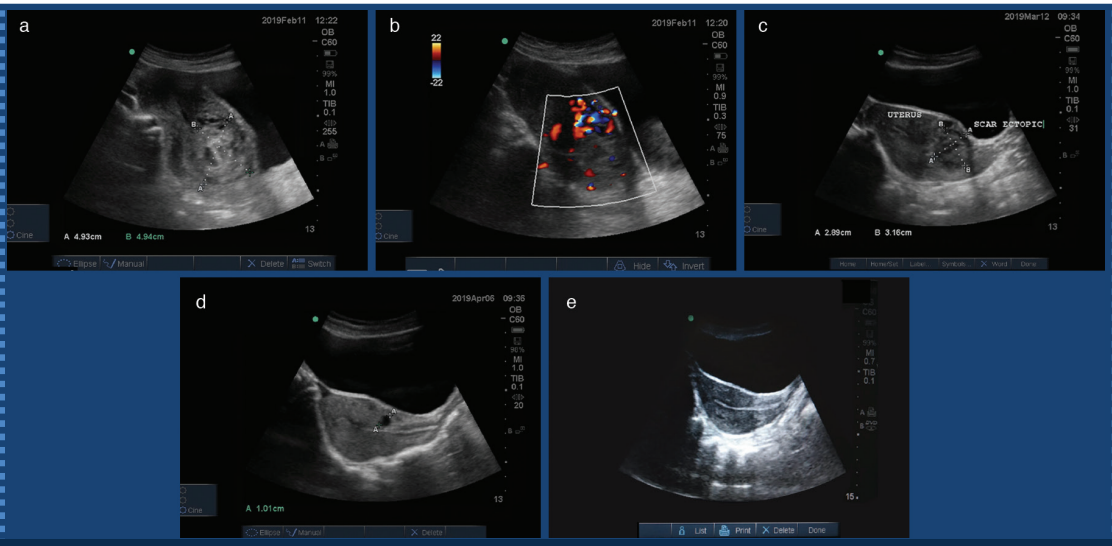
EISSN 1309-0380

Indexed in PubMed and Web of Science

TURKISH-GERMAN GYNECOLOGICAL EDUCATION and RESEARCH FOUNDATION

Journal of the Turkish-German Gynecological Association



Volume 23
Issue 3
September
2022

Cover Picture: Latika Chawla et al. Intra-gestational agents for managing CSP

galenos yayınevi

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Online Publication Date: September 2022

E-ISSN: 1309-0380
International scientific journal published quarterly.

Journal of the
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Aims and Scope

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 publication language of the journal is English. Manuscripts are reviewed in accordance with "double-blind peer review" process for both reviewers and authors.

The target audience of Journal of the Turkish-German Gynecological Association includes gynecologists and primary care physicians interested in gynecology practice. It publishes original works on all aspects of obstertrics and 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, diagnostic puzzle are also published. Suggestions for new books are also welcomed. Journal of the Turkish-German Gynecological Association does not charge any fee for article submission or processing.

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Contents

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Editorial

Dear Colleagues,

It is my great pleasure to introduce the third issue of the "Journal of the Turkish-German Gynecological Association (J Turk Ger Gynecol Assoc)" in the publishing year of 2022. This issue is consisted of eight articles and one review that we hope you will read with interest. Here we share some of our favorite articles that were published in this issue of the journal.

Proper diet and the maintenance of adequate nutritional status play an important role in reproductive function. In recent years, scientific research has increasingly focused on the role of diet in polycystic ovary syndrome (PCOS). You will read an article investigating the relationship between micronutrient intake and androgen levels associated with PCOS.

Cesarean section rates continue to rise globally. In relation to this, the rate of health implications after cesarean section has increased analogously. Every effort for promotion of vaginal birth is of great importance. You will also read an interesting study which analyzed the outcome of trial of labor after cesarean section (TOLAC) in a Western population and identify factors associated with the success of vaginal birth after caesarean section.

You will also have the opportunity to read an experimental study examining the effects of human umbilical cord mesenchymal stem cells (hUCMSCs), amniotic fluid (AF), and a combination of both on the uterus and ovaries in a rat model of abdominal adhesions.

Dear Esteemed Readers,

Clarivate Plc, on July 26, 2022, announced that in the 2023 release of the Journal Citation Reports™, all Web of Science Core Collection™ journals will get a Journal Impact Factor (JIF)™. This will make full transparency possible to the articles and citations that contribute to impact.

The Journal Citation Indicator (JCI) represents the average category-normalized citation impact for papers published in the prior three-year period. As we announced earlier our journal is included in the JCI, a new metric offered by Web of Science, and its score has increased from 0.37 to 0.43. JTGGA became the third-quarter journal according to JCI data.

Citation counts are important metrics for the academics and journals. As we see, JTGGA has also an increase in citation counts. Choice of key words, use of key words in the title, using the same form of your name and surname, presentation of your study at the conferences, sharing your data on social media will all help to increase the citation counts.

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Sincerely,

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

Vitamin B_3 (niacin), B_6, C, and iron intake are associated with the free androgen index, especially in normoandrogenic polycystic ovary syndrome

Andon Hestiantoro[1,2], Brilliant Putri Kusuma Astuti[2], Ericko Ongko Joyo[1], Ririn Rahmala Febri[2], Vita Silvana[1,2], Raden Muharam[1,2]

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Abstract

Objective: Nutritional intake is one of the most common environmental risk factors for polycystic ovary syndrome (PCOS) because it is associated with obesity and insulin resistance. The aim of this study was to determine the relationship between micronutrient intake and androgen levels associated with PCOS.

Material and Methods: This cross-sectional study was performed in patients with PCOS divided into two groups, normoandrogenic (NA) and hyperandrogenic (HA), and healthy controls. Dietary intake assessment was performed using a modified 38-item semi-quantitative food frequency questionnaire. Bivariate, correlation, and multivariate analyses were performed to determine the association between study variables.

