follicles are present in the ovaries. After this age, the disappearance of the follicles accelerates and the curve follows a biphasic pattern (7). The time interval between the beginning of accelerated follicular disappearance and menopause is constant at about thirteen years (7). Menstrual cycles become irregular about 6 years before menopause (9). There is a time period of about 4 years between age 37 when fertility begins to decline and age 41 when fertility practically ends (10).

It is known that the age of menopause in the general population is under 45 in 10% of women and under 40 in 1% of women (11). Thus, if the time interval between the beginning of accelerated follicular disappearance and menopause is constant and about thirteen years it can be speculated that about 10% of women in the general population will suffer from the clinical consequences of impaired fertility in their thirties due to early ovarian aging.

The data from ART cycles with fresh and nondonor oocytes and embryos demonstrate a decrease in embryo implantation, pregnancy and live birth rates per cycle when female partner age exceeds 38 (12). In ICSI cycles of men with obstructive azoospermia, the implantation rate decreases if the female partner age is over 37; this finding also demonstrates the effect of age related decline in oocyte quality on reproductive performance (13). Data obtained from oocyte donation clearly shows that, if oocytes are donated from young women to older women, both embryo implantation and pregnancy rates are restored to normal levels (14). These results suggest that the effect of age on fertility is largely a result of qualitative changes within the aging oocytes, rather than senescent changes in the uterus.

The high rates of pregnancy wastage in older women also indicate the age-related decrease in oocyte quality (15-17). Detection of high abortion rates in oocyte donation cycles if oocytes are donated from older women demonstrates that the age-related factor responsible for pregnancy wastage is also oocyte quality (18). An increased frequency of abnormal chromosome arrangements in human oocytes in older women is reported in several studies (19, 20). Preimplantation genetic diagnosis of embryos in women over 38 shows high rates of aneuploidy, another important evidence of a strong association between advanced maternal age and pregnancy wastage (21).

#### Evaluation of ovarian reserve in infertile patients

An important group of patients that has to be taken into consideration for diminished ovarian reserve are infertile women of advanced age (>35). The proportion of older age infertile women is gradually increasing. Other risk factors for diminished ovarian function in infertile patients are summarized in Table 1.

If 10% of patients enter menopause before the age of 45, then the same proportion of women are expected to experience signs of ovarian aging in their early thirties. Thus, it should be reasonable to test all infertile women over 30 for ovarian

reserve. Ovarian surgeries of any kind, but particularly for ovarian endometriosis, might be detrimental to primordial follicle pool; thus, patients with a history of ovarian surgery need to be evaluated for ovarian reserve regardless of their age. The underlying cause of subfertility might theoretically be a subtle diminished ovarian reserve. For this reason, it will be reasonable to apply ovarian reserve tests liberally to unexplained infertile couples. The patients in whom ovarian reserve tests are indicated are summarized in Table 2.

The effect of diminished ovarian reserve on fertility outcome has largely been evaluated in patients treated with ART. In this group of infertile patients the clinical entities associated with diminished ovarian reserve are poor response to COH, increased need for exogenous gonadotropins, high cancellation rates, low pregnancy and live birth rates in ART.

On the other hand, data regarding the reproductive outcome of ovulatory women in a general infertility population with an abnormal ovarian test is insufficient. Hence, the treatment alternatives to increase the chance to have a baby, especially in patients with an abnormal ovarian reserve test and younger than 35 years of age, are not yet known.

#### Ovarian reserve tests

The ovarian reserve tests are summarized in Table 3. Some of these tests have only been used for research and have not attained common practice.

#### Basal follicle stimulating hormone (FSH) level

Basal or cycle day 3 FSH level is an indirect indicator of ovarian reserve. It reflects the negative feedback effects of inhibin-B and estradiol produced by a cohort of follicles at pituitary level. Most of the studies of basal FSH levels are from ART cycles. The cut-off values for basal FSH vary from 10 to 25 IU/l. The value of basal FSH as a test for ovarian reserve in ART was evaluated

**Table 1. Patients groups at high risk for diminished ovarian reserve**

1.	Family history of early menopause
2.	Past chemotherapy
3.	Past radiotherapy
4.	Past pelvic surgery
5.	History of pelvic infection or tubal disease
6.	History of severe endometriosis
7.	Smoking

**Table 2. Which groups of infertile patients need ovarian reserve tests?**

1.	Patients over 30
2.	Patients with a history of ovarian surgery
3.	Patients with a history of surgery for severe endometriosis
4.	Unexplained infertility
5.	Poor response to ovarian stimulation

**Table 3. Ovarian reserve tests**

1. Basal serum FSH level
2. Basal serum estradiol level
3. Clomiphene citrate challenge test (CC Test)
4. Basal serum inhibin-B level
5. Anti-Mullerian Hormone (AMH)
6. GnRH stimulation test (GAST)
7. Exogenous FSH ovarian reserve test (EFORT)
8. Ultrasonography
• Ovarian volume
• Total antral follicle counts
• Ovarian stromal blood flow
9. Ovarian biopsy

in a meta-analysis of 21 studies (22). The results of receiver-operating curve (ROC) analysis have shown that the performance of basal FSH in ART cycles to predict poor response was moderate, whereas to predict non-pregnancy was poor. In a systemic review, Broekmans et al. (23) found that the cut off FSH levels of > 10U/L had a specificity of 80-90% and a lower sensitivity of 10-30% for the prediction of poor ovarian response to gonadotropins in IVF. The lack of a clear cut-off point with reasonable sensitivity and specificity and inter-cycle variations of FSH measurements also limits the reliability and use of basal FSH in IVF practice. The increase in basal FSH levels is a late indicator of ovarian reserve. Median FSH remained consistently low ( $\leq 5$  U/L) in women  $\leq 35$  years of age and was 6 U/L in 35- to 40-year-olds (24). Prediction of over reserve with only basal FSH may lead to an inappropriate strategy in infertile women, and some with an diminished ovarian reserve cannot take advantage of determining the rapidly closing window of opportunity. Although it is known that the prognosis of ART cycles will be highly negative in patients with high basal FSH levels, it is generally accepted that the predictive value of FSH levels below cut-off values are limited to reflect the outcome of ART cycles. A study evaluating the predictive value of FSH with regard to age showed that the ART performance of the patients over 40 but with normal basal FSH levels were worse than the patients below 40 but with an abnormal basal FSH level (25). That is to say, age reflects oocyte quality whereas basal FSH reflects oocyte number and the outcome of an ART cycle will be better if oocytes can be retrieved despite high basal levels in younger patients. A normal basal FSH level does not negate the effects of chronologic age on oocyte quality, embryo implantation, and pregnancy rates, and expectations should be managed accordingly.

There are only a limited number of studies in which ovarian reserve tests were used to predict fertility prognosis in a general infertility population (26-28). In one of these studies, the predictive value of elevated basal FSH levels during the initial subfertility workup with respect to fecundity has been assessed in a general infertility population (28). Long-term follow-up has shown that the pregnancy rates and time interval to pregnancy were not

different between patients with either normal or high basal FSH levels. It was concluded that screening for high basal FSH levels was of no additional value in a general infertile population.

#### Basal estradiol levels

Early elevations in serum estradiol reflect the advanced follicular development and early selection of a dominant follicle driven by rising FSH levels. A premature estradiol elevation may suppress the FSH levels, masking elevation that might otherwise reveal a low ovarian reserve. Patients with basal estradiol levels of 80 pg/ml or higher during a cycle before IVF achieved a lower pregnancy rate per initiated cycle (14.8% versus 37.0%) and had a higher cancellation rate (18.5% versus 0.4%), compared with those with estradiol levels below 80 pg/ml. Even if FSH > 15 were excluded, elevated basal estradiol levels still correlated with poor ovarian response and higher cancellation rates (29). As an ovarian test basal estradiol level has little value but may provide additional data in basal FSH interpretation. Adding cycle day 3 estradiol measurement to FSH decreases the incidence of false-negative results based on FSH alone.

#### Clomiphene Citrate Challenge Test (CCC Test)

The physiological basis of the CCC test is that, in a group of patients with diminished ovarian reserve but normal FSH levels, CC induced serum FSH rise cannot be suppressed by decreased inhibin secretion from a decreased primordial follicle pool and elevated levels of FSH are measured after CC administration. The test is considered abnormal if any measurement of FSH either on day 3 or on day 10 after CC administration is higher than 10 IU/l. The predictive value of an abnormal CCC test is extremely high with an overall cumulative pregnancy rate of only 1.3%, which is comparable with the 1.5% cumulative pregnancy rate among women with abnormal day 3 FSH values in ART cycles (30). Nevertheless, among older, at-risk patients, the CCC test also identified 29% of patients with compromised fecundity as compared to a rate of 6% for basal FSH screening alone (30). The use of the CCC test for screening ovarian reserve in a general infertile population was assessed only in a large series (26). About 10% of infertile women had an abnormal CCC test result and the fecundity of patients with an abnormal test was extremely decreased.

#### Basal inhibin -B levels

Inhibin-B is a dimeric peptide that is secreted by granulosa cells of preantral and early antral follicles (31). Therefore it is thought to have some value as an ovarian test. Inhibin-B concentrations decline before a rise in basal FSH levels and thus shows the reduction of ovarian reserve earlier than basal FSH (32, 33). As the level of inhibin-B decreases, ovarian response to gonadotropins, the number of oocytes retrieved and pregnancy rates decrease (34). Although there is a correlation between basal inhibin-B levels and ovarian response, it has low sensitivity (60-90%), specificity (40-80%) and positive predictive value (19-22%) even in low threshold values (40-45 pg/mL) (35). In various studies investigating the relationship between basal inhibin-B and ART outcomes, it was concluded that inhibin-B level was not a reliable measure of ovarian reserve and had a poor predictive value for pregnancy (36-39).

### Anti-Mullerian Hormone (AMH)

Anti-mullerian hormone is produced by granulosa cells of preantral and small antral follicles. The secretion begins from the start of primordial follicle growth and continues until the follicles have become capable of responding to FSH, which occurs when the diameter of the follicle reaches 4-6 mm (40). AMH is not expressed in atretic follicles and theca cells (41). The gonadotropin independent expression of AMH results of minimal variation within and between cycles provides advantage over other ovarian reserve markers. Pregnancy, the use of gonadotropin agonists for ovarian suppression, the day of menstrual cycle dose do not affect serum levels (42).

AMH expression is observed as early as the 36<sup>th</sup> gestational week, serum levels are gradually increased in the first 3-4 years of life and become stable until puberty. As the number and quality of the oocytes diminish throughout the woman's reproductive life, serum concentrations of AMH gradually decrease and fall below detectable levels in the menopause (43). Median time of menopause can be predicted by using AMH levels more accurately than inhibin and basal FSH (44). The number of the residual follicular pool correlates with the number of small antral follicles and AMH levels (45-48).

The first study investigating the relation between AMH levels and ovarian response to gonadotropins on ART cycles was performed in 2002. From that time on numerous studies have been performed. In women undergoing ART, low AMH threshold values (0.2-0.7 ng/mL) have 40-97% sensitivity, 78-92% specificity, 22-88% positive predictive value and 97-100% negative predictive value for prediction poor response to stimulation, but do not predict pregnancy (49-52). Almost all studies revealed that there had been a correlation between AMH levels and retrieved oocyte number and AMH seems to be a better marker than age, basal FSH, estradiol, inhibin-B in predicting ovarian response to gonadotropins but, when compared with AFC, it has nearly the same capacity to predict ovarian response (53). In a recent study including 1043 IVF cycles, AMH levels were found to be significantly related with the rate of on going pregnancy both in fresh and frozen embryo transfer cycles (54). In a meta-analysis, a total of 13 studies were analyzed reporting on AMH and 17 on AFC and it was shown that AMH had at least the same level of accuracy and clinical value for the prediction of poor response and non pregnancy as AFC. Both AMH and AFC have limited accuracy for non pregnancy prediction (55). Besides retrieved oocyte number, AMH and AFC are also found to be comparable predictors of the number of good quality embryos available for transfer and freezing (56).

However, AMH determination has some advantages over AFC: 1) It does not have to be carried out on a specific day of the cycle because of stability in serum levels throughout the menstrual cycle. 2) There is no need for a skilled ultrasound operator to count ovarian follicles 3) A possible observer bias in ultrasonographer is eliminated.

In their study, Silberstein et al. (57) found that the serum AMH levels at the time of hCG administration seem to predict not only ovarian reserve, but also embryo morphology. Some studies in the literature have revealed that there is a correlation

between oocyte quality and AMH levels (58-61) but other studies have defended the opposite (39, 62).

