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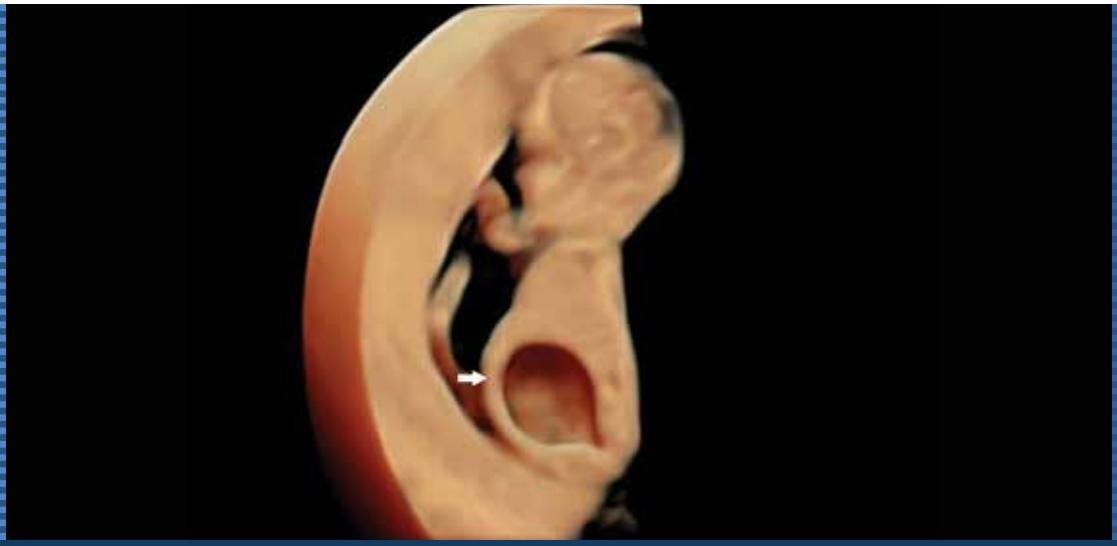

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Cover Picture: HDlive rendering in a fetus at 13 weeks 1 day of gestation showing a megacyst (white arrow) due to lower urinary tract obstruction. *Tonni et al. (Issue: 2, Page: 117)*

Original Investigations

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Journal of the Turkish-German Gynecological Association is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and Turkish-German Gynecological Association and is published quarterly on March, June, September and December.

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 reviewed in accordance with "double-blind peer review" process for both referees and authors.

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STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al, for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/),

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Contents

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Editorial

Dear Colleagues,

It is my great pleasure to present you September 2016 issue of the Journal of the Turkish German Gynecological Association (J Turk Ger Gynecol Assoc).

In this issue, you will find an opportunity to read many high quality manuscripts. One of them is an interesting research article investigating the relationship between the development of intrapartum fetal distress and serum pregnancy-associated plasma protein-A (PAPP-A) levels measured during first-trimester aneuploidy screening tests. You will also read an attractive paper determining predictive values of maternal serum PAPP-A levels, uterine artery Doppler velocimetry, and fetal biometric measurements (FBMs) for poor pregnancy and poor neonatal outcomes.

Substantial controversy exists regarding anesthetic management for patients with preeclampsia or hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome. The choice of the anesthetic method is largely dictated by the composite condition of patients with preeclampsia, their platelet counts, and their coagulation statuses. In one article, with a focus on these issues, a cohort of anesthesiologists, including residents and clinicians, was surveyed to solicit their opinions and practice patterns.

Herein is presented another article determining the optimal cone size to achieve a reliable sensitivity and specificity for clear surgical margins after cold knife conization (CKC). You will also find a review evaluating the current methods of endometrial preparation in frozen-thawed embryo transfer cycles. As usual, we have included a quiz too, which we are sure that you will enjoy solving.

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Best regards,

Prof. Cihat Ünlü, M.D.
Editor in Chief of *J Turk Ger Gynecol Assoc*
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The next generation IUD introduced in Turkey

The intrauterine device (IUD) is the main method of contraception worldwide, including Turkey, according to a cross-sectional study among 6,506 women in 15-49 age group study conducted by Pala et al published in 2008 (1). This study also reported a high incidence of anemia in copper IUD users.

Conventional framed T-shaped devices (TCu380A, Mirena; Bayer HealthCare Pharmaceuticals Inc.; NJ, USA), given their size limitations (32 mm width), whether used in parous or nulliparous women, have several disadvantages as they have to fit the uterine cavity of all women regardless of shape or size in order to be well tolerated. However, a growing body of evidence exists confirming that the uterine cavity size varies significantly amongst women and most devices of singular dimensions do not fit all uterine cavities. Studies of the uterine cavity show that uterine cavities differ from woman to woman ranging from <10 mm to >40 mm. Discrepancy between the IUD and the uterine cavity can often result in partial or total expulsion due to severe uterine contractions. If not expelled, pain, cramping and abnormal bleeding are common complaints caused by malposition, displacement or embedment of the IUD. Malposition and displacement of an IUD, resulting in embedment and/or perforation are being reported more frequently as a consequence of increasing use of 3D sonographic or hysteroscopic methods in the routine clinical setting.

Recently, a frameless copper IUD (GyneFix®) was introduced in Turkey. The IUD consists of a number of copper cylinders attached to a surgical nonabsorbable polypropylene suture thread with a preformed surgical knot on top which, upon contact with the fundus is inserted into the fundus of the uterus. The depth of the insertion is controlled by the specially designed inserter (Figure 1). As the IUD is anchored in the fundus, it cannot be expelled as a consequence of uterine contractions, which can at times be quite severe especially during menstruation. Removal has been found to be easy and with minimal patient discomfort. Return to fertility as with most copper IUDs is almost immediate. Clinical trials conducted in Germany and other regions in Europe have demonstrated a high acceptance and continuation rates by women. Furthermore, due to its small size and lack of transverse arms, the amount of menstrual bleeding that occurs, unlike that seen with framed copper devices such as TCu380A, remains unchanged. The device is only 2 cm long and 2.2 mm in width and uses thick-walled copper cylinders capable of releasing copper from both the internal and external surfaces. As the frameless IUD design lacks a plastic frame and utilizes multiple individual copper cylinders it is completely flexible in utero, resulting in high tolerance and continuations rates reported in clinical studies (>90% over 5 years). By comparison the continuation rates for TCu380A, published in the literature,

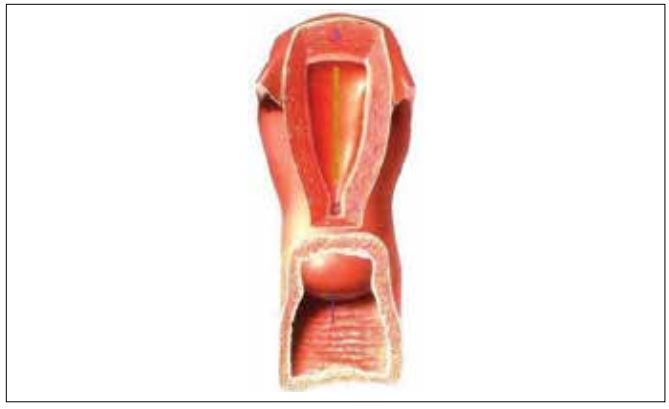

Figure 1. Illustration of the frameless IUD in a uterine cavity

are in the order of 40 to 50% at 5 years. In addition, multicenter efficacy studies confirm equal or slightly higher efficacy compared with the TCu380A IUD in randomized clinical trials (2). One consequence of the anchored design is that it affords release of copper high up in the uterine cavity in the vicinity of the tubal ostia which serves to maximize effectiveness and limit ectopic pregnancies to almost zero, as confirmed in large clinical trials. The lifespan of the different device types can vary between 3 and 10 years. These devices are CE-marked and some of them are currently available for use in Turkey.

Given its novel anchoring and insertion methodology, and the lack of familiarity of most physicians and surgeons with the intrauterine implant (IUI), the insertion technique requires some degree of formalized training. Doctors, even experienced with the conventional IUDs, need to become familiar with the insertion technique. Proper positioning of the anchor in the uterine fundus is required to guarantee optimal performance. But, as the technique is straightforward, proficiency is easily acquired, as I experienced myself. An ultrasound check following insertion shows of the anchor has been properly placed in the fundal myometrium.

Assoc. Prof. Gazi Yıldırım, M.D.

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References

1. Pala K, Dundar N. Prevalence & risk factors of anaemia among women of reproductive age in Bursa, Turkey. Indian J Med Res 2008; 128: 282-6.
2. Wildemeersch D, Jandi S, Pett A, Nolte K, Hasskamp T, Vrijens M. Use of frameless intrauterine devices and systems in young nulliparous and adolescent women: results of a multicenter study. Int J Womens Health 2014; 6: 727-34. [Crossref]

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Uterine cavities are much smaller than what most gynecologists think

In the selection of an appropriate intrauterine device (IUD) little consideration is placed on adequacy of fit. Properly fitting IUDs will likely lead to less adverse effects or patient discomfort resulting in enhanced continuation of use.

We report on a multicenter study conducted at 3 centers Germany and Switzerland in 152 nulliparous women, measuring the maximal width of the uterine cavity using 3D ultrasound.

Measurements were performed by experienced sonographists. The mean width of the uterine cavity in the fundus was 21.6 mm (range 6.0 - 40.0 mm). The median value was 22.0 mm and the interquartile rang (IQR) 18.0 - 24.8 mm, respectively. Eighty-two % of women had a uterine cavity width between 15 mm and 28 mm, 40% <20 mm and 6.6% <15 mm, respectively (Table 1, Figure 1).

Uterine cavities in nulliparous women are narrow and rarely wide enough to fit conventional IUDs. Gross discrepancy between the IUD and the uterine cavity leads to side effects (e.g., expulsion, embedment, bleeding, pain) and early discontinuation. Historically, devices too large for the uterine cavity have been routinely inserted which may account for their 5-year continuation rates being only 40 to 50%. The study suggests that 3D sonography is a precise method to measure the width of the uterine cavity (although 2D may also be suitable) and may result in the selection of a suitable IUD to maximize continuation of use. Measurement of the cavity width is not necessary with a frameless IUD.

Dr. Dirk Wildemeersch, M.D., PhD
Gynecological Outpatient Clinic and IUD Training Center, Ghent, Belgium

Table 1. Maximal fundal width (FUD) in 152 nulliparous women as measured by 3D office sonography

FUD (mm)	3D
N	152
Mean	21.6
SD	5.1
Median	22.0
IQR	18.0 - 24.8
Range	6.0 - 40.0

FUD: maximal fundal width; N: number; SD: standard deviation; IQR: interquartile rang; 3D: three-dimensional

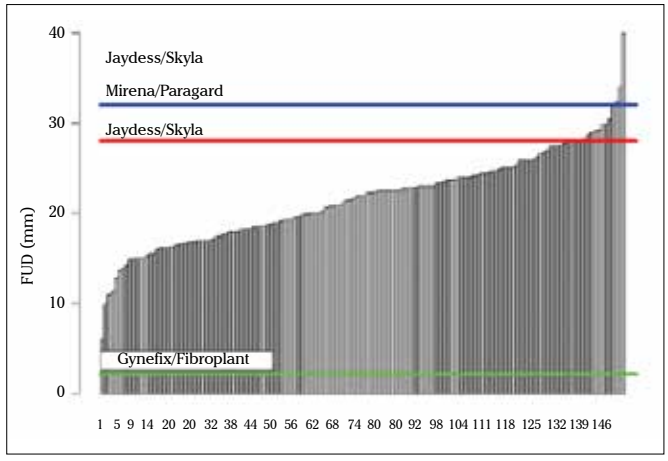

Figure 1. Collated individual maximal fundal widths by 3D sonography in 152 nulliparous women seeking IUD insertion or replacement. For comparison the transverse width for Mirena/Paragard (TCu380A), 32 mm, Jaydess/Skyla, 28 mm, and the frameless GyneFix (copper)/Fibroplant (LNG), 2.2 mm, are included.

References

1. Nolte K, Jandi S. Julen O, Hasskamp T, Wildemeersch D. A multicenter study assessing uterine cavity width in over 150 nulliparous women with IUD or seeking IUD insertion using 3D sonography. Clin Obstet Gynecol Reprod Med 2016; 2: 193-8. [Crossref]

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Anesthetic practices for patients with preeclampsia or HELLP syndrome: A survey

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Abstract

Objective: Substantial controversy exists regarding anesthetic management for patients with preeclampsia or hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome. Experts, researchers, clinicians, and residents in Turkey were surveyed about their practices.

Material and Methods: Questionnaires were distributed to attendees at a national conference, and they were filled out immediately. Anonymous 10-item paper surveys were administered to both residents and non-residents. Descriptive statistics were used in the analysis. Agreement among ≥75% of the respondents was considered a majority opinion. Surveys with missing responses were used to analyze the non-response bias. The Chi-square test was used for comparisons. A historical cohort of obstetricians–gynecologists was used for comparison with anesthesiologists.

Results: Of 339 surveys distributed, 288 were returned (84.9% response rate). Among the returned surveys, the completion rate was 96.1%. The job experience in years among clinicians and residents was 9±5 and 3±1, respectively. General anesthesia was still significantly preferred by 36.1% among patients with preeclampsia with platelet counts of ≥100,000/μL. Compared to obstetricians–gynecologists, anesthesiologists more often preferred general anesthesia. With platelet counts of <50,000/μL or eclampsia, most respondents preferred general anesthesia 94.4% for very low platelets and 89.5% for eclampsia.

Conclusion: A preferential trend toward general anesthesia for patients with preeclampsia or HELLP syndrome exists among anesthesiologists in Turkey, particularly for patients with severe thrombocytopenia and/or eclampsia. There exists a need for well-designed and well-executed prospective clinical trials to provide evidence for the best consensus practice. (J Turk Ger Gynecol Assoc 2016; 17: 128-33)

Keywords: Anesthesia, platelet count, glucocorticoids, HELLP syndrome, preeclampsia, survey

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Introduction

Severe preeclampsia and hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome (hemolysis, elevated liver enzymes, low platelet count) are life-threatening complications during pregnancy (1). Complications and underlying etiopathogenesis of both preeclampsia and HELLP syndrome are globally associated with severe maternal morbidity and mortality. The two syndromes often involve multiple organ systems, including the mother's vasculature and her liver, lungs, kidney, and brain (1). Hence, concerted efforts to enhance the management of these disorders are vital to reduce, to the maximum extent possible, both maternal and fetal morbidity and mortality.

Despite significant advances in our understanding of both preeclampsia and HELLP syndrome, numerous unknowns remain and several controversial issues persist regarding etiology, diagnosis, and best practice management. The literature concerning the two syndromes is extensive but often conflictive. As part of the clinical paradigm, safe and evidence-based anesthetic management of at-risk patients in the peripartum period is of critical importance. When high-grade evidence is lacking, controversy exists. The choice of the anesthetic method is largely dictated by the composite condition of patients with preeclampsia, their platelet counts, and their coagulation statuses. With a focus on these issues, a cohort of anesthesiologists, including residents and clinicians, was surveyed to solicit their opinions and practice patterns.

Material and Methods

A 10-item paper survey was developed to query potentially controversial practices in the anesthetic management of patients with preeclampsia and/or HELLP syndrome. No respondents were compensated for this survey, consisting of single answer multiple-choice questions. Beyond the resident and practice experience questions, additional information about respondents was not requested and surveys were not paired with the clinician or resident, so that survey responses remained anonymous.

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The questionnaires were distributed to attendees at the Turkish Anesthesiology and Reanimation Association 2014 Annual meeting and were filled out immediately. Returned surveys were included in the analysis if at least 90% of the questions were answered. Surveys with missing responses were used to analyze items for non-response bias. To test for bias in non-responses, we compared survey answers between those who completed all the questions with those who did not. An agreement above 75% or more was considered a majority opinion.

Descriptive statistics were used in summarizing the data. The Chi-square test with post hoc analysis was used for comparisons. Two-tailed p values of 0.05 or less were considered as non-chance differences. Responses to the first three questions in the survey were compared to prior responses obtained from obstetricians-gynecologists with international origin during a prior survey-whereby they functioned as a historical control group (2). In order to compare responses between the two professional groups, a weighting was applied according to the overall response rate to both of the surveys, respectively. In order to determine the internal consistency of the survey, answers to questions 1, 7, and questions 2, 5, and 8 were used to compute reliability coefficients. A Cronbach's alpha of >0.7 was considered acceptable (3). The survey appeared to have good internal consistency ($\alpha1=0.805$, $\alpha2=0.875$, respectively). Data analysis and management was performed using IBM SPSS® 21 (IBM Corp.; Armonk, NY, USA).

Results

Of 339 surveys distributed, 288 were returned, for an overall response of 84.9%. Among the returned surveys, the completion rate was 96.1%. In all of the surveys, more than 90% of the questions were answered. The non-response analysis did not indicate differences between those respondents who provided complete data and those with incomplete data. The job experience in years among clinicians and residents was 9±5 and 3±1, respectively. Comparison between the residents and clinicians indicated only limited differences in the responses to questions 1 and 6. Table 1 presents the responses to the survey questions 1 to 8.

When analyzed according to decreasing levels of platelet count in questions 1 to 4, there was a significant inverse relation between the platelet count and the utilization of general anesthesia ($p<0.01$). When compared to obstetricians–gynecologists working with a platelet count of >100,000/μL, anesthesiologists more often preferred general anesthesia ($p<0.001$) (Figure 1). In addition, when the platelet count ranged between 50,000 and 100,000/μL, anesthesiologists preferred general anesthesia more often than the obstetric providers ($p<0.001$) (Figure 2). Below a platelet count of 50,000/μL with severe thrombocytopenia, the responses between anesthesiologists and obstetric providers were similar (Figure 3).

For the last two survey questions (questions 9 and 10), we queried the future use of corticosteroid administration and ultrasound-guided regional anesthetic methods for patients complicated with se-

Table 1. Responses to survey questions 1–8

Questions	General anesthesia	Epidural anesthesia	Spinal anesthesia
Q1: For a patient with severe preeclampsia or HELLP syndrome with a platelet count above 100,000/μL and normal INR, which type of anesthesia do you prefer?	36.11%	10.07%	53.82%
Q2: For a patient with severe preeclampsia or HELLP syndrome with a platelet count between 50,000 and 100,000/μL and normal INR, which type of anesthesia do you prefer?	61.11%	5.9%	32.99%
Q3: For a patient with severe preeclampsia or HELLP syndrome with a platelet count between 25,000 and 50,000/μL and normal INR, which type of anesthesia do you prefer?	94.44%	0.35%	5.21%
Q4: For a patient with severe preeclampsia or HELLP syndrome with a platelet count less than 25,000/μL and normal INR, which type of anesthesia do you prefer?	97.92%	0.35%	1.74%
Q5: A patient with severe preeclampsia or HELLP syndrome with a platelet count below 50,000/μL was transfused with a thrombocyte suspension, and a platelet count above 50,000/μL was attained. The INR was normal. In this patient, which type of anesthesia do you prefer?	67.71%	4.17%	28.13%
Q6: In a patient with eclampsia, which type of anesthesia do you prefer?	89.58%	2.08%	8.33%
Q7: Corticosteroid administration may increase the platelet counts in a patient with preeclampsia or HELLP syndrome. After corticosteroid treatment, a platelet count between 100,000 and 150,000/μL was attained. The INR was normal. In this patient, which type of anesthesia do you prefer?	40.97%	8.33%	50.69%
Q8: Corticosteroid administration may increase the platelet counts in a patient with preeclampsia or HELLP syndrome. After corticosteroid treatment, a platelet count between 50,000 and 100,000/μL was attained. The INR was normal. In this patient, which type of anesthesia do you prefer?	68.06%	4.51%	27.43%

INR: international normalized ratio; HELLP: hemolysis, elevated liver enzymes, low platelets

vere preeclampsia and HELLP syndrome. Half of the respondents agreed that corticosteroid administration via increasing platelet counts might increase the utilization of regional methods (Figure 4). Approximately half of the respondents thought that ultrasound-assisted regional anesthesia methods might decrease complication rates and might increase the procedural safety in this subset of patients (Figure 5).

Discussion

Our understanding of preeclampsia/HELLP syndrome has continued to significantly increase during recent decades. However, the two entities are still classified as a syndrome and not as a single disease entity. A syndrome, by definition, is any combination of signs and symptoms that is indicative of a process. Accordingly, the cause of preeclampsia/HELLP syndrome remains elusive (4). When faced with this dilemma in a clinical setting, clinicians try to improve the outcome for both the mother and

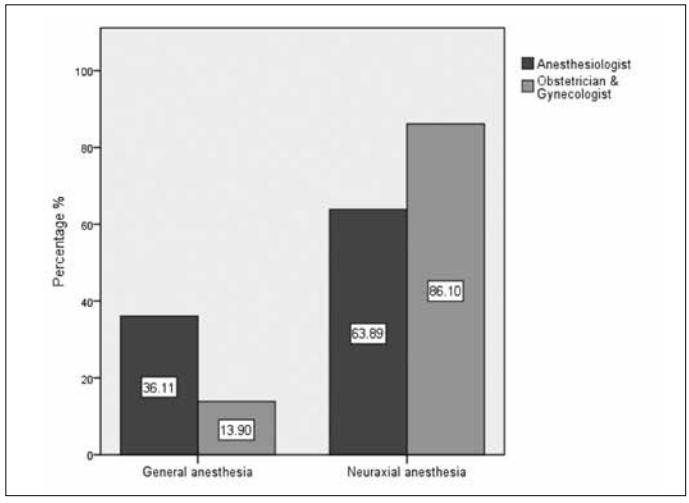

Figure 1. Preference in the type of anesthesia according to anesthesiologists and obstetricians–gynecologists for a platelet count above 100,000/μL

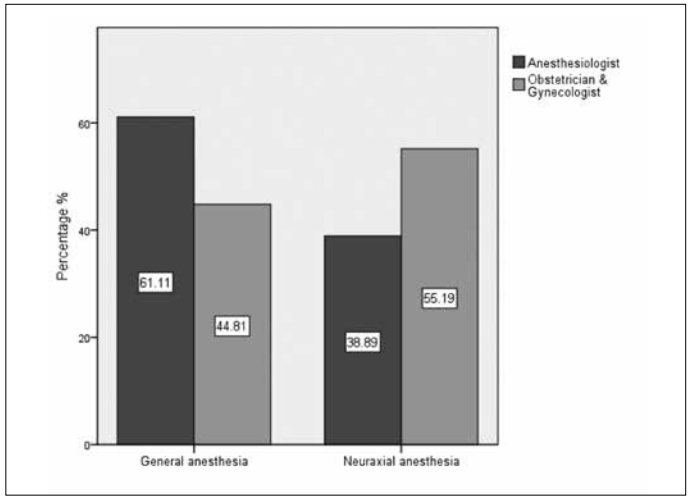

Figure 2. Preference in the type of anesthesia according to anesthesiologists and obstetricians–gynecologists for a platelet count between 50,000 and 100,000/μL

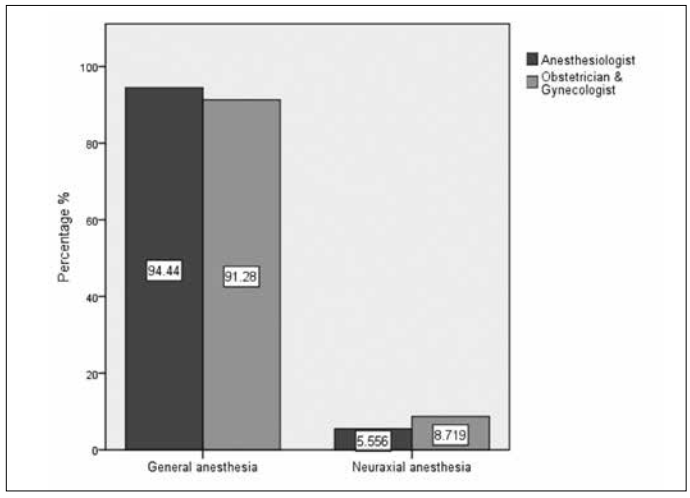

Figure 3. Preference in the type of anesthesia according to anesthesiologists and obstetricians–gynecologists for a platelet count below 100,000/μL

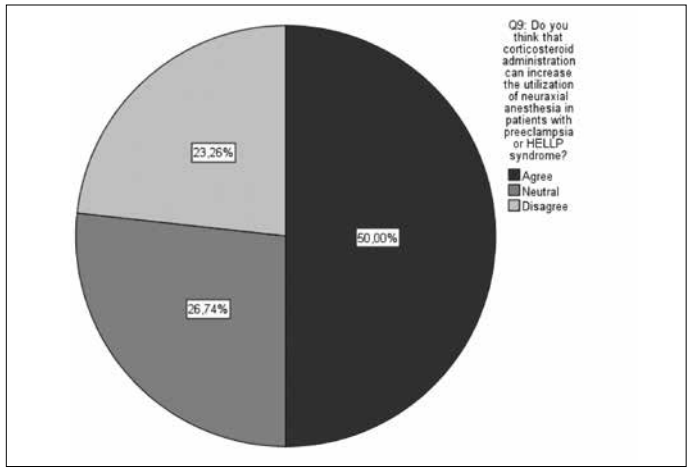

Figure 4. Thoughts on the potential of corticosteroid administration to increase the utilization of neuraxial anesthesia

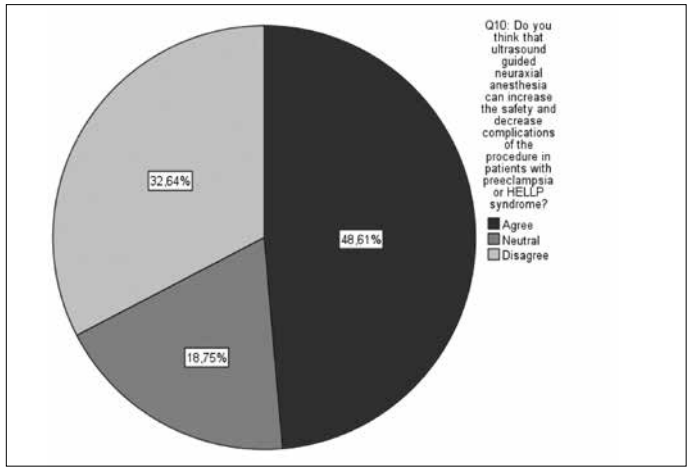

Figure 5. Thoughts on the potential of ultrasound-guided neuraxial anesthesia to decrease complications and increase the safety of the procedure

baby. One excellent intervention for severe thrombocytopenia in the setting of HELLP syndrome has been high dose corticosteroids around the time of delivery. The peripartum period is a critical time, during which there are important opportunities to improve maternal-perinatal outcomes. Anesthetic management is no exception. Rapid resolution of preeclampsia and HELLP syndrome without mortality and with as little severe maternal morbidity as possible is thus highly desirable.

Either general or neuraxial methods may have some disadvantages in patients with preeclampsia. Due to upper airway edema, exacerbated hypertension, failed intubation, and aspiration, general anesthesia carries a significant risk of maternal death. Due to bleeding from the epidural veins, neuraxial anesthesia carries a significant risk of epidural hematoma and, consequently, paraplegia. Since the individualization of care is desirable, the choice of anesthetic method is directed by the maternal condition and laboratory parameters, which include the presence of eclampsia, severity of symptoms, platelet count and possibly complicating coagulopathy. For patients with preeclampsia who are not coagulopathic or thrombocytopenic, the risk of difficult or failed airway management and delayed recognition of maternal stroke during a general anesthetic are exceeded by the potential risk of adverse outcomes from neuraxial anesthesia (5). Moreover, maternal mortality statistics identify general anesthesia as a greater risk to parturients than neuraxial anesthesia (6, 7).

When the platelet count falls below 50,000/μL (severe thrombocytopenia), over 90% of the respondents prefer general anesthesia. However, respondents did not discriminate between platelet count ranges below 25,000/μL and 25,000/μL to 50,000/μL according to their clinical practice, as reflected by survey responses.

Over a platelet count of 50,000/μL, opinions were divided between general and neuraxial methods. A significant 60% of the respondents preferred general anesthesia when the maternal platelet count was between 50,000 and 100,000/μL. It was also interesting and illuminating that when the maternal platelet count exceeds 100,000/μL, approximately 35% of the respondents still preferred general anesthesia.

Moreover, above a platelet count of 100,000/μL, residents were more likely to prefer general anesthesia compared to clinicians ($p=0.02$) (Figure 6). Below a platelet count of 100,000/μL. the responses were similar between clinicians and residents. Accordingly, above a platelet count of 100,000/μL, residents likely are being instructed to utilize neuraxial methods. When compared to obstetric providers, anesthesiologists were less eager to prefer neuraxial methods for obstetric patients with platelet counts above 50,000/μL and above 100,000/μL (Figure 1, 2). Although there appears to be consensus between anesthesiologists and obstetricians–gynecologists when maternal platelet counts decrease below 50,000/μL, otherwise there exists a difference of opinion between the two specialties. Thus more evidence to guide practice and replace opinion is needed.

Altered hemostasis in preeclampsia is more often secondary to severe thrombocytopenia rather than to coagulopathy. Several studies have reported variable incidences of thrombocytopenia (platelet <100,000/μL), prolonged prothrombin time, and a prolonged activated partial thromboplastin time in patients with preeclampsia: 2% to 19%, 0% to 16%, and 1.3% to 2.5%, respectively (8-10). In one retrospective analysis, 8% of parturients exhibited both thrombocytopenia and coagulopathy (11). Studies investigating coagulation in women with preeclampsia using thromboelastography reported that if the platelet count was greater than 100,000/μL (absence of severe or moderate thrombocytopenia), there were no detectable coagulation abnormalities (12, 13). Platelet counts of less than 100,000/μL in women with preeclampsia may be associated with an impaired coagulation function and should prompt additional investigations of the coagulation status (12).

Instead of stratifying patients according to platelet count as a trigger to order coagulation tests, some recommend that it is practical and cost-effective to screen for both platelet count and coagulation studies at the same time (14, 15). As reflected from the current survey findings, a significant proportion of anesthesiologists in Turkey still prefer general anesthesia regardless of a normal coagulation profile and a platelet count exceeding 100,000/μL. With a platelet count above 100,000/μL, balancing the remote risk of maternal death due to epidural hematoma and choosing the greater risk of maternal death via general anesthesia in the clinical setting of preeclampsia/HELLP syndrome does not seem rational. Because there is not an established lower threshold for the platelet count to guarantee no potential for bleeding, practice guidelines simply indicate that the use of platelet count may reduce the risk of neuraxial anesthesia-related complications (5, 16, 17). However, authors in the field generalize safety in the range of a platelet count somewhere between 50,000 and 100,000/ μL. In addition, the most cited cut-off is 70,000 to 80,000/μL (5, 12, 14). The real dilemma, therefore, lies with the management of patients with preeclampsia whose platelet counts range between 50,000 and 100,000/μL. Neuraxial anesthesia is very safe in this platelet range, particularly when the coagulation profile is normal. In this range, two groups of patients raise special issues for clinical practice: patients with preeclampsia and HELLP syndrome. There are several reasons for this. First of all, the clinical course of HELLP syndrome is difficult to predict and variable in onset with usually a rapid progression of the syndrome (18, 19). Once initiated, patients with HELLP syndrome exhibit a 35–50% decrease in platelet count per 24 hours and a mean daily reduction of 40,000/μL (18). Second, HELLP syndrome is a more potent and significant threat to the mother and fetus than preeclampsia. In the English literature, one patient series recorded 77 deaths among 2346 treated patients (ratio of 1:30), 53

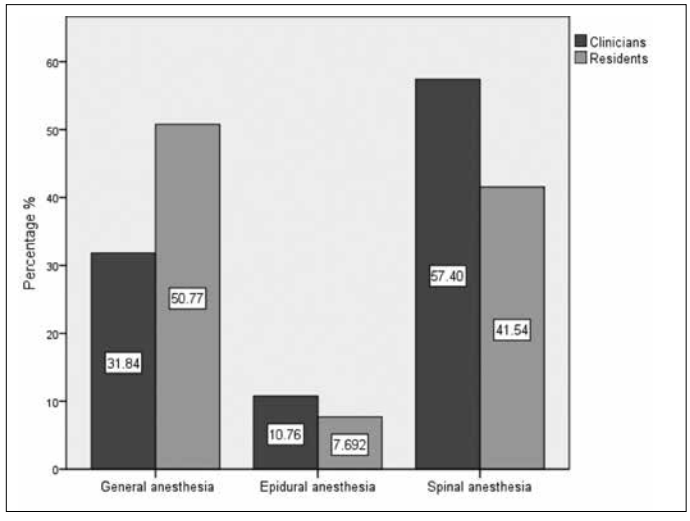

Figure 6. Preference in the type of anesthesia according to residents and clinicians for a platelet count above 100,000/μL

strokes (ratio of 1:44), 178 patients with acute renal failure (1 for every 13 patients with HELLP syndrome), and just over 90 liver ruptures (20). Third, most spinal epidural hematomas occurred in patients with HELLP syndrome and not preeclampsia with no evidence of HELLP syndrome (5, 21-23). Moreover, epidural anesthesia was the preferred method in those complicated cases (24, 25). Vigil-De Gracia and colleagues reported 36 patients with HELLP syndrome with platelet counts below 100,000/μL who received regional anesthesia and no epidural hematoma was suffered by any of the patients (12 patients had platelet counts below 50,000/μL) (26). None of those patients with HELLP syndrome in the study by Vigil-De Gracia (26) had disseminated intravascular coagulation and abnormal indices. Review of other series also revealed similar successful outcomes (27, 28). Fourth, laboratory parameters of patients with HELLP syndrome can continue to deteriorate postpartum, although they usually trend toward normal within 96 hours postpartum (18). Although a cut-off for the risk of spinal epidural hematoma has not been established, a platelet count of 40,000/μL and above has been assigned as the threshold above which there is no risk for postpartum bleeding from surgical sites in patients with HELLP syndrome (29). Spinal epidural hematoma and surgical site bleeding are not the same; therefore, the determined cut-off cannot be absolutely transposed for the risks of neuraxial anesthesia. However, in terms of maternal mortality and morbidity risk due to bleeding, they share a similar background.