Results: There were 79 patients with PCOS, of whom 50 were NA and 29 were HA. There were 66 subjects in the healthy control group. The baseline characteristics in all groups were similar, except for body mass index and hormonal profile which were elevated in the HA group compared to the other groups. There was a significant negative correlation between the free androgen index (FAI) and intake of vitamin B_1, vitamin B_2, niacin, vitamin B_6, calcium, and iron in the NA group, while this association was absent in the HA group. Multivariate linear regression analysis showed that the intake of vitamin B_6, vitamin C, niacin, and iron had a significant effect on the FAI.

Conclusion: There is an effect of micronutrient intake on androgen levels in women with PCOS. The association was more significant in NA PCOS than in the HA PCOS groups. These findings suggest an association between micronutrients, androgens and PCOS at a systemic level. (J Turk Ger Gynecol Assoc 2022; 23: 130-6)

Keywords: Androgens, hyperandrogenism, micronutrients, polycystic ovary syndrome

Received: 04 March, 2022 **Accepted:** 13 June, 2022

Introduction

Polycystic ovary syndrome (PCOS) is a severe health risk for women of all ages, and it has long-term consequences for their health and well-being (1). There are at least four separate phenotypes of PCOS, three of which are PCOS with classic features of hyperandrogenism, while one phenotype of PCOS is characterized by normal androgen levels (2,3). Various studies have identified clinical, biochemical, and even genetic differences between normoandrogenic (NA) and hyperandrogenic (HA) PCOS (3,4). However, no studies have shown the pathophysiological characteristics of PCOS with normal androgen levels. The molecular pathomechanism in PCOS is not well understood due to the diverse character of the disorder. However, it has been suggested that the interaction between hereditary and environmental factors plays a role in the development and variability of PCOS symptoms (5,6).

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DOI: 10.4274/jtgga.galenos.2022.2022-2-1

Among the aforementioned environmental factors, nutritional intake and physical activity are two of the most important predictors of PCOS risk. Given this, there is much research focused on determining the best nutritional intake pattern to include in a PCOS treatment plan (7-9).

Although several studies have been conducted on the impact of macronutrients in PCOS, few studies have looked at the importance and role of micronutrients. Indeed, some current research suggests that appropriate micronutrient intake could help to reduce PCOS symptoms, including insulin resistance and hyperandrogenism (7,10). Unfortunately, there has not been much research into the direct link between micronutrient intake and androgen profiles. To address this knowledge gap, the aim of this study was to investigate the relationship between micronutrient intake and androgen levels associated with PCOS, particularly in the NA group.

Material and Methods

Study designs and ethical consideration

The design of this study was cross-sectional. The authorization to perform this study was obtained from the University of Indonesia Ethical Committee with the approval number 0449/UN2.F1/ETIK/2018. Before subject recruitment and data collection, study objectives were clearly explained to the subjects, and written consent was provided by each individual who agreed to participate. Data processing was conducted anonymously and in strict confidence.

Study population

A total of 145 reproductive age women comprised of 79 PCOS patients and 66 non-PCOS controls were enrolled for the present study. The sample size was calculated according to the sample size formula for the cross-sectional correlation test based on power analysis. The selection of subjects was made according to particular inclusion and exclusion criteria. The inclusion criteria for PCOS subjects were reproductive-age women who have been diagnosed with PCOS according to the Rotterdam criteria. The inclusion criteria for subjects in the control group were reproductive-age women with normal menstrual cycles who did not meet the diagnostic criteria for PCOS. Subjects were excluded if they were pregnant or breastfeeding; on medications known to alter metabolic parameters for the past two months, such as anti-dyslipidemic, anti-diabetic, or hormonal medications; had any endocrine abnormalities, such as diabetes, thyroid diseases, hyperprolactinemia, or Cushing diseases.

The study population was further subdivided into HA and NA groups according to their free androgen index (FAI), with FAI ≥5 used as a cut-off for hyperandrogenism (11). In addition to having FAI <5, subjects in the NA groups had to have normal serum testosterone and sex hormone-binding globulin (SHBG) concentrations, as well as no symptoms of hyperandrogenism, such as hirsutism, acne vulgaris, androgenic alopecia, and acanthosis nigricans (12).

Dietary intake assessment

A dietary intake assessment was performed by experienced staff using a modified 38-item semi-quantitative food frequency questionnaire (SQ-FFQ). The SQ-FFQ assessed daily intake of foods and beverages in the past three months with six possible responses that ranged from never; 1-3 times a month; 1-3 times a week; 4-6 times a week; once a day; or more than once a day, which can be converted into daily servings of 0, 0.5, 2, 5, 7, and 14 times per week, respectively. The quantity of food consumed, both reported in household measures and grams, was converted and homogenized to grams. The mean frequency of food intake was further multiplied by the portion size, which resulted in an estimated weekly intake. The recorded data were analyzed using Nutrisurvey software to estimate micronutrient intakes, according to Indonesian Food Composition Data.

This SQ-FFQ validation study was conducted among a subsample of 40 participants against 30-days repeated 24-hour dietary recall. The participants were asked to fill in both the SQ-FFQ and dietary recall. Then, the means of nutrient intake obtained from both questionnaires were calculated and compared using paired t-test. According to the statistical analysis, the mean intake of nutrients from SQ-FFQ and dietary recall did not differ significantly. Hence, a modified 38-item SQ-FFQ as a dietary assessment tool was valid.