### GnRH stimulation test (GAST)

Administration of GnRH agonists on cycle day 2-3 causes an initial surge of FSH, LH and estradiol. The response of estradiol is an indirect indicator of ovarian reserve. If the follicular cohort is small, GnRH agonists may lead to less estradiol increase. In two prospective studies it is shown that the response of estradiol to GnRH-a stimulation was highly correlated with ovarian response in ART cycles (63, 64).

### Exogenous FSH ovarian reserve test (EFORT)

In the exogenous FSH reserve test, FSH and estradiol, inhibin levels are determined before and 24 hours after administration of 300 IU recombinant FSH on day 3 of the menstrual cycle. Basal FSH and levels and increase in estradiol levels are used to predict ovarian response in ART cycles. In a prospective study investigating the predictive value of EFORT in 52 IVF cycles it was shown that at least 30 pg/mL increase in estradiol levels is a better predictor of ovarian response than basal FSH (65). In another prospective randomized study performed by Kwee et al. (66) CCCT and EFORT were compared in terms of ovarian response in 110 ART cycle and it was found that the inhibin B increment and estradiol increment in the EFORT are the best predictors of the total number of follicles obtained after maximal ovarian hyperstimulation in an IVF treatment; CCCT, basal FSH and estradiol, age show a much lower performance. EFORT and GAST are more complex, expensive and time consuming and the predictive value in ovarian response or pregnancy are not so different from conventional markers. It is not advised to use these tests routinely in the evaluation of ovarian reserve (35).

### Assessment of ovarian reserve by ultrasonography

Comparison of an indirect assessment of ovarian reserve by sonographic measurement of ovarian volume and antral follicle counts with other ovarian reserve tests in ART cycles and their performance to predict response to COH and pregnancy rates have recently been reported in many studies. The most important advantage of ultrasonography is that it can be done in every patient without any additional cost. The sonographic assessment of ovarian reserve is also advantageous in selecting poor responders and choosing appropriate stimulation protocols at the beginning of the cycle.

#### i. Measurement of ovarian volume

The age-related decline in primordial follicle pool is supposed to cause a decrease in ovarian volume. The decrease in ovarian volume is supposed to be more pronounced after the age of 38 till menopause, a time period when the follicular depletion is accelerated. In a study population of women 14 to 45 years of age attending a family planning clinic, no correlation has been detected between age and ovarian volume (67). In a study with healthy and fertile Chinese women it was found that the ovarian volume was not different throughout the whole reproductive period (68). In a similar study population, but in the age group

of 35 to 50 years, the mean ovarian volume was detected to be similar in three age groups of 35 to 39, 40 to 44 and 45 to 49 years and the correlation of decrease in ovarian volume was evident only in the age group of 45 to 49 (69). Interestingly it was found in the same age groups that ovarian volume was decreased in infertile women compared to age-matched fertile women (70). Although the ovarian volume was least in unexplained infertile patients, the difference did not reach significance.

Data on the predictive value of ovarian volume measurement on IVF cycles has demonstrated that although a correlation between response to COH and ovarian volume was present, the predictive value of ovarian volume measurement for pregnancy was poor (71-74). High cancellation rates have also been reported in women with ovaries measuring less than 3 cm<sup>3</sup> (71, 72).

### ii. Antral follicle counts

The age-related decline in the number of antral follicles less than 10 mm measured by ultrasound has been shown in several studies (68, 69, 74). In a study population of fertile women a biphasic pattern has been demonstrated in age related decline in antral follicle counts (75). A yearly decline of 4,8% before the age of 37 was accelerated thereafter to the rate of 11.7%. However, a monophasic yearly decline of 3,8% has been demonstrated in a fertile population in another study (68).

The correlation of antral follicle counts with poor response in IVF has been several studies (74, 76, 77). In a recent study investigating the role of AFC in IVF outcome prediction, it has been shown that antral follicle count was predictive of ovarian response, with a 67% likelihood of poor ovarian response for AFC  $\leq$ 4, also there was a significant linear relationship between AFC, age and live birth which is much more marked for AFC (78). A study comparing the effectiveness of basal and CC induced inhibin-B and FSH, ovarian volume and antral follicle counts to predict the outcome of IVF cycles, reported that ovarian volume was the best parameter to predict poor ovarian response to COH, whereas age and antral follicle counts were found to be better than the other test with respect to predicting pregnancy success (74). In conclusion, it can be suggested that antral follicle counts reflect the ovarian reserve better than ovarian volume in infertile patients.

### iii. Ovarian stromal blood flow

There is a positive and independent correlation between ovarian stromal peak systolic velocity (PSV) measured by transvaginal pulsed Doppler ultrasonography both in the early follicular phase and after pituitary suppression (79, 80). Engman et al. (79) showed that ovarian stromal PSV was the most important single independent predictor of ovarian response in patients with a normal basal serum FSH level, compared to age, FSH/LH ratio, estradiol levels if the the cut-off level for PSV was taken as 10 cm/s. A study using 3D ultrasound reported that ovarian stromal vascularity was associated with a higher number of retrieved oocytes and increased pregnancy rates (81). Contrary to this Jarvela et al. (82) reported quantification of power Doppler signal in the ovaries after pituitary suppression does not provide any additional information to predict the subsequent response to gonadotrophin stimulation during IVF.

In a recent study, early follicular stromal Doppler signals is correlated with ovarian response and basal ovarian reserve parameters, but has no correlation with age or with clinical pregnancy achievement in infertile women undergoing IVF-ET treatment (83). Further studies are needed to clarify the effect of ovarian stromal blood flow on ART outcomes.

### Ovarian Biopsy

Demonstration of primordial follicles depletion in the ovary by ovarian biopsy was studied by several authors. Lass et al. (84) in their investigation attempted to find if there had been correlation between basal estradiol levels, ovarian size and follicular density in 60 infertile women. Computerized image analysis was used to measure the number of follicles per unit volume of ovarian tissue. There was no significant difference between unexplained and tubal infertility patients. They also observed that follicular density diminished significantly with increasing age. A study assessing the accuracy of basal FSH, estradiol, CCCT, GAST in predicting the total number of follicles, which was determined by histological examination of oophorectomy materials in 22 fertile patients older than 35 years, found a positive correlation between only basal estradiol levels and follicle per unit but not with others (85). The uneven distribution of follicles in the ovary makes a large variation even in the same ovary (86). When the random follicular distribution and potential risks of procedure are taken into consideration together, this procedure is not justified on current available data.

### Combination of ovarian reserve tests

None of the tests has a 100% sensitivity and specificity used for poor ovarian response prediction. In order to increase the prognostic reliability of each test, combining the ovarian tests may be considered. A scoring system using the combination of age, AFC, basal FSH, basal AMH, delta E2 and delta inhibin developed by Muttukrishna et al. (87) predicted the ovarian response more accurately than each of the parameters alone. However, in a meta-analysis investigating the performance of the combinations of ovarian reserve tests to predict ovarian response in IVF, the combination of these tests did not perform better compared with AFC alone. According to this meta-analysis there is no advantage in using multivariate model in poor response prediction (88). Addition of age, AFC, basal FSH, inhibin to AMH did not make a significant difference in prognostic reliability of AMH in a recent study (89). The high level correlation of ovarian reserve tests and the differences of chosen thresholds for each test makes analysis difficult.

Although ovarian reserve tests reflect oocyte quantity they do not reflect oocyte quality accurately (90). Age was found better in predicting pregnancy than these tests (90). Women with low ovarian reserve still have a reasonable chance to achieve pregnancy. The increased rates of spontaneous abortus and aneuploidy in young women with poor ovarian reserve suggest that oocyte quality may also contribute in some unexplained infertile women. Counseling and management of the cycle with the knowledge gained only from the ovarian reserve tests is a matter of debate. In fact, many women whose tests results were lower than the cut-off could have pregnancy after IVF.

## Conclusion

Assessment of ovarian reserve should not be neglected in an infertile patient if the age of the patient is above the period when the ovarian reserve is known to be declining.

At present, there is no ideal ovarian reserve test reflecting fertility potential of a woman reliably. Controversial results of the studies make it difficult to compare the efficiency of different tests of ovarian reserve. Data obtained from ART cycles are useful to form models for assessing the efficiency of various tests to predict fertility potential.

None of the tests of ovarian reserve is ideal to predict pregnancy. A woman with an abnormal ovarian test may conceive either spontaneously or by ART.

Although the predictive value (specificity) of an abnormal hormonal parameter (basal or CC induced FSH and inhibin) to detect diminished ovarian reserve is high, their sensitivities are low. The CC test is relatively more sensitive than basal FSH. Among the ultrasound parameters, antral follicle count is the most reliable.

There are only a limited number of studies in which ovarian reserve tests were used to predict fertility prognosis in a general infertility population. The most reliable tests in these patients seem to be AFC and AMH, according to the existing data. The studies in this group of patients will aid in forming screening strategies for asymptomatic cases of diminished fertility due to early ovarian aging in the general population.

AMH has advantages compared with other markers of ovarian reserve tests. It is the earliest marker to change with age and has least inter and intra cycle variability.

## Conflict of interest

No conflict of interest was declared by the authors.

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# Cutaneous umbilical metastases in post-menopausal females with gynaecological malignancies

## *Jinekolojik malignitesi olan menopoz sonrası kadınlarda deriyi tutan umbilikal metastazlar*

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### Abstract

Gynaecological malignancies frequently metastasize to contiguous structures, internal organs and bones. Cutaneous metastasis as a primary or recurrent presentation of these malignancies is rare and only a few cases have been reported in the literature. A twenty year (1991-2010) retrospective search for umbilical metastasis from gynaecological malignancies in our departmental case records showed only four cases. Four post-menopausal females presented with painful cutaneous umbilical (Sister Joseph's) nodules. The clinical examinations of all four patients revealed well delineated nodules of varying sizes and degrees of ulceration. Other findings were matted axillary and inguinal lymph node enlargement, intra-abdominal and pelvic masses, vaginal discharge and vaginal bleeding. Incisional tissue biopsies from the nodules were processed in paraffin wax and stained with haematoxylin and eosin. Histology of the sections showed pigmented skin overlying metastatic malignant tumours consistent with adenocarcinoma from the endometrium and ovary in three cases, and squamous cell carcinoma, large cell keratinizing from the cervix uteri in the fourth case. Gynaecological cancers have a global spread and varied geographic distribution. Cervical cancer is the commonest in our setting and patients often present to hospital with advanced stage disease. Ovarian and endometrial cancers are infrequent and their diagnosis may be delayed by non-specificity of presenting clinical symptoms from other benign tumours at these sites. Although umbilical metastasis is commonly associated with gastro-intestinal malignancies, its presence may be the first harbinger of occult gynaecologic cancer.

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**Key words:** Metastasis, umbilical, cervix uteri, squamous cell carcinoma, endometrial adenocarcinoma

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### Özet

Jinekolojik maligniteler sıklıkla komşu yapılara, iç organlara ve kemiklere metastaz yaparlar. Bu malignitelerin primer veya rekürren prezentasyonu olarak deri metastazları enderdir ve literatürde sadece bir kaç olgu bildirilmiştir. Jinekolojik malignitelerden kaynaklanan umbilikal metastazlar için departmanımızın olgu kayıtlarında yirmi yıllık (1991-2010) retrospektif taramada sadece dört olgu bulundu. Menopoz sonrası dört kadın deriyi tutan ağrılı umbilikal nodüllerle (Rahibe Joseph) başvurmuştu. Dört hastanın klinik muayenesi farklı büyüklükte ve farklı ülserasyon derecelerinde iyi sınırlanmış nodülleri ortaya koymuştu. Diğer bulgular aksiller ve inguinal lenf nodlarında paket halinde büyüme, karn içi ve pelvik kitleler, vajinal akıntı ve vajinal kanama idi. Nodüllerden insizyonla elde edilen doku biyopsileri parafin mumunda işlenmiş ve hematoksilin-eozin ile boyanmıştı. Kesitlerin histolojisi üç olguda endometriyum ve overden köken alan adenokarsinoma ile uyumlu metastatik malign tümör ve üstünü örten pigmente deriyi, dördüncü olguda serviks uteriden köken alan keratinize büyük hücreleri-yassı hücreli karsinomayı ortaya koymuştu. Jinekolojik kanserler dünyanın her yerinde bulunur ve değişken bir coğrafi dağılım gösterir. Bölgemizde en yaygın olanı servikal kanserdir ve hastalar hastaneye sıklıkla ileri bir hastalık aşamasında gelmektedir. Over ve endometriyum kanserleri daha az sıklıktadır ve tanıları bu bölgelerden köken alan diğer iyi huylu tümörlerin klinik belirtilerinin özgün olmayışı nedeniyle gecikebilir. Umbilikal metastaz yaygın olarak gastrointestinal malignitelerle birlikte görülmekle birlikte, varlığı gizli bir jinekolojik kanserin ilk habercisi olabilir.