In patients with severe preeclampsia and HELLP syndrome, the platelet count can be increased to 50,000/μL or above by two means separately or in combination: platelet pack transfusion and potent glucocorticoid administration. Antenatal corticosteroid administration has been reported to increase the platelet count; its use has been debated recently (30-32). It has also been shown to increase the utilization of neuraxial anesthesia among parturients (33). Thus, half of the survey respondents concurred that corticosteroid administration may increase the utilization of neuraxial anesthesia.

Moreover, respondents did not differentiate between the two methods (questions 5 and 8; Table 1) with regard to platelet counts and anesthesia application. However, a combined approach utilizing potent glucocorticoid administration and platelet pack transfusion may provide a more rapid and safer way to increase the platelet count to above 50,000/μL when cesarean surgery is needed to be performed quickly.

For patients with eclampsia, most respondents preferred general anesthesia (Table 1). Although general anesthesia is advocated in patients with eclampsia by some authors, there is minimal evidence to guide practice in the choice of anesthesia for women following an eclamptic convulsion (12). The hypertensive response to intubation has been identified as a direct cause of maternal mortality. Consequently, particular attention and extreme vigilance are required to ablate the hypertensive effect of intubation. Care must also be taken to avoid complications upon emergence from anesthesia, including hypertension, aspiration, and acute pulmonary edema (12). However, if the patient is stable with a normal level of consciousness and no neurological deficits, in the absence of other contraindications, neuraxial anesthesia is an acceptable choice (12).

The most dreaded complication of neuraxial anesthesia among patients with preeclampsia is the development of spinal epidural hematoma. The incidence of the complication is so low that it is difficult to comment on the methods to decrease its occurrence, such as ultrasound-guided neuraxial anesthesia (34). Theoretically, point-of-care ultrasound may decrease the number of attempts and increase the success of neuraxial anesthesia. Specifically, for patients with a borderline coagulation status, such as preeclampsia, ultrasonography may provide extra help and comfort to the anesthesiologist during the procedure

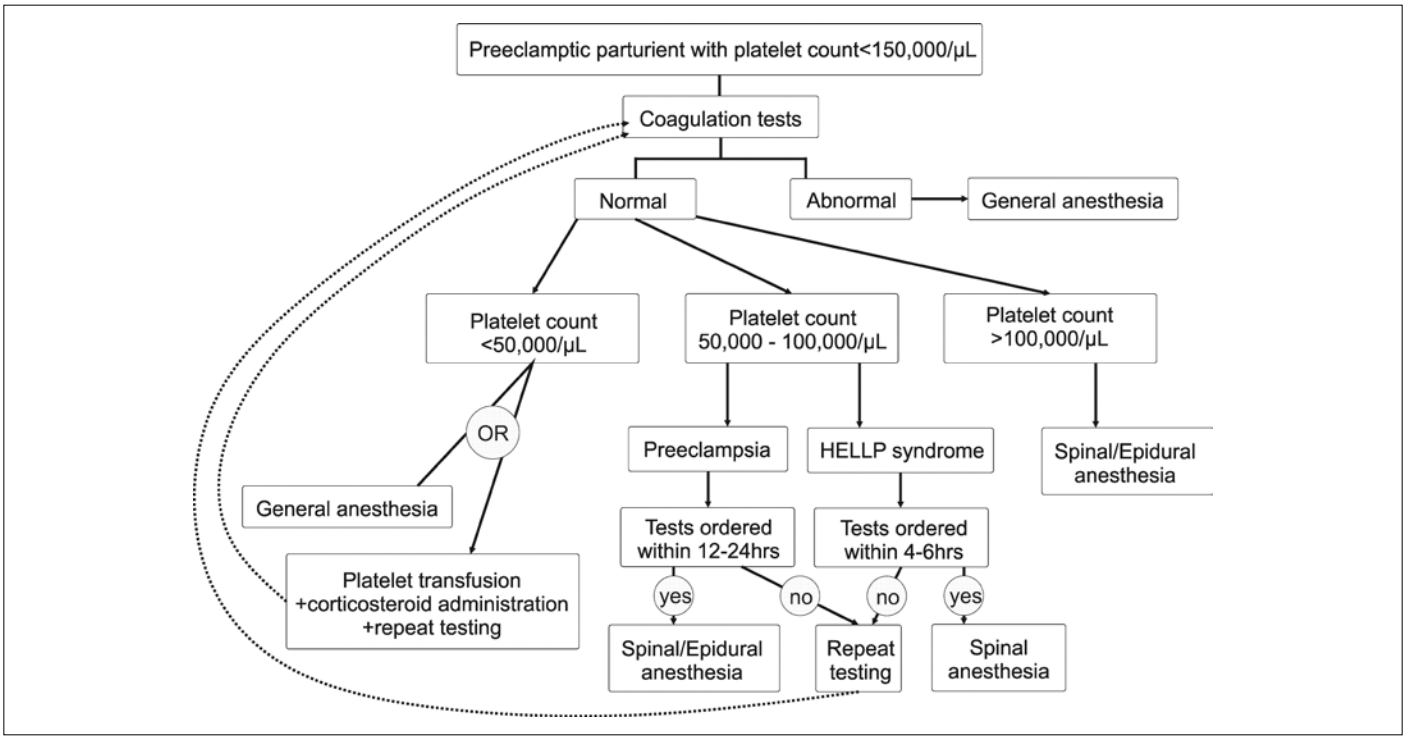

Figure 7. Proposed management algorithm

(34). Accordingly, approximately half of the respondents agreed with this notion and potential (Figure 5).

In conclusion, more evidence from well-conducted prospective randomized trials is needed to clarify many of the issues raised in our survey and in clinical practice. In the interim, based on the available relevant recent literature, a management algorithm is proposed for this very specific at-risk patient group (Figure 7). The marked diversity in anesthesiology practice, as reflected by our survey results, underscores the need for more clarity in obstetric care, which can be principally gained through excellent clinical research.

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References

1. Barton JR, Sibai BM. Gastrointestinal complications of pre-eclampsia. Semin Perinatol 2009; 33: 179-88. [Crossref]
2. Basaran A, Basaran M, Basaran B, Sen C, Martin JN. Controversial clinical practices for patients with preeclampsia or HELLP syndrome: a survey. J Perinat Med 2015; 43: 61-6. [Crossref]
3. Gliem JA, Gliem RR, editors. Calculating, interpreting, and reporting Cronbach's alpha reliability coefficient for Likert-type scales 2003: Midwest Research-to-Practice Conference in Adult, Continuing, and Community Education.
4. Redman CW. Preeclampsia: a multi-stress disorder. Rev Med Interne 2011; 32(Suppl 1): S41-4. [Crossref]
5. Henke VG, Bateman BT, Leffert LR. Focused review: spinal anesthesia in severe preeclampsia. Anesth Analg 2013; 117: 686-93. [Crossref]
6. Hawkins JL, Koonin LM, Palmer SK, Gibbs CP. Anesthesia-related deaths during obstetric delivery in the United States, 1979-1990. Anesthesiology 1997; 86: 277-84. [Crossref]
7. Mhyre JM, Riesner MN, Polley LS, Naughton NN. A series of anesthesia-related maternal deaths in Michigan, 1985-2003. Anesthesiology 2007; 106: 1096-104. [Crossref]
8. FitzGerald MP, Floro C, Siegel J, Hernandez E. Laboratory findings in hypertensive disorders of pregnancy. J Natl Med Assoc 1996; 88: 794-8.
9. Heilmann L, Rath W, Pollow K. Hemostatic abnormalities in patients with severe preeclampsia. Clin Appl Thromb Hemost 2007; 13: 285-91. [Crossref]
10. Sultan P, Butwick A. Platelet counts and coagulation tests prior to neuraxial anesthesia in patients with preeclampsia: a retrospective analysis. Clin Appl Thromb Hemost 2013; 19: 529-34. [Crossref]
11. Valera MC, Parant O, Vayssiere C, Arnal JF, Payrastre B. Physiologic and pathologic changes of platelets in pregnancy. Platelets 2010; 21: 587-95. [Crossref]
12. Dennis AT. Management of pre-eclampsia: issues for anaesthetists. Anaesthesia 2012; 67: 1009-20. [Crossref]
13. Sharma SK, Philip J, Whitten CW, Padakandla UB, Landers DF. Assessment of changes in coagulation in parturients with preeclampsia using thromboelastography. Anesthesiology 1999; 90: 385-90. [Crossref]
14. Ankichetty SP, Chin KJ, Chan VW, Sahajanandan R, Tan H, Grewal A, et al. Regional anesthesia in patients with pregnancy induced hypertension. J Anaesthesiol Clin Pharmacol 2013; 29: 435-44. [Crossref]
15. Metz J, Cincotta R, Francis M, DeRosa L, Balloch A. Screening for consumptive coagulopathy in preeclampsia. Int J Gynaecol Obstet 1994; 46: 3-9. [Crossref]
16. American Society of Anesthesiologists Task Force on Obstetric A. Practice guidelines for obstetric anesthesia: an updated report by the American Society of Anesthesiologists Task Force on Obstetric Anesthesia. Anesthesiology 2007; 106: 843-63.
17. Horlocker TT, Wedel DJ, Rowlingson JC, Enneking FK, Kopp SL, Benzon HT, et al. Regional anesthesia in the patient receiving antithrombotic or thrombolytic therapy: American Society of Regional Anesthesia and Pain Medicine Evidence-Based Guidelines (Third Edition). Regional anesthesia and pain medicine. 2010; 35: 64-101. [Crossref]
18. Martin JN, Jr., Rose CH, Briery CM. Understanding and managing HELLP syndrome: the integral role of aggressive glucocorticoids for mother and child. Am J Obstet Gynecol 2006; 195: 914-34. [Crossref]
19. Sibai BM, Barton JR. Dexamethasone to improve maternal outcome in women with hemolysis, elevated liver enzymes, and low platelets syndrome. Am J Obstet Gynecol 2005; 193: 1587-90. [Crossref]
20. Martin JN, Jr. Milestones in the quest for best management of patients with HELLP syndrome (microangiopathic hemolytic anemia, hepatic dysfunction, thrombocytopenia). Int J Gynaecol Obstet 2013; 121: 202-7. [Crossref]
21. Koyama S, Tomimatsu T, Kanagawa T, Sawada K, Tsutsui T, Kimura T, et al. Spinal subarachnoid hematoma following spinal anesthesia in a patient with HELLP syndrome. Int J Obstet Anesth 2010 19: 87-91. [Crossref]
22. Moen V, Dahlgren N, Irestedt L. Severe neurological complications after central neuraxial blockades in Sweden 1990-1999. Anesthesiology 2004; 101: 950-9. [Crossref]
23. Sibai BM, Taslimi MM, el-Nazer A, Amon E, Mabie BC, Ryan GM. Maternal-perinatal outcome associated with the syndrome of hemolysis, elevated liver enzymes, and low platelets in severe preeclampsia-eclampsia. Am J Obstet Gynecol 1986; 155: 501-9. [Crossref]
24. Lao TT, Halpern SH, MacDonald D, Huh C. Spinal subdural haematoma in a parturient after attempted epidural anaesthesia. Can J Anaesth 1993; 40: 340-5. [Crossref]
25. Yuen TS, Kua JS, Tan IK. Spinal haematoma following epidural anaesthesia in a patient with eclampsia. Anaesthesia 1999; 54: 350-4. [Crossref]
26. Vigil-De Gracia P, Silva S, Montufar C, Carrol I, De Los Rios S. Anesthesia in pregnant women with HELLP syndrome. Int J Gynaecol Obstet 2001; 74: 23-7. [Crossref]
27. Vercauteren M, Jacquemyn Y. Anaesthesia for caesarean section in patients with preterm HELLP syndrome: literature review, personal experience and possible guidelines. Actualitati in anestezie, terapie intensiva si medicina de urgenta; Timisoara, Romania 2008.
28. Tanaka M, Balki M, McLeod A, Carvalho JC. Regional anesthesia and non-preeclamptic thrombocytopenia: time to re-think the safe platelet count. Rev Bras Anestesiol 2009; 59: 142-53. [Crossref]
29. Roberts WE, Perry KG, Jr, Woods JB, Files JC, Blake PG, Martin JN, Jr. The intrapartum platelet count in patients with HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome: is it predictive of later hemorrhagic complications? Am J Obstet Gynecol 1994; 171: 799-804. [Crossref]
30. Basaran A, Basaran M, Sen C. Choice of glucocorticoid in HELLP syndrome - dexamethasone versus betamethasone: revisiting the dilemma. J Matern Fetal Neonatal Med 2012; 25: 2597-600. [Crossref]
31. Wallace K, Martin JN Jr, Tam Tam K, Wallukat G, Dechend R, Lamarca B, et al. Seeking the mechanism(s) of action for corticosteroids in HELLP syndrome: SMASH study. Am J Obstet Gynecol 2013; 208: 380.e1-8. [Crossref]
32. Woudstra DM, Chandra S, Hofmeyr GJ, Dowswell T. Corticosteroids for HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome in pregnancy. Cochrane Database Syst Rev 2010: CD008148. [Crossref]
33. O'Brien JM, Shumate SA, Satchwell SL, Milligan DA, Barton JR. Maternal benefit of corticosteroid therapy in patients with HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome: impact on the rate of regional anesthesia. Am J Obstet Gynecol 2002; 186: 475-9. [Crossref]
34. Ahmad M, Sinha AC. If you don't peep, you may shriek! Ultrasound for regional anesthesia in preeclampsia. J Anaesthesiol Clin Pharmacol 2013; 29: 431-2. [Crossref]

Factors affecting clinical pregnancy rates after IUI for the treatment of unexplained infertility and mild male subfertility

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Abstract

Objective: The aim of the present retrospective study was to evaluate intrauterine insemination (IUI) clinical experiences and to define the variables for predicting success.

Material and Methods: The present study was an observational trial performed in a private IVF center on subfertile couples who had applied for treatment between 2002 and 2012, in which the data of 503 IUI cases were retrospectively reviewed. Couples who had been diagnosed with unexplained and mild male subfertility were included. The primary outcome measure was the clinical pregnancy rate in an attempt to form a predictive model for the odds of a clinical pregnancy. Recorded parameters were used to determine the prediction model.

Results: Utilizing univariate logistic regression analysis, clinical pregnancy was positively associated with the duration of infertility (OR=1.09, p=0.089), secondary infertility (OR=1.77, p=0.050), and +4 sperm motility after preparation (OR=1.03, p=0.091). Following an adjustment analysis involving a multivariate logistic regression, clinical pregnancy was still found to positively associate with secondary infertility (OR=2.51, p=0.008).

Conclusion: IUI success in secondary infertile couples who were in the unexplained infertility and mild male subfertility groups was higher than that in primary infertile couples, and the chances of pregnancy increased as sperm numbers with +4 motility increased. It is difficult to concomitantly evaluate all these parameters and to determine a predictive parameter in IUI independent from other factors. (J Turk Ger Gynecol Assoc 2016; 17: 134-8)

Keywords: Intrauterine insemination, unexplained infertility, male subfertility

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Introduction

Subfertility is defined as the inability to conceive, despite regular sexual intercourse over a period of 12 months, and it affects 10% of couples who wish to have babies. Intrauterine insemination (IUI) is accepted as the first-line treatment for unexplained infertility and mild male factor subfertility (1-3).

In the literature, many factors affecting pregnancy rates following IUI have been reported. For instance, there are many studies related to age (4), the ovarian reserve of females (5) and sperm parameters in males (6), and the duration and type of infertility (7), which are pre-cycle parameters. Furthermore, the effects of cyclic parameters, such as selected ovulation induction protocols and the characteristics of the obtained follicles, have been evaluated on IUI outcomes in different studies (8-10). Despite all these studies, there is still no consensus on parameters that can predict the chances of clinical pregnancy after IUI.

The aim of the present retrospective study was to evaluate IUI clinical experiences at a private IVF center covering a 10-year period and to define the variables for predicting success.

Material and Methods

The present study comprised an observational trial performed in a private in vitro fertilization (IVF) center on subfertile couples who had applied for treatment between 2002 and 2012, and in which the data of 503 IUI cases were reviewed retrospectively. Couples with a female <40 years of age and a male who had been diagnosed with unexplained and mild male subfertility were included in the study. Couples with failure to conceive for >12 months, and with normal sperm param-

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eters, bilateral patent fallopian tubes, and regular menstrual cycles were accepted as cases involving unexplained infertility. Sperm samples that had a concentration above 20 million/mL according to the World Health Organization 1999 criteria, with type a (fast progressive) or type b (medium progressive) motility rates above 50%, with normal morphology and above 14% according to Kruger criteria were accepted as normal (11, 12). If the sperms in a sperm analysis did not satisfy the aforementioned reference criteria, but the total motility count (TMC) was above 5 million, then couples were accepted as cases involving mild male subfertility. Data from couples with any already determined causes of infertility, and cycles with ovarian hyperstimulation syndrome (OHSS) were not included in the evaluation.

Ovulation induction was performed in 38 cases, using oral tablets of clomiphene citrate (Klomen; Kocak Farma; İstanbul, Turkey), which were started on Day 5 of the cycle and given at a 100 mg/day dose for five days. Injectable gonadotropin, rFSH [follitropin alpha (Gonal F, Serono; İstanbul, Turkey), or follitropin beta (Puregon, MSD; İstanbul, Turkey)] was used in 331 cases, and HMG (Menogon, Ferring; İstanbul, Turkey) in 134 cases. Injectable gonadotropin was used on Day 2 or Day 3 of the cycle at doses of 75–150 units. Follicular developments were followed up with transvaginal USG, and dose adjustments were continued until dominant follicle development (max. dose=300 IU). Ovulation triggering was performed using 10000 IU hCG (Pregnyl, MSD; İstanbul, Turkey). Sperms were prepared using the discontinuous gradient method. Briefly, following liquefaction at room temperature, the semen was overlaid on a 40% and 80% PureSperm gradient column and centrifuged at 300 g for 20 min followed by washing the sperm pellet at 500 g in a non-capacitating buffer. A single insemination of 0.4 mL of sperm suspension per cycle was performed at 36 h after hCG administration under ultrasound guidance. Serum samples were obtained 14 days after hCG administration for βhCG levels.

The primary outcome measure of this study was the clinical pregnancy rate, which was gathered in an attempt to form a predictive model for the odds of a clinical pregnancy, defined as observable cardiac activity via a transvaginal ultrasound above six weeks of gestation in women treated by IUI for subfertility. Recorded parameters of maternal age, paternal age, follicle stimulating hormone (FSH) levels, duration of subfertility, type of female subfertility (primary or secondary), type of male subfertility, method of induction (clomiphene citrate or injectable gonadotropin), number of dominant follicles, measured diameter of dominant follicles on the day of hCG, the number of the total motile sperm count and the number of motile sperm counts, i.e., regarding their speeds, were used to determine the prediction model.

The study was ethically approved by the local ethics committee and the written informed consent of all the participants was obtained.

Statistical analysis

For all the statistical analysis, R v.3.1.3 for Windows was used. For the missing variables in the FSH levels, a stochastic regression imputation model was formed, using age and the antral follicle count as covariates. Missing FSH data were then imputed, by a adding a random number error term to the predicted FSH values. The error term was generated from a normal distribution with a mean of zero, and a variance equal to the regression variance, to introduce variability in the predicted values. To assess the association of the odds of clinical pregnancy with the covariates, first a set of univariate logistic regression analyses was carried out, in which the odds of clinical pregnancy was modeled on each covariate, separately. Second, a multivariable logistic regression was used to estimate the adjusted odd ratios between the odds of clinical pregnancy and the covariates of interest. Finally, a backward stepwise selection method was used to obtain the most explanatory model for the odds of clinical pregnancy. The ages of the women were included in the model to prevent a confounding effect. To ensure the final model was a good fit to the dataset at hand, a goodness of fit of the final logistic regression model was assessed using the Deviance and Hosmer–Lemeshow tests. The null hypothesis of no difference between the null and fitted model was rejected at a significance level of 10% according to the Deviance test. P values below 0.10 were considered statistically significant. All the statistical computations, comparisons, and analyses were performed using the Statistical Package for Social Sciences Inc. (SPSS version 15.0 for Windows, SPSS Inc.; IL, USA).

Results

A total of 47 pregnancies were determined in the 503 IUI cycles enrolled in the study. A pregnancy rate of 9.34% per cycle was determined, and the conception rates were 3 cases in the clomiphene citrate (CC) group, 12 in the human menopausal gonadotrophin (hMG) group, and 32 in the FSH group (7.8%, 8.9%, and 9.6%, respectively).

Basal FSH levels, the ages of the males and females, the type and duration of infertility, and male factor characteristics were taken as the basic pre-cycle parameters. The ovulation induction method, the number of follicles obtained, and the diameter of the dominant follicle were evaluated as cycle characteristics. The correlations between the presence of clinical conception and all of these variables were analyzed.

First, a set of univariate logistic regression analyses was carried out, in which the response variable (odds of clinical pregnancy) was modeled separately on each covariate. Table 1 displays the odds ratio estimates (OR), 95% and 90% confidence intervals, and p values resulting from the univariate analyses. Accordingly, clinical pregnancy was positively associated with the duration of infertility (OR=1.09, p=0.089), secondary infertility (OR=1.77, p=0.050), and +4 sperm motility after preparation (OR=1.03, p=0.091).

Second, an adjustment analysis was carried out. Multivariate logistic regression was used to estimate the adjusted OR's between the odds of clinical pregnancy and the covariates of interest. The results of the multivariate analysis are given in Table 2. Accordingly, clinical pregnancy was positively associated with the secondary infertility (OR=2.51, p=0.008).

A backward selection method was employed to obtain the most explanatory model for clinical pregnancy in this study. The ages of the women were included in the model to prevent the pos-

sibility of a confounding effect. Table 3 displays the coefficient estimates, p values, estimated odds ratios, and their confidence intervals in the resultant model. According to the final model, the odds for clinical pregnancy were 1.84 times higher for the women who had a secondary infertility. Furthermore, a one unit increase in +4 sperm motility creates an increase of 1.03 in the odds of achieving a positive outcome.

Discussion

In the literature, conception following IUI shows a wide range of gestational variation rates. Actually, the causes of these conflicting results are due to the selected treatment group, diagnostic criteria, the techniques used, the mean age of the selected cohort, the cause of infertility, and the change in treatment success according to the number of treatment cycles. In 1997, Hannoun et al. (13) published a study showing a treatment success of 5% in a single cycle in a CC+IUI group, and 58.7% in a controlled ovarian stimulation (COS)+IUI group in three cycles. Guzick et al. (14) retrospectively reviewed 45 published articles about unexplained infertility IUI studies, and the author reported clinical gestation rates of 8.3% in a CC+IUI group, and 17.1% in an hMG+IUI group. Goverde et al. (15) reported clinical gestational rates of 7.4% in a natural cycle IUI group, and 8.7% in a COS+IUI group. Finally, a European study conducted between 2001 and 2004 found that the pregnancy rate per IUI cycle was between 11.4% and 12.6% (16). In our study, the obtained clinical gestation rate was 9.34% (7.8% in the CC group, 8.9% in the hMG group, and 9.6% in the rFSH group), and it was within the wide range.

Table 1. Univariate models

Covariate	Coefficient	p	OR	Confidence interval
FSH (basal)	0.024	0.557	1.025	90% (0.957, 1.097)
Female's age	−0.006	0.827	0.994	90% (0.951, 1.039)
Male's age	−0.033	0.230	0.967	90% (0.923, 1.012)
Duration of infertility	0.083	0.089*	1.086	90% (1.002, 1.177)*
Type of infertility (primary/secondary)	0.572	0.050*	1.772	90% (1.096, 2.865)*
Male factor (normal/subfertile)	−0.537	0.385	0.584	90% (0.211, 1.617)
Method of ovulation induction (CC/FSH/hMG)	0.104	0.685	1.109	90% (0.727, 1.694)
Follicle count	0.145	0.519	1.155	90% (0.799, 1.671)
Dominant follicle size	0.004	0.948	1.004	90% (0.913, 1.104)
Sperm count (after preparation)	0.001	0.517	1.001	90% (0.998, 1.005)
Sperm motility +4	0.030	0.091*	1.031	90% (1.009, 1.061)*
Sperm motility +3	−0.005	0.690	0.994	90% (0.972, 1.017)
Sperm motility +2	0.009	0.599	1.009	90% (0.981, 1.037)
TPMSC	0.001	0.739	1.001	90% (0.997, 1.004)

*p values less than 0.10 indicate a significant association.

FSH: follicle stimulating hormone; CC: clomiphene citrate; hMG: human menopausal gonadotrophin; TPMSC: total progressive motile sperm count; OD: odds ratio

In the present study, a negative correlation was observed between the female age, basal FSH levels, and clinical gestational rates, but the difference was not statistically significant. In the present study, we also observed that as the infertility duration became longer, the gestation rates also increased. However, in the literature, many studies have indicated that as the age (5, 8, 17) and infertility duration (7, 17, 18) increase, gestational rates decrease. Also, Ibérico et al. (19) reported in a study published in 2004 that gestational rates in IUI decreased independently of the age through longer infertility durations. Contrary to the literature, the results of our study indicated that, as infertility duration increases, so does the gestational chance, and that no correlation was determined between gestational chance and the female or male ages. These might be explained due to the gestational rates being calculated per treatment cycle instead of per couple in our study. It is possible that, as the infertility duration is increased, the cumulative probability of gestation is increased in couples because of the increased number of IUI cycles.

Table 2. Multivariate model

Covariate	Coefficient	Standard error	z	p
FSH	−0.001	0.052	−0.013	0.989
Female's age	0.002	0.043	0.040	0.968
Male's age	−0.054	0.045	−1.205	0.228
Duration of infertility	−0.008	0.071	−0.110	0.912
Type of infertility (primary/secondary)	0.922	0.349	2.640	0.008*
Male factor (normal/subfertile)	−0.727	0.669	−1.087	0.277
Method of ovulation induction (CC/FSH/hMG)	0.255	0.296	0.863	0.388
Follicle count	0.191	0.240	0.793	0.427
Dominant follicle size	0.015	0.065	0.235	0.814
Sperm count (after preparation)	0.001	0.003	0.406	0.684
Sperm motility +4	0.032	0.034	0.938	0.348
Sperm motility +3	−0.006	0.019	−0.310	0.757
Sperm motility +2	0.029	0.023	1.264	0.206
TPMSC	−0.001	0.004	−0.369	0.712

FSH: follicle stimulating hormone; CC: clomiphene citrate; hMG: human menopausal gonadotrophin; TPMSC: total progressive motile sperm count

Table 3. Final model

Covariate	Coefficient	p	OR	CI
Female's age	−0.021	0.462	0.979	90% (0.932, 1.027)
Type of infertility (primary/secondary)	0.610	0.049*	1.841	90% (1.104, 3.072)*
Sperm motility +4	0.029	0.105*	1.029	90% (0.999, 1.061)*

*Hosmer–Lemeshow test, p=0.414.

Deviance test, p=0.089.

OR: odds ratio; CI: confidence interval

In secondary infertility, it was observed that clinical pregnancy rates were 1.84-fold higher than in primary infertility. There are many studies in the literature supporting this data (20-22). However, contradictory conclusions have also been reported. Ibérico et al. (19) and Dorjpurev et al. (23) proposed that infertility type (primary or secondary) did not affect gestational rates (19, 23).

In the statistical analysis of our cycle data, no statistically significant correlation was shown between the ovulation induction method and the clinical pregnancy rates following IUI. It has been shown in many studies in the literature that the use of FSH and hMG is superior to CC use (24-27). Although we observed that gestational rates were low in the CC group, we could not show the difference due to the small sample size. There was no difference between the FSH and hMG groups, which is a finding consistent with the literature. There are many studies in the literature reporting that the gonadotropin type affected (28, 29) or did not affect (30-32) treatment success rates.

No statistically significant effect was observed between the other examined cycle parameters, the number of obtained follicles, the dominant follicle diameter, and clinical gestation rates. Van Rumste et al. (33) reported that multi-follicular development in IUI did not improve the chances of pregnancy, but rather increased only the number of multiple gestation possibilities. In the meta-analysis containing 11599 cycles, van Rumste (34) reported that the development of two follicles increased the gestation chance more than that with one follicle development, but the development of a higher numbers of follicles did not increase the chances of pregnancy (34). In the same study, van Rumste reported also that the diameter of the dominant follicle did not have a high predictivity. However, Ibérico reported that if the diameter of the dominant follicle was >20 mm, then the pregnancy rates increased (19) accordingly.

There were no statistically significant effects on our clinical pregnancy rates for male factor presence/absence, the number of sperms in the prepared sperms for insemination, and the number of total progressive sperms. It was shown that only a sperm presence with +4 motility increased clinical pregnancy rates, which might be explained due to this study only including an unexplained and mild male subfertile patient group. Cao et al. (35) evaluated 1153 IUI cycles in 2014 and reported that a number of motile sperms lower than 2×10^6 in the insemination had low success rates in IUI, but the author also showed that sperm numbers higher than this is increased the observed pregnancy rates. They concluded that IUI can be performed when the NMSI (number of motile sperm inseminated) exceeds $2x10^6$. As there was no infertile male group in our study, we showed that the number of changes in the subfertile sperm group did not affect pregnancy rates. It was further shown that the conception success rates increased only if the number of sperms with +4 motility increased. As sperm motility has a very high predictive value in an elevated chance of pregnancy, it is accepted as the major determinant of IUI success (36-39).

There are many studies in the literature related to IUI. The strength of our study in the literature is that it reviews IUI procedures that have been performed over a period of 10 years in the same clinic with the same team (one embryologist and one gynecologist).

The most important limitation of our study is that it was a retrospective trial. We evaluated data only as the gestation rate per cycle because we wanted to obtain parameters that had a predictive role only for a single cycle. However, subsequently, this design revealed some findings that were inconsistent with those in the literature, such as age and infertility duration. Further, we could have conducted an evaluation of the success rates for couples. Additionally, in our study, we did not conduct a statistical analysis on multiple gestations.