Laboratory evaluation

Peripheral venous samples were collected and centrifuged to separate the serum for quantitative measurement of testosterone and SHBG concentrations using an ELISA. Serum testosterone and SHBG concentrations were determined using a commercial fluorescence enzyme immunoassay kit according to the manufacturer's instructions: ST AIA-Pack Testosterone (TOSOH, Japan, Cat. No. 0025204) and ST AIA-Pack SHBG (TOSOH Bioscience, Japan), respectively. The assay was of the sandwich-type using a pre-coated 96 well plate and a supply of enzyme-labeled secondary antibodies. The sample required for analysis was 300 μL. The sample cup and test cup were prepared and labelled with ID for each sample before measurement. Then, the sample cup and test cup were inserted into the TOSOH instrument. FAI was calculated as total testosterone to SHBG ratio (both in nmol/L) and was reported as a percentage (%).

Statistical analysis

Statistical analysis was performed with SPSS software, version 22.0 (IBM Corp., Chicago, IL., USA). The Kolmogorov-Smirnov test was used to ensure the normality of data distribution. Descriptive analysis was performed to report the baseline characteristics of our study population, which was presented as mean ± standard deviation for numerical variables. Bivariate analysis of the data was performed using Independent t- or Mann-Whitney U test to determine the mean difference between two numerical variables. The correlation between dependent and independent variables was calculated using Pearson's or Spearman's correlation coefficient. Multiple linear regression analysis was used to determine the micronutrients that were significantly and independently associated with FAI. The significance level was set at 95%, with a p-value of 0.05 or less considered statistically significant.

Results

A total of 145 reproductive age women consented and were involved in this study, consisted of 79 PCOS patients and 66 control subjects. The 79 PCOS patients consisted of 50 NA and 29 HA patients. According to WHO Asia Pacific body mass index (BMI) classification, the mean BMI of subjects in the PCOS group was classified as obese 1 (26.35±5.43 kg/m^2). HA patients also had a slightly higher BMI than NA PCOS (27.57±6.03 kg/m^2 vs 25.66±4.99 kg/m^2). In comparison, the mean BMI of subjects in the control group was classified as overweight (24.02±3.85 kg/m^2). Subjects in the PCOS group also showed a considerably higher FAI compared to subjects in the control group. The mean FAI of subjects in the PCOS group was 14.72±52.70, which was considered hyperandrogenemia. The average FAI of NA PCOS patients was also slightly increased compared to the control group (3.81±2.03% vs 2.27±1.54%). Tables 1 and 2 present the baseline characteristics of the NA PCOS, HA PCOS, and control groups.

The mean levels of various micronutrient intakes in the three groups did not show a significant difference. Tables 3 and 4 present the mean micronutrient intake of the NA PCOS, HA PCOS, and control groups. The FAI in the NA PCOS patient group had a significant negative correlation with the intake of vitamins B_1, B_2, B_6, niacin, calcium, and iron. (r=-0.340, p=0.016; r=0.367, p=0.009; r=-0.356, p=0.011; r=-0.389, p=0.005; r=-0.343, p=0.015; and r=-0.384, p=0.006, respectively). Table 5

Table 1. The characteristics of overall study population (control vs. PCOS)

Characteristics	Control (n=66)	PCOS (n=79)	p
Age (years)	30.00±5.52	29.23±3.66	0.440
Height (m)	1.56±0.04	1.58±0.00	0.158
Weight (kg)	59.14±10.25	66.17±14.74	0.005*
BMI (kg/m^2)	24.02±3.85	26.35±5.43	0.006*
Testosterone level (ng/mL)	0.45±0.37	0.99±2.49	<0.001*
Total testosterone (nmol/L)	1.57±1.28	3.46±8.63	<0.001*
SHBG (nmol/L)	167.25±740.44	49.77±65.86	<0.001*
Free androgen index (%)	2.27±1.54	14.72±52.70	<0.001*

Continuous variables are shown as means ± standard deviations. Independent t-test was performed for the control vs. polycystic ovary syndrome. BMI: Body mass index, SHBG: Sex hormone-binding globulin, *: Indicates statistical significance at the level p<0.05, PCOS: Polycystic ovary syndrome

Table 2. The characteristics of overall study population (control vs normoadrogenic PCOS vs. hyperandrogenic PCOS)

Characteristics	Control (n=66)	Normoandrogenic (n=50)	Hyperandrogenic (n=29)	p
Age (years)	30.00±5.52	29.56±3.53	28.64±3.89	0.506
Height (m)	1.56±0.04	1.57±0.59	1.59±0.65	0.263
Weight (kg)	59.14±10.25	64.06±13.52	69.93±16.28	0.004*
BMI (kg/m^2)	24.02±3.85	25.66±4.99	27.57±6.03	0.008*
Testosterone level (ng/mL)	0.45±0.37	0.52±0.35	1.85±4.03	<0.001*
Total testosterone (nmol/L)	1.57±1.28	1.80±1.22	6.41±14.00	<0.001*
SHBG (nmol/L)	167.25±740.44	62.90±77.94	26.31±21.34	<0.001*
Free androgen index (%)	2.27±1.54	3.81±2.03	34.19±85.44	<0.001*

Continuous variables are shown as means ± standard deviations. ANOVA was performed for the control vs. normoandrogenic PCOS vs. hyperandrogenic PCOS. BMI: Body mass index, SHBG: Sex hormone-binding globulin, *: Indicates statistical significance at the level p<0.05, PCOS: Polycystic ovary syndrome

shows the correlation between FAI and micronutrient intake in NA and HA PCOS. Meanwhile, there was no significant correlation of FAI with any micronutrient intake in the HA PCOS group.

Table 6 shows the results of multivariate linear regression within PCOS groups. Multivariate linear regression analysis of PCOS patients, both NA and HA, showed a significant negative effect of vitamin B_6 intake on FAI. (β=-1.825, $p<0.001$). Meanwhile, intake of vitamin C, iron, and niacin showed a significant positive relationship with FAI in PCOS patients (β=5.844, $p<0.001$; β=2.381, $p=0.020$; β=2.599, $p=0.011$, respectively). There was no significant effect of intake of other micronutrients on FAI.