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**Anahtar kelimeler:** Metastaz, umbilikal, serviks uteri, yassı hücreli karsinoma, endometriyal adenokarsinoma

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### Introduction

There is a recognized global rise in the incidence of gynaecological malignancies, although the frequency of occurrence varies in different geographic regions and these cancers have a predilection for middle-aged to elderly females (1, 2). The commonest in developing countries is carcinoma of the cervix uteri, which remains a major cause of cancer related death

in spite of the tremendous improvements in prevention, early detection and treatment modalities (3). Cancer of the ovary is the second most frequent, however, its diagnosis is often delayed due to the absence of specific clinical symptoms to differentiate it from benign ovarian lesions. The endometrial cancers are less frequent and have a predilection for elderly females (1, 2). These gynaecological cancers are associated with high mortality and morbidity in our setting due to the

patients' late hospital presentation with widespread metastases to contiguous structures intra-abdominally. Cutaneous metastases from mainly ovarian cancers have been reported in a few cases, however, umbilical metastasis from gynaecological cancers is uncommon (4-7). We report four post-menopausal females with umbilical (Sister Joseph's nodule) metastatic nodules from gynaecological malignancies in our hospital. The incision tissue biopsies from the umbilical nodules, cervix uteri and endometrium were fixed in 10% formalin. They were processed in paraffin wax and stained with haematoxylin and eosin and Periodic acid Schiff.

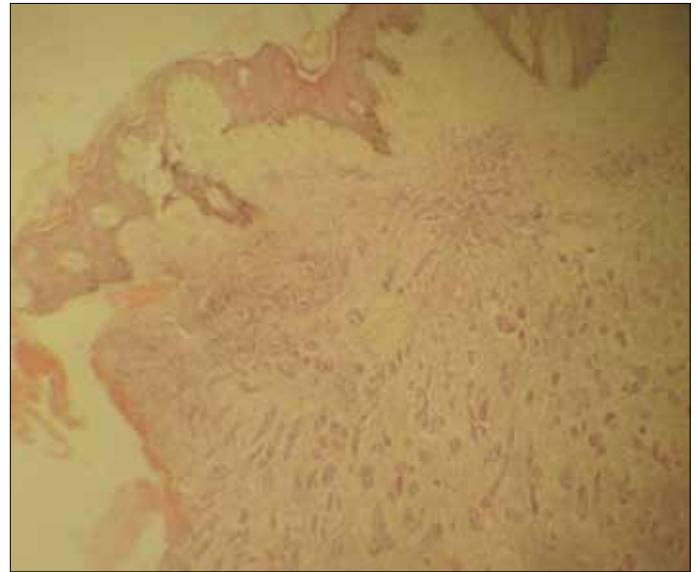
## Case Reports

### Case 1

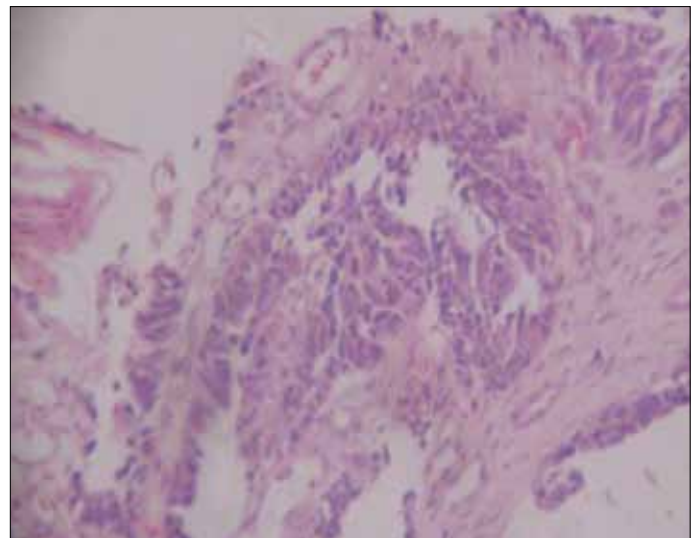
A 50 year-old nulliparous female presented to our gynaecology clinic with a five month history of peri-umbilical nodular growth following an exploratory intra-abdominal surgery at a private clinic. However, there was no clinical diagnosis made or tissue specimen sent for histopathological analysis following the surgery. She first noticed the umbilical nodule a month after surgery. The nodule gradually increased in size and was associated with pain and weight loss. Her clinical examination revealed an emaciated woman with a 5x4cm umbilical nodule, a pelvic mass, a matted right axillary and bilateral inguinal lymph node enlargement. The incisional tissue biopsy taken from the nodule showed keratinized stratified squamous epithelium overlying a malignant tumour. The tumour cells were composed of columnar endometrial cells arranged in glands and focal papillary array in a fibromyxoid stroma. It was diagnosed as metastatic endometrial adenocarcinoma (Figure 1, 2).

### Case 2

A 65 year-old post menopausal housewife presented to the gynaecology clinic of our hospital with a three month history of fungating umbilical growth and progressive weight loss. She gave a medical history of five years of painless abdominal mass which gradually increased in size and a two month history of discharging umbilical sinus. There was no history of associated vagina bleeding or discharge, change in bowel habit or urinary symptoms. She was referred from a private clinic with a clinical diagnosis of ovarian cancer, however she decided to try traditional medication for a month before she finally came to our hospital. Clinical examination at the gynaecology clinic showed a wasted woman having a foul smelling ulcerated umbilical nodule and a non tender mobile right supra-pubic mass which was comparable with a 16-week size uterine gestation. The uterus and cervix were grossly normal and the pouch of Douglas was empty. She also had an abdominal-pelvic scan which showed a complex ovarian mass. Incisional tissue specimen from the umbilical nodule was sent for histopathological diagnosis. It showed pigmented skin overlying a malignant tumour composed of columnar cells having moderately pleomorphic hyperchromatic nuclei and moderate cytoplasm. The tumour cells were arranged in papillary and cystically dilated glandular patterns. It was diagnosed as metastatic ovarian papillary cystadenocarcinoma. However, she refused surgery and opted for more traditional medication at home.



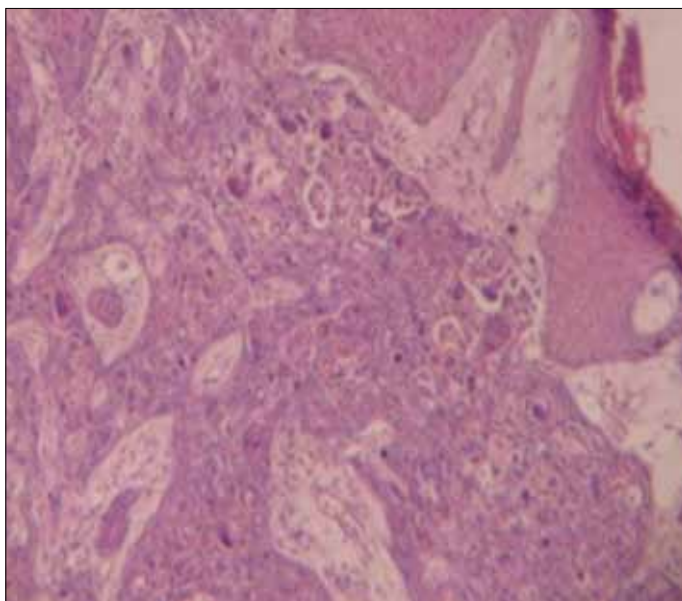
**Figure 1. Keratinized Skin overlying gland forming tumour from the endometrium H&E: mgX40**



**Figure 2. Metastatic endometrial adenocarcinoma with papillary foci. H&E: mg X100**

### Case 3

A 70 year-old elderly grand multi-parous female presented with a six-month history of ulcerated umbilical nodule, vaginal bleeding and vagina discharge. Her examination revealed a 6x4cm bleeding peri-umbilical nodule and a fungating uterine cervix mass which bled spontaneously. Histopathological findings from the incisional tissue biopsies of the nodule and the cervical mass were similar. They showed malignant squamous cells arranged in nests and strands in a fibromyxoid stroma. Some of the tumour cells exhibited individual cell dyskeratosis and keratin formation. Other areas in the nodule showed uninvolved pigmented skin and its appendageal structures. The cervical tissue was diagnosed as squamous cell carcinoma; large cell keratinizing and the nodule was diagnosed as metastatic squamous cell carcinoma (Figure 3).



**Figure 3. Metastatic Squamous cell carcinoma large cell keratinizing H&E: mg X40**

#### Case 4

A 72 year-old, well preserved multi-parous female presented with a three month history of painful subcutaneous peri-umbilical nodule. She was diagnosed with a histologically confirmed stage 1c endometrial carcinoma a year earlier in our hospital. She had a total abdominal hysterectomy as part of her treatment and was also placed on combination chemotherapy. Her follow up at the clinic was uneventful until this current presentation. The histopathological analysis of the incisional tissue biopsy of the nodule showed tumour cells consistent with her primary endometrial cancer. It was diagnosed as metastatic endometrial adenocarcinoma.

#### Discussion

Cutaneous manifestation is associated with a wide variety of systemic diseases, infestations, and infections, while cutaneous metastasis is an uncommon event which is seen in 1-9% of patients with cancer (8, 9). The phenomenon of umbilical metastasis was first noticed by Julia Dempsey (Sister Mary Joseph) in relation to advanced internal malignancies and it represents approximately 10% of cutaneous metastasis. It is an indication of poor prognostic outcome where palliation is the mainstay of patient management. Also, an estimated 1-3% of abdomino-pelvic malignancies arising from the stomach, pancreas, colon, prostate and liver manifest with umbilical metastasis (7, 10-13). Other uncommon primary origins include the breast, lung, ovary and kidney.

The metastatic umbilical nodule is often painful and may be ulcerated or have discharging sinuses as seen in our patients. It should be differentiated from other lesions which may occur at the same site, such as umbilical hernia, pilonidal sinus, endometriosis, foreign body granuloma, fibroma and primary malignancies of the umbilical skin and supporting stroma. The

nodule may also be the first indication of occult or recurrent malignancy. However, 11-30% of cases of umbilical metastasis have no known primary disease (7, 13).

Metastatic lesions from gynaecological malignancies usually spread to contiguous pelvic structures and lymph nodes. Distant metastasis from these tumours spread to the lung, liver, bone and central nervous system (CNS) (1). Cutaneous spread is uncommon, however a few cases have occurred in the head and neck regions, particularly the scalp. Also, a rare umbilical nodule may be the first manifestation of ovarian disease (1, 6, 8).

The exact mechanism of umbilical metastasis is not clear. There are two postulates; one is direct spread, which is the most common. This direct peritoneal spread is further enhanced by the multiple peritoneal folds comprising the umbilical ligament and ligamentum teres. The second is via the rich anastomotic vascular and lymphatic supply at the umbilical region (14).

Over 80% cases of endometrial cancer are seen in postmenopausal females at the time of diagnosis. Some microscopic variants of endometrial cancer may share similarities with non-neoplastic endometrium, while papillary foci may be mistaken for an ovarian lesion (1). Adequate tissue sampling and immuno-histochemical studies will help to differentiate the tissue of origin between the endometrium and ovary.

Cervical cancer is the commonest tumour in reproductive age women in our setting and patients present with advanced stage disease and widespread metastasis. However, our third patient is the only cervical cancer case we have seen with metastatic umbilical nodule.

The tumour types that may metastasize to the umbilicus are the adenocarcinomas, while squamous cell and undifferentiated carcinomas are rather uncommon (5, 15). Ovarian and endometrial cancers are gland forming and this fact may explain their tendency to metastasize to the umbilicus.

Definitive diagnosis of the tumour origin is achieved with tissue biopsy of the metastatic nodule. This is necessary for correct patient treatment and management. The fine needle aspiration cytology technique is also recommended in recurrent lesions with previous histologically confirmed diagnosis (16). However, this may prove unhelpful in our setting where patients would have visited several hospitals and had surgeries without histological diagnosis or confirmation of excised lesions.