In conclusion, our study results, which are consistent with those in the literature, indicates that IUI success in secondary infertile couples who were in the unexplained infertility and mild male subfertility groups was higher than in primary infertile couples and that the chances of pregnancy increased as sperm numbers with +4 motility increased. Success in IUI is multifactorial. Synchronization of the couple and the medical team should be achieved in parallel with all other factors, such as ovaries, fallopian tubes, endometrium, cervix, vagina, and sperms. It is quite difficult to concomitantly evaluate all these parameters and to determine a predictive parameter in IUI independent from other factors. We believe that this is the possible reason for the heterogeneity of the study groups in the IUI trials in the literature. Further prospective, randomized, controlled, and well-planned clinical studies on a large scale and with high subgroup numbers are required on this subject.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Turgut Özal University School of Medicine.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

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References

1. Romundstad LB, Opdahl S, Pinborg A. Which treatment option for couples with unexplained or mild male subfertility? BMJ 2015; 350: g7843. [Crossref]
2. National Institute for Health and Clinical Excellence (NICE) guidelines. 2013 (www.nice.org.uk)
3. Veltman-Verhulst SM, Cohlen BJ, Hughes E, Heineman MJ. Intrauterine insemination for unexplained subfertility. Cochrane Database Syst Rev 2012: CD001838. [Crossref]
4. van der Westerlaken LA, Naaktgeboren N, Helmerhorst FM. Evaluation of pregnancy rates after intrauterine insemination according to indication, age, and sperm parameters. J Assist Reprod Genet 1998; 15: 359-64. [Crossref]
5. Zadehmodarres S, Oladi B, Saeedi S, Jahed F, Ashraf H. Intrauterine insemination with husband semen: an evaluation of pregnancy rate and factors affecting outcome. J Assist Reprod Genet 2009; 26: 7-11. [Crossref]

6. Francavilla F, Sciarretta F, Sorgentone S, Necozione S, Santucci R, Barbonetti A, et al . Intrauterine insemination with or without mild ovarian stimulation in couples with male subfertility due to oligo/astheno-and/or teratozoospermia or antisperm antibodies: a prospective cross-over trial. Fertil Steril 2009; 92: 1009-11. [Crossref]
7. Jeon YE, Jung JA, Kim HY, Seo SK, Cho S, Choi YS, et al. Predictive factors for pregnancy during the first four intrauterine insemination cycles using gonadotropin. Gynecol Endocrinol 2013; 29: 834-8. [Crossref]
8. Nuojua-Huttunen S, Tomas C, Bloigu R, Tuomivaara L, Martikainen H. Intrauterine insemination treatment in subfertility: an analysis of factors affecting outcome. Hum Reprod 1999; 14: 698-703. [Crossref]
9. Steures P, van der Steeg JW, Hompes PG, Bossuyt PM, Habbema JD, Eijkemans MJ, et al; CECERM (Collaborative Effort for Clinical Evaluation in Reproductive Medicine). Effectiveness of intrauterine insemination in subfertile couples with an isolated cervical factor: a randomized clinical trial. Fertil Steril 2007; 88: 1692-6. [Crossref]
10. Practice Committee of the American Society for Reproductive Medicine. Effectiveness and treatment for unexplained infertility. Fertil Steril 2006; 86: S111–S114. [Crossref]
11. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press 1999.
12. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril 1988; 49: 112-7. [Crossref]
13. Hannoun A, Abu-Musa A, Kaspar H, Khalil A. Intrauterine insemination IUI: the effect of ovarian stimulation and infertility diagnosis on pregnancy outcome. Clin Exp Obstet Gynecol 1997; 25: 144-6.
14. Guzick D, Sullivan M, Adamson GD, Cedars M, Falk R, Peterson E, et al. Efficacy of treatment for unexplained infertility. Fertil Steril 1998; 70: 207-13. [Crossref]
15. Goverde AJ, McDonnell J, Vermeiden JP, Schats R, Rutten FF, Schoemaker J. Intrauterine insemination or in-vitro fertilisation in idiopathic subfertility and male subfertility: a randomised trial and cost-effectiveness analysis. Lancet 2000; 355: 13-8. [Crossref]
16. ESHRE Capri Workshop Group. Intrauterine insemination. Hum Reprod Update 2009: 15; 265-77. [Crossref]
17. Dickey RP, Taylor SN, Lu PY, Sartor BM, Rye PH, Pyrzak R. Effect of diagnosis, age, sperm quality, and number of preovulatory follicles on the outcome of multiple cycles of clomiphene citrate-intrauterine insemination. Fertil Steril 2002; 78: 1088-95. [Crossref]
18. Tomlinson MJ, Amissah-Arthur JB, Thompson KA, Kasraie JL, Bentick B. Infertility: Prognostic indicators for intrauterine insemination (IUI): statistical model for IUI success. Hum Reprod 1996; 11: 1892-6. [Crossref]
19. Ibérico G, Vioque J, Ariza N, Lozano JM, Roca M, Llácer J, et al. Analysis of factors influencing pregnancy rates in homologous intrauterine insemination. Fertil Steril 2004; 81: 1308-13. [Crossref]
20. Bhattacharya S, Harrild K, Mollison J, Wordsworth S, Tay C, Harrold A, et al. Clomifene citrate or unstimulated intrauterine insemination compared with expectant management for unexplained infertility: pragmatic randomised controlled trial. BMJ 2008; 337: a716. [Crossref]
21. Guven S, Gunalp GS, Tekin Y. Factors influencing pregnancy rates in intrauterine insemination cycles. J Reprod Med 2008; 53: 257-65.
22. Hughes EG. The effectiveness of ovulation induction and intrauterine insemination in the treatment of persistent infertility: a meta-analysis. Hum Reprod 1997; 12: 1865-72. [Crossref]
23. Dorjpurev U, Kuwahara A, Yano Y, Taniguchi T, Yamamoto Y, Suto A, et al. Effect of semen characteristics on pregnancy rate following intrauterine insemination. J Med Invest 2011; 58: 127-33. [Crossref]
24. Peeraer K, Debrock S, De Loecker P, Tomassetti C, Laenen A, Welkenhuysen M, et al. Low-dose human menopausal gonadotrophin versus clomiphene citrate in subfertile couples treated with intrauterine insemination: a randomized controlled trial. Hum Reprod 2015; 30: 1079-88. [Crossref]
25. Berker B, Kahraman K, Taskin S, Sukur YE, Sonmezer M, Atabekoglu CS. Recombinant FSH versus clomiphene citrate for ovarian stimulation in couples with unexplained infertility and male subfertility undergoing intrauterine insemination: a randomized trial. Arch Gynecol Obstet 2011; 284: 1561-6. [Crossref]
26. Costello MF. Systematic review of the treatment of ovulatory infertility with clomiphene citrate and intrauterine insemination. Aust N Z J Obstet Gynaecol 2004; 44: 93-102. [Crossref]
27. Dickey R, Taylor S, Curole D, Rye P. Sequential clomifene citrate and human menopausal gonadotrophin for ovulation induction: comparison to clomiphene citrate alone and human menopausal gonadotrophin alone. Hum Reprod 1993; 8: 56-9.
28. Rashidi M, Aaleyasin A, Aghahosseini M, Loloi S, Kokab A, Najmi Z. Advantages of recombinant follicle-stimulating hormone over human menopausal gonadotropin for ovarian stimulation in intrauterine insemination: a randomized clinical trial in unexplained infertility. Eur J Obstet Gynecol Reprod Biol 2013; 169: 244-7. [Crossref]
29. Demirol A, Gurgan T. Comparison of different gonadotrophin preparations in intrauterine insemination cycles for the treatment of unexplained infertility: a prospective, randomized study. Hum Reprod 2007; 22: 97-100. [Crossref]
30. Gerli S, Casini ML, Unfer V, Costabile L, Bini V, Di Renzo GC. Recombinant versus urinary follicle-stimulating hormone in intrauterine insemination cycles: a prospective, randomized analysis of cost effectiveness. Fertil Steril 2004; 82: 573-8. [Crossref]
31. Filicori M, Cognigni GE, Pocognoli P, Tabarelli C, Ferlini F, Perri T, et al. Comparison of controlled ovarian stimulation with human menopausal gonadotropin or recombinant follicle-stimulating hormone. Fertil Steril 2003: 80; 390-7. [Crossref]
32. Duran HE, Morshedi M, Kruger T, Oehninger S. Intrauterine insemination: a systematic review on determinants of success. Hum Reprod Update 2002; 8: 373-84. [Crossref]
33. van Rumste MM, den Hartog JE, Dumoulin JC, Evers JL, Land JA. Is controlled ovarian stimulation in intrauterine insemination an acceptable therapy in couples with unexplained non-conception in the perspective of multiple pregnancies? Hum Reprod 2006; 21: 701-4. [Crossref]
34. van Rumste MM, Custers IM, van der Veen F, van Wely M, Evers JL, Mol BWJ. The influence of the number of follicles on pregnancy rates in intrauterine insemination with ovarian stimulation: a meta-analysis. Hum Reprod Update 2008; 14: 563-70. [Crossref]
35. Cao S, Zhao C, Zhang J, Wu X, Zhou L, Guo X, et al. Minimum number of motile spermatozoa are required for successful fertilisation through artificial intrauterine insemination with husband's spermatozoa. Andrologia 2014; 46: 529-34. [Crossref]
36. Ombelet W, Dhont N, Thijssen A, Bosmans E, Kruger T. Semen quality and prediction of IUI success in male subfertility: a systematic review. Reprod Biomed Online 2014; 28: 300-9. [Crossref]
37. Goldman RH, Batsis M, Hacker MR, Souter I, Petrozza JC. Outcomes after intrauterine insemination are independent of provider type. Am J Obstet Gynecol 2014; 211: 492-e1. [Crossref]
38. Miller DC, Hollenbeck BK, Smith GD, Randolph JF, Christman GM, Smith YR, Ohl DA. Processed total motile sperm count correlates with pregnancy outcome after intrauterine insemination. Urology 2002; 60: 497-501. [Crossref]
39. Shulman A, Hauser R, Lipitz S, Frenkel Y, Dor J, Bider D, et al. Sperm motility is a major determinant of pregnancy outcome following intrauterine insemination. J Assist Reprod Gen 1998; 15: 381-5. [Crossref]

The relationship between first-trimester pregnancy-associated plasma protein-A levels and intrapartum fetal distress development

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Abstract

Objective: To investigate the relationship between the development of intrapartum fetal distress and serum pregnancy-associated plasma protein-A (PAPP-A) levels measured during first-trimester aneuploidy screening tests.

Material and Methods: This retrospective study included 283 uncomplicated pregnancies that resulted in full-term live births via spontaneous labor or with the induction by oxytocin. Cases were divided into two groups based on whether their first-trimester PAPP-A multiple of the median (MoM) levels were ≤0.5 (Group 1, n=75) or >0.5 (Group 2, n=208). As primary end points, the rate of cesarean section (C/S), the rate of C/S due to fetal distress, and the umbilical artery blood pH values in cases of C/S for fetal distress were compared between the two groups. Statistical analyses were performed using the Chi-square test and independent samples t-test. P≤0.05 were considered statistically significant.

Results: The mean gestational age at birth and the birth weights were significantly lower in Group 1 than in Group 2 (p=0.002 and p=0.007, respectively). Although the rate of C/S was similar between the groups (p=0.823), the rate of C/S due to fetal distress was significantly higher in Group 1 than in Group 2 (68.4% vs. 42%, respectively; p=0.050) and the mean umbilical artery blood pH value for C/S deliveries indicated by fetal distress was lower (p=0.048) in Group 1 than in Group 2. When the mode of delivery was analyzed according to the application of labor induction, both the C/S delivery rates (31.6% in Group 1 and 31.7% in Group 2; p=0.992) and C/S delivery rates due to fetal distress (66.7% in Group 1 and 46.2% in Group 2; p=0.405) were similar in both groups.

Conclusion: Low PAPP-A levels (≤0.5 MoM) in the first trimester are associated with the risk of intrapartum fetal distress development and the likelihood of C/S for fetal distress. Nonetheless, this risk is not affected by labor induction. (J Turk Ger Gynecol Assoc 2016; 17: 139-42)

Keywords: Cesarean section, fetal distress, pregnancy-associated plasma protein A

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Introduction

Pregnancy-associated plasma protein A (PAPP-A) is one of the parameters in the first-trimester screening test that is used as a biochemical marker to detect aneuploidy in the early weeks of gestation (1). PAPP-A can be detected in the maternal blood 28 days after implantation in singleton pregnancies. The serum PAPP-A level starts to increase during the first trimester, doubling every 3 to 4 days. The rate of increase is gradual until week 36, after which it accelerates, and the maximum serum PAPP-A levels are reached at term (2).

It is thought that PAPP-A released from trophoblastic tissues at abnormal levels early during gestation affects fetal growth negatively by impairing trophoblastic invasion of the decidua, causing abnormal placentation and other pregnancy complications (3). A low PAPP-A concentration is a powerful indicator of potential pregnancy complications, including preeclampsia, intrauterine developmental retardation, gestational hypertension, fetal death, oligohydramnios, and preterm birth (4-6). Because PAPP-A levels during pregnancy are associated with obstetric complications, as well as abnormal placentation, it is thought that fetuses born to mothers with low maternal PAPP-A levels do not tolerate labor stress and also could be more likely to develop intrapartum fetal distress (7).

In this study, we compared the rate of emergency cesarean section (C/S) due to fetal distress and the umbilical arterial blood gas levels in patients with normal or low PAPP-A levels in the first trimester.

Material and Methods

We conducted a retrospective examination of 359 cases of singleton pregnancies in which the first-trimester screening for aneuploidy had been performed in our pregnancy out-

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patient clinic between June 2012 and January 2013. The local ethics committee of the hospital approved the study and each patient signed the informed consent.

In all cases, the mothers were followed for the duration of their pregnancies. Crown-rump length (CRL) measurements (range: 45–84 mm) were obtained and the nuchal translucency (NT) test was performed on the live singleton fetuses confirmed to be between 11 weeks and 13 weeks and 6 days old by ultrasound. The serum levels of PAPP-A and free hCG were tested in the maternal blood and adjusted according to the maternal weight, insulin-related diabetes, and smoking habits. Multiple of the median (MoM) values were calculated.

Cases with abnormal fetal karyotypes, pregnancy complications (preterm delivery, pregnancy induced hypertension/preeclampsia, gestational diabetes, intrauterine growth restriction, and placental abruption) and systemic diseases (chronic hypertension, type 1 and type 2 diabetes mellitus) or in which the mothers elected to have C/S deliveries (due to past C/S or presentation abnormalities) were excluded from the study (n=76).

The final cohort included the results from 283 cases in which first-trimester screening for fetal aneuploidy had been conducted and also the delivery had occurred at term (≥37 weeks gestation) following either spontaneous labor or labor induction with oxytocin for cases in which the gestation reached beyond the 41st week without the spontaneous onset of labor, or there was early membrane rupture, oligohydramnios, or non-reassuring fetal well-being tests.

The cases were divided into two groups based on their first-trimester PAPP-A multiples of their median (MoM) values as ≤0.5 MoM (Group 1) or >0.5 MoM (Group 2).

The demographic features, birth weight, gender, delivery modes, and indications for C/S were recorded, and the umbilical arterial blood gas pH values were compared between the two groups in cases where fetal distress had developed. Fetal distress was determined using fetal monitoring based on the American Congress of Obstetricians and Gynecologists intrapartum fetal heart rate trace management protocol (8). C/S decision was made by the same authors (AFA and HLK). According to the mentioned management protocol, category II (intermediate) cases were evaluated and under surveillance, and if the fetal heart rate (FHR) accelerations were absent and absent/minimal FHR variability occurred and did not improve, or FHR tracing progressed to a category III (abnormal) pattern, then delivery was considered. General anesthesia was administered in all the cases in which C/S was performed because of fetal distress. There was not any instrumental operative delivery.

Statistical analyses were performed using SPSS ver. 21.0 (IBM Corp.; California, USA). Descriptive characteristics were expressed as numbers and percentages, the mean±standard deviation (SD) (minimum–maximum), or as the median (interquartile ranges [IQRs]) (minimum–maximum) according to whether the variables were distributed as normal using the Shapiro-Wilk test. Inter-group comparisons were made using the Pearson Chi-square test for the categorical variables, and with the independent samples t-test for continuous data. Statistical significance was defined as $p \leq 0.05$.

Results

PAPP-A levels were ≤0.5 MoM in 75 (26.5%) cases (Group 1) and >0.5 MoM in 208 (73.5%) subjects (Group 2). The mean PAPP-A levels was 0.38±0.10 MoM for Group 1 and 1.14±0.63 MoM for Group 2 ($p<0.001$).

The age, gravidity, and parity were similar among the groups ($p>0.05$). (Table 1) The mean gestational age at birth and the birth weights were significantly lower in Group 1 than Group 2 ($p=0.002$ and $p=0.007$, respectively).

The cesarean delivery rate was 24.4% (69/283) in all the subjects. Although the rate of C/S were similar among the groups ($p=0.823$), the rate of C/S due to fetal distress was significantly higher in Group 1 (68.4% (13/19) vs 42% (21/50), $p=0.050$) (Table 1). In addition, the mean umbilical artery blood gas pH value for C/S deliveries indicated by fetal distress was also significantly lower in Group 1 ($p=0.048$). According to the ACOG management protocol, the category III (abnormal) FHR pattern rate was 30.8% (4/13) in Group 1 and 33.3% (7/21) in Group 2 ($p=0.877$).

The induction of labor was applied in 19 (25.3%) subjects in Group 1 and in 41 (19.7%) subjects in Group 2 and the rate of labor induction were similar between the groups ($p=0.307$). When the mode of delivery was analyzed according to applying labor induction, both C/S delivery rates ((6/19 (31.6%) in Group 1, 13/41 (31.7%) in Group 2; $p=0.992$)) and the rate of C/S delivery due to fetal distress ((4/6 (66.7%) in Group 1, 6/13 (46.2%) in Group 2; $p=0.405$)) were not different in both groups.

Discussion

PAPP-A, which was first obtained from the plasma of pregnant women in 1974, is a protein released from the placenta, and its concentration in maternal blood reflects placental activity (9, 10). PAPP-A is a specific protease for insulin-like growth factor binding protein-4 (IGFBP-4), and thus plays a role in fetal growth and the development and many physiopathologic events related to IGF-1 and -2 (11). Furthermore, it was found that PAPP-A plays an exclusive role in the autocrine and paracrine regulation of the trophoblastic invasion of the decidua (12, 13). Low PAPP-A levels are thought to be attributable to sequestration by binding proteins for free IGFs, and this can negatively affect fetal growth and cause obstetric complications (4, 12).

Ucella et al. (7) found that the rate of non-elective C/S deliveries indicated by fetal distress was higher among mothers with low PAPP-A levels (16.2%) than among mothers with normal PAPP-A (7.9%) levels. Thus, they suggested that low PAPP-A levels may be not only related to antenatal complications (preeclampsia, intrauterine growth retardation, preterm delivery, and loss of pregnancy), but also may be a risk factor for acute intrapartum fetal distress related to abnormal placentation and placental dysfunction, leading to more emergency C/S deliveries (7).

In this study, we investigated whether fetal distress developed more frequently during labor in cases with low maternal PAPP-A levels in the first trimester and examined the umbilical arterial blood gas levels to evaluate whether there was an indirect correlation between low PAPP-A levels and the placental reserve during active labor.

Table 1. Demographic features and delivery results in groups based on PAPP-A levels

Parameter		PAPP-A ≤0.5 MoM (n=75)	PAPP-A >0.5 MoM (n=208)	p
Maternal age, years*		27.1±5.1 (18–40)	26.6±5.1 (18–42)	0.433
Gravidity, n**		2 (2) (1–5)	2 (2) (1–9)	0.766
Parity, n**		1 (1) (0–3)	1 (1) (0–3)	0.120
Gestational age at delivery, weeks*		38.3±4.2 (37.1–41.5)	39.3±1.6 (37.0–42.1)	0.002
Mean birth weight, g*		3213 (±515) (2190–4310)	3377(±494) (2180–5010)	0.007
Infant gender, n	Male	37	109	0.136
	Female	38	99	
C/S delivery rate, n (%)		19 (25.3%)	50 (24.0%)	0.823
C/S due to fetal distress, n (%)		13/19 (68.4%)	21/50 (42%)	0.050
Umbilical artery blood gas pH value for C/S due to fetal distress*		7.28 (±0.09) (7.07–7.41)	7.34 (±0.03) (7.00–7.43)	0.048
Labor induction, n (%)		19 (25.3%)	41 (19.7%)	0.307
C/S rate in labor induction applying group, n (%)		6/19 (31.6%)	13/41 (31.7%)	0.992
C/S rate due to fetal distress in labor induction group, n (%)		4/6 (66.7%)	6/13 (46.2%)	0.405

*Mean±SD (Min–Max)
**Median (IQR) (Min–Max)
PAPP-A: pregnancy-associated plasma protein A; n: number; C/S: cesarean section; MoM: multiple of the median

Our findings are consistent with the results reported by Ucella et al. (7) and demonstrate a higher rate of C/S delivery because of fetal distress during active labor in cases with low PAPP-A levels (≤0.5 MoM) measured during the first trimester.

Ucella et al. (7) also found that the umbilical arterial blood pH was significantly lower in cases where a C/S was performed because of fetal distress in cases of low PAPP-A levels compared to cases with normal PAPP-A levels (pH: 7.19 vs pH: 7.26, respectively). We also analyzed the umbilical cord blood pH immediately postpartum in cases where a C/S was performed because of fetal distress, but we could not found any significant differences between the groups. Although the intrapartum wellbeing of the fetus during labor is generally followed with fetal electronic monitoring (FEM), in almost 50% of the fetuses where fetal distress is detected with FEM, the oxygenation of the fetus is normal (14-16).

In conclusion, PAPP-A levels between the 11th and 14th weeks of gestation likely reflect placental function, and the risk of developing intrapartum fetal distress and the risk of C/S due to fetal distress are higher in cases with low (≤0.5 MoM) PAPP-A levels. Nonetheless, this risk is not affected by labor induction by oxytocin. The PAPP-A level measured during aneuploidy screening in the first trimester of pregnancy can help predict the development of intrapartum fetal distress. Our study is limited by its retrospective design; thus, prospective studies are required to verify our results.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Yıldırım Beyazıt University School of Medicine.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

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Author Contributions: *Concept - A.F.Y., E.İ.S., G.F.Y.A.; Design - A.F.Y., E.İ.S., G.F.Y.A.; Supervision - A.F.Y., E.İ.S., G.F.Y.A., H.L.K.; Resources - G.F.Y.A., H.L.K., E.E.T., A.F.D.; Materials - H.L.K., E.E.T., A.F.D.; Data Collection and/or Processing - E.İ.S., G.F.Y.A., E.E.T., A.F.D.; Analysis and/or Interpretation - H.L.K., E.E.T.; Literature Search - H.L.K., E.E.T., A.F.D.; Writing Manuscript - A.F.Y., E.İ.S., G.F.Y.A., A.F.D.; Critical Review - A.F.Y., E.İ.S., G.F.Y.A., H.L.K., E.E.T., A.F.D.*

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References

1. Ball RH, Caughey AB, Malone FD, Nyberg DA, Comstock CH, Saade GR, et al; First and Second Trimester Evaluation of Risk (FASTER) Research Consortium. First and second- trimester evaluation of risk for Down syndrome. Obstet Gynecol 2007; 110: 10-7. [Crossref]
2. Smith R, Bischof P, Hughes G, Klopper A. Studies on pregnancy- associated plasma protein A in the third trimester of pregnancy. Br J Obstet Gynaecol 1979; 86: 882-7. [Crossref]
3. Huynh L, Kingdom J, Akhtar S. Low pregnancy-associated plasma protein A level in the first trimester. Can Fam Physician 2014; 60: 899-903.
4. Kale İ, Gülerman HC, Moraloğlu Ö, Gürlek B, Yenicesu O, Sarıkaya E, et al. Association between low levels of maternal serum PAPP-A in the first trimester and complications of pregnancy. Turkiye Klinikleri J Gynecol Obst 2012; 22: 25-35.

5. Spencer K, Cowans NJ, Molina F, Kagan KO, Nicolaides KH. First trimester biochemical markers of aneuploidy and the prediction of small-for-gestational age fetuses. Ultrasound Obstet Gynecol 2008; 31: 15-9. [Crossref]
6. Smith GC, Shah I, Crossley JA, Dobbie R. Pregnancy- associated plasma protein A and alpha-fetoprotein and prediction of adverse perinatal outcome. Obstet Gynecol 2004; 104: 30-6.
7. Ucella S, Colombo GF, Bulgheroni CM, Serati M, Bogani G, Salvatore S, et al. First- trimester maternal serum screening and risk for fetal distress during labor. Am J Obstet Gynecol 2009; 201: 1661-6. [Crossref]
8. American College of Obstetricians and Gynecologists. Practice bulletin no. 116: Management of intrapartum fetal heart rate tracings. Obstet Gynecol 2010; 116: 1232-40. [Crossref]
9. Lin TM, Halbert SP, Kiefer D, Spellacy WN, Gall S. Characterization of four pregnancy-associated plasma proteins. Am J Obstet Gynecol 1974; 118: 223-36. [Crossref]
10. Hughes G, Bischof P, Wilson G, Klopper A. Assay of a plasental protein to detrmine fetal risk. BMJ 1980; 8: 671-3. [Crossref]
11. Lawrence JB, Oxvig C, Overgaard MT, Sotturp-Jensen L, Gleich GJ, Hays LG,et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. Proc Natl Acad Sci USA 1999; 96: 3149-53. [Crossref]
12. Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Conner JM. Early pregnancy levels of pregnancy-associated plasma protein A and the risk of intrauterine growth restriction, premature birth, preclampsia and stillbirth. J Clin Endocrinol Metab 2002; 87: 1762-7. [Crossref]
13. Clemmons DR. Role of insulin-like growth factor binding proteins in controlling IGF actions. Mol Cell Endocrinol 1998; 140: 19-24. [Crossref]
14. Benian A, Uludağ S, Atiş A, Gök M, Madazli R. Analysis of umbilical cord blood acid-base status at birth. Cerrahpaşa J Med 2002; 33: 236-44.
15. Gilstrap LC, Hauth JC, Toussaint S. Second stage fetal heart abnormalities and neonatal acidosis. Obstet Gynecol 1984; 63: 209-13.
16. Paul RH, Suidan AK, Yeh S, Schifrin BS, Hon EH. Clinical fetal monitoring.VII. The evaluation and significance of intrapartum baseline FHR variability. Am J Obstet Gynecol 1975; 123: 206-10. [Crossref]

Predictive values of maternal serum PAPP-A level, uterine artery Doppler velocimetry, and fetal biometric measurements for poor pregnancy and poor neonatal outcomes in pregnant women

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Abstract

Objective: To determine predictive values of maternal serum PAPP-A (msPAPP-A) levels, uterine artery Doppler velocimetry, and fetal biometric measurements (FBMs) for poor pregnancy and poor neonatal outcomes.

Material and Methods: This prospective cohort study was conducted on singleton pregnancies followed until delivery. Pregnancy and neonatal outcomes were evaluated with respect to the msPAPP-A level at the 11th–14th weeks, uterine artery Doppler velocimetry at the 15th–18th weeks, and FBMs at the 20th–24th and 28th–32nd weeks of pregnancy.

Results: One hundred fifty-eight women constituted the study group; 17 (10.75%) of them had at least one poor pregnancy outcome. The cut-off point of 0.72 multiple of the median (MoM) for the PAPP-A level achieved a sensitivity of 82.4% and a specificity of 29.8% for poor pregnancy outcomes. The mean birth weight was significantly lower in the subgroup with a higher mean pulsatility index of uterine arteries (UAPImean≥1.19) (p=0.025) as well as in the subgroup with a higher mean resistance index of uterine arteries (UARImean≥0.62) (p=0.013). When the subgroup of pregnant women under the risk of early-onset IUGR according to FBMs was compared to the low-risk group, statistically significant differences were seen in terms of pregnancy outcomes (p=0.045) and birth weight (p=0.011).

Conclusion: Maternal serum PAPP-A level and FBMs could be used for predicting pregnancy outcomes, while uterine artery Doppler velocimetry and FBMs could be used for predicting neonatal outcomes, specifically the birth weight. (J Turk Ger Gynecol Assoc 2016; 17: 143-9)

Keywords: Pregnancy-associated plasma protein-a, ultrasonography, Doppler, color, fetal biometry, pregnancy outcome, fetal development

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Introduction

Preeclampsia and intrauterine growth restriction (IUGR), as well as related perinatal death or preterm birth before the 32nd week of pregnancy, are very closely related with the underlying potential placental pathology. In the last 25 years, many studies have been conducted regarding the early recognition of placental insufficiency. Doppler flow measurements of uterine and umbilical arteries, as well as maternal serum alpha fetoprotein (AFP) and human chorionic gonadotropin (hCG) levels, during the first and second trimesters of pregnancy are most frequently studied to predict placental insufficiency and its effects on the fetus (1).

Uterine artery Doppler velocimetry results have demonstrated that hemodynamic changes detectable in the uterine artery as early as in the first trimester of pregnancy are associated with an increased risk of preeclampsia and IUGR (2-4). This association can also be demonstrated in the second and third trimesters (5-7).

Several studies have recently shown that low serum levels of pregnancy-associated plasma protein A (PAPP-A) might be associated with poor pregnancy and poor neonatal outcomes (1, 8, 9). PAPP-A, which was first purified from the serums of pregnant women in 1974, is a member of the metzincin family of metalloproteinases (10, 11). PAPP-A is an insulin-like growth factor-binding protein (IGFBP)-specifically, a protease. IGFBPs bind to insulin-like growth factor (IGF) 1 and 2 and disconnect these proteins from cell surface receptors; thus, low levels of serum PAPP-A are associated with low levels of bioactive IGF (11).

A low level of maternal serum PAPP-A is an important sign of early placental insufficiency in the first three months of pregnancy; however, the effects on the fetus reaches recognizable levels in the second trimester. Nevertheless, growth restriction determined in the second trimester is also directly associated with poor pregnancy and poor neonatal outcomes. Detailed ultrasonography for fetal biometric measurements (FBMs) and Doppler flow measurements that would be performed in the second trimester in patients with low levels of serum PAPP-A,

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which is measured routinely within the scope of first-trimester aneuploidy screening at the 11th–14th weeks of pregnancy may be beneficial in predicting poor pregnancy and poor neonatal outcomes and in taking necessary measures (12, 13).

The present study aimed to determine the predictive value of maternal serum PAPP-A levels measured in the first trimester, uterine artery Doppler velocimetry performed during the second trimester, and FBMs in the second and third trimesters for poor pregnancy and poor neonatal outcomes in pregnant women.

Material and Methods

This prospective cohort study was conducted in a single university–based pregnancy clinic in the Department of Gynecology and Obstetrics in Turkey between July 2013 and July 2015, with approval of the hospital ethics committee. The singleton pregnant women, who presented to our pregnancy clinic, were enrolled into the study from September 2013 to May 2015 after their informed consents were obtained. The study complied with the Declaration of Helsinki and ethical standards. Pregnant women were excluded from the final analyses if they missed follow-up visits or had complicated pregnancies, such as abortion or iatrogenic termination of the pregnancy.

The women included in the study were followed up after the confirmation of pregnancy until delivery. Initially, all the study participants underwent the first-trimester aneuploidy screening test at the 11th–14th weeks of pregnancy, including maternal serum PAPP-A, β-hCG, and nuchal translucency measurements. Subsequently, color pulsed Doppler ultrasound examination of the bilateral uterine arteries was performed at the 15th–18th weeks of pregnancy. The women with diastolic notches, unilateral or bilateral, in the uterine artery Doppler waveforms were recorded. The pulsatility index (PI) and resistance index (RI) of both uterine arteries were measured and their arithmetic means were calculated. FBMs, including fetal biparietal diameter (BPD), head circumference (HC), femur length (FL), and abdominal circumference (AC), were measured at the 20th–24th and 28th–32nd weeks of pregnancy, and the HC/AC ratio was calculated. Poor pregnancy outcomes, such as pregnancy-induced hypertension, preeclampsia, and preterm birth, and poor neonatal outcomes as well as birthweight measured at delivery were noted. Women were divided into two groups according to the pregnancy or neonatal outcomes: the group with and the group without poor outcomes. These two groups were compared with each other with respect to the test results specified above, in order to determine the predictive values of the analyses.

All the clinic-demographic characteristics and test results were recorded in a prospectively formed electronic database. All the ultrasonographic measurements were performed by the same physician, using color Doppler ultrasound (Voluson 730 Expert, General Electric Healthcare; Chicago, USA) and the data were assessed by a single researcher.

Pregnant women were considered to be under risk of early-onset IUGR when the estimated gestational age according to the ultrasonographic FBMs at the 20th–24th and 28th–32nd weeks of pregnancy was more than one-week behind the gestational age calculated according to the last menstrual period (LMP) and when HC/AC ratio was over 1.15 and 1.10 at the 20th–24th and 28th–32nd weeks of pregnancy, respectively (12, 13). This group was compared with the group of pregnant women in whom the estimated gestational age according to the FBMs was consistent with the gestational age calculated according to the LMP, with respect to the test results as well as pregnancy outcomes, including the birth weight.

Pregnancy-induced hypertension (PIH) was diagnosed if the arterial blood pressure of the patient was 140/90 mmHg and higher, whereas the diagnosis of preeclampsia was made if a high blood pressure was accompanied by proteinuria ≥300 mg in 24 hours (14). Birth before the 37th week of pregnancy was considered as a preterm birth. The threat of preterm labor was diagnosed when there were regular uterine contractions (4 per 20 minutes) without any cervical dilatation before the 37th week of pregnancy. Preterm premature rupture of the membranes (PPROM) was defined as rupture of the membranes before the onset of labor before the 37th week of pregnancy. Low birth weight was defined as a birth weight <2500 g.

A prospective cohort study design was chosen to conduct the study in order to minimize a potential bias that could result from the nature of the trial. In addition, ultrasonographic measurements and the data assessment were performed by two different researchers.

Data were analyzed by Chi-square, Mann–Whitney U test, and Student t-test using SPSS (Statistical Package for Social Sciences version 15.0, SPSS Inc.; Chicago, USA) program. A value of $p<0.05$ was considered as statistically significant. The parameters assumed to predict pregnancy outcomes were evaluated by receiver operating characteristic (ROC) curves.