Discussion

Vitamins, particularly vitamins A and C, act as antioxidants and play an essential role in suppressing chronic inflammation linked to PCOS (13). However, our study did not demonstrate any significant mean differences of any micronutrient intake in PCOS women compared with normal women. To the best of our knowledge, our study is the first study that compares the intakes of vitamin A, calcium, and iron in women with PCOS and normal women. As for vitamin B_1, vitamin B_2, niacin, vitamin $B_{6,}$ vitamin C, and folate, Szczuko et al. (14) have conducted a systematic review study and reported that vitamin C intake was lower in women with PCOS than in normal women, while for other micronutrients, no difference was found. Meanwhile, Zaeemzadeh et al. (15) found that the dietary intake of zinc was significantly lower in PCOS women with metabolic syndrome than in control groups. However, our results did not indicate a lower vitamin C and zinc intake in the PCOS group.

In another study, Szczuko et al. (16) found plasma levels of vitamin C in PCOS women were higher than in non-PCOS women, while plasma levels of vitamin B in PCOS women were lower than in non-PCOS women. Given that our study did not confirm significant differences in intakes of any micronutrients

Table 3. Micronutrients' intake in control and overall PCOS subjects

Micronutrient	Control (n=66)	PCOS (n=79)	p
Vitamin A (mg)	1035.23±1153.58	1114.67±1332.35	0.694
Vitamin B_1 (mg)	0.51±0.35	0.53±0.40	0.946
Vitamin B_2 (mg)	1.06±0.74	1.11±0.86	0.949
Vitamin B_6 (mg)	0.92±0.65	0.95±0.68	0.883
Vitamin C (mg)	6.17±7.02	6.39±7.19	0.789
Calcium (mg)	381.97±255.58	350.77±264.11	0.347
Zinc (mg)	9.64±7.09	9.95±7.06	0.871
Iron (mg)	5.72±4.19	5.57±4.11	0.816
Niacin (mg)	10.85±8.70	11.24±9.76	0.970
Folic acid (mg)	136.57±134.32	150.07±144.07	0.512
Continuous variables are shown as means ± standard deviations. Independent t-test was performed for the control vs. polycystic ovary syndrome, Indicates statistical significance at the $p<0.05$ level, PCOS: Polycystic ovary syndrome			

Table 4. Micronutrients' intake in control, normoandrogenic PCOS, and hyperandrogenic PCOS subjects

Micronutrient	Control (n=66)	Normoandrogenic (n=50)	Hyperandrogenic (n=29)	p
Vitamin A (mg)	1035.23±1153.58	881.54±937.27	1530.97±1785.45	0.517
Vitamin B_1 (mg)	0.51±0.35	0.50±0.37	0.57±0.44	0.691
Vitamin B_2 (mg)	1.06±0.74	1.03±0.78	1.24±0.99	0.610
Vitamin B_6 (mg)	0.92±0.65	0.92±0.63	1.01±0.77	0.914
Vitamin C (mg)	6.17±7.02	6.16±7.10	6.80±7.44	0.655
Calcium (mg)	381.97±255.58	349.45±292.37	353.11±209.25	0.431
Zinc (mg)	9.64±7.09	9.19±6.24	11.29±8.28	0.421
Iron (mg)	5.72±4.19	5.23±3.79	6.18±4.64	0.522
Niacin (mg)	10.85±8.70	10.67±8.11	12.24±12.28	0.970
Folic acid (mg)	136.57±134.32	132.36±120.20	181.87±177.03	0.512
Continuous variables are shown as means ± standard deviations. ANOVA was performed for the control vs. normoandrogenic PCOS vs. hyperandrogenic PCOS, $p<0.05$ indicates statistical significance at the 0.05 level, PCOS: Polycystic ovary syndrome				

studied, including vitamins B and C, we suspected that the effect of vitamin C on PCOS incidence is due to its concentration in serum rather than its intake. The concentration of vitamin C, regardless of the amount of intake, in women with PCOS is related to the individual response to oxidative stress. During oxidative stress and activation of anti-inflammatory reactions, the concentration of ascorbic acid and cortisol in rat plasma increases but decreases in the adrenal glands (17,18).

Our study revealed significant correlations between the estimated intake of several micronutrient (vitamin C, B_6, niacin, iron) and the FAI in women with PCOS. We demonstrated that vitamin C intake was positively associated with the FAI in women with PCOS. Szczuko et al. (16) also confirmed a positive correlation between plasma vitamin C levels and total testosterone. Vitamin C has antioxidant properties that can suppress chronic inflammation in PCOS. In addition, ascorbic acid is found in large quantities in the pituitary gland, so it is thought to have an important role in the secretion of the some hormones including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin. Furthermore, studies have shown that treatment with ascorbic acid increases FSH and testosterone levels, but these studies have not been performed in healthy women (19). This supports and may explain our study's finding that vitamin C intake affects androgens in women with PCOS.

Our study showed that niacin (B_3) intake had a significant effect on FAI in women with PCOS. This can be explained by studies on PCOS mouse models showing that the metabolite N_1-methyl nicotinamide, a metabolite of a niacin-derived compound, helps ameliorate hyperandrogenism and ovarian adenosine 5'monophosphate-activated protein kinase (AMPK) via aldehyde oxidase 1, which plays a role in detoxifying the enzymes that metabolize it (20,21). As niacin may activate AMPK (22), we speculate that decreased AMPK activity due to niacin deficiency is closely related to increased frequency of gonadotropin-releasing hormone pulsatility and increased LH production in the pituitary (23,24) as well as AMPK-dependent steroidogenesis disorders in the ovaries (25,26), in PCOS subjects. However, other studies have shown that niacin level has a negative correlation with SHBG (16). The kynurenine pathway uses tryptophan from food to produce niacin. The majority of the kynurenine pathway occurs in the liver and, to a lesser extent, in extrahepatic organs (27). This may explain the stronger correlation between niacin intake and FAI in the NA group, as demonstrated in this study.