#### Conclusion

Metastatic gynaecological malignancies should be a differential for postmenopausal females presenting with umbilical nodules. Adequate tissue sampling from the nodule is necessary for the typing and characterization of tumour origin as well as further patient management. Cervical squamous cell carcinoma, endometrial and ovarian adenocarcinoma may present with umbilical metastasis in the advanced stage of the diseases.

#### Conflict of interest

No conflict of interest was declared by the authors.

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# Advanced stage micropapillary serous borderline ovarian tumor in a postmenopausal woman: a case report

## *Postmenopozal bir kadında ileri evre mikropapiller seröz borderline over tümörü: olgu sunumu*

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### Abstract

Serous borderline ovarian tumors (SBOT) generally occur in young women, present at early stages and are associated with an excellent prognosis. However, there are rare subtypes of SBOT which may exhibit a more aggressive course. In contrast with other types of SBOT, the micropapillary variant SBOT (SBOT-MP) tends to present at advanced stages. Herein, we present a rare case of a SBOT-MP that occurred in a 66-year-old woman, who had tumoral involvement on bilateral ovaries, sigmoid serosa and a positive peritoneal cytology. The currently recommended treatment options for these cases are also discussed. (J Turkish-German Gynecol Assoc 2012; 13: 208-11)

**Key words:** Borderline ovarian tumor, peritoneal implant, micropapillary, advanced stage, postmenopause

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### Özet

Seröz borderline over tümörleri (SBOT) genellikle genç kadınlarda ortaya çıkmakta, erken evrede tanı almakta ve çok iyi bir prognoza sahip olmaktadır. Ancak, SBOT'nin daha agresif bir seyir gösterebilen nadir alt tipleri mevcuttur. Diğer SBOT'lerin aksine, SBOT'nin mikropapiller varyantı (SBOT-MP) ileri evrelerde ortaya çıkma eğilimindedir. Bu yazıda, 66 yaşındaki postmenopozal bir kadında ortaya çıkan, bilateral overlerde ve sigmoid kolon serozasında tutulumu ve pozitif peritoneal sitolojiye sahip nadir bir SBOT-MP olgusu sunulmaktadır. Ayrıca, bu tip olgular için güncel tedavi önerileri de tartışılmaktadır.

(J Turkish-German Gynecol Assoc 2012; 13: 208-11)

**Anahtar kelimeler:** Borderline over tümörü, peritoneal implant, mikropapiller, ileri evre, postmenopoz dönem

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### Introduction

Serous tumors account for 60% of all epithelial ovarian tumors, and are classified into three groups: benign, borderline and malignant. Serous borderline ovarian tumors (SBOT) constitute 9-15% of all serous neoplasms. With respect to invasive ovarian cancer, borderline tumors generally occur in younger women at childbearing age. Fortunately, these tumors are generally diagnosed at early stages, and have a favorable prognosis (1).

However, there are subtypes of SBOTs that are associated with a more aggressive behavior and an unfavorable outcome. Herein, we report a case of a bilaterally located micropapillary variant of SBOT resembling an invasive ovarian malignancy at an advanced stage with serosal sigmoid implants and positive peritoneal cytology in a postmenopausal woman. The currently recommended treatment approaches for these tumors are also discussed.

### Case Report

A 66-year-old woman who had been postmenopausal for 22 years applied to our institution with pelvic pain and abdominal distention that initially occurred four months before admission. Her past medical and family histories were unremarkable. A pelvic mass predominantly located in the right lower quadrant was palpated on pelvic examination. On transvaginal ultrasonography (TVUS), she was found to have bilateral cystic pelvic masses with peripheral solid areas, each measuring 10 cm in diameter. Pelvic computerized tomography (CT) revealed bilateral heterogeneous pelvic masses with irregular surface contours and solid components. Circulating serum tumor markers were within the normal reference range (CA125: 22.2, CA19-9: 0.56, CA15-3: 8.7, AFP: 2.2, CEA: 1.7).

Exploratory laparotomy was performed. Minimal peritoneal fluid accumulation was observed, which was sampled for cytological analysis. Solid-cystic masses on both ovaries were

*The case has been previously presented in part as an abstract in a local obstetrics and gynecology meeting (Zekai Tahir Burak Gunleri 2011, Antakya, Turkey).*

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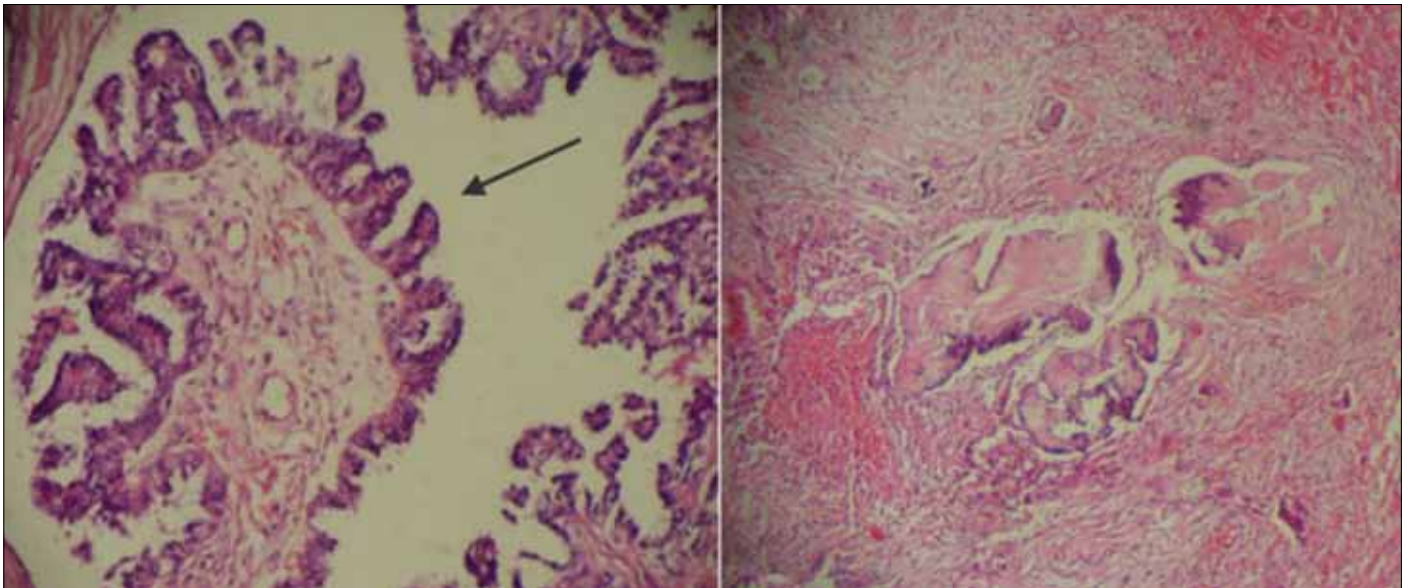
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present, each measuring about 10 cm in size. There were multiple tumoral implants on the sigmoid colon serosa, the largest measuring 5 mm. Other peritoneal surfaces were macroscopically normal.

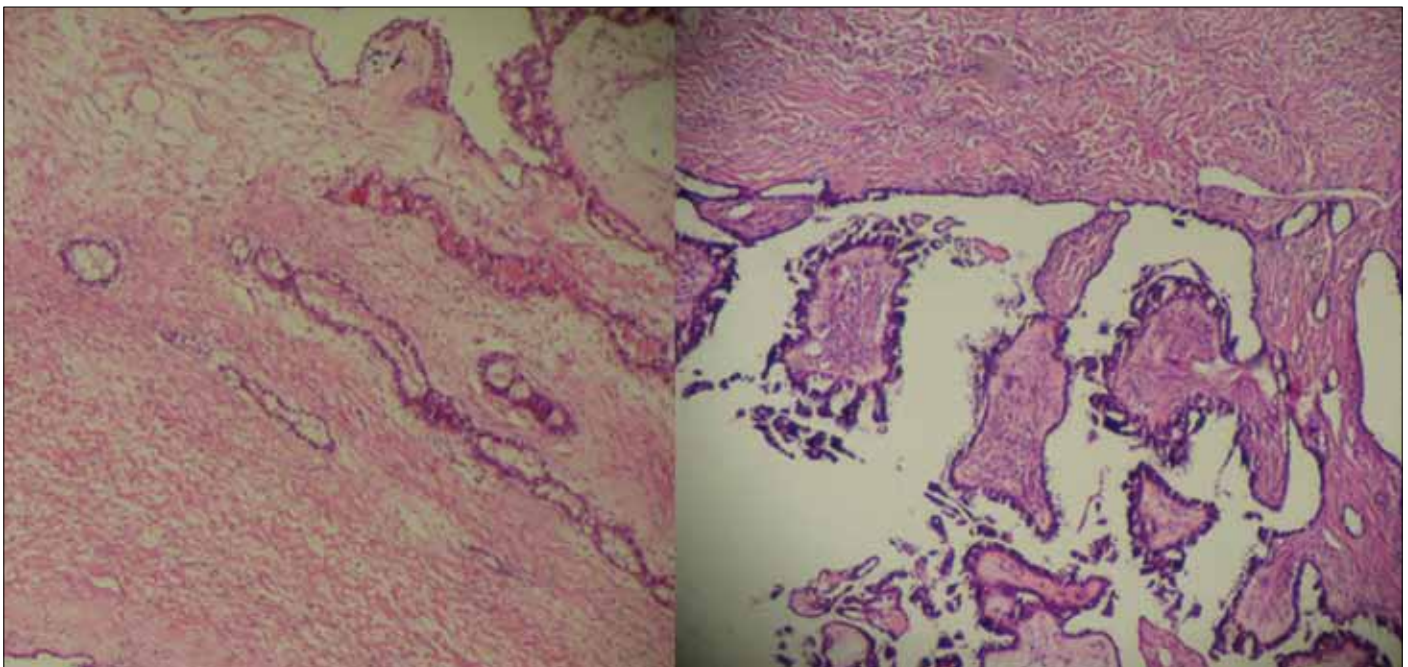
Bilateral salpingo-oophorectomy was performed initially, and the masses were sent for frozen section analysis. Both of the masses were reported to be serous ovarian tumors with at least borderline histology. Total abdominal hysterectomy, omentectomy, bilateral pelvic and paraaortic lymph node dissection and appendectomy were performed for staging purposes. Tumoral implants were excised from the sigmoid colon serosa and the

defects were primarily repaired. The patient had an uneventful postoperative course and was discharged from the hospital on the third postoperative day.

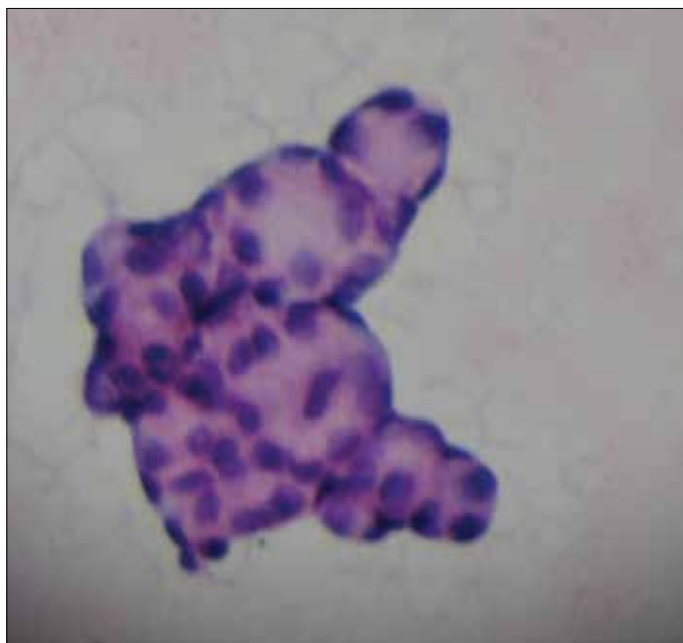
On final pathological examination, a micropapillary variant of SBOT on both ovaries and peritoneal implants on sigmoid serosa were reported (Figure 1). The implants on the sigmoid serosa were non-invasive (Figure 1). The tumor had both endophytic and exophytic invasion patterns (Figure 2). Cytological washings were also positive for tumor cells (Figure 3). Pelvic and paraaortic lymph nodes, omentum and appendix were free of any tumoral involvement. After consultation with the medical



**Figure 1.** Micropapillary pattern of the tumor (left image) (H&E x200) and Non-invasive peritoneal implant on sigmoid serosa (right image) (H&Ex40)



**Figure 2.** Endophytic (left) and exophytic (right) growth pattern of the tumor (H&E x40)



**Figure 3. Cytologic examination demonstrating tumor cells forming a papillary pattern (H&E x400)**

oncology department, adjuvant chemotherapy with paclitaxel and carboplatin was planned.