Results

A total of 175 singleton pregnant women were enrolled in the study. Of these pregnancies, three were terminated due to missed abortion and one was terminated due to Trisomy 21. Thirteen patients were lost to follow-up at different phases of the study. The remaining 158 patients formed the study group. Table 1 summarizes the clinicodemographic characteristics of the study participants. Over the course of the follow-up period, a total of 17 (10.75%) pregnant women were determined to have at least one of the poor pregnancy outcomes (Table 2).

An ROC curve generated for the first-trimester maternal serum PAPP-A values is presented in Figure 1. Considering the cut-off value as a 0.72 multiple of the median (MoM), (Standard Error, SE: 0.039) (95% CI 0.646–0.798), the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) achieved for the poor pregnancy outcomes were 82.4%, 29.8%, 67%, and 56%, respectively.

In the ROC curve analysis of the arithmetic mean of PI of the right and left uterine arteries (UAPImean), the cut-off value was considered to be 1.08 with 58.8% sensitivity, 48.2% specificity, 76% PPV, and 55% NPV for poor pregnancy outcomes (Standard Error, SE: 0.039) (95% CI 0.646–0.798) (Figure 2). Pregnancy outcomes were compared between the two groups formed based

Table 1. Clinicodemographic characteristics of the study participants (n=158)

Characteristics	Descriptive values (mean±SD or n)	Additional explanation
Age	27.9±4.4 years	Range: 17–41
Weight	62.8±10.1 kg	Range: 42–108
Smoking	8 women (5.06%)	2–8 cigarettes day
First pregnancy	64 women (40.5%)	-
Previous poor pregnancy outcome	18 women (11.4%)	PIH, Preeclampsia, GDM, Preterm birth, PPROM, IUGR, Trisomy 21, Fetal anomaly
Concomitant disease	13 women (8.2%)	Hypo-hyperthyroidism, FMF, Psoriasis, Cardiac valve diseases, Chronic HT, Asthma, Thalassemia carrier
Medications	7 women (4.4%)	Levothyroxine, Colchicine, Alfa methyldopa

PIH: pregnancy-induced hypertension; GDM: gestational diabetes mellitus; PPROM: preterm premature rupture of the membranes; IUGR: intrauterine growth restriction; FMF: familial Mediterranean fever; HT: hypertension

Table 2. Numeric and proportional distribution of poor pregnancy outcomes determined during follow-up

Outcomes	Number of patients (n=17)	Percentage (%) (n=158)
PIH/Preeclampsia	5 (3/2)	3.16
Threat of preterm labor	1	0.63
PPROM	4	2.53
Preterm birth <37th week	14	8.86
Preterm birth <32nd week	-	-
Need for neonatal intensive care	7	4.43
Low birth weight	7	4.43
Placental abruption (ablatio placentae)	-	-

PIH: pregnancy-induced hypertension; PPROM: preterm premature rupture of the membranes

on this cut-off value; however no statistically significant difference was determined (p=0.582) (Table 3). After calculating the UAPImean for each pregnant woman, the mean value of the entire group was calculated as 1.18. The entire group was divided into two subgroups according to the UAPImean value: ≤1.18 or ≥1.19. There was no statistically significant difference between the two subgroups in terms of poor pregnancy outcomes (p=0.723), but the mean birth weights showed a significant difference (p=0.025), being lower in the subgroup with an UAPImean of ≥1.19 than that of the subgroup with an UAPImean ≤1.18 (Table 4).

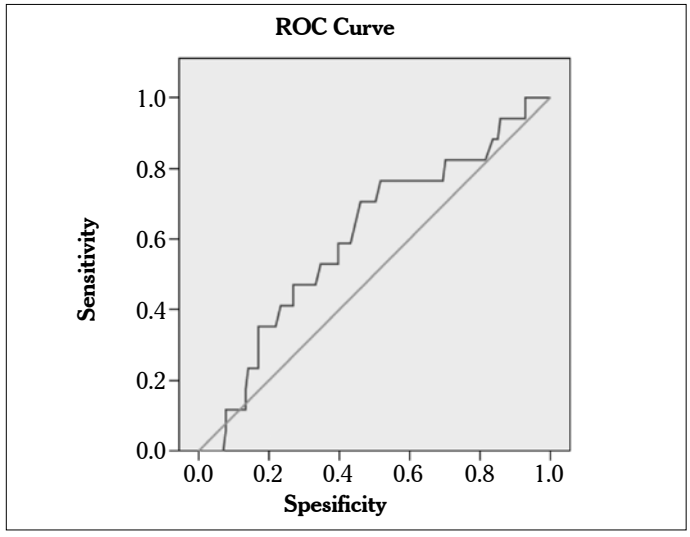

Figure 1. ROC curve for maternal serum PAPP-A level

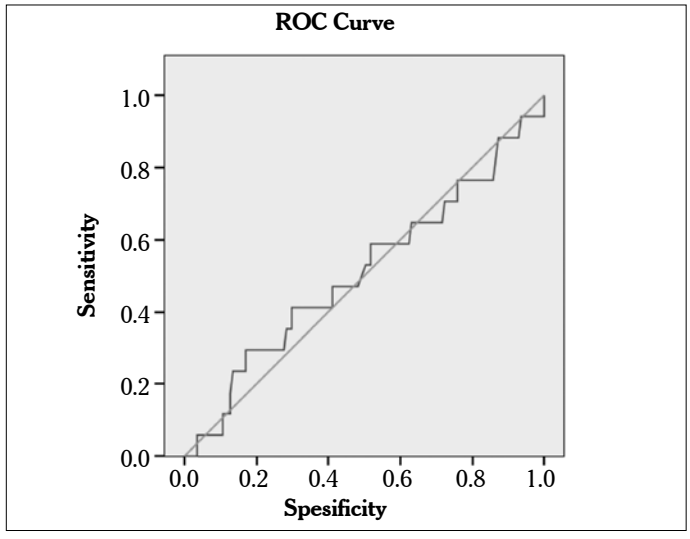

Figure 2. ROC curve for UAPImean

Table 3. Comparison of pregnancy outcomes between two subgroups formed according to the cut-off value of UAPImean determined by the ROC curve analysis

Outcomes	Number of patients (n=17)	Percentage (%) (n=158)
<1.08 (n=75)	7 (9.3%)	68 (90.7%)
≥1.08 (n=83)	10 (12%)	73 (88%)

*Chi-square test: p=0.582.

UAPImean: arithmetic mean of the pulsatility indices of the right and left uterine arteries; ROC curve: receiver operating characteristic curve

The mean RI (UARImean) value of both uterine arteries was calculated for each pregnant woman, and then an ROC curve was drawn (Figure 3). The sensitivity, specificity, PPV, and NPV values for the poor pregnancy outcomes were 58.8%, 34%, 36%, and 47%, respectively, when the cut-off value was taken as 0.582 (Standard Error, SE: 0.039) (95% CI 0.646–0.798). After calculat-

Table 4. Comparison of pregnancy outcomes and birth weight between two subgroups formed according to the UAPImean of the entire group

	Pregnancy outcome* (n=158)		Birth weight (g) (n=158)		
UAPImean	Poor (n=17)	Not poor (n=141)	Mean±SD**	Median	Range
≤1.18 (n=90)	9 (10%)	81 (90%)	3320.2±411.1	3350	2400–4105
≥1.19 (n=68)	8 (11.8%)	60 (88.2%)	3165.7±445.8	3145	1860–4600

*Chi-square test: p=0.723 for comparison of the pregnancy outcomes between the two subgroups.

**T-test: p=0.025 for comparison of the mean birth weight between the two subgroups.

UAPImean: arithmetic mean of the pulsatility indices of the right and left uterine arteries; SD: standard deviation

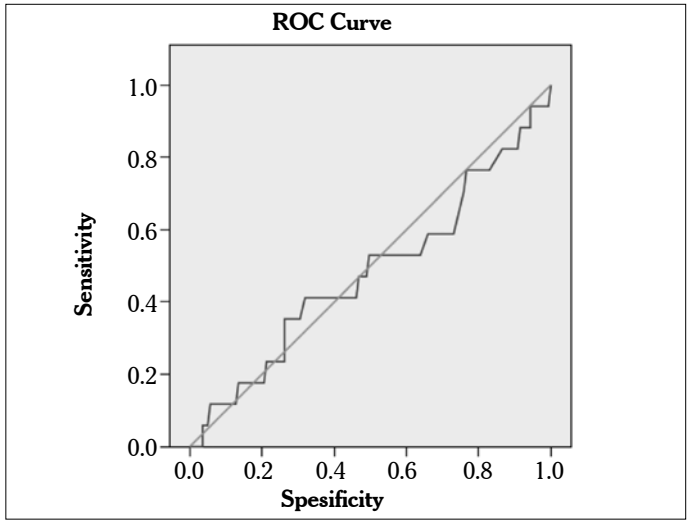

Figure 3. ROC curve for UARImean

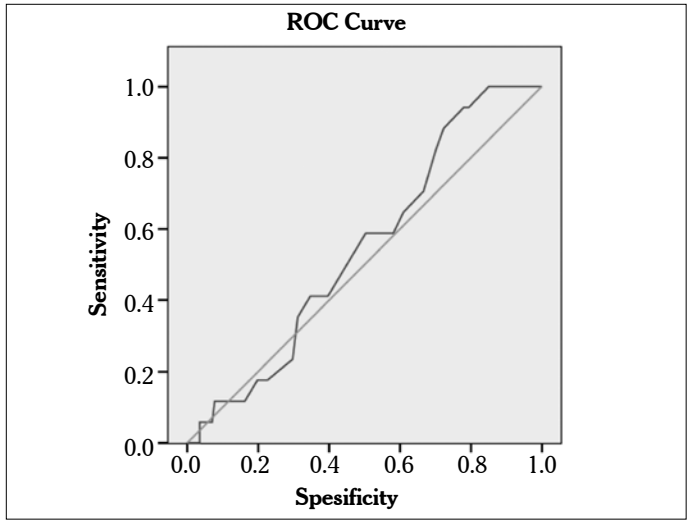

Figure 4. ROC curve for HC/AC ratio measured at the 20th–24th weeks of pregnancy

ing the UARImean for each pregnant woman, the mean value of the entire group was calculated as 0.62 and the entire group was divided into two subgroups according to the UARImean values: ≥0.62 or <0.62. No statistically significant difference was determined between the two subgroups in terms of pregnancy outcomes (p=0.797), while the mean birth weight was significantly higher in the subgroup with UARImean <0.62 than that of the subgroup with an UARImean of ≥0.62 (p=0.013) (Table 5). There was no significant difference between the women with unilateral uterine artery notch and the women with bilateral uterine artery notch in terms of pregnancy outcomes.

Receiver operating characteristic curves were drawn for HC/AC ratio measured at the 20th–24th and 28th–32nd weeks of pregnancy (Figure 4, 5, respectively). Taking the cut-off value of HC/AC ratio as 1.135 for the 20th–24th weeks of pregnancy (Standard Error, SE: 0.039) (95% CI 0.646–0.798), the sensitivity, specificity, PPV, and NPV for poor pregnancy outcomes were 58.8%, 49.6%, 56%, and 49%, respectively. The cut-off value of HC/AC ratio for the 28th–32nd weeks of pregnancy was taken as 1.075 (Standard Error, SE: 0.039) (95% CI 0.646–0.798) with 52.9% sensitivity, 41.1% specificity, 59% PPV, and 67% NPV.

Pregnant women considered to be under the risk of early-onset IUGR (inconsistent FBMs subgroup), as specified in the Material and Methods section, were compared with the pregnant women with whom the estimated gestational age according to the

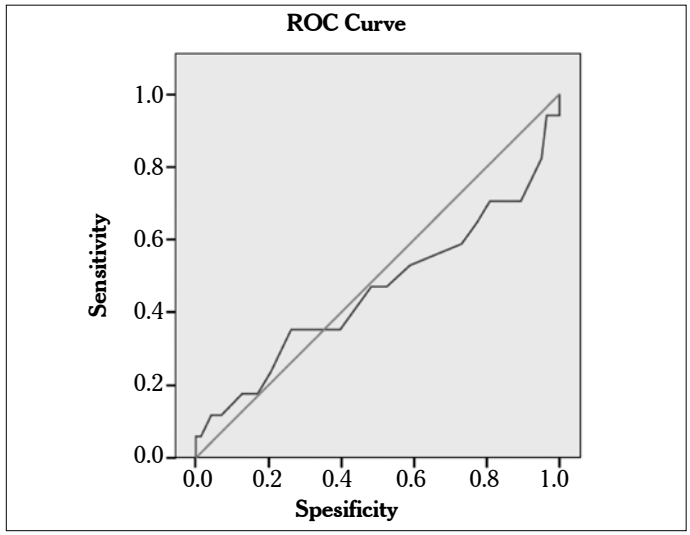

Figure 5. ROC curve for HC/AC ratio measured at the 28th–32nd weeks of pregnancy

fetal biometric measurement was consistent with the gestational age calculated according to the last menstrual period (consistent FBMs subgroup), in terms of the pregnancy outcomes and birth weight (Table 6). Statistically significant differences were

determined between the two subgroups in terms of pregnancy outcomes (p=0.045) and birth weight (p=0.011).

Discussion

Impaired placentation is one of the most important causes of poor pregnancy outcomes for mother and/or the baby. Researches on the etiology of unpleasant outcomes of pregnancy, such as preeclampsia, IUGR, and preterm birth, highlight the importance of normal placental development. Markers that could indicate these changes prior to the onset of disorder will provide us a chance to take early preventive measures and even to prevent this in the future. For this reason, the world of perinatology has carried out many studies, particularly in recent years, on numerous placental biochemical markers, various Doppler ultrasound techniques and parameters, and various ultrasonographic methods to evaluate fetal and placental development in order to determine placental defects (1, 8, 10, 11). We aimed to determine the predictive value of the maternal serum PAPP-A level of the first trimester, uterine artery Doppler velocimetry of the second trimester, and FBMs of the second and third trimester for poor pregnancy and poor neonatal outcomes which would probably occur due to placentation defect in pregnant women, and we found that the early prediction of unfavorable maternal and unfavorable neonatal outcomes of placental insufficiency might be possible using some test results, such as FBMs or an assessment of the HC/AC ratio, even though there is no method that can be used alone as a screening test.

Table 5. Comparison of pregnancy outcomes and birth weight between two subgroups formed according to the UARImean of the entire group

	Pregnancy outcome* (n=158)		Birth weight (g)** (n=158)
UARImean	Poor (n=17)	Not poor (n=141)	Mean±SD**
<0.62 (n=79)	8 (10.1%)	71 (89.1)	3338.3±413.5
≥0.62 (n=79)	9 (11.4%)	70 (%88.6)	3169.1±435.8

*Chi-square test: p=0.797 for comparison of the pregnancy outcomes between the two subgroups.
**T-test: p=0.013 for comparison of the mean birth weight between the two subgroups.
UARImean: arithmetic mean of the resistance indices of the right and left uterine arteries

The association between low hCG (<0.5 MoM) and PAPP-A (<0.4 MoM) levels, which are measured on the 10th–14th weeks of pregnancy, and the complications of pregnancy has been demonstrated previously (15, 16). In the present study as well, poor pregnancy outcomes could be predicted with 82.4% sensitivity and 29.8% specificity when the cut-off value was taken as 0.72 MoM in the ROC curve drawn for PAPP-A, which was measured in the 11th–14th weeks of pregnancy.

Doppler ultrasonography of the uterine arteries is the other method used for the early prediction of pathological placentation and can be performed at various weeks of pregnancy. In the present study, a Doppler ultrasound of uterine arteries was performed on the 15th–18th weeks of pregnancy, the mean PI value of both sides was calculated (UAPImean), and then an ROC curve was drawn. We achieved a sensitivity of 58.8% and a specificity of 48.2% for poor pregnancy outcomes when the cut-off value was taken as 1.08. The average value of the entire group of pregnant women for UAPImean was found to be 1.18±0.40, with no significant difference determined between pregnancy outcomes when the group was divided into two based on this value. However, a statistically significant difference was determined in terms of the mean birth weight (p=0.025). The results, thus, indicate that increased uterine artery PI might cause a decrease in birth weight. Cooper et al. (17) conducted a study in 229 pregnant women and underlined the value of an increased mean uterine artery PI measured at the 22nd week of pregnancy in predicting preterm birth, the small for gestational age (SGA), and a low birth weight, particularly in pregnant women with a PAPP-A value lower than 0.4 MoM. Pilalis et al. (18) evaluated 878 pregnant women on the 11th–14th weeks of pregnancy and emphasized that each of the Doppler ultrasound of uterine arteries and the PAPP-A value is an independent factor for predicting SGA and that the combination of both is more effective for prediction. The present study, showing an inverse relation between uterine artery PI and birth weight, differs from the other studies in that uterine artery PI has been evaluated within a different gestational age period of pregnancy. Nevertheless, it is necessary to underline that a normal PI does not exclude obstetric complications, as was demonstrated in almost all studies.

RI is another parameter assessed by Doppler ultrasound of the uterine arteries. In the present study, the mean RI of the uterine arteries (UARImean) was calculated for each pregnant woman and then an ROC curve was drawn. We were able to predict poor pregnancy outcome with 58.8% sensitivity and 34% specificity when the cut-off value was taken as 0.582. The average

Table 6. Comparison of pregnancy outcomes and birth weight between two subgroups formed according to fetal biometric measurements

UAPImean	Pregnancy outcome* (n=158)		Birth weight (g) (n=158)		
	Poor (n=17)	Not poor (n=141)	Mean±SD**	Median	Range
Consistent (n=130)	11 (8.5%)	119 (91.5%)	3292.1±440.7	3313.5	1860–4600
Inconsistent (n=28)	6 (21.4%)	22 (78.6%)	3075.7±341.1	3110	2500–3740

*Chi-square test: p=0.045 for comparison of the pregnancy outcomes between the two Subgroups.
**Mann–Whitney U test: p=0.011 for comparison of the mean birth weight between the two Subgroups.

value of UARImean was calculated for the whole group and the group was divided into two according to this value (0.62±0.10). We failed to determine a significant difference between the groups in terms of obstetric complications; however, there was significant difference between the mean birth weights of the groups (p=0.013). This result suggests that increased uterine artery RI, as well as increased PI, might cause a decrease in birth weight. The literature includes studies propounding that increased uterine artery RI and uterine notch can predict preeclampsia and SGA (19, 20). In these studies, which were performed in large population groups and usually in the 18th–24th weeks of pregnancy, the sensitivity reached 63–95% when RI was above the 95th percentile or when the limit values of 0.56–0.58 were used for RI (21, 22). The RI found in the present study is close to those determined in earlier studies, although the cut-off value 0.582 was measured in a different gestational age period of pregnancy. The present study found no significant difference between the women with unilateral uterine artery notch and the women with bilateral uterine artery notch in terms of pregnancy outcomes.

Ultrasonographic biometry has always been an important tool in monitoring fetal development. In the present study, the pregnant women with whom estimated gestational age that was calculated based on ultrasonographic FBMs performed at the 20th–24th and 28th–32nd weeks of pregnancy was more than 1 week behind the gestational age calculated according to the last menstrual period and the pregnant women with an HC/AC ratio over 1.15 and 1.10 at the 20th–24th and 28th–32nd weeks of pregnancy, respectively, were considered to be under a risk of early onset fetal growth restriction. This high-risk group was compared with the other group of pregnant women and statistically significant differences were determined in terms of pregnancy outcomes (p=0.045) and mean birth weight (p=0.011). In the literature, studies on this subject are not so many, excluding two separate studies conducted by Fox et al. (12, 13) suggesting an association between a HC/AC ratio >90th percentile and poor pregnancy outcomes and the study conducted by Colley et al. (23) determining a weak correlation between the HC/AC ratio and the ponderal index. In addition to the literature information, the present study indicated that it is possible to obtain significant results in terms of pregnancy outcomes and birth weight in the light of precise FBMs and an assessment of the HC/AC ratio (23, 24).

Our study has some limitations. First, power calculation was not performed, and we had a small sample size in terms of the subgroup with poor pregnancy outcomes. Second, we did not combine the results of the tests to analyze the predictive values of the combinations of current biochemical and ultrasonographic methods.

In conclusion, we aimed to determine the predictive value of maternal serum PAPP-A levels, uterine artery Doppler velocimetry, and FBMs for poor pregnancy and poor neonatal outcomes in pregnant women and found that serum PAPP-A levels and FBMs could be used for predicting pregnancy outcomes, while uterine artery Doppler velocimetry and FBMs could be used for predicting neonatal outcomes, specifically the birth weight. Therefore, an early prediction of unfavorable maternal and neonatal outcomes of placental insufficiency might be possible using some test results, such as FBMs or by the assessment of the HC/AC ratio, even though there is no method that can be used alone as a screening test. Therefore, different combinations of current biochemical and ultrasonographic methods may be necessary. It is clear that a screening test including certain combinations of biochemical and ultrasonographic markers with certain cut-off values, which will be created in multicenter studies with larger study populations, is needed in the future in order to predict obstetric complications earlier.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Dokuz Eylül University School of Medicine.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

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

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References

1. Smith GC, Shah I, Crossley JA, Aitken DA, Pell JP, Nelson SM, et al. Pregnancy-associated plasma protein A and alpha-fetoprotein and prediction of adverse perinatal outcome. Obstet Gynecol 2006; 107: 161-6. [Crossref]
2. Van den Elzen HJ, Cohen-Overbeek TE, Grobbee DE, Quartero RW, Wladimiroff JW. Early uterine artery Doppler velocimetry and the outcome of pregnancy in women aged 35 years and older. Ultrasound Obstet Gynecol 1995; 5: 328-33. [Crossref]
3. Martin AM, Bindra R, Curcio P, Cicero S, Nicolaides KH. Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler at 11-14 weeks of gestation. Ultrasound Obstet Gynecol 2001; 18: 583-6. [Crossref]
4. Dugoff L, Lynch AM, Cioffi-Ragan D, Hobbins JC, Schultz LK, Malone FD, et al. First trimester uterine artery Doppler abnormalities predict subsequent intrauterine growth restriction. Am J Obstet Gynecol 2005; 193: 1208-12. [Crossref]
5. Campbell S, Diaz-Recasens J, Griffin DR, Cohen-Overbeek TE, Pearce JM, Willson K, et al. New doppler technique for assessing uteroplacental blood flow. Lancet 1983; 1: 675-7.[Crossref]
6. Harrington K, Cooper D, Lees C, Hecher K, Campbell S. Doppler ultrasound of the uterine arteries: the importance of bilateral notching in the prediction of pre-eclampsia, placental abruption or delivery of a small-for-gestational-age baby. Ultrasound Obstet Gynecol 1996; 7: 182-8. [Crossref]
7. Steel SA, Pearce JM, McParland P, Chamberlain GV. Early Doppler ultrasound screening in prediction of hypertensive disorders of pregnancy. Lancet 1990; 335: 1548-51. [Crossref]
8. Huang T, Hoffman B, Meschino W, Kingdom J, Okun N. Prediction of adverse pregnancy outcomes by combinations of first and second trimester biochemistry markers used in the routine prenatal screening of Down syndrome. Prenat Diagn 2010; 30: 471-7. [Crossref]
9. Salvig JD, Kirkegaard I, Winding TN, Henriksen TB, Torring N, Uldbjerg N. Low PAPP-A in the first trimester is associated with reduced fetal growth rate prior to gestational week 20. Prenat Diagn 2010; 30: 503-8. [Crossref]

10. Berry E, Aitken DA, Crossley JA, Macri JN, Connor JM. Screening for Down's syndrome: changes in marker levels and detection rates between first and second trimesters. Br J Obstet Gynaecol 1997; 104: 811-7. [Crossref]
11. Consuegra-Sanchez L, Fredericks S, Kaski JC. Pregnancy-associated plasma protein A: Has this biomarker crossed the boundary from research to clinical practice? Drug News Perspect 2009; 22: 341-8. [Crossref]
12. Fox NS, Shalom D, Chasen ST. Second-trimester fetal growth as a predictor of poor obstetric and neonatal outcome in patients with low first-trimester serum pregnancy-associated plasma protein-A and a euploid fetus. Ultrasound Obstet Gynecol 2009; 33: 34-8. [Crossref]
13. Fox NS, Huang M, Chasen ST. Second-trimester fetal growth and the risk of poor obstetric and neonatal outcomes. Ultrasound Obstet Gynecol 2008; 32: 61-5. [Crossref]
14. ACOG Committee on Obstetric Practice. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. Int J Gynaecol Obstet 2002; 77: 67-75.
15. Morris RK, Cnossen JS, Langejans M, Robson SC, Kleijnen J, Ter Riet G, et al. Serum screening with Down's syndrome markers to predict pre-eclampsia and small for gestational age: systematic review and meta-analysis. BMC Pregnancy Childbirth 2008; 8: 33. [Crossref]
16. Gagnon A, Wilson RD, Audibert F, Allen VM, Blight C, Brock JA, et al. Obstetrical complications associated with abnormal maternal serum markers analytes. J Obstet Gynaecol Can 2008; 30: 918-49. [Crossref]
17. Cooper S, Johnson JA, Metcalfe A, Pollard J, Simrose R, Connors G, et al. The predictive value of 18 and 22 week uterine artery Doppler in patients with low first trimester maternal serum PAPP-A. Prenat Diagn 2009; 29: 245-8. [Crossref]
18. Pilalis A, Souka AP, Antsaklis P, Basayiannis K, Benardis P, Haidopoulos D, et al. Screening for pre-eclampsia and small for gestational age fetuses at the 11-14 weeks scan by uterine artery Dopplers. Acta Obstet Gynecol Scand 2007; 86: 530-4. [Crossref]
19. Arduini D, Rizzo G. Normal values of Pulsatility Index from fetal vessels: a cross-sectional study on 1556 healthy fetuses. J Perinat Med 1990; 18: 165-72. [Crossref]
20. Dane B, Dane C, Kiray M, Cetin A, Koldas M, Erginbas M. Correlation between first-trimester maternal serum markers, second-trimester uterine artery doppler indices and pregnancy outcome. Gynecol Obstet Invest 2010; 70: 126-31. [Crossref]
21. Fonseca E, Yu CK, Singh M, Papageorghiou AT, Nicolaides KH. Relationship between second-trimester uterine artery Doppler and spontaneous early preterm delivery. Ultrasound Obstet Gynecol 2006; 27: 301-5. [Crossref]
22. Jacobson SL, Imhof R, Manning N, Mannion V, Little D, Rey E, et al. The value of Doppler assessment of the uteroplacental circulation in predicting preeclampsia or intrauterine growth retardation. Am J Obstet Gynecol 1990; 162: 110-4. [Crossref]
23. Colley NV, Tremble JM, Henson GL, Cole TJ. Head circumference/abdominalcircumference ratio, ponderal index and fetal malnutrition. Should head circumference/abdominal circumference ratio be abandoned? Br J Obstet Gynaecol 1991; 98: 524-7. [Crossref]
24. Chambers SE, Hoskins PR, Haddad NG, Johnstone FD, McDicken WN, Muir BB. A comparison of fetal abdominal circumference measurements and Doppler ultrasound in the prediction of small-for-dates babies and fetal compromise. Br J Obstet Gynaecol 1989; 96: 803-8. [Crossref]

The effect of adenomyosis on the outcomes of laparoscopic hysterectomy

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Abstract

Objective: The presence of adenomyosis (ADS) may increase complication rates associated with laparoscopic hysterectomy (LH) due to an increased weight of the uterus, increased vascularization of the uterus, impaired myometrial tissue, and presence of additional gynecological pathologies such as leiomyoma or endometriosis. The aim of the present study was to evaluate perioperative and early postoperative parameters in patients with or without adenomyotic lesions.

Material and Methods: The study included patients who underwent LH in a university hospital. Patient data were retrieved from the hospital records and reviewed retrospectively. Sixty-one patients (85.9%) without adenomyotic lesions comprised the control group. Ten patients with adenomyotic lesions (14.1%) were regarded as the study group.

Results: In this study, the mean age of the patients was 50.93±9.39 years. The mean uterus size was significantly higher in patients with ADS (p=0.02). There was no statistically significant difference in perioperative variables such as delta hemoglobin (Hb), insertion of pelvic drainage catheter, and invasive assessment of the urinary tract between both the groups (p=0.27, p=1.0, and p=0.67, respectively). The difference between the groups in terms of postoperative blood transfusion was not statistically significant (p=0.25). There was no statistically significant difference in the postoperative maximum body temperature, length of hospital stay, and duration of urinary catheterization between both the groups (p=0.77, p=0.36, and p=0.75, respectively).

Conclusion: LH appears to be a safe alternative for patients with ADS. Large-scale, prospective, and randomized trials are required in order to suggest the routine use of LH in patients preoperatively diagnosed with ADS. (J Turk Ger Gynecol Assoc 2016; 17: 150-4)

Keywords: Adenomyosis, laparoscopic hysterectomy, outcomes

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Introduction

Adenomyosis (ADS) is a benign gynecological condition where ectopic endometrial glands invade into the myometrium, resulting in hyperplasia of the adjacent smooth muscle (1). The prevalence of ADS in hysterectomy specimens was found to be 28.2% in a study (2). Studies have demonstrated the occurrence of invasive adenomyotic lesions associated with increased levels of matrix metalloproteinases (MMPs) and showed increased levels of vascular endothelial growth factor (VEGF) in patients with ADS (3, 4). The progressive enlargement of the uterus due to adenomyotic lesions is considered to cause symptoms such as abnormal uterine bleeding (AUB), dysmenorrhea, and uterine tenderness (5). ADS is often accompanied by endometriosis and leiomyomas (5). However, there is a debate over the definitive diagnosis of ADS in the preoperative period based on demographic features and patient symptoms (6).

There is currently no diagnostic imaging method or standard treatment approach for ADS (7). Although medical therapies involving antiestrogenic drugs, gonadotropin-releasing hormone agonists (GnRH-a), and levonorgestrel-releasing intrauterine systems are used to control the symptoms of ADS as it is an estrogen-dependent condition, hysterectomy still remains the definitive treatment for this disease (5, 8). Abdominal, vaginal, or laparoscopic hysterectomy (LH) can be performed in patients that are considered to have ADS (5).

LH is a popular technique. Because it has better cosmetic results and short recovery time (9).Performing LH is popular among surgeons because it facilitates faster recovery, faster return to work, and better cosmetic outcomes (9). Wu et al. (10) declared that LH is performed in 12% of cases. LH has a rate of 10% among other hysterectomy methods. Overall, LH is not preferred due to higher rates of major surgical complications (11). On the other hand, complication rates in LH increase as the uterus size increases (12). Supracervical LH can be performed in patients with benign uterine conditions such as ADS. However, Sasaki et al. (13) argued that postoperative persistent vaginal bleeding may present due to residual adenomyotic foci in the endometrial tissue remaining in the cervical stump or adenomyotic lesions that may get implanted on the stump during morcellation after supracervical LH.

The presence of ADS may increase complication rates associated with LH due to the increased weight of uterus, in-

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creased vascularization of uterus, impaired myometrial tissue, and presence of additional gynecological pathologies such as leiomyoma or endometriosis. The aim of the present study was to evaluate perioperative and early postoperative parameters in patients with or without adenomyotic lesions.

Material and Methods

The study included patients who underwent LH in the Department of Obstetrics and Gynecology at Düzce University School of Medicine between August 1, 2012, and December 1, 2015. Patient data were retrieved from the university records and retrospectively reviewed. Out of the 94 patients who underwent LH, 71 were found to be eligible for our study. The study group comprised patients who were found to have adenomyotic lesions in the postoperative histopathologic examination of tissue sections, and the control group comprised patients who did not have any adenomyotic lesions.

All patients underwent bimanual pelvic examination, transvaginal ultrasonography (USG), cervico-vaginal smear, and endometrial tissue sampling in order to rule out the presence of any malignancy before surgery (9). The patients that had undergone cesarean section, hysterotomy, adnexal surgery, appendectomy, rectosigmoid surgery, or similar surgery in the pelvic area in the past were considered to have a positive history of pelvic surgery. The patients with uterovaginal prolapse, descensus uteri, and any defect in the anterior or posterior compartment of the vagina in the preoperative period were considered to have prolapse of the pelvic organs. The patients who were found to have focal/generalized and simple/complex endometrial hyperplasia with or without atypical changes in the preoperative examination of the endometrial tissue samples were classified under endometrial hyperplasia, which was used as an indication for surgery. The patients with a preoperative diagnosis of AUB who were found to have a demonstrable uterine pathology such as accompanying leiomyoma of the uterus, endometrial polyps, or endometrial hyperplasia were classified under the relevant uterine pathology as an indication for surgery. The patients with uterine bleeding but without a demonstrable cause were preoperatively classified as AUB.