One possible link between vitamin B_6 and androgens is homocysteine. Several B vitamins and zinc play a role in the elimination of homocysteine from circulation. The re-

Table 5. Correlation analysis between micronutrients intake and FAI in PCOS groups

Characteristics	Normoandrogenic PCOS (n=50)		Hyperandrogenic PCOS (n=29)	
	r	p	r	p
Vitamin A (mg)	- 0.255	0.074	- 0.097	0.617
Vitamin B_1 (mg)	- 0.340	0.016*	0.104	0.592
Vitamin B_2 (mg)	- 0.367	0.009*	- 0.016	0.934
Vitamin B_6 (mg)	- 0.356	0.011*	0.102	0.600
Vitamin C (mg)	- 0.199	0.165	0.117	0.547
Calcium (mg)	- 0.389	0.005*	0.012	0.952
Zinc (mg)	- 0.277	0.052	0.070	0.717
Iron (mg)	- 0.343	0.015*	0.126	0.515
Niacin (mg)	- 0.384	0.006*	0.156	0.419
Folic acid (mg)	- 0.271	0.057	0.106	0.585

Pearson correlation analysis was performed between estimated micronutrients intake and free androgen index. r is the Pearson correlation coefficient which shows the strength of the association between micronutrients intake and androgen levels. *: Indicates statistical significance at the $p<0.05$ level, FAI: Free androgen index, PCOS: Polycystic ovary syndrome

Table 6. The results of multivariate linear regression within PCOS groups

Variables	Unstandardized β ± SE β	Standardized β	p	95% CI range for ExpB
Vitamin B_6	-140.473±38,183	-1.825	<0.001*	(-216.465)-(-64.481)
Vitamin C	6,022±1,030	5,844	<0.001*	3,968-8,075
Iron	9,694±4,071	2,381	0.020*	1,583-17,806
Niacin	4,131±1,589	2,599	0.011*	0.965-7,298

Multivariate linear regression was performed. *: Indicates statistical significance at the $p<0.05$ level, PCOS: Polycystic ovary syndrome, CI: Confidence interval

methylation process involves folate, B_2, B_3, and zinc, while the transsulfuration process involves B_6 and zinc. Meanwhile, it has been reported that blood homocysteine levels were negatively correlated with SHBG concentrations (28). Therefore, the association of FAI with vitamins B_2, B_3, B_6, folate, and zinc may be mediated by SHBG concentrations.

Iron dysregulation can lead to reduced circulating levels of total testosterone. The association between iron and testosterone was weaker in overweight or obese patients than in normal weight patients (29). This is similar to findings from our study where there was a significant correlation between iron intake and FAI in women with PCOS, and this correlation was found to be weaker in HA PCOS patients who were predominantly overweight or obese.

A meta-analysis by Janjuha et al. (12), who conducted studies in normal populations, stated that they did not find a significant effect of micronutrient supplementation, including vitamin A, vitamin C, iron, and zinc, on sex hormones. In contrast to Janjuha et al. (12), our study of women with PCOS confirmed an association between androgens and intake of several types of micronutrients. Furthermore, regarding the fact that the HA phenotype was associated with insulin resistance, whereas the NA phenotype was not (30), we also report a stronger correlation in NA PCOS than HA PCOS.

Study Limitations

Our study still has some limitations that need to be considered when interpreting the results. Our study was conducted with a small sample and had cross-sectional design. It would be better if a longitudinal study design were carried out to determine the causal relationship between androgens and micronutrients in women with PCOS. It should also be noted that this study examined the estimated intake of micronutrients, not the pre-intake serum levels and post-intake serum levels of micronutrients, which of course, were influenced by absorption, transport, and demand.

Conclusion

This study observed an association between the FAI and estimated intake of some micronutrients, such as vitamin B_6, vitamin C, niacin, and iron. These findings suggest a role for micronutrients in the pathology of PCOS and androgens at the systemic level. We suggest future studies should be performed with a larger number of samples, measuring nutrient levels in plasma directly and focusing on its association with the increased role of homocysteine in PCOS.

Ethical Committee Approval: *The authorization to perform this study was obtained from the University of Indonesia Ethical Committee with the approval number 0449/UN2.F1/ETIK/2018.*

Informed Consent: *Written consent was provided by each individual who agreed to participate.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept: A.H.; Design: A.H.; Data Collection or Processing: B.P.K.A., R.R.F.; Analysis or Interpretation: A.H., E.O.J., R.R.F., V.S., R.M.; Literature Search: A.H., E.O.J.; Writing: E.O.J.*

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

Financial Disclosure: *The authors declared that this study received no financial support.*

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Risk factor analysis in women who underwent trial of labor after cesarean section: a multicenter study in Germany

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Abstract

Objective: Rising caesarean delivery (CD) rates throughout the world are accompanied with high rates of severe maternal complications. The aim of the present study was to analyze the outcome of trial of labor after caesarean section (TOLAC) in a Western population and identify factors associated with the success of vaginal birth after caesarean section (VBAC).

Material and Methods: A retrospective study was performed at two large obstetric departments in Germany from 2008 to 2018. Women with singleton pregnancies, a history of only one previous CD with a low transverse incision, a viable fetus in cephalic presentation, and gestational age >32 weeks were included in the study. The characteristics and outcome of successful VBAC and failed TOLAC were compared. A subgroup analysis addressed gestational age, interpregnancy interval, fetal macrosomia, body mass index, and maternal age.

Results: Of 1,546 patients, 62.3% achieved VBAC while 37.7% had a secondary CD. Independent factors associated with the success of TOLAC were a history of vaginal birth in previous pregnancies ($p<0.001$) and the use of oxytocin ($p<0.001$), whereas preterm birth between gestational week 32 and 37 signified a higher risk of failed TOLAC ($p=0.04$). The success of VBAC did not differ significantly for patients older than 40 years of age, those with a shorter interpregnancy interval than 12 months, and fetal macrosomia with birth weight exceeding 4000 grams. Maternal and neonatal outcomes were poorer in women with failed TOLAC.