## Discussion

Borderline ovarian tumors (BOT) were initially described by Taylor et al. (2) in 1929. As these tumors are most commonly diagnosed at reproductive ages, fertility sparing is important for most of the patients. Subsequent pregnancies with normal obstetric outcomes may be achieved, especially in younger patients (3). In the case presented here, a serous type BOT with micropapillary pattern occurred in a 66-year-old postmenopausal woman, which is a rare presenting age for BOTs.

Serous BOTs constitute the most common type among BOTs. These are well-differentiated tumors and generally have an indolent growth pattern. However, they have a higher rate of recurrence than other types of BOT and progression to invasive carcinoma is not uncommon (4). SBOTs are bilateral in 30% of cases, and extra-ovarian spread may develop in about 30% of these (5). Interestingly, our case had multiple peritoneal implants on the sigmoid serosa, but other peritoneal surfaces were free of tumoral involvement. SBOTs are usually detected at early stages (6). The presenting stage is considered the most important factor for predicting recurrence. Our case presented as stage III-B disease. Positive peritoneal cytology rates increase with advancing tumor stage (7). In our case, cytology was also positive.

According to histopathological features, SBOTs are classified into two groups: typical non-invasive proliferative type SBOT and micropapillary SBOT (SBOT-MP) type, which is also termed micropapillary serous carcinoma (MPSC). SBOT-MPs are further classified into non-invasive and invasive types. Invasive SBOT-MP is the most common histologic type (8). It is characterized by

small micropapillae that infiltrate the underlying ovarian stroma (Figure 1). As in primary invasive ovarian tumors, invasive SBOT-MPs may be associated with peritoneal implants. Some researchers consider the presence of invasive peritoneal implants as a poor prognostic sign (9). The case presented here had non-invasive implants on the sigmoid serosa, which may be associated with a better prognosis than cases with invasive implants.

Unlike the proliferative type SBOTs, SBOT-MPs have a higher risk of presenting at an advanced stage, having bilateral ovarian involvement, ovarian capsule invasion, positive peritoneal cytology, stromal microinvasion and extra-ovarian implants (particularly invasive) (10). Moreover, SBOT-MPs with an exophytic surface growth pattern have a higher association with extra-ovarian surface implants and recurrence (9). Thus, it is important to distinguish proliferative SBOT and SBOT-MP tumors at the time of initial diagnosis. In our case, micropapillary histology, bilateral ovarian involvement, positive peritoneal cytology and presence of serosal implants placed the patient at high risk for recurrence.

Treatment of BOTs mainly consists of surgical resection of the tumor and its macroscopic implants, and may be performed via either laparoscopy or laparotomy. During surgery, pelvic structures, all peritoneal surfaces including sub-diaphragmatic area and intestinal serosa should be thoroughly examined to rule out extra-ovarian disease. Fertility sparing surgery in terms of cystectomy or unilateral oophorectomy is an acceptable option in cases with future fertility desire and low risk for recurrence. There are also publications suggesting that lymph node dissection may be avoided in such cases (11). However, for cases of SBOT with completed childbearing, a complete surgical staging approach with pelvic and paraaortic lymph node dissection is recommended for both diagnostic and therapeutic purposes, as nearly 30 percent of women with SBOTs have extraovarian disease spread. Moreover, 6 to 27 percent of those with a frozen section diagnosis of serous borderline tumor will be upgraded to invasive cancer on final pathological examination (12). As previously mentioned, our case had multiple high risk factors for recurrence; thus, complete staging surgery was undertaken. Especially for postmenopausal patients with SBOT-MP, we recommend an extensive surgical intervention, as for invasive serous ovarian carcinomas. These cases should also have long-term follow-up visits, as there may be recurrences even 10 years after initial treatment (13).

Although considered ineffective by some authors, chemotherapy is commonly used in advanced stages of disease, especially with extraovarian implants, as done in our case (14). Well-designed future studies are needed to clarify the role of chemotherapy in the treatment of SBOT at advanced stages.

In summary, we presented a case of bilateral SBOT-MP tumor with extraovarian spread in a postmenopausal patient. Complete staging procedures with optimal cytoreductive efforts are strongly recommended in these cases in order to achieve high cure rates and minimal disease related morbidity and mortality.

## Conflict of interest

No conflict of interest was declared by the authors.

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# Management of retained products of conception with marked vascularity

## *Belirgin damarlanması olan döllenme ürünü kalıntılarına yaklaşım*

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### *Abstract*

To discuss the management options of retained products of conception (RPOC) with increased vascularity where simple dilatation and curettage may lead to life threatening haemorrhage and endanger the life of the patient. We present a series of three cases that had RPOC with hyper vascularity and were managed with different approaches. A brief review of management options is discussed. Out of three cases of RPOC with hyper vascularity, two cases underwent hysterectomy and one case was managed with methotrexate. From our study and review of literature, we conclude that evaluation of vascularity in all cases of RPOC is mandatory prior to Dilatation&Curettage in order to avoid the dreaded complication of massive haemorrhage.

(J Turkish-German Gynecol Assoc 2012; 13: 212-4)

**Key words:** Retained products of conception, increased vascularity, Doppler study, management options

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### *Özet*

Basit dilatasyon ve küretajın yaşamı tehdit eden hemorajiye yol açabildiği ve hastanın yaşamını tehlikeye attığı artmış damarlanması olan döllenme ürünü kalıntılarının (retained products of conception; RPOC) tedavi seçeneklerini tartışmak. Aşırı damarlanmalı RPOC'u olan ve farklı yaklaşımlarla tedavi edilmiş üç olguluk bir seriyi sunmaktayız. Tedavi seçeneklerinin kısa bir değerlendirilmesi tartışılmaktadır. Aşırı damarlanması olan üç RPOC olgusunun ikisine histerektomi yapılmış ve bir olgu metotreksat ile tedavi edilmiştir. Kendi çalışmamız ve literatürün gözden geçirilmesi ile şu sonuca ulaştık: korkulan komplikasyon olan massif hemorajiden sakınmak için Dilatasyon ve Küretajdan önce bütün RPOC olgularında damarlanmanın değerlendirilmesi zorunludur.

(J Turkish-German Gynecol Assoc 2012; 13: 212-4)

**Anahtar kelimeler:** Döllenme ürünü kalıntıları, artmış damarlanma, doppler çalışması, tedavi seçenekleri

**Geliş Tarihi:** 14 Şubat 2012

**Kabul Tarihi:** 20 Nisan 2012

### **Introduction**

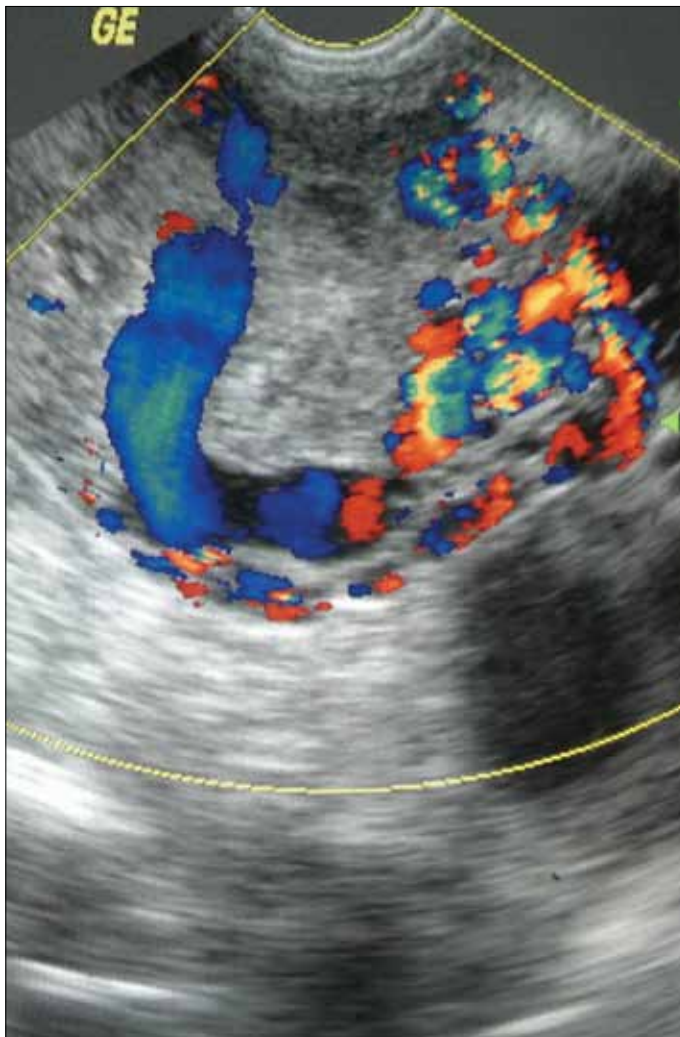
Various medical and surgical methods have been employed in the treatment of retained products of conception (RPOC). Amongst the surgical methods the universally accepted technique is simple dilatation and curettage. However, it is estimated that nearly 20% of RPOC's have increased vascularity and in such cases simple dilatation and curettage (D&C) may lead to massive haemorrhage (1).

Various causes of increased vascularity of RPOC's include arteriovenous malformations, placental polyp and excessive myometrial invasion by the trophoblasts. Due to excessive trophoblastic invasion of myometrium, the physiological myometrial arteriovenous shunting in the placental bed persists, leading to prominent vascularity. We report a series of three cases of hyper vascular RPOC's and the different ways in which they were managed. We aimed to report these cases because as a routine, it is not the practice to do a Doppler study in cases with RPOCs. It was only when patients started bleeding heavily during the D&C procedure that arteriovenous malformations were suspected and a Doppler ultrasound was done. Evaluation of blood supply to RPOC with colour Doppler ultrasonography prior to D&C may prevent the dreaded complications of massive haemorrhage.

### **Case Reports**

#### **Case 1**

A 39 year-old female, P3L2A1, presented to us with recurrent episodes of bleeding per vagina after medical termination of pregnancy (MTP) at 11 weeks of pregnancy. Post MTP ultrasonography findings were suggestive of retained products of conception. She had D&C twice, but bleeding persisted, for which she received two doses of methotrexate Injection.  $\beta$ -hcg levels were not done prior to admission to our hospital. Her last child was 12 yrs old, delivered by caesarean section. At the time of admission, vital signs were stable, she was not bleeding, and mild pallor was present. On vaginal examination the external os was closed. The uterus was 6-8 weeks pregnant uterus size and bilateral fornices were free. Her hemoglobin was 9.4% gm, and coagulation profile was within normal limits. Ultrasonography and Colour Doppler showed a heterogeneous mass 2.5 cmx2 cm size with prominent vessels in the myometrium suggestive of either retained products of conception with AV malformations or Invasive mole (Figure 1 and 2).  $\beta$ -hcg levels were 22.3 miu/ml. D &C was attempted but she started bleeding profusely and the procedure was abandoned, and bleeding stopped within five minutes. She received two units of blood transfusion. In view of recurrent

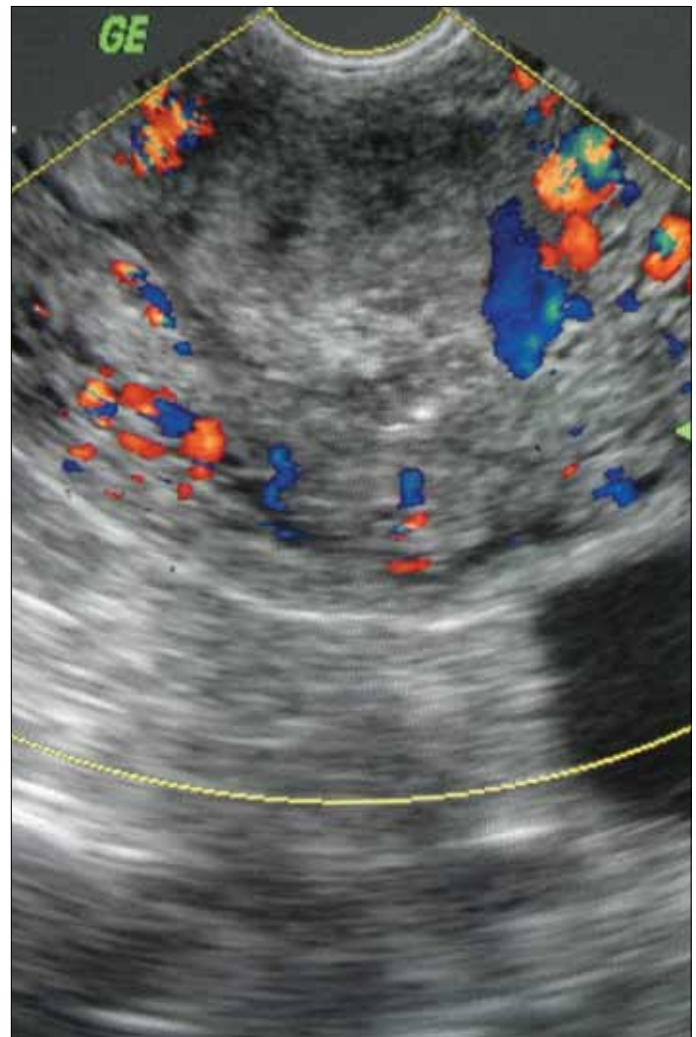


**Figure 1. Showing RPOC with AV Malformation**

bleeding and doubtful diagnosis of retained products of conceptions with Arteriovenous malformations or invasive mole or possibility of rare persistent trophoblastic tumor, the decision for hysterectomy was taken. Intra-operatively there was leash of vessels on the right side and anterior surface of uterus in the isthmic region. She had excessive bleeding during surgery and received three units of blood. On cut section the uterus showed a mass about 3x3cm in size with dilated vessel channels in the isthmo-cervical area. Histopathological report came out as products of conception. Post operative period was uneventful.