Bowel preparation involved the administration of oral laxatives and rectal enema 1 day before the surgery. The anesthesiologist recorded the operation time based on the patient follow-up forms. Delta hemoglobin (Hb) was defined as the difference between preoperative and postoperative Hb levels (9). A pelvic drainage system was placed before the completion of surgery in some patients, if deemed necessary by the attending surgeon. The diagnosis of postoperative fever was confirmed only when the body temperature was ≥100.4°F (38°C) in 2 subsequent measurements within 4- to 6-hour intervals 24 hours after the surgery or if body temperature persisted at ≥101°F (38.3°C) in the postoperative period (14). In patients without fever, the highest body temperature measured in the postoperative period was recorded as the "maximum body temperature." Massive hemorrhage, need for reoperation due to other reasons, urinary tract injuries, and bowel and major vessel injuries were considered as major complications.

The patients were re-assessed every 24 hours in the postoperative period. The time to remove urinary catheters and the discharge time were recorded as the time interval during which they occurred, namely, 0–24, 24–48, or 48–72 hours. The patients without the need for opioids, exhibiting urinary retention, and mobile patients who were able to dress on their own were discharged from the hospital (15).

Dr. A.Y. was involved in all the surgeries considered in this study, and LH was performed with the technique used by this surgeon. The patients who underwent surgery using other techniques and patients who underwent supracervical LH or non-gynecologic additional procedures, patients whose medical records could not obtained, and patients who were found to have a malignancy in the histopathologic examination of frozen sections or during preoperative/postoperative histopathologic examination were excluded from our study. The operation time was missing for 1 patient. The excised specimens were examined, and the pathology reports were issued by the same pathology department.

All procedures were performed under general anesthesia: the patients were placed in the lithotomy position. Urinary catheterization and insertion of a nasogastric tube were performed in all the patients. A manipulator was placed in the uterine cavity. The surgeon remained on the left-hand side of the patient during surgery. After creating pneumoperitoneum and a 10-mm trocar sleeve placement, a 10-mm telescope was passed through this sleeve. Two 5-mm trocar sleeves were placed in the right and left hypochondrium regions lateral to the rectus abdominis muscle and inferior epigastric vessels in the outer one-third of the distance between the anterior superior iliac spine and umbilicus. A 5- or 10-mm trocar sleeve was placed 3 cm below the left costal margin at the midclavicular line (Palmer's point). A vessel-sealing device (LigaSure™, Covidien; Boulder, Colorado, USA) or ultrasonic scalpel (Harmonic Ace®, Ethicon Endo-Surgery Inc.; Cincinnati, OH, USA) was passed through the trocar placed at the Palmer's point. A 5-mm dissector and grasping forceps were passed through the trocars placed in the two lower quadrants. A manipulator was used to elevate the uterus from the pelvic floor during surgery, while paying particular attention to the possible projection of the ureter below the peritoneum. Hemostasis was achieved in the infundibulopelvic ligament in patients that underwent salpingo-oophorectomy and in the utero-ovarian ligament in patients that did not undergo salpingo-oophorectomy. The bladder was dissected from the lower segment of the uterus and superior portion of the vagina. The uterine arteries were skeletonized, coagulated, and separated on both sides using LigaSure. The vaginal wall was completely separated from the cervix using Harmonic Ace® above the sacrouterine ligaments and vaginal fornices delineated with a manipulator. The vaginal cuff was stitched with absorbable sutures having increased strength using the transvaginal or laparoscopic approach.

For a large uterus (weight >280 g or gestation size >12 weeks) during inspection before surgery, the operation proceeded with the method used by Yavuzcan et al. (9) in their previous study.

Patients in whom the trajectory of the ureter could not be clearly observed or in patients with a high index of suspicion

for injury to the urinary tract or patients that had a difficult bladder dissection underwent diagnostic cystoscopy and/or ureteral catheterization at the end of the procedure. These patients were recorded as "patients that underwent invasive assessment of the integrity of the urinary tract." Surgical procedures to treat stress urinary incontinence, corrective surgery for the prolapse of pelvic organs, and perineoplasty procedures were regarded as additional genitourinary surgical procedures.

In the present study, the histopathological examination of multiple sections obtained from the hysterectomy specimens revealed the presence of ADS in 10 patients (14.1%), which comprised the study group. The remaining 61 patients (85.9%) without ADS comprised the control group.

Descriptive statistics included mean, standard deviation, and ratio. Data from the t-test performed on the independent samples was used in the analysis of the qualitative data, and Fisher's exact test was used to compare the quantitative data between both the groups. SPSS version 19.0 (IBM SPSS Statistics for Windows, Version 19.0, IBM Corporation; New York, USA) software package was used in the statistical analysis. In our case, $p<0.05$ was considered to be statistically significant.

Results

In this study, the mean age of the patients was 50.93±9.39 years. The indication for surgery was uterine fibroids in 23 patients (32.4%) and AUB in 14 patients (19.7%), and prediagnosis of ADS in the preoperative period happened in only 1 patient (1.4%). Out of the patients that underwent LH, 14 patients (19.7%) also underwent additional surgery to their genitourinary system. The characteristics of the patients are presented in Table 1.

Out of the patients who exhibited adenomyotic lesions, 6 patients (60%) had concurrent leiomyoma, 4 patients (40%) had chronic cervicitis, and 2 patients (20%) had salpingitis isthmica nodosa (SIN). Out of the 61 patients (85.9%) without adenomyotic lesions in the control group, 41 patients (67.2%) had concurrent chronic cervicitis, 29 patients (47.5%) had leiomyoma, and 5 patients (8.1%) had 1 of the subtypes of endometrial hyperplasia.

The mean uterus size was significantly higher in patients with adenomyotic lesions (290.30±179.41 vs. 173.52±138.49 g, $p=0.02$). There was no statistically significant difference in the perioperative variables such as delta Hb, insertion of pelvic drainage catheter, and invasive assessment of the urinary tract between both the groups ($p=0.27$, $p=1.0$, and $p=0.67$, respectively) (Table 2).

Blood transfusion was required in the postoperative period in 2 patients (20%) with adenomyotic lesions in the study group and 5 patients (8.1%) without adenomyotic lesions in the control group; however, the difference between the groups was not statistically significant ($p=0.25$). There was no statistically significant difference in the postoperative maximum body temperature, length of hospital stay, and duration of urinary catheterization between both the groups ($p=0.77$, $p=0.36$, and $p=0.75$, respectively) (Table 3).

Table 1. General patients' characteristics

	n=71/ (100%)	Mean±SD	Min. - Max.
Age	71 (100%)	50.93±9.39	38-79
Parity	71 (100%)	3.38±2.00	0-9
Gravida	71 (100%)	3.87±2.70	0-17
Past history of pelvic surgery (+)	20 (28.2%)		
Use of morcellation	5 (7%)		
Additional genitourinary surgery	14 (19.7%)		
Mean operation time, t (min)γ	70 (98.5%)	158.39±45.54	75-300
Major complication	3 (4.2%)		
Indications			
• Leiomyoma	23 (32.4%)		
• AUB	14 (19.7%)		
• Adnexial mass	7 (9.9%)		
• Prolapse of the pelvic organs	6 (8.5%)		
• Endometrial hyperplasia	5 (7.0%)		
• Pelvic mass	4 (5.6%)		
• Cervical premalignant lesion	3 (4.2%)		
• ADS+chronic pelvic pain	1 (1.4%)		
• Other	8 (11.2%)		

γmissing data in 1 patient
SD: standard deviation; AUB: abnormal uterine bleeding; ADS: adenomyosis

Table 2. Comparison of perioperative variables

	ADS (-) n=61 (85.9%)	ADS (+) n=10 (14.1%)	p
Weight of uterus (gr.)α	173.52±138.49	290.30±179.41	0.02
Delta Hb (gr/dL)α	1.64±1.11	2.14±2.36	0.27
Pelvic drainage catheter[a]	18 (29.5%)	3 (33.3%)	1.0
Inv. assess. of urinary tract.[a]	12 (19.6%)	1 (10%)	0.67

αMean±Standard Deviation
[a]nv (%)
*$p<0.05$ was considered statistically significant.
Inv. assess. of urinary tract.: patients that underwent invasive assessment of the integrity of the urinary tract; ADS: adenomyosis; Hb: hemoglobin

Table 3. Comparison of postoperative variables

	ADS (-) (n=61)	ADS (+) (n=10)	p
Length of Hospital Stay (days)α	3.38±1.62	2.90±0.73	0.36
Urinary Catheterization (days)α	1.23±0.66	1.30±0.48	0.75
Postoper. max. body temp. (°C)α	36.78±0.50	36.82±0.27	0.77
Blood Tx. Requirement[a]	5 (8.1%)	2 (20%)	0.25

αMean±Standard Deviation
[a]n (%)
*$p<0.05$ was considered statistically significant.
Postoper. max. body temp.: postoperative maximum body temperature
ADS: adenomyosis

Discussion

The definitive diagnosis of ADS is based on the examination of hysterectomy materials in the postoperative period (16). There are ongoing studies attempting to accurately diagnose ADS using noninvasive and minimally invasive methods (16). USG and magnetic resonance imaging (MRI) can be helpful in the preoperative period (16). Also, Exacoustos et al. (17) reported that the coronal section of the uterus obtained by three-dimensional transvaginal ultrasound (3D-TVS) permits the accurate evaluation and measurement of the JZ, and its alteration has good diagnostic accuracy for ADS. In the present study, only 1 patient (1.4%) was diagnosed with ADS in the preoperative period. A hysteroscopic biopsy can diagnose ADS (18). Traditionally, laparoscopy has no place in the diagnosis of ADS (16). On the other hand, minimally invasive procedures may be used to treat ADS. In a recent study it was estimated that laparoscopic or robotic adenomyomectomy was feasible and safe for women with severely symptomatic ADS who requested uterine preservation (19). ADS does not usually cause marked pathological changes in the serosa of the uterus. On the other hand, Graziano et al. (16) reported that an irregular structure of the uterus, blue discoloration after performing the methylene blue dye test, and decreased resistance of the uterus during manipulation could be characteristic features of ADS.

Laparoscopic findings of ADS are probably associated with underlying endometrial pathological lesions and altered uterine vascularization. ADS islets invading deep into the endometrial line are scattered within the muscle structure of the uterus (20). Although various definitions have been made regarding the distance of invasion below the endometrial line, definitive criteria are still debated (16). The posterior wall of the uterus is commonly involved in ADS, which exhibits irregular enlargement (1). In the present study, the mean weight of the uterus was significantly higher in patients with adenomyotic lesions (290.30±179.41 g vs. 173.52±138.49 g, p=0.02). Uterine fibroids are common findings accompanying ADS. The occurrence of uterine fibroids is another condition that causes the enlargement of the uterus. Vercellini et al. (20) reported that ADS is accompanied by uterine fibroids and menorrhagia in 23% patients. Similarly, in the present study, 60% patients with adenomyotic lesions had concurrent uterine leiomyoma. It is also known that an increasing weight of the uterus decreases visibility and unfavorably influences the performance of endoscopic devices (21). Increased weight of the uterus is also associated with increased perioperative blood loss during LH (22). The structure of vessels supplying a large uterus becomes thicker (22). Goteri et al. (4) claimed that there has been a marked increase in the development of microvessels in patients with ADS. The lesions of ADS have been shown to be hypervascularized tissues due to high VEGF content (23). Considering all these factors, increased perioperative blood loss and increased need for postoperative blood transfusion can be expected during LH in patients with ADS. However, there was no statistically significant difference in delta Hb (p=0.27) and need for blood transfusion (p=0.25) between patients with or without adenomyotic lesions in our study.

Urinary tract injury occurred in 1.3% of LH operations, with ureteral injuries almost as common as bladder injuries (24). American College of Obstetrics and Gynecology (ACOG) recommended the use of cystoscopy in patients with a high index of suspicion for urinary tract injuries (25). The highest risk of ureter trauma occurs in the neighborhood of the uterine artery (24). Manoucheri et al. (26) claimed that performing cranial deviation of the uterus with upward pressure on the uterine manipulator before cauterizing or incising uterine vessels or using upward traction on the uterine specimen with a laparoscopic tenaculum in lieu of a uterine manipulator are important for avoiding ureter injuries. Traction and countertraction are two important steps for maintaining hemostasis at the uterine artery level while performing LH (27). The difficulty of manipulating the uterus reported among the findings of ADS in the study by Graziano et al. (16) is possibly associated with the increased level of MMPs and increased angiogenesis (3). MMPs are proteolytic enzymes, which have the potential capacity of degrading the extracellular matrix (ECM) components (28). In particular, MMP-2 and MMP-9 disintegrate type-IV collagen, which is the main component of the basement membrane (29). A study has demonstrated a marked increase in MMP-2 and MMP-9 levels in the ADS tissue (3). An invasive assessment of the urinary tract, placement of pelvic drainage system to monitor postoperative hemorrhage, and performing prolonged urinary catheterization due to urinary tract injury can be expected in patients with ADS having difficulty in uterine manipulation and additional gynecologic pathologies such as leiomyoma and endometriosis; however, the present study found no difference in these parameters between patients with or without adenomyotic lesions (p=0.67, p=1.0, and p=0.75, respectively).

Further, VanEvert et al. (30) reported that LH is associated with a reduction in postoperative wound infections. Brummer et al. (31) reported that a febrile event is less common with a more experienced LH surgeon. The experience of the surgeon who has performed fewer than 30 LHs was an independent risk factor for febrile events. All of the operations were performed under the management of the same experienced surgeon in the present study, so that none of the patients had postoperative fever and there was no difference between both the groups in terms of postoperative maximum body temperature (p=0.77). In addition, the length of hospital stay did not differ significantly between patients with or without adenomyotic lesions (p=0.36).

According to the results of the present study, LH appears to be a safe alternative in patients with ADS. Large-scale, prospective, and randomized trials are required in order to suggest the routine use of LH in patients preoperatively diagnosed with ADS.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Düzce University School of Medicine (2016/32).*

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References

1. Ferenczy A. Pathophysiology of Adenomyosis. Hum Reprod Update 1998; 4: 312-22. [Crossref]
2. Parazzini F, Mais V, Cipriani S, Busacca M, Venturini P; GISE. Determinants of Adenomyosis in women who underwent hysterectomy for benign gynecological conditions: results from a prospective multicentric study in Italy. Eur J Obstet Gynecol Reprod Biol 2009; 143: 103-6. [Crossref]
3. Li T, Li YG, Pu DM. Matrix metalloproteinase-2 and -9 expression correlated with angiogenesis in human Adenomyosis. Gynecol Obstet Invest 2006; 62: 229-35. [Crossref]
4. Goteri G, Lucarini G, Montik N, Zizzi A, Stramazzotti D, Fabris G, et al. Expression of vascular endothelial growth factor (VEGF), hypoxia inducible factor-1 alpha (HIF-1 alpha), and microvessel density in endometrial tissue in women with adenomyosis. Int J Gynecol Pathol 2009; 28: 157-63. [Crossref]
5. Pepas L, Deguara C, Davis C. Update on the surgical management of Adenomyosis. Curr Opin Obstet Gynecol 2012; 24: 259-64. [Crossref]
6. Vercellini P, Viganò P, Somigliana E, Daguati R, Abbiati A, Fedele L. Adenomyosis: epidemiological factors. Best Pract Res Clin Obstet Gynaecol 2006; 20: 465-77. [Crossref]
7. Struble J, Reid S, Bedaiwy MA. Adenomyosis; A Clinical Review of a Challenging Gynecologic Condition. J Minim Invasive Gynecol 2016; 23: 164-85. [Crossref]
8. Scarperi S, Pontrelli G, Campana C, Steinkasserer M, Ercoli A, Minelli L, et al. Laparoscopic Radiofrequency Thermal Ablation for Uterine Adenomyosis. JSLS 2015; 19: pii: e2015.00071
9. Yavuzcan A, Cağlar M, Ustün Y, Dilbaz S, Kumru S. Evaluation of the outcomes of laparoscopic hysterectomy for normal and enlarged uterus (>280 g). Arch Gynecol Obstet 2014; 289: 831-7. [Crossref]
10. Wu JM, Wechter ME, Geller EJ, Nguyen TV, Visco AG. Hysterectomy rates in the United States, 2003. Obstet Gynecol 2007; 110: 1091-5. [Crossref]
11. Kluivers KB, Johnson NP, Chien P, Vierhout ME, Bongers M, Mol BW. Comparison of laparoscopic and abdominal hysterectomy in terms of quality of life: a systematic review. Eur J Obstet Gynecol Reprod Biol 2008; 136: 3-8. [Crossref]
12. Bonilla DJ, Mains L, Whitaker R, Crawford B, Finan M, Magnus M. Uterine weight as a predictor of morbidity after a benign abdominal and total laparoscopic hysterectomy. J Reprod Med 2007; 52: 490-8.
13. Sasaki KJ, Cholkeri-Singh A, Sulo S, Miller CE. Persistent bleeding after laparoscopic supracervical hysterectomy. JSLS 2014;18: pii: e2014.002064.
14. Lachiewicz MP, Moulton LJ, Jaiyeoba O. Infection Prevention and Evaluation of Fever After Laparoscopic Hysterectomy. JSLS 2015;19: pii: e2015.00065.
15. Jørgensen H, Fomsgaard JS, Dirks J, Wetterslev J, Andreasson B, Dahl JB. Effect of peri- and postoperative epidural anaesthesia on pain and gastrointestinal function after abdominal hysterectomy. Br J Anaesth 2001; 87: 577-83. [Crossref]
16. Graziano A, Lo Monte G, Piva I, Caserta D, Karner M, Engl B, et al. Diagnostic findings in adenomyosis: a pictorial review on the major concerns. Eur Rev Med Pharmacol Sci 2015; 19: 1146-54.
17. Exacoustos C, Brienza L, Di Giovanni A, Szabolcs B, Romanini ME, Zupi E, Arduini D. Adenomyosis: three-dimensional sonographic findings of the junctional zone and correlation with histology. Ultrasound Obstet Gynecol 2011; 37: 471-9. [Crossref]
18. Dakhly DM, Abdel Moety GA, Saber W, Gad Allah SH, Hashem AT, Abdel Salam LO. Accuracy of Hysteroscopic Endomyometrial Biopsy in Diagnosis of Adenomyosis. J Minim Invasive Gynecol 2015: pii: S1553-4650(15)01663-5.
19. Chong GO, Lee YH, Hong DG, Cho YL, Lee YS. Long-Term Efficacy of Laparoscopic or Robotic Adenomyomectomy with or without Medical Treatment for Severely Symptomatic Adenomyosis. Gynecol Obstet Invest 2016; 81: 346-52. [Crossref]
20. Vercellini P, Parazzini F, Oldani S, Panazza S, Bramante T, Crosignani PG. Adenomyosis at hysterectomy: a study on frequency distribution and patient characteristics. Hum Reprod 1995; 10: 1160-2.
21. Zeng W, Chen L, Du W, Hu J, Fang X, Zhao X. Laparoscopic hysterectomy of large uteri using three-trocar technique. Int J Clin Exp Med 2015; 86319-26.
22. Dassel MW, O'Hanlan KA, Shwayder JM. Ultrasonographically calculated uterine mass as a predictor for surgical outcomes in total laparoscopic hysterectomy. J Gynecol Surg 2015; 31: 128-34. [Crossref]
23. Ota H, Igarashi S, Tanaka T. Morphometric evaluation of stromal vascularization in the endometrium in adenomyosis. Hum Reprod 1998; 13: 715-9. [Crossref]
24. Tan-Kim J, Menefee SA, Reinsch CS, O'Day CH, Bebchuk J, Kennedy JS, Whitcomb EL. Laparoscopic Hysterectomy and Urinary Tract Injury: Experience in a Health Maintenance Organization. J Minim Invasive Gynecol 2015; 22: 1278-86. [Crossref]
25. AAGL Practice Report: Practice guidelines for intraoperative cystoscopy in laparoscopic hysterectomy. AAGL Advancing Minimally Invasive Gynecology Worldwide. J Minim Invasive Gynecol 2012; 19: 407-11. [Crossref]
26. Manoucheri E, Cohen SL, Sandberg EM, Kibel AS, Einarsson J. Ureteral injury in laparoscopic gynecologic surgery. Rev Obstet Gynecol 2012; 5: 106-11.
27. Jelovsek JE, Chiung C, Chen G, Roberts SL, Paraiso MF, Falcone T. Incidence of lower urinary tract injury at the time of total laparoscopic hysterectomy. JSLS 2007; 11: 422-7.
28. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002; 2: 161-74. [Crossref]
29. Laios A, Mohamed BM, Kelly L, Flavin R, Finn S, McEvoy L, et al. Pre-Treatment of platinum resistant ovarian cancer cells with an MMP-9/MMP-2 inhibitor prior to cisplatin enhances cytotoxicity as determined by high content screening. Int J Mol Sci 2013; 14: 2085-103. [Crossref]
30. VanEvert JS, Smeenk JM, Dijkhuizen FP, de Kruif JH, Kluivers KB. Laparoscopic subtotal hysterectomy versus laparoscopic total hysterectomy: a decade of experience. Gynecol Surg 2010; 7: 9-12. [Crossref]
31. Brummer TH, Jalkanen J, Fraser J, Heikkinen AM, Kauko M, Mäkinen J, et al. FINHYST, a prospective study of 5279 hysterectomies: complications and their risk factors. Hum Reprod 2011; 26: 1741-51. [Crossref]

A novel approach using a minimal number of injections during the IVF/ICSI cycle: Luteal half-dose depot GnRH agonist following corifollitropin alfa versus the corifollitropin alfa with a GnRH-antagonist cycle

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Abstract

Objective: Corifollitropin alfa is a good choice for assisted reproductive technology (ART) cycles because fewer injections are needed than with other agents. In this retrospective cohort, we analyzed luteal injected half-dose depot gonadotropin hormone-releasing hormone (GnRH) agonist cycles in women who received corifollitropin alfa and those who underwent a conventional corifollitropin alfa cycle with a GnRH antagonist.

Material and Methods: In this retrospective cohort, we analyzed luteal injected half-dose depot GnRH agonist cycles in women who received corifollitropin alfa and those who underwent a conventional corifollitropin alfa cycle with a GnRH antagonist at the Division of Reproductive Endocrinology and IVF Unit, Obstetrics and Gynecology Department, Başkent University School of Medicine, Adana, Turkey, from March 2014 to August 2015. The patient's baseline characteristics were similar between the two groups. Forty-five patients underwent the long protocol, in which a half-dose of depot GnRH agonist was administered on day 21 of the preceding cycle. Forty-nine patients underwent the GnRH-antagonist protocol. Corifollitropin alfa was administered on the menstrual cycle day 3.

Results: The mean ages of the two groups were similar (32.77±5.55 vs. 34.2±4.51 years ["for the long- and antagonist-protocol groups, respectively"]). The total number of retrieved oocytes, the fertilization rate, and the number of transferred embryos were similar between the two groups. The only significant difference between the two protocols was the number of injections during the controlled ovarian stimulation (COH) cycle, which included the depot-agonist injection in the long-protocol group (4.46±1.64 vs. 5.71±2.51, p=0.006). The clinical pregnancy and implantation rates were similar in the two protocols (16/45 [35.6%] vs. 16/49 [32.7%] for the intention to treat and 32.5±6.82% vs. 36.25±8.58%, respectively).

Conclusion: Our results show that ART cycles could be performed with fewer injections using corifollitropin alfa and a half-dose of depot GnRH agonist. (J Turk Ger Gynecol Assoc 2016; 17: 155-8)

Keywords: Corifollitropin alfa, IVF/ICSI outcomes, depot GnRH agonist, GnRH antagonist

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Introduction

The clinical excellence of an assisted reproductive technology (ART) program is demonstrated by the live birth of a healthy baby. The many painful injections administered throughout this difficult procedure seem unimportant after a healthy newborn appears. Most women suffering from infertility tolerate this painful time. However, some women drop out of treatment because of the injections (1). Mild ovarian stimulation protocols with clomiphene citrate/letrozole are significant considering that fewer injections are given and that the *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatment has a lower cost (2-3). Corifollitropin alfa is a good choice for ART cycles because fewer injections are needed than with other agents. Although the total cost of a corifollitropin alfa cycle is higher than that for the conventional controlled ovarian hyperstimulation (COH) protocol, women tend to select corifollitropin alfa because of the lower number of injections.

Over the last decade, the gonadotropin hormone-releasing hormone (GnRH) antagonist cycle treatment protocol has been favored in ART programs. Although a meta-analysis of cycle outcomes comparing GnRH agonist and antagonist protocols reported similar results, women undergoing the GnRH-antagonist program are administered fewer injections and the protocol is easier to perform than agonist protocols (4-5). However, some clinicians continue to prefer the long GnRH agonist cycle. Depot GnRH agonists have been used in ART

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programs before their integration into daily use and have resulted in reasonable outcomes (6-9). Furthermore, the drug industry may force the use of these innovative options, making them more popular. The depot form of recombinant follicle-stimulating hormone (FSH; corifollitropin alfa) is another innovative option that allows for fewer injections during ART cycles.
In the present study, we compared the outcomes of a protocol combining the oldest version of the long protocol, which includes the depot form of GnRH agonist, with corifollitropin alfa to the outcomes of the GnRH-antagonist protocol, which is a rising star of the last decade.

Material and Methods

In this retrospective cohort, we analyzed luteal injected half-dose depot GnRH agonist cycles in women who received corifollitropin alfa and those who underwent a conventional corifollitropin alfa cycle with a GnRH antagonist at the Division of Reproductive Endocrinology and IVF Unit, Obstetrics and Gynecology Department, Başkent University, Adana, Turkey, from March 2014 to August 2015. This study was approved by the Ethics committee of Başkent University. Ninety-four normal responding women were analyzed in this cohort. Women suspected and/or defined as potential hyper-responders with polycystic ovary syndrome (PCOS) and/or polycystic ovaries (PCO) were excluded and were not administered corifollitropin alfa because of the increased risk of ovarian hyperstimulation syndrome (OHSS).
Forty-five patients underwent the long protocol, in which a half-dose of depot GnRH agonist (1.9 mg leuprolide acetate; Lucrin; Abbott France, Rungis Complexe, France) was administered on day 21 of the preceding cycle. If no cysts ≥2 cm were detectable and estradiol (E2) was <50 pg/mL, weekly gonadotropin stimulation with 150 μg corifollitropin alfa (150 μg Elonva, MSD; Haarlem, The Netherlands) was administered on the menstrual cycle day 3 after ovarian suppression was achieved. The estradiol and follicular monitoring continued until human chorionic gonadotropin (hCG) administration criteria were met and at least three follicles had maximum diameters >17 mm. Forty-nine patients underwent the GnRH-antagonist protocol. Corifollitropin alfa (Elonva 150 μg; MSD, The Netherlands) was administered on the menstrual cycle day 3. A GnRH antagonist (Orgalutran, MSD; The Netherlands) was added to this regimen on the last day of weekly gonadotropin administration, which was day 6 of stimulation. The hCG administration was applied with the guidance of ultrasound and estradiol monitoring until at least two or three follicles had maximum diameters >17 mm. The oocyte retrieval was performed 35–36 h after the hCG injection performed with a 17-gage needle under sedation. Embryos were transferred on day 3. All the patients had luteal support with 90 mg daily progesterone administered intravaginally (Crinone 8% gel, Merck Serono; Darmstadt, Germany) and 0.1 mg/mL triptorelin on day 3 after embryo transfer. Clinical pregnancy was defined as the presence of at least one gestational sac, with detectable fetal cardiac activity by transvaginal ultrasonography. The data expresses the means±SD. The baseline differences between the two groups were analyzed by Student's t test. Pearson's Chi-square test and Fisher's exact test were used to compare the ratios between groups. A value of p less than 0.05 was considered statistically significant. The data was analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 17.0; SPSS, Inc.; Chicago, IL, USA).

Results

We performed 94 cycles with corifollitropin alfa in normal responding women during the 17 months of the study. The patients' baseline characteristics were similar between the two groups (Table 1). The mean ages of the two groups were similar (32.77±5.55 vs. 34.2±4.51 years ["for the long- and antagonist-protocol groups, respectively"]). Antimüllerian hormone (AMH) levels and mean antral follicle counts (AFC) of one ovary were also similar between the two groups (2.41±0.9 vs. 2.45±0.45 ng/mL and 5.14±2.15 vs. 4.91±2.41, respectively) (Table 1). Although the mean E2 level on the day of hCG administration tended to be higher in women undergoing the depot-agonist protocol, the difference was not significant (2073.62±194.18 vs. 1626.5±188.94 pg/mL). The total number of retrieved oocytes, the fertilization rate, and the number of transferred embryos were similar between the two groups (Table 1). The only significant difference between the two protocols was the number of injections during the COH cycle, which included the depot-agonist injection in the long-protocol group (4.46±1.64 vs. 5.71±2.51, p=0.006) (Table 1).
The clinical pregnancy and implantation rates were similar in the two protocols (16/45 [35.6%] vs. 16/49 [32.7%] for the intention to treat and 32.5±6.82% vs. 36.25±8.58%, respectively) (Table 2). Five and nine cycles were cancelled in the depot-agonist and GnRH-antagonist groups, respectively (Table 2). No moderate or severe ovarian OHSS occurred in either group.

Discussion

Our results show that IVF/ICSI cycles could be performed with fewer injections using corifollitropin alfa and a half-dose of depot GnRH agonist. The corifollitropin alfa and GnRH-antagonist cycle was introduced so that fewer injections would be needed. Our results show that the half-dose depot GnRH agonist plus corifollitropin alfa protocol resulted in fewer injections than were required in the GnRH-antagonist program in which corifollitropin alfa was used.
The combination of the depot form of GnRH agonist and corifollitropin alfa is a satisfactory option for patients who plan to drop out of IVF treatment because of the fear of multiple injections. Use of a COH strategy with fewer injections is associated with >50% reduction in dropout rate (1). Although our long protocol with a depot GnRH agonist is not considered to be a mild stimulation, it was superior to mild stimulation, as approximately only four injections were administered, the patients were satisfied, and a greater number of cryopreserved embryos were obtained. Another disadvantage of the protocol is the increased risk for OHSS, which we did not encounter because of our proper selection of normal responding patients.
Depot GnRH agonists have been used since the last decade

Table 1. Demographic and cycle characteristics of half-dose depot GnRH-agonist and GnRH-antagonist protocols in which corifollitropin alfa was used

	Depot GnRH agonist+ corifollitropin alfa group (n=45)	GnRH antagonist+ corifollitropin alfa group (n=49)	p
Age (years)	32.77±5.55	34.2±4.51	0.17
BMI (kg/m^2)	24.95±0.9	25.12±0.8	0.88
Duration of infertility (years)	5.23±3.01	4.72±3.46	0.45
AMH (m IU/L)	2.41±0.9	2.45±0.45	0.96
Antral follicle count	5.14±2.15	4.91±2.41	0.66
Follicle count >14 mm	11.18±5.3	10.79±6.51	0.75
E2 level (pg/mL) on hCG administration day	2073.62±194.18	1626.5±188.94	0.1
Progesterone level (ng/mL) on hCG administration day	1.07±0.22	0.88±0.07	0.42
Endometrial thickness (mm)	12.57±1.77	10.92±1.9	0.33
COH duration	9±2.29	8.36±2.58	0.21
Number of injections in the COH cycle	4.46±1.64	5.71±2.51	0.006
Retrieved oocytes (no.)	12.22±7.08	12.95±7.95	0.64
Metaphase II oocytes (no.)	9.81±6.29	10.8±6.82	0.47
Fertilization rate (%)	61.58±3.14	71.56±11.96	0.42
Embryo (no.)	6.65±4.95	6.29±3.63	0.7
Transferred embryos (no.)	1.48±0.55	1.63±048	0.22
Grade 1 embryos transferred (no.)	0.65±0.69	0.89±0.68	0.11
Grade 2 embryos transferred (no.)	0.8±0.85	0.69±0.86	0.57
Cryopreserved embryos (no.)	5.27±2.37	3.75±3.06	0.074

BMI: body mass index; AMH: antimüllerian hormone; COH: controlled ovarian hyperstimulation; E2: estradiol; hCG: human chorionic gonadotropin; GnRH: gonadotropin hormone-releasing hormone

Table 2. IVF/ICSI outcomes of half-dose depot GnRH-agonist and GnRH-antagonist protocols in which corifollitropin alfa was used

	Depot GnRH agonist+ corifollitropin alfa group (n=45)	GnRH antagonist+ corifollitropin alfa group (n=49)	
Positive β-hCG	18 (40%)	18 (36.7%)	0.93
CPR	16 (35.6%)	16 (32.7)	0.95
BPR	2 (4.44%)	2 (4.08%)	NS
Implantation rate (%)	32.5±6.82	36.25±8.58	0.73
Singleton pregnancy rate	13/16 (81.25)	12/16 (75%)	NS
Twin pregnancy rate	3/16 (18.75%)	4/16 (25%)	NS
OHSS rate	0	0	NS
Cancel rate	5 (11.1%)	9 (18.4%)	0.46

CPR: clinical pregnancy rate; BPR: biochemical pregnancy rate; GnRH: gonadotropin hormone-releasing hormone; IVF/ICSI: in vitro fertilization/intracytoplasmic sperm injection; hCG: human chorionic gonadotropin; OHSS: ovarian hyperstimulation syndrome

of the 20th century. At that time, the depot agonist was recognized as advantageous because of the attendant pituitary suppression and the patient-friendly requirement of only a single injection. Hesitation to use a depot agonist was related to the concern that it might lead to pituitary oversuppression, which could cause a luteal phase defect because of the absence of pituitary luteinizing hormone (10, 11). However, the use of half-dose depot GnRH for the COH cycle has resulted in reasonable IVF outcomes in infertile women (8-9). Therefore, we used half doses of the depot form of GnRH agonist to suppress the ovary. Furthermore, the ultra-long protocol, which includes 3 months of depot GnRH agonist injections to suppress the pituitary and endometriosis, seems to be the best choice in women with endometriosis undergoing IVF/ICSI cycles (12-14). One randomized study that compared depot forms of a GnRH agonist and antagonist reported similar IVF/ICSI outcomes (15). No depot

GnRH antagonists are marketed in Turkey, but they could be included in another simple protocol with corifollitropin alfa.