Conclusion: Nearly two thirds of women with a history of CD achieve VBAC in Germany. Previous vaginal birth and the augmentation of labor with oxytocin are positively associated with the achievement of VBAC and no major perinatal complications. The decision to have a TOLAC should be encouraged in the majority of patients. Further studies are needed to evaluate the feasibility of TOLAC in preterm delivery. (J Turk Ger Gynecol Assoc 2022; 23: 137-44)

Keywords: VBAC, TOLAC, predictors, risk factors, maternal outcome, neonatal outcome

Received: 12 January, 2022 **Accepted:** 15 June, 2022

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2022.2022-1-2

Introduction

The frequency of caesarean deliveries (CD) has risen markedly in the last few decades. In Europe, CD rates have increased from 11.2% of all deliveries in 1990 to 25.0% in 2014 (1). The most frequent indication for CD is prior CD, which contributes strongly to the overall increase in CD rates (2). Trial of labor after caesarean section (TOLAC) is a crucial strategy to reduce CD rates. Vaginal birth after caesarean section (VBAC) is achieved in 60% to 83.3% of cases (3,4). VBAC is a medically safe procedure. The fall in VBAC rates worldwide from 24% to 8% is a matter of public and professional concern (5). The drop in VBAC rates has been accompanied by large numbers of elective repeat caesarean section (ERCS) (6).

CD is known to be associated with severe maternal complications, including a high risk of mortality (7) compared to vaginal deliveries. The numerous benefits of vaginal birth, such as rapid maternal recovery, fewer maternal complications in future pregnancies, lower risk of childhood diseases, such as allergies and asthma, are also worthy of note (8). A number of studies focusing on the outcome of TOLAC, published in the last few years, have yielded diverse results. However, VBAC was shown to be relatively safe for mother and child compared to ERCS (9). Successful VBACs are also associated with lower overall morbidity rates (10) compared to ERCS. Nevertheless, a failed VBAC increases the risk of perinatal and maternal complications compared to ERCS (11).

Several attempts have been made to identify clinical factors associated with successful TOLAC. One of the aims of these investigations was to create validated risk scores for the likelihood of VBAC (12). Factors such as ethnicity, prior vaginal delivery or VBAC, cervical length, head-perineum distance, maternal age, inter-delivery interval, neonatal weight, and body mass index (BMI) were investigated. Risk scores might help physicians and expectant mothers to decide in favor of or against TOLAC, but have not been established at present. In view of the absence of an international consensus concerning VBAC and the frequent modification of guidelines every few years, the outcome of TOLAC must be re-evaluated in the light of recent data.

The purpose of the present study was to assess the possibility of vaginal delivery in women who underwent TOLAC, identify predictors and risk factors that could influence the success of a planned VBAC, and present maternal and neonatal outcomes of successful and failed TOLAC. Data from two large tertiary care academic hospitals in Germany were analyzed in order to issue recommendations for counseling candidates for TOLAC.

Material and Methods

A retrospective multicenter investigation was conducted at two large obstetrics departments (the academic teaching hospitals of Klinikum Leverkusen and the University Hospital of Luebeck) with facilities for high-risk pregnancies in Germany, from January 2008 to January 2018. Written informed consent was obtained from all patients. The study was in compliance with the Helsinki Declaration and was approved by the University of Luebeck Faculty of Medicine Ethics Committee (approval number: 19-285A). Inclusion criteria were singleton pregnancy, a history of only one previous CD with a low transverse incision, a viable fetus in cephalic presentation, intention to deliver vaginally, and patients >32 weeks of gestation (a vaginal delivery under this gestational age was not favored at these institutions).

A computer-based search yielded 4,139 patients with one previous caesarean section in their medical history. All patients gave their consent to attempt TOLAC. Approximately a half of the patients had undergone an ERCS, while the other half wished to attempt TOLAC. In addition to ERCS, exclusion criteria were emergencies before labor, intrauterine growth restriction, fetal anomalies, and multiple gestation. Finally, 1,546 patients (607 from Leverkusen and 939 from Luebeck) fulfilled the inclusion criteria (Figure 1).

Patients were divided into a successful VBAC group (group 1) and a failed TOLAC group with secondary CD (group 2). Success rates and risk factors were studied in both groups. Patient characteristics are summarized in Table 1. The period of investigation extended from the start of regular labor pain to birth. Maternal surveillance data, such as analgesia for labor, sanguineous or green amniotic fluid, the use of oxytocin, and labor induction with prostaglandin were analyzed.

A subgroup analysis was performed to identify specific risk factors for successful TOLAC. According to the international classification, "severely obese" is defined as a BMI ≥35 kg/m^2 (13). The following factors were analyzed in both groups: an interpregnancy interval shorter than 12 months, women older than 40 years of age, BMI >35 kg/m^2, fetal macrosomia with birth weight exceeding 4000 grams, preterm delivery between gestational week 32 and 37, post-term TOLAC beyond 40+0 weeks of gestation, and neonatal umbilical cord blood pH below 7.10. The BMI limit of 35 kg/m^2 was selected in order to facilitate comparison of our data with the published literature. Maternal and neonatal outcomes were also analyzed (Table 2). Both institutions had the same standards of care.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA).