#### Case 2

A forty year-old Para 4 with 4 live issues and one abortion presented to our outpatient department with history of recurrent episodes of bleeding per vaginum for the previous three months with no preceding history of amenorrhea. She had had four caesarean sections. On admission her vital signs were stable and bleeding per vagina was present. Urine pregnancy test was weakly positive. Her Hb was 5% gm, coagulation profile was within normal limits. Per speculum examination showed a normal cervix and vagina with bleeding from the external cervical os. Per vaginal



**Figure 2. Showing RPOC with hypervascularity**

examination showed a retroverted uterus, 6-8 weeks size with bilateral fornices free. Ultrasonography showed a mass of size 6x4 cm in the cervix and lower endometrial cavity and on Doppler flow there was increased vascularity in the mass and surrounding myometrium and cervix. Her  $\beta$ -hcg levels were 39 miu/ml. endometrial aspirates was taken and malignancy was ruled out. In view of the mass invading the myometrium and increased vascularity in the mass and myometrium she was taken up for hysterectomy. On cut section of the uterus there was a friable growth in the isthmus and cervix suggestive of retained products of conception. Histopathology examination confirmed the diagnosis of cervical RPOC. Her post operative period was uneventful.

#### Case 3

A 25 year-old P2L2 with previous two normal deliveries presented to us with history of amenorrhea two months and bleeding per vagina since the previous week. On examination her vital signs were stable and per vaginal bleeding was present. Per speculum examination showed a normal cervix and bleeding from the external cervical os. Per vaginal examination revealed a 6-8

weeks size uterus. Hb was 10% gm and all other investigations were within normal limits. Ultrasonography showed a mass of size 2.0x2.5 cm in the lower uterine segment suggestive of retained products of conception. She was taken up for D&C but she started bleeding profusely and the procedure had to be abandoned because of heavy bleeding. Bleeding stopped within 10 minutes of abandoning the procedure. Post procedure Doppler ultrasonography showed increased vascularity in the RPOC and the surrounding myometrium. She was planned for conservative management and an injection of methotrexate 1mg/kg intramuscular was given. One week after methotrexate injection a repeat Doppler study showed no vascularity in the myometrium and regression of RPOC.

## Discussion

Hyper vascular RPOC's present a clinical challenge because simple dilatation and curettage in such cases may lead to massive haemorrhage necessitating life saving hysterectomy. The risk factor of developing excessive vascularity in RPOC's includes implantation of the embryo in the lower part of the uterus and a history of multiple D&C's (2, 3). Interestingly, in our series, 2 out of the 3 cases gave a history of multiple D&C and two cases had an embryo implanted in a relatively lower part of the uterus. Since the endometrium is thin and decidual formation tends to be insufficient in the lower part of the uterus, embryo implantation in this part leads to excessive trophoblastic invasion thereby causing increased vascularity. Tomoko et al. (4) in 2011 reported two cases of hypervascular RPOC's. The diagnosis of hypervascularity was confirmed on colour Doppler ultrasonography. Vascularity was reduced with uterine artery embolization followed by D&C, while the second case of hypervascular placental polyp was managed conservatively. They concluded that simple D&C in cases of hypervascular RPOC should be deferred until its vascularity is sufficiently reduced. Arora et al. (5) reported three cases of uterine AV malformations with recurrent vaginal bleeding. One case presented with bleeding three weeks after medical termination of pregnancy. Repeat D&C was done with the diagnosis of incomplete abortion which led to massive torrential bleeding for which she was transfused three units of blood. With the suspicion of AV malformation, colour Doppler study was done, which con-

firmed the diagnosis. Joseph et al. (6) reported a case with a heavy intractable bleeding per vaginum, following dilatation and curettage for an incomplete abortion. A provisional clinical diagnosis of gestational trophoblastic disease was made. After radiological investigations this was diagnosed as uterine Arterio-venous malformation. The patient was treated by uterine artery embolization, because she wanted to preserve fertility. In our series of three cases, in the first case emergency life saving hysterectomy had to be done while in the second case, in view of the patient's age, completed family and assumed risk of excessive bleeding, D&C was not attempted and she was taken up for elective hysterectomy. The third patient was managed conservatively with methotrexate injection.

## Conclusion

From our experience and review of literature, we conclude that in all cases of RPOC's, vascularity should be evaluated with colour Doppler prior to attempting dilatation and curettage in order to avoid the complications of massive haemorrhage and hysterectomy.

## Conflict of interest

No conflict of interest was declared by the authors.

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# A new way of setting rFSH deposit: a case of severe injection error in IVF/ICSI cycle ending with live birth

## *Depo rFSH yerleřtirmenin yeni bir yolu: canlı doğumla sonuçlanan IVF/ICSI döngüsünde bir ciddi enjeksiyon hatası olgusu*

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### Abstract

We present a case with a severe injection error: a 25-year old woman with secondary infertility caused by a male factor was enrolled in our IVF/ICSI-ET program. Stimulation was performed in a long- protocol and ovarian stimulation, using rFSH follitropin beta, starting on the third day of the menstrual cycle. The rFSH dose per day was 900 IU-0 IU-0 IU-0 IU. Due to normal ovarian response and follicle growth, stimulation was continued and there was no detriment in oocyte quality and no symptoms of OHSS. Following blastocyte transfer cesarean section was unpreventable at 37+5 weeks of gestation due to an impacted transverse lie. Different stimulation protocols are needed for appropriate treatment of various patients provided that the administration of treatment was done correctly. In the case of injection errors, continuing stimulation protocol seems to be achievable in certain cases considering hormone levels and the process of follicle growth. (J Turkish-German Gynecol Assoc 2012; 13: 215-7)

**Key words:** Assisted reproductive technique, severe injection error, long protocol, rFSH, live birth

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### Özet

Ciddi enjeksiyon hatası olan bir olgu sunuyoruz: Erkek faktörünün yol açtığı ikincil infertilitesi olan 25 yaşındaki bir kadın IVF/ICSI-ET programımıza alındı. Stimulasyon uzun protokol ile gerçekleştirildi ve over stimulasyonu, rFSH follitropin beta kullanılarak, menstrüel döngünün üçüncü günü başlatıldı. Günlük rFSH dozu şöyleydi: 900 IU-0 IU-0 IU-0 IU. Normal over yanıtı ve folikül büyümesi nedeniyle stimulasyona devam edildi, oosit kalitesinde bozulma ve OHSS semptomları yoktu. Blastokist transferini takiben sezaryenle doğum, kalıcı transvers duruş nedeniyle 37+5'inci gebelik haftasında önlenemez durumdaydı. Çeşitli hastaların uygun tedavisi için, tedavi uygulamasının doğru bir şekilde yapılmasını sağlayan, farklı stimulasyon protokollerine gerek duyulmaktadır. Enjeksiyon hataları durumunda, hormon düzeyleri ve folikül büyümesi süreci göz önüne alınarak, stimulasyon protokolünün sürdürülmesi bazı olgularda başarılabılır görünmektedir.

(J Turkish-German Gynecol Assoc 2012; 13: 215-7)

**Anahtar kelimeler:** Yardımlı üreme tekniği, ciddi enjeksiyon hatası, uzun protokol, rFSH, canlı doğum

**Geliş Tarihi:** 18 Kasım 2011

**Kabul Tarihi:** 03 Ocak 2012

### Introduction

Woman suffering fertility problems and their partners are confronted with significant psychosocial consequences. The prevalence of negative emotions, psychological distress and changes in inter-partner relationships have been reported (1). These emotional feelings may worsen during ovarian stimulation for different reasons. One reason seems to be the need of self-injections, the impact on everyday life by the injection regimen and, especially, concerns relating to the correct practical application of gonadotrophins. It seems important to recognise that only 29% of patients actually report an injection error to their physician or nurse (2).

These injection errors need to be analysed individually and can hardly be categorised. However, the outcome of infertility treatment depends on the correct application of gonadotrophin products, which in turn could gain an influence on oocyte quality.

Indeed, this hypothesis is supported by the literature. In detail, influence on the zona score of mature gametes was affected by stimulation details (3). Furthermore, ovarian hyperstimula-

tion syndrome may be determined and high estradiol levels could be one of the causes of smooth endoplasmic reticulum clusters in MII human oocytes, which are associated with lower chances of successful pregnancy (4).

The study was approved by our institutional review board and informed consent of the patient had been obtained.

### Case Report

In 2010, a 25-year old woman with secondary infertility caused by a male factor-OAT was enrolled in our IVF/ICSI-ET program. Sperm analyses in detail were: concentration 2.5 millions/mL, progressive motility 12% (0% fast progressive) normal morphology 1%. She had a history of spontaneous pregnancy and delivery in 2005 with the same male partner. The patient presented with a normal AMH level of 1.67 ng/mL, 75 kg body weight and a BMI of 27. Serum FSH concentration was 5.8 mU/mL measured in precycle.

Stimulation was performed in a long- protocol, undergoing down-regulation with the GnRH-analogue buserelin acetate (Metrelef®, Ferring, Germany, 0.15 mg, 3x2 puffs/day) started

on the 21<sup>st</sup> day of precycle and ovarian stimulation using rFSH follitropin beta (Puregon®, Organon, Netherlands) started on the third day of the menstrual cycle. Hormone levels measured on the second day of the menstrual cycle: estradiol 20.7 pg/mL, progesterone 0.4 ng/mL, LH 2.2 mU/mL. Luteal phase support was via progesterone 400 mg vaginal suppositories. The recommended daily dose of rFSH, started on the third day of menstrual cycle, was 200 IU-200 IU-150 IU-150 IU. Follow-up was planned on the 5<sup>th</sup> day of stimulation measuring the ovarian follicles and estradiol, progesterone and LH hormone levels. The actual rFSH dose per day, injected by the male partner, was 900 IU-0 IU-0 IU-0 IU. On the fifth day of stimulation the five leading follicles reached 12mm in 2D plane, hormone- levels: estradiol 405.0 pg/mL, progesterone 0.6 ng/mL, LH 1.8 mU/mL. rFSH stimulation was continued using 200 IU-150 IU-150 IU. After these three days of stimulation the leading follicle reached 16.5 mm; hormone levels: estradiol 1994.0 pg/mL, progesterone 0.6 ng/mL, LH 3.9 mU/mL. Stimulation was continued for another two days using 150 IU-150 IU of rFSH. Estradiol level reached on the day of β-hCG application-chorionic gonadotrophin alpha (Ovitrelle® 250µg, Merck Serono)-was 3608 pg/mL, progesterone level was 1.1ng/mL, LH level was 2.1 mU/mL and the two leading follicles reached 20mm, measured in 2-D plane. Finally, 13 oocytes were retrieved and a single blastocyst- transfer using a blastocyst of optimal quality of inner cell mass and good quality of trophoctoderm (Vab) was performed; all the rest stopped development. 10 days after embryo transfer, β-hCG reached 248 mU/mL, progesterone was 11 ng/mL and at 6+6 weeks of gestation an intact pregnancy with CRL 6mm was diagnosed. As a result of major relationship problems an appointment for medically induced abortion was made, although psychological support is offered routinely to all patients undergoing an IVF/ ICSI- cycle at our clinic. Although the consent form was already signed, the patient ultimately decided not to perform the abortion. At eleven weeks of gestation the patient suffered a psychological breakdown with suicidal thoughts and was transferred to the General Psychological Hospital in Linz, Upper Austria. After five days of inpatient treatment an adjustment disorder with depressive reaction was diagnosed and the patient could be discharged without medical treatment. At 37+5 weeks of gestation the patient presented with an impacted transverse lie and a cesarean section was performed. The birth weight of the neonate was 3600 g, Apgar score was 8/9/10 and pH was 7.29 and 7.32, respectively.