The fundamental limitation of this study was the retrospective design. We did not perform a randomized trial because we did not want drug industry support for two reasons. First, our institute must pay all costs, including the costs of the COH cycles and patient insurance, when a randomized trial is conducted, making it too expensive to design a sufficiently powered randomized study with high patient enrollment without industry support. Moreover, trials supported by a pharmaceutical company would be criticized as being biased in favor of a specific drug.

This is the first study to demonstrate similar IVF/ICSI results from a minimum-injection COH protocol compared with those from a more conventional method. This minimum-injection protocol is a welcome development that was appreciated by our patients. Our study focused on the long protocol, which most clinicians do not prefer. Additional well-designed randomized trials that compare depot GnRH agonists and antagonists with corifollitropin alfa are expected to demonstrate good IVF/ICSI outcomes.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Başkent University School of Medicine.*

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References

1. Verberg MF, Eijkemans MJ, Heijnen EM, Broekmans FJ, de Klerk C, Fauser BC, et al. Why do couples drop-out from IVF treatment? A prospective cohort study. Hum Reprod 2008; 23: 2050-5. [Crossref]
2. Verberg MF, Macklon NS, Nargund G, Frydman R, Devroey P, Broekmans FJ, et al. Mild ovarian stimulation for IVF. Hum Reprod Update 2009; 15: 13-29. [Crossref]
3. Mohsen IA, El Din RE. Minimal stimulation protocol using letrozole versus microdose flare up GnRH agonist protocol in women with poor ovarian response undergoing ICSI. Gynecol Endocrinol 2013; 29: 105-8. [Crossref]
4. Al-Inany HG, Abou-Setta AM, Aboulghar M. Gonadotrophin-releasing hormone antagonists for assisted conception. Cochrane Database Syst Rev 2006; CD001750. [Crossref]
5. Franco JG Jr, Baruffi RL, Mauri AL, Petersen CG, Felipe V, Cornicelli J, et al. GnRH agonist versus GnRH antagonist in poor ovarian responders: a meta-analysis. Reprod Biomed Online 2006; 13: 618-27. [Crossref]
6. Balasch J, Gomez F, Casamitjana R, Carmona F, Rivera F and Vanrell JA. Pituitary-ovarian suppression by the standard and half-doses of D-Trp-6-luteinizing hormone-releasing hormone depot. Hum Reprod 1992; 7: 1230-4.
7. Olivennes F, Righini C, Fanchin R, Torrisi C, Hazout A, Glissant M, et al. A protocol using a low dose of gonadotrophin-releasing hormone agonist might be the best protocol for patients with high follicle-stimulating hormone concentrations on day 3. Hum Reprod 1996; 11: 1169-72. [Crossref]
8. Janssens RM, Lambalk CB, Vermeiden JP, Schats R, Bernards JM, Rekers-Mombarg LT, et al. Dose-finding study of triptorelin acetate for prevention of a premature LH surge in IVF: a prospective, randomized, double-blind, placebo-controlled study. Hum Reprod 2000; 15: 2333-40. [Crossref]
9. Dal Prato L, Borini A, Trevisi MR, Bonu MA, Sereni E, Flamigni C. Effect of reduced dose of triptorelin at the start of ovarian stimulation on the outcome of IVF: a randomized study. Hum Reprod 2001; 16: 1409-14. [Crossref]
10. Devreker F, Govaerts I, Bertrand E, Van den Bergh M, Gervy C, Englert Y. The long-acting gonadotropin-releasing hormone analogues impaired the implantation rate. Fertil Steril 1996; 65: 122-6. [Crossref]
11. Albuquerque LE, Saconato H, Maciel MC, Baracat EC, Freitas V. Depot versus daily administration of GnRH agonist protocols for pituitary desensitization in assisted reproduction cycles: a Cochrane Review. Hum Reprod 2003; 18: 2008-17. [Crossref]
12. Barnhart K, Dunsmoor-Su R, Coutifaris C. Effect of endometriosis on in vitro fertilization. Fertil Steril 2002; 77: 1148-55. [Crossref]
13. Surrey ES, Silverberg KM, Surrey MW, Schoolcraft WB. Effect of prolonged gonadotropin-releasing hormone agonist therapy on the outcome of in vitro fertilization-embryo transfer in patients with endometriosis. Fertil Steril 2002; 78: 699-704. [Crossref]
14. Sallam HN, Garcia-Velasco JA, Dias S, Arici A. Long-term pituitary down-regulation before in vitro fertilization (IVF) for women with endometriosis. Cochrane Database Syst Rev 2006; CD004635. [Crossref]
15. Vlaisavljevic V, Reljic M, Lovrec VG, Kovacic B. Comparable effectiveness using flexible single-dose GnRH antagonist (cetrorelix) and single-dose long GnRH agonist (goserelin) protocol for IVF cycles--a prospective, randomized study. Reprod Biomed Online 2003; 7: 301-8. [Crossref]

Optimal cone size to predict positive surgical margins after cold knife conization (CKC) and the risk factors for residual disease

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Abstract

Objective: To determine the optimal cone size to achieve a reliable sensitivity and specificity for clear surgical margins after cold knife conization (CKC).

Material and Methods: The medical reports of patients who had high-grade cervical intraepithelial lesions, carcinoma in situ, or stage 1A1 microinvasive carcinoma in their CKC specimens between June 2008 and January 2015 were reviewed retrospectively.

Results: In total, 315 women fulfilled the inclusion criteria. The mean age of the patients was 40.7 years. The conization results were microinvasive carcinoma and high-grade squamous lesion (HSIL) for 8 and 307 patients, respectively. Ninety-nine patients had positive surgical margins. Eighty-one patients with positive cone margins underwent the repeat excisional procedure and 35 of them showed residual disease. In the univariate analyses, the patient age, menopausal status, and mean cone height parameters showed statistically significant differences between the patients with positive and negative margins. Also, residual disease was associated with the menopausal status and age of the patients.

Conclusion: There is no optimal cone depth that is applicable for all patients. The most important predictors for positive margins are the menopausal status of the patient and that more than two quadrants are involved. However, the menopausal status and age of the patients were still predictors for residual disease. (J Turk Ger Gynecol Assoc 2016; 17: 159-2)

Keywords: Cold knife conization, residual disease, surgical margin

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Introduction

Cervical cancer is the most preventable gynecological cancer; the treatment of high-grade cervical lesions leads to a decreased incidence of cervical cancer (1). The diagnosis and surgical treatment of high-grade cervical preinvasive lesions depends on the colposcopic findings (2). The main purpose of the treatment is to remove the cervical lesion with adequate surgical margins as well as the whole transformation zone (TZ) (3). Studies have shown that the most important predictor for residual disease or disease recurrence is positive cone margins (4, 5). Positive surgical margins after loop electrosurgical excision procedures (LEEPs) are seen more frequently and have been extensively studied compared with cold knife conization (CKC) (6). Margin-positive patients are more likely to have recurrent disease, and the recurrence is earlier than seen in margin-negative patients (7). When there is no visible lesion in the ectocervix or when the lesion is located in the endocervical canal, it can be challenging to achieve clear margins without compromising future obstetric outcomes. Also, a deeper cone height and repeat excisions after positive surgical margins are associated with adverse obstetric outcomes (8). There is no clear definition of the optimal cone size in the treatment of high-grade cervical intraepithelial lesions. The depth of the cone specimen is usually determined in the operating room according to the age, parity, fertility desire, and initial colposcopic findings of the patient. The objective of the present study was to determine the optimal cone size needed to achieve a reliable sensitivity and specificity for clear surgical margins.

Material and Methods

After approval of the Institutional Review Board, the medical records of patients who had undergone a CKC procedure in Zekai Tahir Burak Women's Health Training and Research Hospital between June 2008 and January 2015 were reviewed. We included only the patients with preceding colposcopic biopsy results showing high-grade cervical lesions [i.e., cervical intraepithelial neoplasia (CIN) 2/3 or high-grade squamous lesion (HSIL)]. The patients whose conization results showed lesser abnormalities [i.e., CIN-1, low-grade squamous in-

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traepithelial lesion (LSIL), or normal epithelium] and invasive carcinomas were excluded, so we collected only the follow-up data of the patients with conization results of CIN 2/3 and carcinoma *in situ* (CIS). Written informed consents were obtained from all subjects either before the colposcopic procedures or CKC procedures.

All of the excisional procedures were performed in the operating room using a scalpel under general or regional anesthesia. As the standard procedure, the hemostatic sutures were applied lateral margins of the cervix using no. 0 polyglactin (Vicryl; Ethicon, Cincinnati USA); endocervical curettage was applied and the base of the CKCs were cauterized using a high-voltage spray mode. In the presence of a visible lesion in the cervix, the conization margins were adjusted accordingly, otherwise a standard cone-shaped specimen was removed with the intent of including the entire TZ. A silk suture was placed at the 12 o'clock position of the cone specimen for orientation and the specimens were transported to the pathology depart ment in a formalin solution container. Specimens were divided into four quadrants and each quadrant was examined in at least three consecutive slices. The surgical margins were considered as positive if the lesion was cut-through or closer than 1 mm to the margin.

The cone volume was calculated using the radius (r) and height (H) reported in the pathology result after formalin fixation, by the formula ($\pi.r^2.H/3$). If the base of the cone was elliptical rather than circular, then the mean of the two perpendicular diameters was used to calculate the radius of the cone base. The patient characteristics, dimensions of the conization specimens, and surgical margin status (endocervical and ectocervical) were analyzed in a descriptive manner. The associations between the cone margin status and mean cone diameter, cone height, and cone volume were investigated.

The Statistical Package for the Social Sciences (SPSS) version 21 for Macintosh (SPSS Inc.; Chicago, IL, USA) was used for data interpretation. Differences in the means of the continuous variables were assessed using the Mann–Whitney U test or independent samples t-test; the difference in the categorical variables was assessed using the Chi-square test. A multivariate logistic regression model was used to calculate the odds ratios when the univariate analyses showed a significant difference of the variables. $P<0.05$ was considered statistically significant.

Results

Overall, 315 women fulfilled the inclusion criteria (CKC result CIN2/3 or microinvasive carcinoma) among 486 CKCs. All the patients had a prior high-grade cervical cytology result. The mean age of the patients was 40.7 years. Patients' ages ranged from 23 to 73 years. In total, 240 women were premenopausal, while the remaining 75 women were postmenopausal. Eight conizations showed microinvasive carcinoma, while the remaining 307 patients had HSIL in the conization specimens. In total, 216 women (68.6%) had clear margins, while the remaining 99 women (31.4%) had positive margins. Positive margins were ectocervical, endocervical, or both for 22 (7%), 75 (23.8%), and 2 (0.6%) patients, respectively. The study design is summarized in Figure 1.

In the univariate analyses, the patient age, menopausal status, and mean cone height parameters showed statistically significant differences between the patients with positive and negative margins: patients with positive margins had smaller cone heights than those with negative margins (13.7 mm vs. 15.1 mm, respectively; $p<0.05$). Also, patients with positive margins tended to be older and postmenopausal. Twenty-seven percent of the premenopausal patients had positive margins, whereas 46.7% of the postmenopausal patients had positive surgical margins in the CKC specimens ($p<0.01$). Age and menopausal status were included in the multivariate analysis; the age of the patients was not an independent risk factor associated with margin status, whereas menopausal status was still found to be an independent risk factor associated with positive margin status. The detailed demographic and clinical characteristics of the patients are shown in Table 1. However, the cone volume, cone diameter, and cone height were not associated with the margin status of the conization specimens. The only pathological factor that was associated with the margin status was the number of quadrants involved in the conization specimen. If three or more quadrants were involved with HSIL, the risk of positive surgical margins was 2.71 times higher than for the patients with two or more involved quadrants. A receiver operating curve (ROC) was created for the association between cone height and margin status, and it was found that a 21-mm cone height provided 93% sensitivity and 71% specificity to achieve clear surgical margins (Table 2). However, the area under the ROC was calculated as 0.567, which means it was not statistically significant (Figure 2). Eighty-one (82%) of the patients with positive cone margins underwent repeat excisions; 49 of them underwent hysterectomy, while the remaining 32 of them underwent reconization. A total of 35 patients had residual disease in their re-excision specimens. The clinicopathological characteristics of the patients according to the residual disease status are shown in Table 3.

In the univariate analyses, residual disease was associated with the menopausal status and age of the patients; 17 of 49

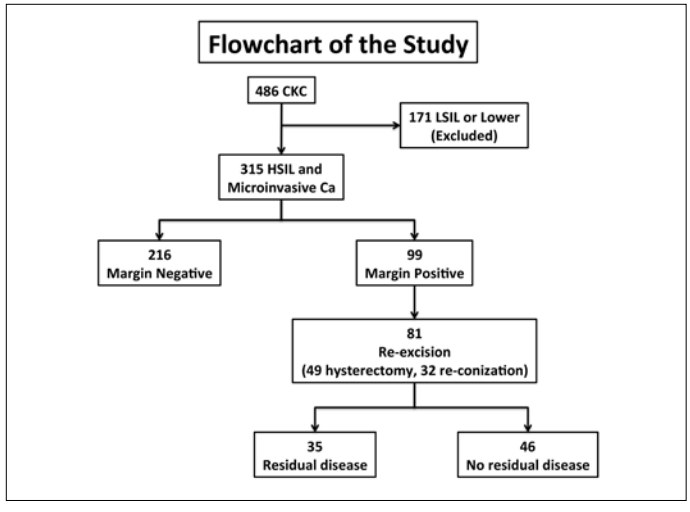

Figure 1. Flowchart of the study

CKC: cold knife conization; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; microinvasive ca: microinvasive carcinoma

Table 1. Detailed demographic and clinical features of the patients with negative and positive conization margins

	Margin Negative (N=216)	Margin Positive (N=99)	p	OR
Age (Mean±SD)	39.3±9.3	43.5±9.3	<0.01[a]	
Menopausal Status			<0.01[b]	1.8
Premenopausal N=240 (%)	176 (73%)	64 (27%)		
Postmenopausal N=75 (%)	40 (53.3%)	35 (46.7%)		
Preceding Cytology			>0.05[b]	
ASC-US	33 (15.3%)	5 (5%)		
LSIL	18 (8.3%)	9 (9%)		
HSIL	146 (67.6%)	74 (74.7%)		
ASC-H	12 (5.5%)	5 (5%)		
AGC	2 (1%)	0		

ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells cannot exclude HSIL; AGC: atypical glandular cells; OR: odds ratio

[a]Student's t-test was used.

[b]Fisher's exact test was used.

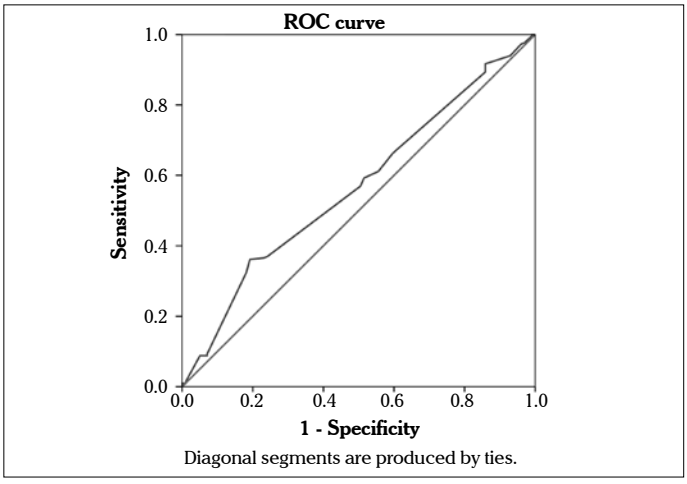

Figure 2. ROC curve

(35%) premenopausal women had residual disease, while 18 of 32 (56%) postmenopausal women had residual disease in their re-excision specimens (p<0.05). The mean age of the patients with and without residual disease was 47.4±9 and 42.6±9 years, respectively (p<0.05).

The number of positive quadrants was not associated with residual disease status. Also, the diameter, depth, and volume of the conization specimen were not a predictor for residual disease.

Discussion

Approximately one-third of the CKC specimens had positive surgical margins in the present study. The prevalence of positive margins reported in the previous studies was similar to our study, Costa et al. (9) and Sun et al. (10) reported the prevalence of positive cone margins in the CKC specimens in their study as 27%. In the former study, the authors reported that the cone size was not an independent risk factor for positive cone margins. In the latter study, Sun et al. (10) found that the conization depth and multiquadrant involvement were significant factors associated with positive margins in both univariate and multivariate analyses. Kliemann et al. (2) reported similar results in their study. They found the cone height and lesion size as independent prognostic factors for positive margins. Positive margins were associated

Table 2. Pathological characteristics of margin-positive and margin-negative patients

	Margin Negative (N=216)	Margin Positive (N=99)	p	OR
Mean Cone Diameter (mm)	28.3	29	>0.05[a]	
Mean Cone Volume (mm^3)	3559	3556	>0.05[a]	
Mean Cone Height (mm)	15.1	13.7		
<21 mm (N=288)	196 (68%)	92 (32%)	<0.05[a]	
≥21 mm (N=27)	20 (74%)	7 (26%)		
Positive quadrants	N (%)	N (%)		
1 quadrant	99 (45.8%)	1 (3%)		
2 quadrants	62 (28.7%)	28 (28.3%)		
3 quadrants	31 (14.4%)	36 (36.4%)		
4 quadrants	24 (11.1%)	32 (32.3%)	p<0.001[b]	
≥3 quadrants	55 (25.5%)	68 (68.7%)	p<0.001[c]	2.71

[a]Independent samples t-test was used.

[b]Fisher's exact test was used.

[c]Chi-square test was used.

OR: odds ratio

Table 3. Clinicopathological characteristics of the patients according to residual disease status

	Residual disease		
	Negative (N=46)	Positive (N=35)	p
Age (mean±SD)	42.5±9	47.3	<0.05[a]
Menopausal status			<0.05[b]
Postmenopausal (N=32)	14 (43.7%)	18 (56.3%)	
Premenopausal (N= 49)	32 (65.3%)	17 (34.7%)	
Positive quadrants			<0.05[b]
<3 (N=26)	16 (61.5%)	10 (38.5%)	
≥3 (N=55)	30 (54.5%)	25 (45.5%)	
Conization			>0.05[c]
Diameter (mean)	30.4 mm	28.3 mm	
Depth (mean)	13.9 mm	14.5 mm	
Volume (mean)	3757 mm^3	3943 mm^3	

[a]Independent samples t-test was used.

[b]Fisher's exact test was used.

[c]Chi-square test was used.

with a shallow cone height and the menopausal status of the patient. In the majority of cases, the operator cannot see a visible lesion in the cervix and performs the conization with the intent to remove the entire TZ and the lesion.

We found that a 21 mm cone height provided 93% sensitivity and 71% specificity to achieve clear surgical margins. The cervix has varying size and shape among women, and there are no objective criteria that help the surgeon to achieve clear surgical margins.

In a recent study that aimed to identify the predictors of residual disease after cervical conization, the authors concluded that positive ECC and a volume of disease 50% or greater were predictors of residual disease, whereas more than two involved quadrants was not associated with positive margin status (11). However, Tasci et al. (12) reported that more than two involved quadrants was one of the most important factors for residual disease after cervical conization. Nevertheless, we did not demonstrate a relationship between the number of quadrants involved and residual disease. In the present study, the patient's age and menopausal status were significantly related with residual disease.

There are several studies that investigate the optimal cone depth to achieve clear surgical margins. Papoutsis et al. (13) reported that the optimal cut-off value of cone depth to achieve clear surgical margins is 10 mm; however, Kliemann et al. (2) showed that the mean depth of cone specimens were 17.1 mm and 22.4 mm among the patients with positive and negative surgical margins, respectively. We found that cone depth was significantly different between margin-positive and margin-negative patients, but multivariate analysis showed that cone depth alone was not an independent predictor of margin status. Also, we did not find cone diameter and cone volume as a predictor of a positive surgical margin. The liberal excision of deep cone specimens should be avoided because greater cone heights are associated with a greater risk of stenosis (14), bleeding (15), and poor obstetric outcome (16). Therefore, cone depth should be individualized for each patient considering the age of the patient, size of the cervix, and TZ.

The main limitation of the present study is its retrospective design. In addition, the CKC procedures were performed by several different surgeons, including less experienced ones, which may lead to an increased positive margin status.

In conclusion, there is no optimal cone depth that is applicable for all patients. A significant proportion of patients with HSIL will have a positive surgical margin after CKC, and the most important predictors for positive margins are the menopausal status of the patient and more than two quadrants involved. However, the menopausal status and age of the patients are still predictors for residual disease.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Zekai Tahir Burak Women's Health Training and Research Hospital (Approval date and number: 31.07.2015/14).*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - M.Ö., E.K., N.Ç.; Design - M.Ö., N.Ç.; Supervision - M.M.M., T.G.; Resources - M.Ö., K.D.S.; Materials - M.Ö., E.K.; Data Collection and/or Processing - E.K., N.Ç., K.D.S.; Analysis and/or Interpretation - M.Ö.; Literature Search - M.Ö.; Writing Manuscript - M.Ö., K.D.S.; Critical Review - M.M.M., T.G.*

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References

1. Petry KU. Management options for cervical intraepithelial neoplasia. Best Pract Res Clin Obstet Gynaecol 2011; 25: 641-51. [Crossref]
2. Kliemann LM, Silva M, Reinheimer M, Rivoire WA, Capp E, Dos Reis R. Minimal cold knife conization height for high-grade cervical squamous intraepithelial lesion treatment. Eur J Obstet Gynecol Reprod Biol 2012; 165: 342-6. [Crossref]
3. Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D; 2006 American Society for Colposcopy and Cervical Pathology-sponsored Consensus Conference. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. Am J Obstet Gynecol 2007; 197: 346-55. [Crossref]
4. Kietpeerakool C, Khunamornpong S, Srisomboon J, Siriaunkgul S, Suprasert P. Cervical intraepithelial neoplasia II-III with endocervical cone margin involvement after cervical loop conization: is there any predictor for residual disease? J Obstet Gynaecol Res 2007; 33: 660-4. [Crossref]
5. Woelber L, Choschzick M, Eulenburg C, Hager M, Jaenicke F, Gieseking F, et al. Prognostic value of pathological resection margin distance in squamous cell cancer of the vulva. Ann Surg Oncol 2011; 18: 3811-8. [Crossref]
6. Kietpeerakool C, Srisomboon J, Ratchusiri K. Clinicopathologic predictors of incomplete excision after loop electrosurgical excision for cervical preneoplasia. Asian Pac J Cancer Prev 2005; 6: 481-4.
7. Miyoshi Y, Miyatake T, Ueda Y, Morimoto A, Yokoyama T, Matsuzaki S, et al. Prediction, based on resection margins, of long-term outcome of cervical intraepithelial neoplasia 3 treated by Shimodaira-Taniguchi conization. Arch Gynecol Obstet 2012; 285: 1427-32. [Crossref]
8. Simoens C, Goffin F, Simon P, Barlow P, Antoine J, Foidart JM, et al. Adverse obstetrical outcomes after treatment of precancerous cervical lesions: a Belgian multicentre study. BJOG 2012; 119: 1247-55. [Crossref]
9. Costa S, De Nuzzo M, Terzano P, Santini D, De Simone P, Bovicelli A, et al. Factors associated with cone margin involvement in CIN patients undergoing conization-equivalent electrosurgical procedure. Acta Obstet Gynecol Scand 2000; 79: 586-92. [Crossref]
10. Sun XG, Ma SQ, Zhang JX, Wu M. Predictors and clinical significance of the positive cone margin in cervical intraepithelial neoplasia III patients. Chin Med J (Engl) 2009; 122: 367-72.
11. Diaz ES, Aoyama C, Baquing MA, Beavis A, Silva E, Holschneider C, et al. Predictors of residual carcinoma or carcinoma-in-situ at hysterectomy following cervical conization with positive margins. Gynecol Oncol 2014; 132: 76-80. [Crossref]
12. Tasci T, Turan T, Ureyen I, Karalok A, Kalyoncu R, Boran N, et al. Is there any predictor for residual disease after cervical conization with positive surgical margins for HSIL or microinvasive cervical cancer? J Low Genit Tract Dis 2015; 19: 115-8. [Crossref]
13. Papoutsis D, Rodolakis A, Mesogitis S, Sotiropoulou M, Antsaklis A. Appropriate cone dimensions to achieve negative excision margins after large loop excision of transformation zone in the uterine cervix for cervical intraepithelial neoplasia. Gynecol Obstet Invest 2013; 75: 163-8. [Crossref]
14. Baldauf JJ, Dreyfus M, Ritter J, Meyer P, Philippe E. Risk of cervical stenosis after large loop excision or laser conization. Obstet Gynecol 1996; 88: 933-8. [Crossref]
15. Girardi F, Heydarfadai M, Koroschetz F, Pickel H, Winter R. Cold-knife conization versus loop excision: histopathologic and clinical results of a randomized trial. Gynecol Oncol 1994; 55: 368-70. [Crossref]
16. Andia D, Mozo de Rosales F, Villasante A, Rivero B, Diez J, Perez C. Pregnancy outcome in patients treated with cervical conization for cervical intraepithelial neoplasia. Int J Gynaecol Obstet 2011; 112: 225-8. [Crossref]

Could S6K1 immunopositivity be used to distinguish early and advanced stages of endometrioid endometrial adenocarcinoma?

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Abstract

Objective: To assess whether the immunopositivity of S6K1, a crucial effector of the mTOR signaling pathway, varies between early-stage low-grade and advanced-stage high-grade endometrial endometrioid adenocarcinoma (EEA) as well as to discuss its prognostic significance.

Material and Methods: A total of 22 normal endometrial tissue samples (Control group) and 41 EEA specimens (Study group) were enrolled in the study, and all the samples underwent immunohistochemical staining for S6 kinase alpha (S6K1). The study group was further evaluated in two subgroups; stage 1A, grade 1 (Group 1) and stage ≥1A, grade 2 or 3 (Group 2). Group 2 patients were considered as a poor prognosis for EEA. The samples were examined by two independent pathologists. Statistical analyses were performed using the Student's t-test for continuous variables, the Chi-square test for categorical variables, and one-way analysis of variance for the comparison of multiple variables.

Results: The immunopositivity rate for all the included EEA patients was 56.1%, whereas none of the 22 normal endometrial tissue samples revealed immunoreactivity for S6K1. The immunopositivity rates were significantly different between Groups 1 and 2 [38.1% (8/21) and 75.0% (15/20), respectively, p=0.039]. When S6K1 positivity was used as a criterion of poor prognosis in EEA, the sensitivity, specificity, positive predictive value, and negative predictive value were calculated to be 62%, 75%, 72%, and 65%, respectively (OR: 4.9 and 95% CI: 1.3–18.7).

Conclusion: S6K1 was positive in the majority of EEAs and malignancies at an advanced stage. Higher grade disease had a significantly higher rate of S6K1 positivity. S6K1 immunopositivity appears to be a promising method to predict poor prognosis in EEA.

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Introduction

Endometrial cancer (EC) is the most common gynecological malignancy in developed countries, with the most frequent histological type being endometrial endometrioid adenocarcinoma (EEA), which accounts for 70%–80% of all cases (1). EEAs are different from non-EEAs in molecular and clinical pathology and are associated with less aggressive behavior and better prognosis as well as earlier diagnosis (2). Thus, a surgical approach is sufficient in early-stage EEA; however, adjuvant treatment is recommended for women with a moderate to high risk of recurrence (3, 4). Patients with advanced or recurrent disease have a poor median survival; therefore, cytotoxic or radiation therapy should accompany surgery in those with invasion of more than 50% of myometrium or with advanced disease, including early stage at grade 2 or 3. It appears clinically important to identify the markers that determine the need for adjuvant therapy to surgery and that predict a poor prognosis in patients with EEA. Moreover, novel therapeutic approaches, such as molecular targeted therapy, which have a lower side-effect profile and systemic toxicity, are necessitated, particularly in patients with advanced or recurrent disease (5, 6). The mammalian target of the rapamycin (mTOR) signaling pathway is directly involved in many cell signaling pathways and mainly regulates three important downstream substrates: eukaryotic initiation factor 4E (eIF4E), eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), and P70 ribosomal protein S6 kinase alpha (S6K1) (5-7). Activation of the mTOR signaling pathway and aberrations of the mTOR, including PI3K amplification/mutation and S6K1 overexpression, have been reported in a set of malignancies and in some types of gynecologic cancers (8, 9). In the present study, our aim was to investigate the expression patterns of S6K1, a crucial effector of mTOR signaling, in tis-

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sues of non-pathologic endometrium, early-stage (IA, Grade 1) and advanced-stage (IA, grade 2–3, and IB, II, III, IV) EEA, using indirect immunohistochemistry.

Material and Methods

Clinical samples

This is a cross-sectional study conducted between January 2003 and December 2011. Ethical approval from the Institutional Review Board was granted before the initiation. Patients who underwent surgery for EEA and in whom diagnosis was made upon pathological examination, were extracted retrospectively from the clinic's patient database. The control group consisted of patients undergoing surgery for non-endometrial benign gynecological diseases. Patients who had received treatment that could potentially affect S6K1 immunostaining, such as chemotherapy, radiotherapy, and hormone replacement therapy (HRT) or oral contraceptive pills, and those with concurrent malignancies, cardiac hypertrophy, and type 2 diabetes and obesity, were excluded from the study. All the specimens were re-evaluated by a single pathologist, and all the pathological diagnoses were confirmed by another pathologist before the study. During these examinations, cases without sufficient tissue samples were excluded. Patients diagnosed as EEA by pathological examination were divided into two subgroups: Group 1; grade 1, stage 1A EEA patients, and, Group 2; grade 2 or 3, stage ≥1A EEA patients. Stages for endometrial cancer were determined according to the clinical criteria established by the International Federation of Gynecology and Obstetrics (FIGO) 2014 (10). Clinical and pathology data from the included patients were recorded.

Details of the antibody used and analysis of the immunohistochemistry

4 micron thick cross-sections were taken from suitable paraffin blocks. These cross-sections, in conjunction with positive controls, were incubated for 17 hours at 55^0C, and, after standard deparaffinization and rehydration processes, were applied. Afterwards, the immunohistochemical staining process was manually performed in accordance with the suggested procedure with a 1/100 dilution of polyclonal Rabbit P70S6K1 (Anti-S6K1 antibody (ab47504), Abcam; Cambridge, MA, USA) primer antibody. HeLa cells were used as positive control materials.

Evaluation of the staining

The immunohistochemical results were evaluated under a microscope independently by two pathologists who did not have any knowledge of the clinical outcome and who scored the results semi-quantitatively. Any discrepancies between the staining intensity score results between the two pathologists were noted and referred to as the interobserver difference. We used a scoring method referred to as the expression index (EI), which was based on two characteristics: overall stain intensity and the percentage of neoplastic tissue that was stained. The staining intensity was scored as 0 (no staining), 1+ (weakly positive), 2+ (moderately positive), 3+ (strongly positive). The most frequently observed scores were recorded for areas painted in varying intensities. An EI was calculated by multiplying the staining intensity score and the percentage of positively stained cells (ranging from 0 to 300).

Then, the resulting staining intensity scores and the percentage of positive staining were averaged between the two pathologists and were recorded. In the present study, the interobserver differences were less than 5%.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows 15.0 software (SPSS Inc.; Chicago, IL, USA). Descriptive statistics were given as the mean, standard deviation, frequency, and percentage. Statistical analysis was performed using the Student's t-test for comparison of two continuous variables, one-way ANOVA for comparison of multiple variables, and the x^2 test for categorical variables. When there was a need for a non-parametric test, the Mann–Whitney U and Kruskal–Wallis tests were performed. Statistical significance was defined as $p<0.05$. A nominal two-sided p value was considered for all comparisons.