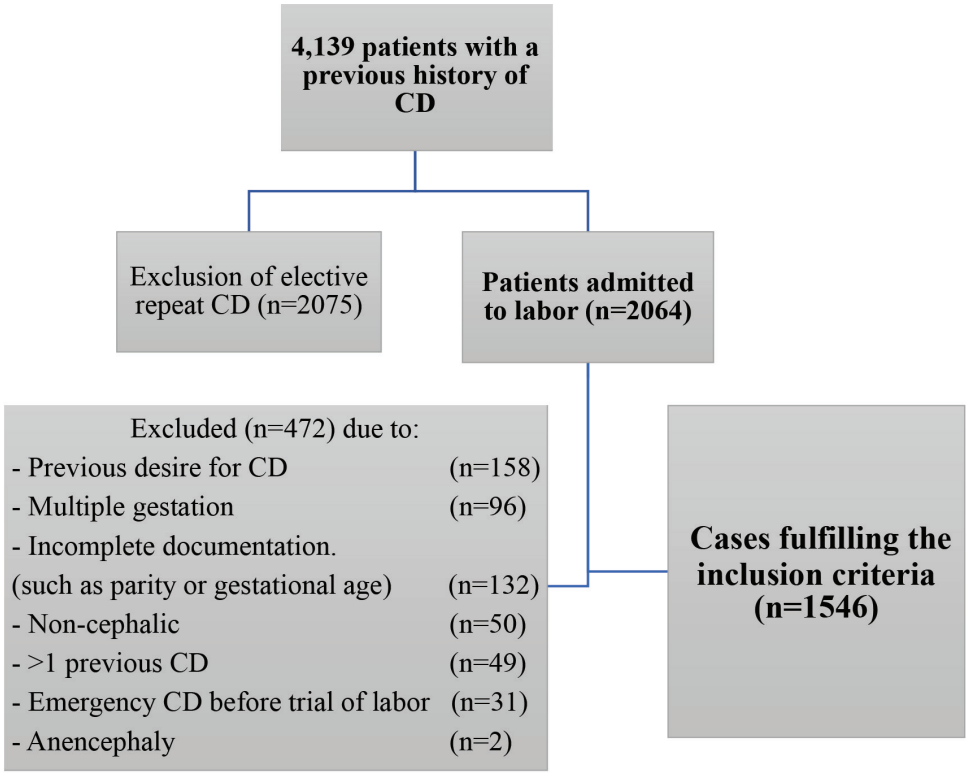

Figure 1. Flowchart throughout the recruitment phase of the study

CD: Caesarean delivery

Table 1. Patient characteristics according to birth mode

	n	Group 1 (n=963)	Group 2 (n=583)	Total	p[a]
Age (years)	1545	32.29±5.02	32.82±5.03	32.49±5.03	**0.051**
Parity	1546	2.48±0.864	2.23±0.683	2.38±0.809	**<0.001**
BMI (kg/m^2)	922	25.66±5.84	26.21±5.92	25.66±5.84	**0.003**
Gestational age (weeks)	1546	39.25±1.78	38.70±2.54	39.05±2.11	**0.004**
Gestational diabetes	1544	100 (10.4%)	71 (12.2%)	171 (11.1%)	**0.274**
Hypertension, pregnancy-related disease	1544	48 (5.0%)	37 (6.4%)	85 (5.5%)	**0.253**
Previous vaginal birth	1312	269 (33.3%)	78 (15.5%)	347 (26.4%)	**<0.001**
Birth weight at prior CD (grams)	524	2969.29±819.95	3202.70±776.44	3052.14±811.76	**<0.001**
Neonatal weight (grams)	1546	3357.82±529.06	3321.67±670.91	3344.19±586.64	**0.766**
Obstructed labor history	529	47 (13.8%)	47 (24.9%)	94 (17.8%)	**0.008**
Fetal distress history	529	97 (28.5%)	56 (29.6%)	153 (28.9%)	**0.212**
Prostaglandin used	1546	278 (28.9%)	199 (34.1%)	477 (30.9%)	**0.030**
Oxytocin used	1544	397 (41.3%)	173 (29.7%)	570 (36.9%)	**<0.001**
Epidural anesthesia	1546	393 (25.4%)	197 (12.7%)	590 (38.2%)	**<0.001**
Oxytocin and prostaglandin used	1546	115 (11.9%)	63 (10.8%)	178 (11.5%)	**0.498**
Sanguineous or green amniotic fluid	1546	27 (2.8%)	48 (8.2%)	75 (4.9%)	**<0.001**
Cervical opening at admission (cm)	603	2.02±2.24	1.15±1.50	1.71±2.05	**<0.001**
Smoking during pregnancy	942	63 (11.0%)	45 (12.2%)	108 (11.5%)	**0.589**

[a]P-value was calculated by χ^2 test (for qualitative variables) or t-test (for continuous variables) to test the difference between the two groups, BMI: Body mass index, CD: Caesarean delivery

Continuous data are reported as mean and standard deviation. Categorical variables are shown as numbers of patients and percentages and the χ^2 test or Fisher's exact test was used. Normal distribution of data was assessed using a one-sample Kolmogorov-Smirnov test. Quantitative variables were compared by Student's t-test. P-values less than or equal to 0.05 were considered to be statistically significant.

Results

Baseline characteristics and maternal surveillance were homogeneous in the two groups (963 women in group 1 vs. 583 women in group 2) (Table 1). The success rate of intended and completed VBAC was 62.3% and the rate of secondary secondary CD was 37.7%. Success rates were 60.7% in Luebeck and 64.6% in Leverkusen. Vacuum or forceps extraction accounted for 13.8% (133/963) of vaginal deliveries. Episiotomy was used in about 67% of assisted and 20% of spontaneous deliveries. Nearly one third (n=477, 30.9%) of the patients had labour induced with prostaglandin.

Prior vaginal birth was a strong independent factor associated with successful TOLAC (p<0.001). Epidural anesthesia and the induction of labor with prostaglandin or oxytocin were significantly more common in women with successful VBAC than in those who had a repeat CD (p<0.001). Failed TOLAC was associated with a history of caesarean section due to obstructed labor. Further parameters are shown in Table 1.