## Discussion

The application of 900 IU rFSH represents a severe injection error which could only be done by using the injection pen twice and the pen even needs to be reloaded. The application was performed by the male partner of our patient. Both, patient and male partner, had not recognised that the injection pen was blank after the first day of stimulation although they had received an appropriate course of instruction for self-injection. As a consequence, a three-day period without gonadotrophin stimulation ensued. In addition to regular follicle recruitment and usual serum hormone levels, the stimulation protocol was continued.

The injection error presented involves two separate problems, namely OHSS and follicle growth arrest. Because of the relatively short elimination (terminal) half-life (t1/2) of rFSH of about 30-40h, daily injections are needed to keep the serum concentration above the threshold required for follicular recruitment and ongoing maturation. Peak serum FSH levels are reached 10-12 h after application and then decline until the next injection; thus, usually 3- 5 days of treatment are needed to reach FSH steady-state levels during ovarian stimulation (5, 6). Following these pharmacokinetics, FSH serum concentration seemed above the threshold in this case, because of the high peak serum level after application of 900 IU, even considering the short elimination half-life. As a consequence, there was no follicle growth arrest and continuing the stimulation protocol showed a normal follicle growth. Thus, there was no detriment to oocyte quality.

The response to gonadotrophins is highly variable, and a certain proportion of women exhibit an unexpectedly poor response to stimulation. Ovarian response shows a strong correlation with serum anti-Müllerian hormone (AMH) levels (7). Regardless of the antral follicle count, anti-Müllerian hormone seems to be the most significant predictor of poor response in controlled ovarian stimulation (8), which was within normal, age-related range for our patient (9).

Other patients demonstrate an exaggerated response and are at risk of developing an OHSS. Risk factors for the development of OHSS include young age, low body mass index, previous OHSS, high antral follicle count, polycystic ovaries and elevated peak serum estradiol (10, 11). The pathophysiology of OHSS is controversial, but circulating estradiol levels have been suggested to be of predictive value for OHSS, regardless of the actual role

**Table 1. Stimulation protocol including hormone levels, follicle size in 2-D plane and rFSH IU used (follitropin beta, Puregon®, Organon, Netherlands)**

Day of menstrual cycle	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
rFSH (IU)		900	-	-	-	200	150	150	150	150	>
Estradiol (pg/mL)	20.7					405			1994		3608
Progesterone (ng/mL)	0.4					0.6			0.6		1.1
LH (mU/mL)	2.2					1.8			3.9		2.1
Follicle (mm)						12			16.5		20.5
						11			16		20
									16		19

of estradiol in the pathogenesis (12). In our case, the estradiol level reached 3608 pg/mL and no OHSS could be recognised. Specific subgroups of women may even benefit from LH activity supplementation during ovarian stimulation, such as patients older than 35 years and patients with suboptimal IVF outcome and normogonadotrophic patients with 'steady response' (13). As a consequence, different stimulation protocols are needed for appropriate treatment of different patients, provided that administration of treatment was performed correctly. Injection errors seem to occur in about 2.3% of IVF/ICSI cycles. Even if the pregnancy rate following an injection error is only 7% in our own unpublished data, continuing the stimulation protocol seems to be achievable in certain cases.

#### Conflict of interest

No conflict of interest was declared by the authors.

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## ERRATUM

Author names of the article titled *"Iliofemoral-popliteal deep vein thrombosis at 35<sup>th</sup> week of pregnancy: treated with cesarean section and vena cava blockage plus thrombectomy-Gebeliğin 35. haftasında iliofemoral-popliteal derin ven trombozu olan sezaryen seksiyö, vena kava blokajı ve trombektomi ile tedavi edilen olgu sunumu"* which was published in the Journal of the Turkish-German Gynecological Association's 13<sup>th</sup> Volume, 2<sup>nd</sup> issue, on pages 139-141 from the year 2012 were published incorrect due to a technical error and the correct versions are as stated below. We hereby correct this error and apologize from our readers.

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# Metastatic vulvo-vaginal choriocarcinoma mimicking a Bartholin cyst and vulvar hematoma-two unusual presentations

## *Bartolin kistini ve vulvar hematomaı taklit eden metastatik vulvovajinal koryokarsinom-İki olağandışı olgu sunumu*

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### Abstract

Metastatic choriocarcinoma may present solely as a vulvo-vaginal growth. It may pose initial diagnostic dilemmas and thus treatment delay. Two cases of metastatic choriocarcinoma which presented as vulvo-vaginal swelling are described here. Both the cases were initially misdiagnosed. Later, unresponsiveness to treatment alerted us to the possibility of metastatic choriocarcinoma. Combination chemotherapy was started following diagnosis by serum  $\beta$ -HCG titer. In spite of initial responsiveness in both cases, one could not be saved due to poor compliance. Suspicion of metastatic choriocarcinoma should be kept in mind while dealing with any recent onset vulvovaginal swelling following a pregnancy. It may initially mislead the clinician due to its apparent benign appearance.

(J Turkish-German Gynecol Assoc 2012; 13: 218-20)

**Key words:** Choriocarcinoma, metastasis, vulvo-vaginal

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### Özet

Metastatik koryokarsinom yalnızca vulvovajinal tümör olarak görülebilir. Bu durum, ilk tanıda çelişkilere ve dolayısıyla tedavide gecikmeye yol açabilir. Bu çalışmada, vulvovajinal tümör şeklinde prezente olan iki metastatik koryokarsinom vakası tanımlanmıştır. Her iki vaka da başlangıçta yanlış tanı aldı. Daha sonra, tedaviye cevap alınmaması bizi metastatik koryokarsinom ihtimaline karşı alarma geçirdi. Hastanın serum  $\beta$ -HCG değerleri ölçülüp tanısı konduktan sonra kombine kemoterapi uygulanmaya başlandı. İki vakada da başlangıçta tedaviye cevap alınmamasına rağmen, bir tanesi düşük uyumdan dolayı kurtarılmadı. Hamilelik sonrası yakın zamanda vulvovajinal şişme görülmesi durumunda metastatik koryokarsinom şüphesi akılda tutulmalıdır. Bariz benign görünümünden dolayı başlangıçta hekimi yanlış yönlendirebilir. (J Turkish-German Gynecol Assoc 2012; 13: 218-20)

**Anahtar kelimeler:** Koryokarsinom, metastaz, vulvovajinal

**Geliş Tarihi:** 21 Eylül 2011

**Kabul Tarihi:** 29 Ekim 2011

### Introduction

Choriocarcinoma is a highly vascular tumour of the trophoblast with immense metastatic potential to the lung, liver, brain or vulva. Next to the lung, vulvo-vaginal metastasis comprises 30% of all metastatic incidences (1).

Metastasis in this region is often misleading in its initial appearance. Sometimes antecedent pregnancy remains uninformative. Recent onset vulvo-vaginal swelling may be the sole clinical presentation (1). This may confuse the clinician. Here we present two cases of vulvo-vaginal metastasis of choriocarcinoma which were misdiagnosed initially. We present these two cases to remind clinicians about its entity, diagnostic difficulties and treatment options.

### Case Reports

#### Case 1

Mrs. A.K., 22 years old, attended out patient department (OPD) with a vulvo-vaginal swelling measuring about 7x5 cm<sup>2</sup>. She had a history of uncomplicated home delivery two

months previously. The swelling was angry looking, bluish red in color along with infected crusts and oozing surfaces (Figure 1). It was tender and firm. Treatment was initiated on the line of an old infected vulvar hematoma with local wound care, antibiotics and blood transfusion. Despite this, the swelling gradually increased in size. A suspicion of choriocarcinoma arose. Serum  $\beta$ -hCG titer was measured. The value was 1.2x10<sup>5</sup> I.U. /mL, which confirmed the diagnosis. Combination chemotherapy with MAC (Methotrexate, Actinomycin and Cyclophosphamide) regimen was started. There was 50% shrinkage of the mass following the first course. Serum  $\beta$ -hCG titer had been reduced to 60.200 I.U. /mL. She left the hospital after the first course despite repeated counseling and did not come back for follow up. She was admitted three weeks later with massive vaginal bleeding and a very poor general condition. She developed convulsions and died on the next day in spite of aggressive supportive measures.

#### Case 2

Mrs. P.M. a 25 year-old primipara attended OPD with a swelling over the left vulva measuring 4x3 cm<sup>2</sup> which she

had noticed two months previously. She had a history of full term vaginal delivery three and half years earlier. The swelling was tense, tender, cystic and infected looking. It was provisionally diagnosed as an infected Bartholin cyst and surgical intervention was planned. We encountered unexpected, profuse, uncontrollable hemorrhage during the procedure. However, the bleeding was managed at that time by deep mattress sutures but it recurred after two weeks. On repeated enquiry, she then revealed a history of Medical termination of pregnancy (MTP) at 8 weeks gestation performed surgically four months earlier. A suspicion of choriocarcinoma arose and her serum  $\beta$ -hCG titer was estimated. It had a value of  $1.5 \times 10^5$  I.U./ml. Other relevant investigations were carried out, however they revealed no other distant metastasis except a sonological finding of a bulky uterus. The first course of chemotherapy was started with EMACO (Etoposide, Methotrexate, Actinomycin, Cyclophosphamide and Oncovin) regimen. The mass shrunk in size but vaginal bleeding was still continuing from the internal os. We planned a laparotomy due to persistence of bleeding. Laparotomy findings revealed a bulky uterus of approximate 10 weeks size along with gross adhesions to surrounding structures. Careful bilateral internal iliac artery ligation was performed. A second course of chemotherapy was started at a later date. Her  $\beta$ -hCG titer declined gradually, with improvement of general condition. She received 6 courses of combination chemotherapy and recovered completely. She is now on follow up and doing well.

## Discussion

These two similar cases are presented because of their initial diagnostic dilemmas and subsequent management protocol. Vulvo-vaginal metastasis of trophoblastic tumour may occur even after first trimester loss. Gary L Goldberg in omit similar study showed that, out of five cases of vulvo-vaginal metastasis, two developed following spontaneous abortion (2).



**Figure 1.** Metastatic vulvovaginal choriocarcinoma mimicking an old infected vulvar hematoma

In present study, the first case was treated conservatively for a week as an old infected vulvar hematoma without any improvement. Initially its malignant nature was not considered. Perhaps, with an earlier appropriate intervention, we might have gained the confidence of her relatives and could have saved her life. The second case was erroneously diagnosed as infected Bartholin cyst which bled profusely during surgical intervention. As these sites which the tumor itself are hypervascular in nature, any local surgical intervention may precipitate life threatening hemorrhage. Here the question arises whether to perform biopsy or is it needed at all for confirmation of the diagnosis. Essentially, diagnosis of metastatic choriocarcinoma is based on history, clinical presentation and allied elevated serum  $\beta$ -HCG titer. Biopsy from the local metastatic sites is not mandatory for the diagnosis. Song et al in their study also supported this view (1).

Chemotherapy is the treatment of choice with a favorable prognosis (3). Regarding prognostic scoring, vulvo-vaginal metastasis should be considered as a poor prognostic factor. Different studies in this context thus directly recommended combination chemotherapy as their first choice (2, 3). The problem lies in the interim period regarding control of bleeding from these hypervascular sites. Options like intravaginal packing; hemostatic, deep, figure-of-eight mattress suturing or more extensive surgical interventions like hysterectomy along with ligation of feeding vessels had been advocated by different investigators (1, 2). The method of intravaginal packing and its subsequent removal needs special mention. Packing should be initiated cautiously with soaked roller gauze under direct vision after covering the oozing nodules with separate soaked gauze-pieces. Packing may be offered also following hemostatic suturing. Abrasions, during changing of pack, may incite fresh bleeding. So, placing the fingers first between the packing and covering gauze and subsequently proceeding to pack-removal might prevent such a complication.

Even both procedures might not be able to control bleeding, especially where there is pelvic involvement, as in the second case, or in cases of large, friable metastatic foci. More extensive procedures such as abdominal hysterectomy with simultaneous ligation of ovarian and internal iliac artery may be needed in these circumstances. However, radiological embolisation of feeding vessels can be an effective alternative in these circumstances and if opted, can avoid the need for complicated surgical procedures (1, 2).