Results

Immunohistochemical staining for S6K1 expression was performed on paraffin-embedded biopsy specimens from a total of 63 patients, including 22, 21, and 20 cases in the control group and Groups 1 and 2, respectively. Of all the EEA cases, 56.1% were immunostaining positive, whereas none of the 22 normal gynecologic endometrial tissue samples revealed immunoreactivity for S6K1. Figures 1 and 2 show the activation status of immunocytochemical staining for S6K1, whereas Figure 3 shows the EI status of S6K1 protein in all groups. Comparisons of the patient age and EI and S6K1 results between the groups are presented in Table 1. Accordingly, EI was higher in early-stage patients than in advanced-stage patients, but not very significant ($p=0.107$). The percentage of S6K1 immunopositive tissues was significantly higher in advanced-stage EEAs than in early-stage EEAs (75% vs. 38.1%, respectively; $p=0.039$), indicating that as the stage of the disease advanced, the chance of having a positive immunostaining increased. When S6K1 positivity was used as a criterion of advanced stage in EEA, the sensitivity, specificity, positive predictive value, and negative predictive value were calculated to be 62%, 75%, 72%, and 65%, respectively (OR: 4.9 and 95% CI: 1.3–18.7).

Table 1. Comparisons between the groups in terms of age and EI and S6K1 immunostaining

Characteristics	Group 1 (n=21)	Group 2 (n=20)	p
Age, years	62±10.8	66.3±9.3	0.189*
EI	17.6±49.2	14.1±24.8	0.107*
S6K1 immunostaining (-)	13 (31%)	5 (12%)	
S6K1 immunostaining (+)	8 (20%)	15 (37%)	0.039**

Data are presented as the mean±standard deviation and number (percent).
The p value was determined by using *Mann-Whitney U test and **Chi-square test.
EI: expression index

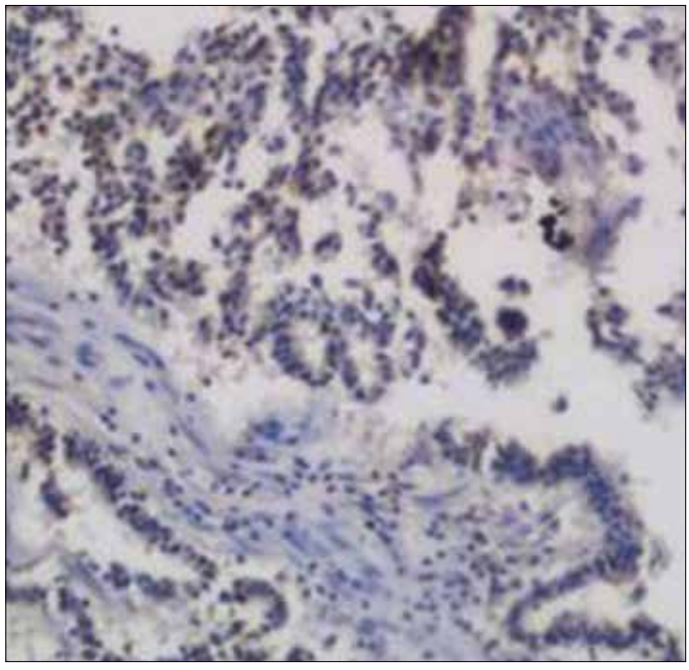

Figure 1. Activation status of immunocytochemical staining for P70S6K1 in endometrial endometrioid adenocarcinoma. Weak staining with ×200 magnification.

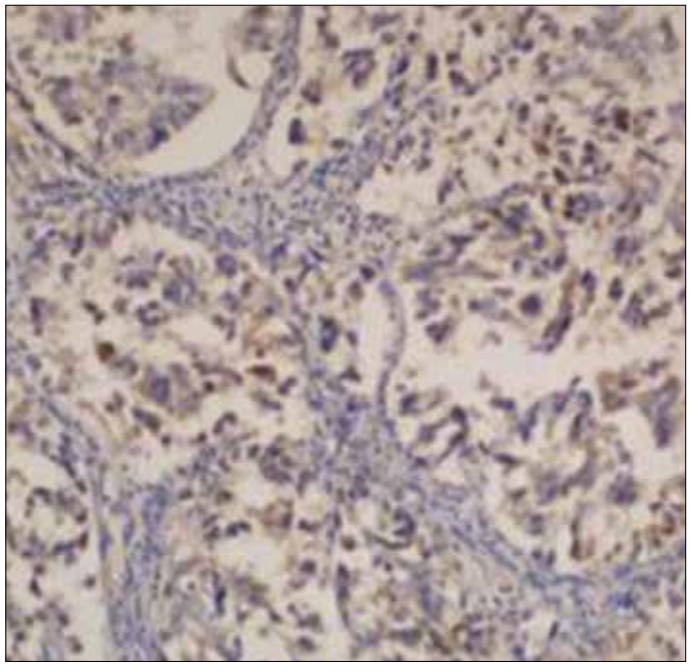

Figure 2. Activation status of immunocytochemical staining for P70S6K1 in endometrial endometrioid adenocarcinoma. Moderate staining with ×100 magnification.

Discussion

In this immunohistochemistry study, we examined whether S6K1, a downstream target of mTOR, was activated in EEA and whether its expression correlated with disease stage. There was no S6K1 immunostaining positivity in any specimen of the 22 normal tissue-containing group. However, S6K1 immunopositivity rates in Groups 1 and 2 were 38.1% and 75%, respectively (p=0.039), suggesting that the percentage of S6K1 immunopositive tissues was significantly higher in advanced-stage EEAs than in early-stage EEAs. Tumors positive for S6K1 expression were significantly more likely to be at an advanced stage than those negative for S6K1 staining, indicating that as the stage of the disease advanced, the chance of having a positive immunostaining result increased. The sensitivity and specificity rates of S6K1 immunostaining positivity in determining poor prognosis were 62% and 75%, respectively.

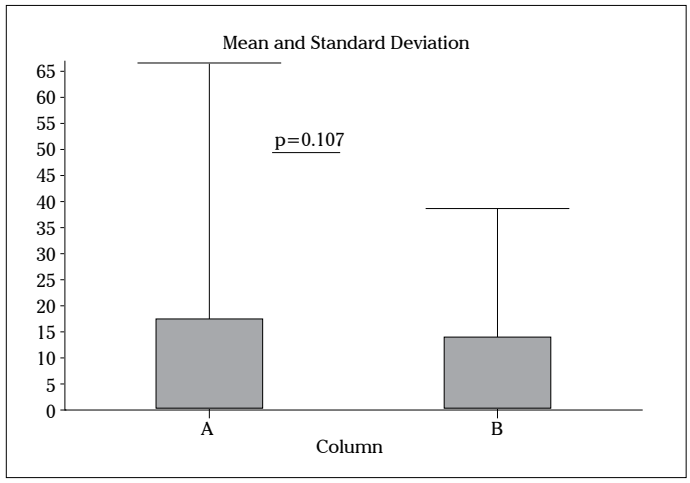

Figure 3. Expression index status of S6K1 protein in Group 1 and Group 2

Inter- and intra-cellular signaling is essential for the maintenance of cellular life. Protein kinases regulate many cell signaling pathways involved in protein phosphorylation/activation (11). Serine/threonine kinases constitute an important group in cytoplasmic protein kinases. Phosphatase and tensin homologue deleted on chromosome ten (PTEN)/phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR is considered to be a crucial regulatory pathway of protein translation, and mTOR is a 289 kDa serine/threonine protein kinase located in the center of this pathway (12). mTOR exists in two distinct complexes: mTORC1 and mTORC2. mTORC1 is sensitive to rapamycin and is regulated by multiple signals, such as growth factor, nutrients, energy status, oxygen, and cellular stress. mTORC1 phosphorylates and activates S6K1 and 4EBP1, which in turn, enhances protein synthesis, proliferation, cell survival, ribosome biogenesis, angiogenesis, migration, invasion, and metastasis. (8, 12, 13).

The deregulation of multiple elements of the mTOR pathway, such as amplification or mutation of PI3K, loss of PTEN function, and overexpression of AKT, S6K1, 4EBP1, and eIF4E, has been reported in numerous types of human diseases, including cancer, cardiac hypertrophy, type 2 diabetes, and obesity. (8, 12, 14). Aberrations of the mTOR signaling pathway are common in several types of human cancers, such as breast, ovarian, prostate, bladder, thyroid, colon, and head and neck cancers (8, 15-18). Activation of the PI3K/AKT/mTOR pathway and increased mTOR signaling have been reported to be associated with chemoresistance, aggressive disease, and poor prognosis in many cancer types, irrespective of the tumor type (19-23). *In vivo* studies support the

hypothesis that a loss of PTEN and subsequent AKT activation result in the activation of Estrogen Receptor-α (ERα)-dependent pathways that play a pivotal role in the neoplastic process (24). In a recent study, nuclear phosphorylated (p) Ser(167)–ERα was reported to significantly positively correlated with p-MAPK and p-S6K1 and with a significantly shorter relapse-free survival in EEA (25). Another study reported that extracellular signal-regulated kinase (ERK1/2) and p-AKT can be useful in the differential diagnosis of benign vs. malignant endometrial lesions as well as early- vs. advanced-stage EEA (26). Similarly, our results suggest that S6K1 immunopositivity could be used as a predictive test in EEAs and is a promising prognostic indicator of advanced stage and higher grade disease. On the other hand, mTOR inhibition has been associated with the diminished development and progression of hyperplastic lesions (27) in endometrial cancer cell lines. To date, six phase II trials assessing the use of rapalogs in recurrent endometrial carcinoma have been published (28). Surprisingly, two studies conducted in patients with endometrial cancer revealed no statistically significant correlation between activity of the PI3K/AKT/mTOR pathway and clinicopathological characteristics, including stage, grade, and lymph node involvement. (29, 30). In a very recent study, S6K1 expression has been reported to be a promising biomarker of sensitivity (31). Based on the results of our study, we demonstrated that only malignant tissue cell lines are associated with S6K1 immunostaining, whereas benign pathologies are not. Moreover, S6K1 expression is associated with advanced stage and a poor prognosis of disease. Given the uncertainty with respect to the adjuvant therapy options and fertility-sparing surgery in women with stage IA, grade 2 disease, S6K1 may cease the scientific debate as an indicator of poor prognosis. S6K1 immunostaining tests in women with EEA might surrogate advanced stage and poor prognosis and hence may be used to determine the appropriate therapeutic approach in this patient population.

In our study, EI, which is a scoring method based on overall stain intensity and the percentage of neoplastic tissue that is stained, was higher in early-stage patients than in advanced-stage patients, but not significantly so (p=0.107). Even if pathological specimens were evaluated independently by two pathologists in a blinded fashion and their results were averaged to minimize interobserver variability, the semi-quantitative scoring method might have influenced the results. Studies investigating the relationship between staining patterns and the stage or grade of the disease with larger patient numbers and using quantitative interpretation methods may indicate significant differences.

There is a very limited number of studies in the available literature investigating the mTOR pathway, S6K1 signaling, and endometrial carcinomas, which thus constitutes a substantial strength of this study. However, our study is limited by a number of matters. First, the number of the patients in our study was relatively low. The second is the lack of the treatment outcomes and survival period of the included patients. In this study, we excluded patients with certain clinical conditions, such as type 2 diabetes and obesity, which are well-known risk factors for EEA. This situation might be perceived to have biased the results of the study. However, it has been known that the mTOR pathway is mediated by a wide variety of cellular signal communications, which include hormones, such as insulin and growth factors; nutrients, such as amino acids and glucose; and cellular stress conditions. mTOR integrates signals from a variety of "energy balancing" hormones, such as leptin, insulin, and ghrelin, although its action varies in response to these distinct hormonal stimuli as well as across different neuronal populations, and it has roles in the regulation of body weight, energy expenditure, and glucose/lipid homeostasis (32). Moreover, the mTOR pathway has been associated with obesity in numerous studies, in which the risk of obesity was correlated with the overactivation of the mTOR-Raptor-S6K1 signaling pathway and a decrease in AKT levels. On the other hand, insulin resistance is a major aspect of type 2 diabetes, which results from impaired insulin signaling in target cells, and it was reported that the mTORC2-mediated phosphorylation of PKB-Ser473 was unperturbed in type 2 diabetes (33). From this aspect, we intended to avoid the confounding effects of clinical situations that may potentially interact with the mTOR pathway, such as obesity and type 2 diabetes, and to evaluate the association between endometrium carcinoma and S6K1 expression.

In conclusion, although S6K1 immunostaining was weak-moderate in both early and advanced stages, advanced stage and higher grade disease is significantly more likely to reveal S6K1 immunopositivity than early-stage, low-grade disease. S6K1 immunopositivity appears to be a promising method to predict poor prognosis in EEA. There is a need for more comprehensive studies on this subject.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Gülhane Military Medical Academy, Haydarpaşa Training and Research Hospital (11.11.2010, Project Number: 101).*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - İ.G., Ö.Ö.; Design - İ.G., Ö.Ö.; Supervision - İ.G., M.M.; Resources - Ö.Ö., Z.K.; Materials - Ö.Ö., Z.K.; Data Collection and/or Processing - İ.G., Ö.Ö.; Analysis and/or Interpretation - İ.G., D.D.; Literature Search - Ö.Ö., Z.K.; Writing Manuscript - Ö.Ö., İ.G.; Critical Review - M.M., D.D.*

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References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics. CA Cancer J Clin 2009; 59: 225-49. [Crossref]
2. Amant F, Moerman P, Neven P, Timmerman D, Van LE, Vergote I. Endometrial cancer. Lancet 2005; 366: 491-505. [Crossref]
3. Cengiz M, Singh AK, Grigsby PW. Postoperative vaginal brachytherapy alone is the treatment of choice for grade 1-2, stage IC endometrial cancer. Int J Gynecol Cancer 2005; 15: 926-31. [Crossref]
4. Horowitz NS, Peters WA 3rd, Smith MR, Drescher CW, Atwood M, Mate TP. Adjuvant high dose rate vaginal brachytherapy as treatment of stage I and II endometrial cancer. Obstet Gynecol 2002; 99: 235-40. [Crossref]

5. Thanapprapasr D, Thanapprapasr K. Molecular therapy as a future strategy in endometrial cancer. Asian Pac J Cancer Prev 2013; 14: 3419-23. [Crossref]
6. Umene K, Banno K, Kisu I, Yanokura M, Nogami Y, Tsuji K, et al. New candidate therapeutic agents for endometrial cancer: potential for clinical practice (review). Oncol Rep 2013; 29: 855-6.
7. Dancey J. mTOR signaling and drug development in cancer. Nat Rev Clin Oncol 2010; 7: 209-19. [Crossref]
8. Pópulo H, Lopes JM, Soares P. The mTOR Signalling Pathway in Human Cancer. Int J Mol Sci 2012; 13: 1886-918. [Crossref]
9. Xu G, Zhang W, Bertram P, Zheng XF, McLeod H. Pharmacogenomic profiling of the PI3K/PTENAKT-mTOR pathway in common human tumors. Int J Oncol 2004; 24: 893-900.
10. FIGO Committee on Gynecologic Oncology. FIGO staging for carcinoma of the vulva, cervix, and corpus uteri. Int J Gynaecol Obstet 2014; 125: 97-8. [Crossref]
11. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science 2002; 298: 1912-34. [Crossref]
12. Zhou H, Huang S. The complexes of mammalian target of rapamycin. Curr Protein Pept Sci 2010; 11: 409-24. [Crossref]
13. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol 2011; 12: 21-35. [Crossref]
14. Djordjevic B, Hennessy BT, Li J, Barkoh BA, Luthra R, Mills GB, et al. Clinical assessment of PTEN loss in endometrial carcinoma: immunohistochemistry outperforms gene sequencing. Mod Pathol 2012; 25: 699-708. [Crossref]
15. Xing M. Genetic alterations in the phosphatidylinositol-3 kinase/Akt pathway in thyroid cancer. Thyroid 2010; 20: 697-706. [Crossref]
16. Nawroth R, Stellwagen F, Schulz WA, Stoehr R, Hartmann A, Krause BJ, et al. S6K1 and 4E-BP1 are independent regulated and control cellular growth in bladder cancer. PLoS One 2011; 6: e27509. [Crossref]
17. Gao W, Li JZ, Chan JY, Ho WK, Wong TS. mTOR Pathway and mTOR Inhibitors in Head and Neck Cancer. ISRN Otolaryngol 2012; 2012: 953089. [Crossref]
18. Karlsson E, Pérez-Tenorio G, Amin R, Bostner J, Skoog L, Fornander T, et al. The mTOR effectors 4EBP1 and S6K2 are frequently coexpressed, and associated with a poor prognosis and endocrine resistance in breast cancer: a retrospective study including patients from the randomised Stockholm tamoxifen trials. Breast Cancer Res 2013; 15: R96. [Crossref]
19. Kremer CL, Klein RR, Mendelson J, Browne W, Samadzedeh LK, Vanpatten K, et al. Expression of mTOR signaling pathway markers in prostate cancer progression. Prostate 2006; 66: 1203-12. [Crossref]
20. Faried LS, Faried A, Kanuma T, Aoki H, Sano T, Nakazato T, et al. Expression of an activated mammalian target of rapamycin in adenocarcinoma of the cervix: A potential biomarker and molecular target therapy. Mol Carcinog 2008; 47: 446-57. [Crossref]
21. Faried LS, Faried A, Kanuma T, Sano T, Nakazato T, Tamura T, et al. Predictive and prognostic role of activated mammalian target of rapamycin in cervical cancer treated with cisplatin-based neoadjuvant chemotherapy. Oncol Rep 2006; 16: 57-63. [Crossref]
22. Salvesen HB, Carter SL, Mannelqvist M, Dutt A, Getz G, Stefansson IM, et al. Integrated genomic profiling of endometrial carcinoma associates aggressive tumors with indicators of PI3 kinase activation. Proc Natl Acad Sci U S A 2009; 106: 4834-9. [Crossref]
23. Darb-Esfahani S, Faggad A, Noske A, Weichert W, Buckendahl AC, Müller B, et al. Phospho-mTOR and phospho-4EBP1 in endometrial adenocarcinoma: association with stage and grade in vivo and link with response to rapamycin treatment in vitro. J Cancer Res Clin Oncol 2009; 135: 933-41. [Crossref]
24. Vilgelm A, Lian Z, Wang H, Beauparlant SL, Klein-Szanto A, Ellenson LH, et al. Akt-mediated phosphorylation and activation of estrogen receptor alpha is required for endometrial neoplastic transformation in Pten+/- mice. Cancer Res 2006; 66: 3375-80. [Crossref]
25. Kato E, Orisaka M, Kurokawa T, Chino Y, Fujita Y, Shinagawa A, et al. Relation between outcomes and expression of estrogen receptor-α phosphorylated at Ser(167) in endometrioid endometrial cancer. Cancer Sci 2014; 105: 1307-12. [Crossref]
26. Gungorduk K, Ertas IE, Sahbaz A, Ozvural S, Sarica Y, Ozdemir A, et al. Immunolocalization of ERK1/2 and p-AKT in normal endometrium, endometrial hyperplasia, and early and advanced stage endometrioid endometrial adenocancer and their prognostic significance in malignant group. Eur J Obstet Gynecol Reprod Biol 2014; 179: 147-52. [Crossref]
27. Milam MR, Celestino J, Wu W, Broaddus RR, Schmeler KM, Slomovitz BM, et al. Reduced progression of endometrial hyperplasia with oral mTOR inhibition in the PTEN heterozygote murine model. Am J Obstet Gynecol 2007; 196: 247.e1-5. [Crossref]
28. Lheureux S,Wilson M, Mackay H. Recent and current phase II clinical trials in endometrial cancer: review of the state of art. Expert Opin Investigating Drugs 2014; 23: 773-92. [Crossref]
29. No JH, Jeon YT, Park IA, Kang D, Kim JW, Park NH, et al. Expression of mTOR protein and its clinical significance in endometrial cancer. Med Sci Monit 2009; 15: BR301-5.
30. Mori N, Kyo S, Sakaguchi J, Mizumoto Y, Ohno S, Maida Y, et al. Concomitant activation of AKT with extracellular-regulated kinase 1/2 occurs independently of PTEN or PIK3CA mutations in endometrial cancer and may be associated with favorable prognosiss. Cancer Sci 2007; 98: 1881-8. [Crossref]
31. Santacana M, Coronado P, Matias-Guiu X, Romero I, Casado A, Gil-Moreno A, et al. Biological Effects of Temsirolimus on the mTOR Pathway in Endometrial Carcinoma: A Pharmacodynamic Phase II Study. Int J Gynecol Cancer 2016. [Crossref]
32. Hu F, Xu Y, Liu F. Hypothalamic Roles of mTOR Complex I: Integration of nutrient and hormone signals to regulate energy homeostasis. Am J Physiol Endocrinol Metab 2016. [Crossref]
33. Rajan MR, Nyman E, Kjolhede P, Cedersund G, Stralfors P. Systems-Wide Experimental and Modeling Analysis of Insulin Signaling through FOXO1 in Human Adipocytes, Normally and in Type 2 Diabetes. J Biol Chem 2016. [Crossref]

Methods for endometrial preparation in frozen-thawed embryo transfer cycles

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Abstract

Frozen-thawed (FT) embryo transfer is a procedure used for the storage and transfer of excess embryos obtained during in vitro fertilization–intracytoplasmic sperm injection cycles. In recent years, improvements in laboratory conditions and limitations on the number of embryos to be transferred have led to a progressive increase in FT embryo transfer cycles. However, the best solution for endometrial preparation in these cycles is still a matter of debate.

In this study, we aimed to review the current methods of endometrial preparation in FT embryo transfer cycles. In light of the current literature, it is hard to determine which method is the best for endometrial preparation. It is therefore necessary to conduct randomized controlled studies in a prospective design, which will also evaluate the above-mentioned factors. (J Turk Ger Gynecol Assoc 2016; 17: 168-71)

Keywords: Thawing, embryo cryopreservation, endometrial preparation, frozen-thawed embryo transfer, transdermal estradiol

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Introduction

Frozen-thawed (FT) embryo transfer is a procedure used for the storage and transfer of excess embryos obtained during in vitro fertilization (IVF)–intracytoplasmic sperm injection (ICSI) cycles. In recent years, improvements in laboratory conditions and limitations on the number of embryos to be transferred have led to a progressive increase in FT embryo transfer cycles. Another preferred practice to prevent multiple pregnancies in IVF cycles is to transfer a single embryo and freeze all surplus embryos (1). However, the best solution for endometrial preparation in these cycles is still a matter of debate (2).

Frozen-thawed embryo transfer prevents embryo waste and increases the probability of pregnancy in a single stimulated cycle. Protocols applied in FT cycles aim for endometrial preparation only and are therefore simpler than complicated protocols that aim to develop many follicles. As the treatment for subfertility increases, so does the importance of FT embryo transfer; however, there is no consensus about which method is the best (3, 4).

Pregnancy rates following FT embryo transfer have been found to be higher than those following fresh embryo transfer (5). Further, FT embryo transfer increases the cumulative pregnancy rate and decreases the cost; in addition, it is easy to perform and can be applied in a shorter time duration when compared to repetitive fresh embryo transfers (5). Using frozen excess embryos obtained as a result of the time-to-time implementation of in vitro maturation (IVM) in patients with polycystic ovaries, successful pregnancies have been achieved (6). Therefore, studies have concentrated on factors affecting the success rate of FT embryo transfer cycles.

Various cycle protocols are used for the preparation of the endometrium in an FT embryo transfer cycle. In one of these procedures, the transfer time is determined either by the natural course of a cycle [i.e., in an ovulatory patient exhibiting a natural (spontaneous) cycle] or by inducing ovulation during the course of a natural cycle. The second procedure involves the artificial preparation of the endometrium through the administration of exogenous estrogen and progesterone, which can be performed with or without the association of a gonadotropin-releasing hormone agonist. In the third procedure, the cycle is stimulated by gonadotropins and ovulation is induced by recombinant-human chorionic gonadotropin (r-Hcg) or hCG; however, this practice is no longer applied (7, 8).

Embryo transfer in a natural (spontaneous) cycle

Both embryo and endometrial development have to be synchronized in FT embryo transfer cycles in order to maximize the pregnancy rate (9). This synchronization can be achieved in several ways. The easiest is the endocrinological preparation of the endometrium during the natural cycle using the patient's own follicular sex steroids. In this application, the timing for embryo transfer (ET) is determined by either investigating the spontaneous luteinizing hormone (LH) surge or by the administration of exogenous human chorionic gonadotrophin (hCG) to start luteinization (10). Success of the natural cycle depends on the accurate determination of the ovulation time and the precise estimation of endometrial receptivity (11, 12).

Thawing and transfer procedures have to be performed during this receptive period. In the FT cycles performed during a natural cycle, urine or blood LH level is regularly analyzed and followed up. Ovulation is estimated to occur 36 to 40 hours

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after the determination of the blood LH surge (13). Urine LH increases 21 hours after the detection of the blood LH surge, and this fact has to be taken into consideration when interpreting the increase in urine LH (14).

Another problem in determining the time of the spontaneous LH surge is the variability of this increase, both among cycles and patients (15). At least one measurement, and preferably two measurements, has to be performed daily in order to accurately determine the LH surge. The threshold values of urine LH kits are highly variable, corresponding to an approximately 30% risk of a false-negative result; furthermore, patients state that it is hard to interpret the test results (16).

In order to avoid the difficulties of LH follow-ups, ovulation is frequently triggered by the administration of hCG during natural cycles; this is called the modified natural cycle. This approach does not require LH measurement, but the development of a dominant follicle has to be regularly investigated and followed up with ultrasonography (USG) in order to determine the suitable time for hCG administration (17). When the dominant follicle has sufficiently matured and has reached the proper size (17–18 mm), hCG is administered to the patient for the final oocyte maturation and ovulation. Ovulation occurs about 36 to 38 hours after hCG administration (13). The administration of hCG to induce ovulation in a natural cycle (or modified natural cycle) has been reported to negatively affect the rates for ongoing pregnancies (14.3% vs 31.1%) (18).

In natural or modified natural cycles, the embryo transfer is performed three to five days after ovulation, depending on when the embryos were frozen (19).

Ovulation may occur unexpectedly while planning a natural cycle, which can lead to difficulties in adjusting the time of thawing and transferring the embryos. When an unexpected early ovulation occurs, the cycle is generally cancelled. In a study by Weissman et al. (20), the LH surge was determined on the day of hCG administration during a modified natural cycle, and the cycle had to be cancelled. However in previous studies, an LH surge on the day of hCG administration was shown not to exert any negative effect (21, 22).

In most studies related to natural cycle applications, USG and the evaluation of estradiol, progesterone, and LH levels were used in combination in order to determine the time of thawing and transferring the embryos. With regard to timing, this approach is more reliable, but it is expensive and problematic. Alternatively, the follow-up of the LH surge, using urine LH kits, is an easier and more straightforward method; however, it carries with it a higher risk of a faulty test result. Cycle cancellation may be inevitable in 7% to 12% of natural cycles due to undetected ovulation (18).

Fewer laboratory analyses and USG follow-ups are required for determining the time of hCG administration in the modified natural cycle, and it is therefore less troublesome for both the physician and the patient. In studies comparing the natural cycle with the modified natural cycle, no marked differences were determined between the two procedures with regard to clinical pregnancy, ongoing pregnancy, and live births (20, 23). In two studies investigating the effects of luteal support in the natural cycle, luteal support was shown not to affect the results with regard to clinical pregnancy (Odds ratio (OR) 0.80, 95% confidence interval (CI) 0.61–1.0 vs OR 1.1, 95% CI 0.79–1.5). However, luteal support was shown to positively affect the results relating to a pregnancy continuing (OR 1.5, 95% CI 0.58–4.0 vs OR 0.82, 95% CI 0.62–1.1) (20, 24).

Artificial endometrium preparation by the exogenous administration of estrogen–progesterone

Another frequently used method for endometrium preparation is with the exogenous administration of estrogen and progesterone (with or without a gonadotrophin-releasing-hormone (GnRH) agonist), also called the artificial cycle, and is frequently used as an alternative for the natural cycle. Rates of clinical pregnancy and chemical pregnancy were shown not to differ in artificial cycles with regard to the administration of a GnRH agonist (25).

In order to mimic the endocrine conditions of the endometrium of a normal cycle in an artificial cycle, estrogen and progesterone are administered consecutively. Estrogen administration is started at the beginning of the cycle, causing endometrial development while suppressing dominant follicle development. Estradiol is introduced before the fourth day of the cycle. The earlier estradiol is commenced, the less the risk there is of unwanted follicular development and ovulation. Estrogen administration is continued until the endometrium reaches a thickness of 8 mm (determined using an ultrasonographic examination), and progesterone is then combined to initiate the secretory changes. Thus, an attempt is made to mimic the physiologic mid-cycle estrogen–progesterone transition (26, 27).

Estrogen can be administered as an oral tablet, transdermal plaster, or transvaginal ring. The most widely used forms are oral micronized estradiol or transdermal estradiol. Serum estradiol levels and endometrial thickness were not found to differ between these two applications (28). Some of the orally administered estradiol valerate is converted to estrone in the intestinal system (29). Estradiol and estrone are then transferred to the liver via the portal system, and converted there to estriol. During this transportation process, circulatory estrogen activity decreases by 30% (30).

The commonly used forms are currently estradiol valerate and micronized estrogens. Estradiol valerate (Progynova, Shering; Berlin, Germany) is administered throughout the cycle as follows: 1 mg on days 1 to 5, 2 mg on days 6 to 9, 6 mg on days 10 to 13, 2 mg on days 14 to 17, 4 mg on days 18 to 26, and 1 mg on days 27 and 28 (31) (Figure 1).

Estrogen can also be administered by the transdermal route, meaning it cannot be metabolized in the liver, which results in estradiol valerate concentrations exceeding those of estrone. In other words, a more physiological estradiol/estrone ratio (approximately 1) exists. When estrogen is administered orally, this ratio is 0.2, and therefore is not in accordance with the physiological values (32).

Transdermal estrodiol valerate (Estraderm, CIBA Pharmaceutical Co.; Summit, NJ, USA) plasters are applied on the lower abdominal region, whereby between day 1 and 6 of the cycle, plasters are applied as a dose of 50 micrograms once every three days; between day 7 and 9, one plaster of 100 micrograms; on days 10 and 11, a 200 microgram plaster; and between day 12 and 14, four plasters, each of 100 micrograms (400 micrograms total) are used. Between day 15 and 17, the dose is decreased to 100 micrograms, but is then increased again to 200 micrograms between day 18 and 28 of the cycle. In this protocol, an estradiol concentration exceeding 200 pg/mL is accepted to be sufficient (33). However, transdermal estrogen application can cause fluctuations in estrogen concentrations, and it may sometimes be difficult to maintain a constant steroid level. Another reason for preferring the transdermal route to oral administration is the unchanged serum lipid levels, coagulation factors, and renin substrate (34).

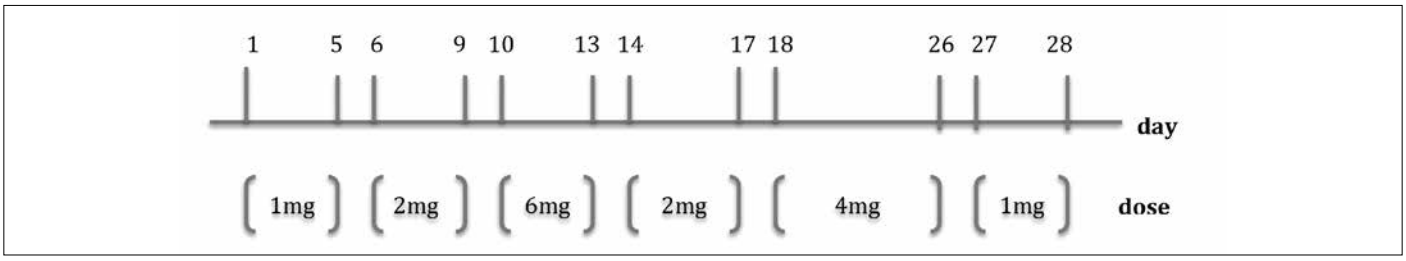

Figure 1. Doses and days of estrogen in the artificial cycle. This figure shows the days and doses of estrogen administration in patients with an artificial endometrial preparation.

Pregnancy rates were not found to differ between oral and transdermal administration (35). However, transvaginal estrogen reaches only 25% of the circulatory level of the same dose of estrogen administered orally; furthermore, it damages the effect of the vaginal progesterone used for luteal support, making transvaginal estrogen a less favored application (36).