Follow-up should be carried out as usual with the level of serum  $\beta$ -hCG titer along with clinical correlation. Prognosis is usually good provided interim bleeding episodes are tackled efficiently and long term follow up is accepted.

## Conclusion

While dealing with a case of vulvo-vaginal swelling with a history of antecedent pregnancy, one should always remember the remote possibility of a metastatic choriocarcinoma and investigate accordingly. Prompt diagnosis and early treatment with combination chemotherapy may thus save many lives by keeping its probability in mind.

**Conflict of interest**

No conflict of interest was declared by the authors.

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## What is your diagnosis?

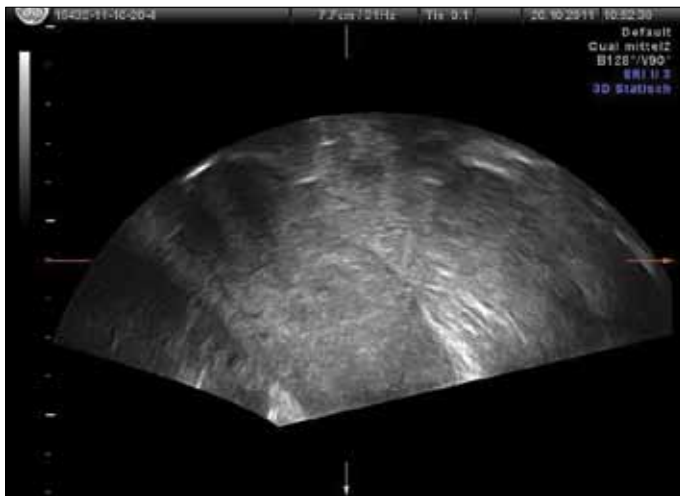


Figure 1. Sagittal transvaginal two dimensional sonography, showing a diffusely enlarged cervix with heterogeneous echogenicity. The tumor margins are clearly delineated

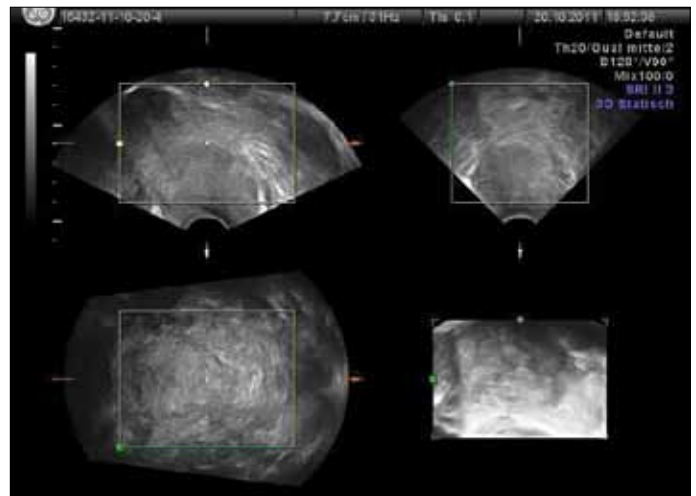


Figure 2. Three-dimensional sonography of barrel shaped cervical cancer in multiplanar sections

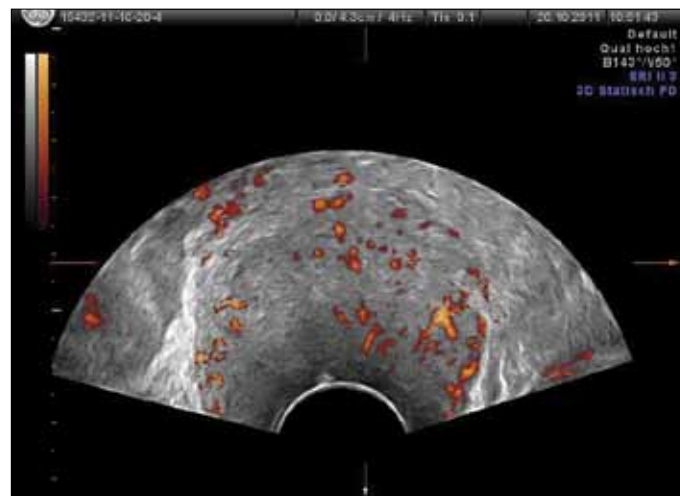


Figure 3. Three-dimensional power Doppler vascularisation of cervical cancer

Cancer of the cervix is the second most common cancer in women worldwide, with about 500 000 new cases and 250 000 deaths each. Among these case 80% occur in low-income countries. Cervical cancer cases (99%) are linked to genital infection with human papillomavirus, which is the most common viral infection of the reproductive tract.

Ultrasound has been used to evaluate the size and locoregional extent of the tumor. In the early stage of cervical carcinoma, the primary lesion is difficult to depict with any imaging modality, including transvaginal US. With disease progression, the barrel shaped bulky cervical cancer can appear as a hypoechoic lesion with well defined margins, or the disease may manifest as an enlarged cervix with heterogeneous echogenicity (see the images).

The prognosis for patients with cervical cancer is markedly affected by the extent of disease at the time of diagnosis. The majority (>90%) of these cases can and should be detected early through the use of the Pap test and human papillomavirus (HPV) testing. The current death rate is far higher than it should be, which shows that, even today, the Pap test and HPV testing are not done on approximately 33% of eligible women (1). The clinical stage, however, as a prognostic factor must be supplemented by several gross and microscopic pathologic findings in surgically treated patients. These include: volume and grade of tumor, histologic type, lymphatic spread, and vascular invasion. In a large surgicopathologic staging study of patients with clinical stage IB disease reported by the Gynecologic Oncology Group, the factors that predicted most prominently for lymph node metastases and a decrease in disease-free survival were capillary-lymphatic space involvement by tumor, increasing tumor size, and increasing depth of stromal invasion, with the latter being most important and reproducible (2, 3). In a study of 1.028 patients treated with radical surgery, survival rates correlated more consistently with tumor volume (as determined by precise volumetry of the tumor) than clinical or histologic stage (4). The preoperative diagnosis of tumor geography may play an important role in management of cervical cancer. Trimboş et al. (5) evaluated the prognostic significance of tumor geography, defined as

exophytic or barrel-shaped growth, in bulky (>4 cm) cervical cancer. For this purpose, they evaluated four hundred women with cervical cancer, treated by primary radical hysterectomy. In 58 patients, the tumor was defined as bulky exophytic and in 51 patients as bulky barrel shaped. There were no differences among these groups in terms of operating time, blood loss during surgery or complications at 3 or 6 months postoperatively. Bulky exophytic tumors had an identical overall survival as compared to small-diameter (<4 cm) tumors. The overall survival of bulky barrel-shaped tumors was significantly worse. The same was found for disease-free survival.

As the tumor geography may predict the outcome, preoperative ultrasound should be performed in all patients with suspected cervical cancer.

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# JTGGA CME/CPD CREDITING



## Questions on the article within the scope of CME/CPD

1. Which of the below does not increase the risk of diminished ovarian reserve?
  - a) Smoking
  - b) Pelvic surgery history
  - c) Severe endometriosis
  - d) Alcohol
  - e) Family history of premature menopause
2. Which of the statement is wrong?
  - a) Menstrual cycles become irregular about 6 years before menopause
  - b) At a mean age of 37-38 years only about 25 thousands of follicles are present in the ovaries
  - c) Primordial follicle number reaches its maximum level at birth.
  - d) In 1% of women age of menopause is under 40 years
  - e) Ovarian reserve test should be performed to all infertile women over 30 years
3. In which of the following ovarian reserve tests the results are independent from the day of measurement?
  - a) FSH
  - b) AMH
  - c) Estradiol
  - d) Inhibin B
  - e) Antral follicle count
4. Which of the following ovarian reserve tests accurately predict live birth?
  - a) Antral follicle count
  - b) Basal FSH
  - c) AMH
  - d) Clomiphene citrate challenge test
  - e) None
5. Which of the following statement is not true for ovarian reserve tests?
  - a) Inhibin-B is a dimeric peptide that is secreted by granulosa cells of preantral and early antral follicles
  - b) Performance of the combination of ovarian reserve tests to predict ovarian response in IVF is reported better compared with AFC alone
  - c) In IVF, CC test is relatively less sensitive than basal FSH in poor response prediction
  - d) The most reliable predictor of poor ovarian response to gonadotropins among sonographic markers is AFC
  - e) FSH has a low sensitivity but high specificity to predict poor ovarian response
6. What is the minimal amount of increase in estradiol levels after administration of 300 IU recombinant FSH in Exogenous FSH ovarian reserve test (EFORT) to interpret the result as normal?
  - a) 10 pg / mL
  - b) 20 pg / mL
  - c) 30 pg / mL
  - d) 50 pg / mL
  - e) 100 pg / mL

# JTGGA CME/CPD CREDITING



## Answer form for the articles within the scope of CME/CPD

1<sup>st</sup> Question

A	B	C	D	E
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4<sup>th</sup> Question

A	B	C	D	E
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2<sup>nd</sup> Question

A	B	C	D	E
---	---	---	---	---

5<sup>th</sup> Question

A	B	C	D	E
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3<sup>rd</sup> Question

A	B	C	D	E
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6<sup>th</sup> Question

A	B	C	D	E
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People who answer these questions will receive "2 TMA-CME/CPD credits"

## TMA-CME CREDITING BOARD ENQUIRY FORM

JTGGA MANUSCRIPT 2012/3

DATE

TR Identification Number

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*This form will not be reviewed if TR Identification Number is not stated.*

Name

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Surname

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Signature

..... The City You Work In .....

Your Institution

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**IMPORTANT NOTE:** You may apply for Turkish Medical Association CME/CPD credits by answering the questions in the front page, filling in your personal information and sending this form to "Abdi İpekçi Cad. No: 2/7 34367 Nişantaşı, İstanbul" by post. This form should arrive to the above-mentioned address latest by November 30<sup>th</sup>, 2012.

## CONGRESS CALENDAR

- 13-15 September 2012 **NESA Palmaplanas Innovation Days 2012**  
Palma de Mallorca, Spain  
[www.uspnesadays.com](http://www.uspnesadays.com)
- 13-15 September 2012 **AGOS (American Gynecological & Obstetrical Society) Annual Meeting**  
Washington (Ritz Carlton), DC USA  
[www.agosonline.org](http://www.agosonline.org)
- 26 -30 September 2012 **8<sup>th</sup> Obstetrics and Gynecologic Ultrasonography Congress and Ian Donald Advanced Ultrasound Course**  
Lykia World Oludeniz Hotel-Antalya  
[www.usgkongre2012.org](http://www.usgkongre2012.org)
- 27-30 September 2012 **5.TSRM**  
Sheraton Çeşme Hotel-İzmir  
[www.2012.tsrn.org.tr](http://www.2012.tsrn.org.tr)
- 3-6 October 2012 **AUGS (American Urogynecologic Society) Meeting**  
Chicago, IL, USA  
[www.augs.org](http://www.augs.org)
- 7-12 October 2012 **XX FIGO World Congress**  
Rome, Italy  
[www.figo.org](http://www.figo.org)
- 11-14 October 2012 **The 8<sup>th</sup> National Congress of Turkey Maternal Fetal Medicine and Perinatology Association**  
İstanbul, Turkey  
[www.tmfptkongre2012.org](http://www.tmfptkongre2012.org)
- 8-12 November 2012 **XVII. COGI (Controversies in Obstetrics, Gynecology & Infertility)**  
Lisbon, Portugal  
[www.congressmed.com/cogilisbon/](http://www.congressmed.com/cogilisbon/)
- 9-11 November **2<sup>nd</sup> Asian Conference on Endometriosis**  
İstanbul, Turkey  
[www.ace-2012.org](http://www.ace-2012.org)
- 29 Nov-1 Dec 2012 **Update on Cervical Cancer**  
Las Vegas, NV USA  
[www.acog.org](http://www.acog.org)
- 6-7 December 2012 **4<sup>th</sup> Annual Workshop on Video Assisted and Robotic Laparoscopy and Hysterectomy with Comprehensive Hands-on Laparoscopic Suturing**  
The Roosevelt Hotel, New York, NY USA  
[www.aagl.org](http://www.aagl.org)
- 12-15 December 2012 **Pelvic Anatomy and Gynecologic Surgery Symposium**  
Las Vegas, NV USA  
[www.pags-cme.org/](http://www.pags-cme.org/)