Progesterone can either be administered in artificial cycles using the intramuscular route, or as vaginal suppositories or vaginal gels. With regard to the pregnancy rates in donor cycles, Glujovsky et al. (37) could not detect any difference between vaginal and intramuscular administration. The starting time for progesterone administration depends on endometrial thickness but not on the duration of estrogen administration. Progesterone administration can only be commenced when the endometrium thickness exceeds 8 mm (38, 39).

Natural progesterone (Federa, Sterop; Brussels, Belgium) or micronized progesterone (Utrogestan, Piette; Brussels, Belgium) are the most generally used progesterone preparations. The administration of natural progesterone is commenced intramuscularly in a dose of 50 mg on day 14 of the cycle, and is continued in a dose of 100 mg daily between days 15 and 26. Micronized progesterone is administered vaginally in a dose of 100 mg or 200 mg on day 14 of the cycle, and is continued in a dose of 300 mg or 600 mg daily between days 15 and 26 (40).

Orally administered micronized progesterone was shown not to be suitable for preparing the endometrium for implantation. None of the biopsy investigations was observed to be compatible in this respect (41). Vaginal progesterone was determined to form a secretory phase of endometrium that resembles that of the natural cycle (42). In intramuscular progesterone administration, 43% of the endometria cases were found to be in accordance with the cycle phase; in the rest of the cases, increased asynchronous maturation was detected (43).

In artificial preparation, the time for thawing and transferring the embryos is planned according to the commencement of progesterone support. The exogenous administration of estrogen and progesterone does not guarantee the complete suppression of the pituitary gland; in other words, a dominant follicle may develop. The developing follicle may also undergo spontaneous luteinization, which leads to the early exposure of the endometrium to progesterone, and thus incorrect calculations for thawing and transfer times. For these reasons, GnRH agonists can be added to the treatment protocols in order to downregulate the pituitary, thus preventing follicular development. Both of the artificial cycles are less physiological due to exogenous drug administration; however, they are practical and easy to apply, and hence preferred both by physicians and patients. However, with regard to overall ongoing pregnancy rates, it has not yet been totally clarified which procedure offers the superior method (44). Table 1 shows the alternative estrogen and progesterone application methods.

Which method is preferable?

When eight retrospective studies, including 8152 cycles, and one randomized controlled study including 111 cycles were investigated in the literature, no differences were observed between the natural cycles and artificial cycles with regard to clinical pregnancy (OR 1.2, 95% CI 0.86–1.6), ongoing pregnancy (OR 1.2, 95% CI 0.95–1.5), or live births (OR 1.2, 95% CI 0.93–1.6) (45). In four studies comparing natural cycles with GnRH agonist-supported artificial cycles, clinical pregnancy and live births did not differ (46, 47-49). Furthermore, clinical pregnancy was determined not to differ between the artificial cycles and GnRH-supported artificial cycles (44, 50, 51).

Some studies have shown that endometrial thickness positively affects pregnancy rates in FT cycles (39, 52, 53). Complete downregulation cannot be guaranteed in artificial cycles, and early luteinization may therefore exist in 5% of cases. Estradiol levels are higher in the artificial cycles than in natural cycles, and consequently, the endometrium has been reported to be thicker in the artificial cycles (30); however, in some studies, such a difference has not been detected (54). Non-physiologically high estradiol levels have been claimed to cause endometrial damage and variances in the implantation window (55). Broadly, this is the main problem in IVF applications. If this claim were taken into account, low pregnancy rates would be expected in the artificial cycles because of high E_2 levels. However, such a negative effect has not been shown in the artificial cycles, and this claim of a negative effect needs to be investigated in further studies.

Pregnancy rates do not differ between the natural and artificial cycles; therefore, laboratory conditions, social status, and physicians' and patients' preferences are effective for making a decision in the choice between these two procedures. In a study by Gelbaya et al. (56), the natural cycles and downregulated artificial cycles did not differ from each other in this respect.

Clinical pregnancy, ongoing pregnancy, and live birth rates have not been found to differ between endometrial preparations induced by human menopausal gonadotrophin (HMG) or by exogenously administered estrogen–progesterone in patients with polycystic ovary syndrome (PCOS) or those with anovulation; however, a thin endometrium was more frequently observed in HMG-induced cycles (57, 58).

Some studies have shown that long-term GnRH agonist administration before IVF/ICSI in infertile women with endometriosis or adenomyosis significantly increases the chances of pregnancy (59). It may be true for FT embryo transfer cycles. In a study

Table 1. Alternative ways of administering estrogen and progesterone

		Dose/Days of Cycle					
Estrogen	Oral (daily)	1 mg (1–5)	2 mg (6–9)	6 mg (10–13)	2 mg (14–17)	4 mg (18–26)	1 mg (27–28)
	Transdermal (patch) once every 3 day	50 μg (1–6)	100 μg (7–9)	200 μg (10–11)	400 μg (12–14)	100 μg (15–17)	200 μg (18–28)
Progesterone	Intravaginal (daily)	----------		100 or 200 mg (14)		300 or 600 mg (15–26)	
	Intramuscular (daily)	----------		50 mg (14)		100 mg (15–26)	
	Oral	Inefficient					

involving patients administered a GnRH agonist combined with estrogen and progesterone, clinical pregnancy, implantation, and ongoing pregnancy rates were 51.35%, 32.56%, and 48.91%, respectively, which were significantly higher than the rates in patients administered only estrogen and progesterone (24.83%, 16.07%, and 21.38%, respectively) (60).

Briefly, in light of the current literature, it is difficult to determine which method is better for endometrial preparation. All current procedures appear to be equally successful with regard to ongoing pregnancy rates. Most studies are retrospective, and this may lead to prejudice during the comparisons. The preferences of patients and the impact of costs have not been taken into consideration during studies. Therefore, it is necessary to conduct randomized controlled studies in a prospective manner, which should also evaluate the above-mentioned factors.

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References

1. Gurgan T, Demirol A. Why and how should multiple pregnancies be prevented in assisted reproduction treatment programmes? Reprod Biomed Online 2004; 9: 237-44. [Crossref]
2. Le Lannou D, Griveau JF, Laurent MC, Gueho A, Veron E, Morcel K. Contribution of embryo cryopreservation to elective single embryo transfer in IVF–ICSI. Reprod Biomed Online 2006; 13: 368-75. [Crossref]
3. Ghobara T, Vanderkerchove P. Cycle regimens for frozen-thawed embryo transfer. The Cochrane Library 2008. [Crossref]
4. Weissman A, Levin D, Ravhon A, Eran H, Golan A, Levran. What is the preferred method for timing natural cycle frozen–thawed embryo transfer? Reprod Biomed Online 2009; 19: 66-71. [Crossref]
5. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen–thawed embryo transfer in normal responders. Fertil Steril 96; 2: 344-8. [Crossref]
6. Demirol A, Guven S, Benkhalifa M, Sari T, Gurgan T. Successful birth following transfer of frozen–thawed embryos produced from in-vitro matured oocytes. Reprod Biomed Online 2010; 21: 215-8. [Crossref]
7. Wright KP, Guibert J, Weitzen S, Davy C, Fauque P, Olivennes F. Artificial versus stimulated cycles for endometrial preparation prior to frozen–thawed embryo transfer. Reprod Biomed Online 2006; 13: 321-5. [Crossref]
8. Imbar T, Hurwitz A. Synchronization between endometrial and embryonic age is not absolutely crucial for implantation. Fertil Steril 2004; 82: 472-4. [Crossref]
9. Ming L, Liu P, Qiao J, Lian Y, Zheng X, Ren X, et al. Synchronization between embryo development and endometrium is a contributing factor for rescue ICSI outcome. Reprod Biomed Online 2012; 24: 527-31. [Crossref]
10. Groenewoud ER, Macklon NS, Cohlen BJ. Cryo-thawed embryo transfer: natural versus artificial cycle. A non-inferiority trial (ANTARCTICA trial). BMC Women's Health 2012; 12: 27. [Crossref]
11. Harper MJ. The implantation window. Baillieres Clin Obstet Gynaecol 1992; 6: 351-71. [Crossref]
12. Tabibzadeh S. Molecular control of the implantation window. Hum Reprod Update 1998; 4: 465-71. [Crossref]
13. Andersen AG, Als-Nielsen B, Hornnes PJ, Andersen LF. Time interval from human chorionic gonadotrophin (HCG) injection to follicular rupture. Hum Reprod 1995; 10: 3202-5.
14. Miller PB, Soules MR. The usefulness of a urinary LH kit for ovulation prediction during menstrual cycles of normal women. Obstet Gynecol 1996; 87: 13-7. [Crossref]
15. Park SJ, Goldsmith LT, Skurnick JH, Wojtczuk A, Weiss G. Characteristics of the urinary luteinizing hormone surge in young ovulatory women. Fertil Steril 2007; 88: 684-90. [Crossref]
16. O'Connor KA, Brindle E, Miller RC, Shofer JB, Ferrell RJ, Klein NA, et al. Ovulation detection methods for urinary hormones: precision, daily and intermittent sampling and a combined hierarchical method.Hum Reprod 2006; 21: 1442-52. [Crossref]
17. Sathanandan M, Macnamee MC, Rainsbury P, Wick K, Brinsden P, Edwards RG. Replacement of frozen-thawed embryos in artificial and natural cycles: a prospective semi-randomized study. Hum Reprod 1991; 6: 685-7.
18. Fatemi HM, Kyrou D, Bourgain C, Van den Abbeel E, Griesinger G, Devroey, P. Cryopreserved-thawed human embryo transfer: spontaneous natural cycle is superior to human chorionic gonadotropin–induced natural cycle. Fertil Steril 2010; 94: 2054-8. [Crossref]
19. Paulson RJ. Hormonal induction of endometrial receptivity. Fertil Steril 2011; 96: 530-5. [Crossref]
20. Weissman A, Horowitz E, Ravhon A, Steinfeld Z, Mutzafi R, Golan A, et al. Spontaneous ovulation versus HCG triggering for timing natural-cycle frozen–thawed embryo transfer: a randomized study. Reprod Biomed Online 2011; 23: 484-9. [Crossref]
21. Griesinger G, Weig M, Schroer A, Diedrich K, Kolibianakis EM. Mid-cycle serum levels of endogenous LH are not associated with the likelihood of pregnancy in artificial frozen-thawed embryo transfer cycles without pituitary suppression. Human Reprod 2007; 22: 2589-93. [Crossref]

22. Groenewoud ER, Kollen BJ, Macklon NS, Cohlen BJ. Spontaneous LH surges prior to HCG administration in unstimulated-cycle frozen–thawed embryo transfer do not influence pregnancy rates. Reprod Biomed Online 2012; 24: 191-6. [Crossref]
23. Chang EM, Han JE, Kim YS, Lyu W, Lee WS, Yoon TK. Use of the natural cycle and vitrification thawed blastocyst transfer results in better in-vitro fertilization outcomes. J Assist Reprod Genet 2011; 28: 369-74. [Crossref]
24. Tomás C, Alsbjerg B, Martikainen H, Humaidan P. Pregnancy loss after frozen-embryo transfer—a comparison of three protocols. Fertil Steril 2012; 98: 1165-9. [Crossref]
25. Nekoo EA, Chamani M, Tehrani ES, Rashidi BH, Tanha FD, Kalantari V. Artificial Endometrial Preparation for Frozen-Thawed Embryo Transfer with or without Pretreatment with Depot Gonadotropin Releasing Hormone Agonist in Women with Regular Menses. J Fam Reprod Health 2015; 9: 1-4.
26. El-Toukhy T, Coomarasamy A, Khairy M, Sunkara K, Seed P, Khalaf Y, et al. The relationship between endometrial thickness and outcome of medicated frozen embryo replacement cycles. Fertil Steril 2008; 89: 832-9. [Crossref]
27. Jaroudi KA, Hamilton C J, Willemsen WN, Sieck UV, Roca G. Artificial endometrial stimulation for frozen embryo replacement. Fertil Steril 1991; 55: 835-7. [Crossref]
28. Hancke K, More S, Kreienberg R, Weiss JM. Patients undergoing frozen-thawed embryo transfer have similar live birth rates in spontaneous and artificial cycles. J Assist Reprod Genet 2012; 29: 403-7. [Crossref]
29. Ryan KJ, Engel lL. The interconversion of estrone and estradiol by human tissue slices 1, 2, 3. Endocrinology 1953; 52: 287-91. [Crossref]
30. van Keep PA, Utian WH, Vermeulen A. Potency and hepato-cellular effects of oestrogens after oral, percutaneous, and subcutaneous administration. In The Controversial Climacteric. Springer Netherlands 1982; pp. 103-25. [Crossref]
31. Devroey P, Pados G. Preparation of endometrium for egg donation. Hum Reprod Update 1998; 4: 856-61. [Crossref]
32. Powers MS, Schenkel L, Darley PE, Good WR, Balestra JC, Place VA. Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17β-estradiol: comparison with conventional oral estrogens used for hormone replacement. Am J Obstet Gynecol 1985; 152: 1099-106. [Crossref]
33. Chetkowski RJ, Meldrum DR, Steingold KA, Randle D, Lu JK, Eggena P, et al. Biologic effects of transdermal estradiol. N Eng J Med 1986; 314: 1615-20. [Crossref]
34. Powers MS, Schenkel L, Darley PE, Good WR, Balestra JC, Place VA. Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17β-estradiol: comparison with conventional oral estrogens used for hormone replacement. Am J Obstet Gynecol 1985; 152: 1099-106. [Crossref]
35. Rosenwaks Z, Navot D, Veeck L, LIU HC, Steingold K, Kreiner D, et al. Oocyte Donation. Ann NY Acad Sci 1988; 541: 728-41. [Crossref]
36. Stumpf PG. Selecting constant serum estradiol levels achieved by vaginal rings. Obstet Gynecol 1986; 67: 91-4.
37. Glujovsky D, Pesce R, Fiszbajn G, Sueldo C, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. The Cochrane Library 2010. [Crossref]
38. Nawroth F, Ludwig M. What is the 'ideal'duration of progesterone supplementation before the transfer of cryopreserved–thawed embryos in estrogen/progesterone replacement protocols? Hum Reprod 2005; 20: 1127-34. [Crossref]
39. El-Toukhy T, Coomarasamy A, Khairy M, Sunkara K, Seed P, Khalaf Y, et al. The relationship between endometrial thickness and outcome of medicated frozen embryo replacement cycles. Fertil Steril 2008; 89: 832-9. [Crossref]
40. Pados G, Camus M, Van Waesberghe L, Liebaers I, Van Steirteghem A, Devroey P. Oocyte and embryo donation: evaluation of 412 consecutive trials. Hum Reprod 1992; 7: 1111-7.
41. Devroey P, Palermo G, Bourgain C, Van Waesberghe L, Smitz J, Van Steirteghem AC. Progesterone administration in patients with absent ovaries. Int J Fertil 1988; 34: 188-93.
42. Bourgain C, Devroey P, Van Waeshberghe L, Smitz J, Van Steirteghem AC. Effects of natural progesterone on the morphology of the endometrium in patients with primary ovarian failure. Hum Reprod 1990; 5: 537-43.
43. Pados G, Devroey P. Luteal phase support. Assist Reprod Rev 1992; 2: 148.
44. Dal Prato L, Borini A, Cattoli M, Bonu MA, Sciajno R, Flamigni C. Endometrial preparation for frozen-thawed embryo transfer with or without pretreatment with gonadotropin-releasing hormone agonist. Fertil Steril 2002; 77: 956-60. [Crossref]
45. Groenewoud ER, Cantineau AE, Kollen BJ, Macklon NS, Cohlen BJ. What is the optimal means of preparing the endometrium in frozen–thawed embryo transfer cycles? A systematic review and meta-analysis. Hum Reprod Update 2013; dmt030. [Crossref]
46. Al-Shawaf T, Yang D, Al-Magid Y, Seaton A, Iketubosin F, Craft I. Infertility: Ultrasonic monitoring during replacement of frozen/thawed embryos in natural and hormone replacement cycles. Hum Reprod 1993; 8: 2068-74.
47. Queenan Jr JT, Veeck LL, Seltman HJ, Muasher SJ. Transfer of cryopreserved-thawed pre-embryos in a natural cycle or a programmed cycle with exogenous hormonal replacement yields similar pregnancy results. Fertil Steril 1994; 62: 545-50. [Crossref]
48. TA G, Hunter HR. Cryopreserved-thawed embryo transfer in natural or down-regulated hormonally controlled cycles: A retrospective study. Digest of the World Core Medical Journals (Obstetrics/Gynecology) Z1 2006.
49. Hill MJ, Miller KA, Frattarelli JL. A GnRH agonist and exogenous hormone stimulation protocol has a higher live-birth rate than a natural endogenous hormone protocol for frozen-thawed blastocyst-stage embryo transfer cycles: an analysis of 1391 cycles. Fertil Steril 2010; 93: 416-22. [Crossref]
50. Simon A, Hurwitz A, Zentner BS, Bdolah Y, Laufer N. Transfer of frozen-thawed embryos in artificially prepared cycles with and without prior gonadotrophin-releasing hormone agonist suppression: a prospective randomized study. Human Reprod 1998; 13: 2712-7. [Crossref]
51. El-Toukhy T, Taylor A, Khalaf Y, Al-Darazi K, Rowell P, Seed P, et al. Pituitary suppression in ultrasound-monitored frozen embryo replacement cycles. A randomised study. Human Reprod 2004; 19: 874-9. [Crossref]
52. Richter KS, Bugge KR, Bromer JG, Levy MJ. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos. Fertil Steril 2007; 87: 53-9. [Crossref]
53. Kumbak B, Erden HF, Tosun S, Akbas H, Ulug U, Bahçeci M. Outcome of assisted reproduction treatment in patients with endometrial thickness less than 7 mm. Reprod Biomed Online 2009; 18 : 79-84. [Crossref]
54. Tomás C, Alsbjerg B, Martikainen H, Humaidan P. Pregnancy loss after frozen-embryo transfer—a comparison of three protocols. Fertil Steril 2012; 98: 1165-9. [Crossref]
55. Adams SM, Terry V, Hosie MJ, Gayer N, Murphy CR. Endometrial response to IVF hormonal manipulation: Comparative analysis of menopausal, down regulated and natural cycles. Reprod Biol Endocrinol 2004; 2: 21. [Crossref]
56. Gelbaya TA, Nardo LG, Hunter HR, Fitzgerald CT, Horne G, Pease EE, et al. Cryopreserved-thawed embryo transfer in natural or down-regulated hormonally controlled cycles: a retrospective study. Fertil Steril 2006; 85: 603-9. [Crossref]
57. Yu J, Ma Y, Wu Z, Li Y, Tang L, Li Y, et al. Endometrial preparation protocol of the frozen-thawed embryo transfer in patients with polycystic ovary syndrome. Arch Gynecol Obstet 2015; 291: 201-11. [Crossref]
58. Wright KP, Guibert J, Weitzen S, Davy C, Fauque P, Olivennes F. Artificial versus stimulated cycles for endometrial preparation prior to frozen–thawed embryo transfer. Reprod Biomed Online 2006; 13: 321-5. [Crossref]
59. Devlieger R, D'Hooghe T, Timmerman D. Uterine adenomyosis in the infertility clinic. Hum Reprod Update 2003; 9: 139-47. [Crossref]
60. Niu Z, Chen Q, Sun Y, Feng Y. Long-term pituitary downregulation before frozen embryo transfer could improve pregnancy outcomes in women with adenomyosis. Gynecol Endocrinol 2013; 29: 1026-30. [Crossref]

What is your diagnosis?

A 25-year-old primigravida presented at 35 weeks of gestation with progressive, severe, bilateral pitting, lower limb edema with excessive weight gain. This was associated with itching, scaling, weeping, crustation, ulceration, and discoloration. She had no history of varicose veins or treatment for venous disorders. Her general examination findings were unremarkable. The skin of both lower limbs showed eczematous scales, oozing, crusts, and brownish pigmentation that involved the dorsum of both feet and ankles up to the knees with scattered areas of ulcerations (Figure 1). All her prenatal investigation results were within normal limits, and a color duplex examination of the lower limbs ruled out any vascular disorder. Treatment included elevation of the legs, intermittent compression therapy, and application of mid-potency steroids and systemic and topical antibiotics. However, the skin lesions progressed to mimic cellulitis. At 38 weeks of gestation, a decision of pregnancy termination was taken, which was followed by a dramatic improvement within 1 week after cesarean section (Figure 2).

Figure 1. The skin of both lower limbs shows the typical picture of severe stasis dermatitis (eczematous, crusts, and brownish discoloration) that involved the dorsum of both feet and ankles up to the knees with scattered areas of ulceration.

Figure 2. One week after cesarean section, both lower limbs showed dramatic improvement of stasis dermatitis with few residual areas of brownish discoloration due to hemosiderin deposition.

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Answer

Lower extremity gestational edema (GE) is an almost universal finding in late pregnancy (i.e., physiologic), occurring secondary to increased venous pressure in the legs, the obstruction of lymphatic flow, and reduced plasma colloid osmotic pressure (1). Stasis dermatitis/eczema is an inflammatory dermatosis, commonly affecting the lower extremities; it occurs in patients with chronic venous insufficiency, often in association with varicose veins and dependent chronic edema. Clinical features include erythema, scaling, weeping, crusting, hyperpigmentation, lipodermatosclerosis, and ulcerations (2). Thus, as a diagnosis of exclusion, our patient was managed as a case of "idiopathic eczema/dermatitis."

The mechanism by which venous stasis causes eczema/dermatitis is yet to be elicited. The hypoxia/stasis theory has suggested that increased hydrostatic pressure leads to decreased oxygen content of pooled blood, resulting in hypoxic damage of the overlying skin (3). This may, in part, explain the rapid development of stasis dermatitis with severe GE. In spite of intensive treatment, the skin lesions exhibited progressive acute inflammation and exudation that mimicked severe cellulitis. Diagnostic skin biopsies are rarely indicated (4); thus, it was not recommended. At this stage, a decision of pregnancy termination was taken to eliminate the venous congestion in the legs caused by the pressure exerted mechanically by the uterus onto the inferior vena cava and iliac veins (5). Surprisingly, the patient showed a dramatic improvement in her condition within 1 week after cesarean section.

In conclusion, our presented case suggests that the rapid development of this type of "stasis dermatitis" and its dramatic improvement after pregnancy termination supports our speculation to be as a new subtype termed "gestational stasis dermatitis" induced by severe GE. Pregnancy termination is indicated in such cases.

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References

1. Gabbe SG, Niebyl JR, Simpson JL, (editors) Obstetrics: normal and problem pregnancies, 3rd ed. New York (NY): Churchill Livingstone, 1996.
2. Mc Koy K. Stasis dermatitis. Available from: http://www.merckmanuals.com/professional/dermatologicdisorders/dermatitis/stasis dermatitis.html. Accessed on January 2016.
3. Flugman SL, Elston D M. Stasis Dermatitis. Medscape, Available from http://emedicine.medscape.com/article/1084813-overview. [citation updated: Aug 30, 2012] Retrieved May 5, 2015.
4. Flugman SL, Clark, RA. Stasis dermatitis workup. Available from: http://emedicine.medscape.com/article/1084813-workup#aw2aab6b5b2 [updated July 21, 2014] Retrieved November 13, 2015.
5. Bracero LA, Didomenico A. Massive vulvar edema complicating preeclampsia: a management dilemma. J Perinatol 1991; 11: 122-5.

Does size matter?

To the Editor,

In 1995, I described a modified Cesarean Section (CS), the so-called "Misgav Ladach" method (1) which gained international usage, including both in Turkey (2) and in Germany (3). There are scores of publications from many countries, all showing without exception a notably shorter operative time, and fewer complications of wound healing and reduced hospital stay. Although all display these benefits, the results also demonstrate large statistical variations. These variations are probably due to the lack of standardization of the surgical method, as only studies using a standardized method enable comparison of outcomes among different surgeons and institutions (4).

The opening of the uterus in the classical Misgav Ladach CS, is done by cutting a small transverse incision in the middle of the lower segment and stretching the opening bi-digitally, transversely. In this way, the resulted bleeding is minimal. This enables suturing the uterus in one layer and although controversial, one layer may result in less ruptures during repeated pregnancies (5).

As the uterus contracts immediately after CS, suturing is used mainly for immediate hemostasis. The suture material cannot contract with the uterus therefore; the more suture material left behind the more foreign body reaction, which might result in increased pain, irritation to the bladder and a weaker scar.

For many years, I was suturing the uterus with a one-layer continuous locking stitch (6), using an 80 mm needle, PGA size 1. Using a big needle enables stitching further away from the incision line resulting in excellent hemostasis with minimal steps and less suture material, rarely needing extra single hemostatic sutures.

When using a smaller needle, the surgeon must stitch closer to the incision line, demanding more time and steps, and in case of suturing the uterus in one layer, this could result in the necessity for an additional second layer or single extra stitches for hemostasis.

Standardization of CS is needed in order to be able to compare the outcome among different surgeons and institutions. Comparing outcome of surgeries even performed using the same standardized method but with different sized needles, might lead to different outcome and therefore inaccurate conclusions.

Therefore, it seems that the size of the needle does matter and I highly suggest to routinely using a standardized-sized 80 mm needle.

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References

1. Stark M, Chavkin Y, Kupfersztain C, Guedj P, Finkel AR. Evaluation of combinations of procedures in cesarean section. Int J Gynaecol Obstet 1995; 48: 273-6. [Crossref]
2. Naki MM, Api O, Celik H, Kars B, Yaşar E, Unal O. Comparative study of Misgav-Ladach and Pfannenstiel-Kerr cesarean techniques: a randomized controlled trial. J Matern Fetal Neonatal Med 2011; 24: 239-44. [Crossref]
3. Heidenreich W, Borgmann U. [Results of the Misgav Ladach caesarean section]. Zentralbl Gynakol 2001; 123: 634-7. [Crossref]
4. Stark M, Gerli S, Di Renzo GC, The importance of analyzing and standardizing surgical methods, in: J Minim Invasive Gynecol 2009; 16: 122-5. [Crossref]
5. Hudić I1, Fatusić Z, Kamerić L, Misić M, Serak I, Latifagić A. Vaginal delivery after Misgav-Ladach cesarean section--is the risk of uterine rupture acceptable? J Matern Fetal Neonatal Med 2010; 23: 1156-9. [Crossref]
6. Holmgren G, Sjöholm L, Stark M. The Misgav Ladach method for cesarean section: method description. Acta Obstet Gynecol Scand 1999; 78: 615-21. [Crossref]

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CONGRESS CALENDAR

INTERNATIONAL MEETINGS

September 8-10, 2016	**ESGO-European Society of Gynaecological Oncology** Antalya, Turkey http://www.esgo.org
September 21-24, 2016	**The International Congress on Antiphospholipid Antibodies (aPL)** İstanbul, Turkey www.apsistanbul2016.org
September 21-25, 2016	**22nd IFFS World Congress** New Delhi NCR, India http://www.iffs2016.com
October 2-5, 2016	**ESGE-25th Annual Congress of the ESGE** Brussels http://www.esgecongress.eu
October 15-19, 2016	**ASRM- 72nd Annual Meeting of the ASRM** Salt Lake City, UT, USA https://www.asrm.org
October 26-28, 2016	**Update in Obstetrics, Gynecology and Reproductive Medicine** Barcelona, Spain http://www.comtecmed.com
November 10-13, 2016	**The 24th World Congress on Controversies in Obstetrics, Gynecology & Infertility (COGI)** Amsterdam, Netherlands http://congressmed.com/cogi
November 19-21, 2016	**Türkçe Konuşan Ülkelerin 1. Jinekoloji Kongresi,** Bakü, Azerbeycan www.1tgc.org

NATIONAL MEETINGS

5-9 October 2016	**14. Ulusal TJOD Kongresi** Antalya Kaya Palazzo Otel http://www.tjodkongre2016.org
6-8 October, 2016	**I. Uluslararası ve II. Ulusal Kadın Hastalıkları ve Ana Çocuk Sağlığı Kongresi,** Kuşadası, Antalya www.kadinvecocuksagligi.org
6-8 October, 2016	**Gebelik, Doğum ve Lohusalık Kongresi** Ankara www.korukdg2016.org
6-8 October, 2016	**6. İstanbul Üniversitesi Kadın Doğum Günleri** Harbiye Askeri Müze, İstanbul www.istanbulkadindogum2016.org
14-15 October, 2016	**34. Zeynep Kamil Jineko/Patoloji Kongresi** (TJOD Istanbul Anadolu Şb Desteği ile) www.zeynepkamilkongre2016.org
20-22 October, 2016	**3. Uluslararası 4. Ulusal Ebelik Kongresi,** Ankara www.ebko2016.org
28-30 October, 2016	**Türkiye Maternal Fetal Tıp ve Perinatoloji Derneği X. Ulusal Kongresi** Harbiye, Istanbul www.tmftp2016.org
17 - 20 November, 2016	**7. Ulusal Üreme Endokrinoloji ve İnfertilite Kongresi** (TSRM 2016) www.tsrm.org

JTGGA CME/CPD CREDITING

Answer form for the article titled "*Methods for endometrial preparation in frozen-thawed embryo transfer cycles*" within the scope of CME/CPD

1. Which of the following is not true for frozen-thawed (FT) embryo transfer?
   a. Improvements in laboratory conditions have led to a progressive increase in FT embryo transfer cycles.
   b. Limitations for the number of embryos to be transferred have led to a progressive increase in FT embryo transfer cycles.
   c. FT doesn't increase the probability of pregnancy in a single stimulated cycle.
   d. FT embryo transfer prevents embryo waste.
   e. The preferred practice to prevent multiple pregnancy in IVF cycles is to transfer single embryo and freeze all surplus embryos.

2. Which of the following is not a contrubuting factor to the significance of frozen-thawed (FT) embryo transfer?
   a. FT embryo transfer prevents embryo waste.
   b. Pregnancy rates following FT embryo transfer are higher than fresh embryo transfer
   c. Protocols applied for endometrial preparation in FT cycles are simpler than the complicated protocols that aim to develop many follicles.
   d. FT embryo transfer increases the cumulative pregnancy rate.
   e. There is no consensus about which preparation method of endometrium is better.

3. Which of the following statements is incorrect for embryo transfer in a natural (spontaneous) cycle.
   a. Success of natural cycle depends on the accurate determination of the ovulation time
   b. Timing for embryo transfer (ET) is determined by investigating the spontaneous luteinizing hormone (LH) surge.
   c. Timing for embryo transfer (ET) is also determined by the administration of exogenous human chorionic gonadotropin (hCG) to start luteinization.
   d. Ovulation is estimated to occur 36 to 40 hours after the determination of the blood LH surge.
   e. Urine LH increases 21 hours before the detection of the blood LH surge.

4. Which of the following statement is not true for artificial cycles?
   a. In order to mimic the endocrine conditions of the endometrium of a normal cycle in an artificial cycle, estrogen and progesterone are administered simultaneously.
   b. Estrogen administration is started at the beginning of the cycle, causing endometrial development, while suppressing dominant follicle development.
   c. The earlier estradiol is commenced, the less the risk there is of unwanted follicular development and ovulation.
   d. Estrogen administration is continued until the endometrium reaches a thickness of 8 mm (determined using an ultrasonographic examination), and progesterone is combined to initiate the secretory changes.
   e. In an artificial cycle, an attempt is made to mimic physiologic mid-cycle estrogen-progesterone transition

5. Which of the following statement is not true for estrogen adminstration in artificial cycles?
   a. Estrogen can be administered as an oral tablet, transdermal plaster or transvaginal ring.
   b. The commonly used forms are currently Estradiol valerate and micronized estrogens
   c. A more physiological estradiol/estrone ratio (approximately 1) exists when estrogen is administered orally.
   d. Transdermal estrogen application can cause fluctuations in estrogen concentrations, and it may sometimes be difficult to maintain a constant steroid level.
   e. Another reason of preferring the transdermal route to oral administration is the unchanged serum lipid levels, coagulation factors, and renin substrate

6. Which of the following statement is not true for progesterone adminstration in artificial cycles?
   a. Progesterone can either be administered in artificial cycles using the intramuscular route, or as vaginal suppositories or vaginal gels.
   b. Natural progesterone or micronized progesterone are the most generally used progesterone preparations.
   c. The starting time for progesterone administration depends on the duration of estrogen administration but not on the endometrial thickness.
   d. Progesterone administration can only be commenced when endometrium thickness exceeds 8 mm.
   e. Vaginal progesterone was determined to form a secretory phase of endometrium that resembles that of the natural cycle.

JTGGA CME/CPD CREDITING

Questions on the article titled "*Methods for endometrial preparation in frozen-thawed embryo transfer cycles*" within the scope of CME/CPD

1st Question				
A	B	C	D	E

2nd Question				
A	B	C	D	E

3rd Question				
A	B	C	D	E

4th Question				
A	B	C	D	E

5th Question				
A	B	C	D	E

6th Question				
A	B	C	D	E

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