The time period from the beginning of regular labor to parturition was significantly shorter (5.8±3.1 vs. 7.9±5.5 hours; p<0.001) in women who had a spontaneous birth prior to cesarean delivery. The time period from the start of regular labor to parturition was also significantly shorter (5.3±3.1 hours vs 8.0±5.5 hours; p<0.001) in women with a previous VBAC. This significance existed in the subgroup of patients with a history of fetal distress (6.8±5.0 hours; p=0.031) but not in those with a history of obstructed labour (8.8±5.7 hours). Women who delivered before 37 completed weeks of pregnancy had a significantly shorter duration of delivery after the start of regular contractions (4.9±3.3 hours) compared to those with term pregnancies (7.8±5.4 hours; p<0.001).

The subgroup analysis (Table 3) yielded no significant difference in the success of VBAC in patients older than 40 years of age, an interpregnancy interval less than 12 months, and fetal macrosomia with birth weight exceeding 4000 grams. Women with a post-term pregnancy exceeding 40+0 weeks of gestation had greater chances of a successful VBAC. Failed

Table 2. Maternal and neonatal outcome

	n	Group 1	Group 2	Total	p
Blood loss (mL)	940	267.98±363.89	431.49±200.28	332.34±319.98	**<0.001***
Uterine rupture	1546	3 (0.3%)	17 (2.9%)	20 (1.3%)	**<0.001****
Postpartum hysterectomies	1546	2 (0.2%)	1 (0.2%)	3 (0.2%)	**0.893****
Apgar score at 1 minute (mean)	1545	8.68±1.03	8.14±1.67	8.48±1.33	**<0.001***
Apgar score at 5 minutes (mean)	1545	9.69±0.83	9.25±1.19	9.52±1.00	**<0.001***
Apgar score at 10 minutes (mean)	941	9.86±0.80	9.59±0.91	9.75±0.85	**<0.001***
Umbilical cord pH	937	7.29±0.08	7.30±0.10	7.29±0.09	**<0.001***
pH <7.10	937	6 (1.1%)	14 (3.8%)	20 (2.1%)	**0.005*****
Transfer to neonatal intensive care unit	607	25 (2.6%)	17 (2.8%)	42 (2.6%)	**0.401*****
5 minute Apgar score below 6	1545	5 (0.5%)	17 (2.9%)	15 (1.0%)	**0.020*****
5 minute Apgar score below 7	1545	7 (0.7%)	17 (2.9%)	24 (1.6%)	**0.001*****

*Student's t test, **Fisher's exact test, ***χ^2 test

Table 3. Subgroup analysis

	n	Group 1 (n=963)	Group 2 (n=583)	Total	p
Age ≥40 years	1546	71 (7.4%)	57 (9.8%)	128 (8.3%)	**0.096*****
BMI ≥35 kg/m^2	922	54 (9.7%)	28 (8.2%)	82 (9.1%)	**0.428*****
Interpregnancy interval less than 12 months	603	24 (2.5%)	15 (2.7%)	39 (2.6%)	**0.893*****
Fetal macrosomia (birth weight >4000 g)	1546	91 (9.4%)	73 (12.5%)	164 (10.6%)	**0.054*****
Post-term pregnancy (>40+0 weeks)	1546	205 (33.4%)	337 (38.5%)	582 (37.3%)	**<0.001*****
Preterm birth (32-37 weeks of gestation)	1546	83 (8.6 %)	103 (17.7%)	186 (12.0%)	**<0.001*****

*Student's t test, **Fisher's exact test, ***χ^2 test, BMI: Body mass index

TOLAC was associated with preterm birth between 32 and 37 weeks of gestation.

Patients with failed TOLAC experienced significantly greater blood loss and had higher uterine rupture rates than those with successful VBAC (Table 2). Only five patients (25%) with uterine rupture were given oxytocin. Neonatal outcomes were significantly poorer in the failed TOLAC group. Analytically, an umbilical cord blood pH <7.1 was significantly more common in women with failed TOLAC compared to those who had a normal or operative vaginal delivery by VBAC ($p<0.01$). Additionally, significant differences were noted in the rates of 5 minute Apgar scores below 6 ($p<0.02$) or 7 ($p<0.001$), and Apgar score at 10 minutes ($p<0.001$) (Table 2).

Discussion

To our knowledge, the present study is the largest investigation conducted in Germany on outcomes and risk factors for TOLAC. Baseline characteristics and maternal surveillance were similar in the successful VBAC group and the failed TOLAC group. Approximately two thirds of patients who attempted TOLAC (62.3%) achieved VBAC safely. This is in line with the data reported in a large cohort study (6) comprising 143,970 patients from England (63%), but slightly lower than the rates reported in previous studies.

The majority of investigations report successful VBAC in 60% to 83.3% of cases (3,4). A relatively high success rate of 91.0% was reported in one study (14). These variations are probably due to differences in healthcare systems or selection criteria. At our institutions, a trial of TOLAC was offered to all women with no contraindications in accordance with international standards (15-18). The two institutions involved in the present study did not differ in terms of structure. The difference in success rates between Luebeck (60.7%) and Leverkusen (64.6%) suggests that the management of TOLAC is a multifactorial issue.

In keeping with previous studies (19,20), our data showed that a previous vaginal birth is a strong predictor of the success of VBAC. Information about previous vaginal births must be included in any consultation of a patient asking for VBAC. The likelihood of successful VBAC is approximately threefold higher in patients with a previous vaginal delivery (21). Moreover, a previous vaginal delivery is associated with a six- to ten-fold greater likelihood of achieving VBAC (22).

We observed an association between the use of epidural anesthesia and the success of VBAC, which is contrary to the data reported in many studies (23,24). Furthermore, the indication for previous CD (obstructed, or history of fetal distress or malpresentation, for example) may be an important factor in the success of TOLAC. In our analysis, a history of obstructed labor, rather than a history of malpresentation, was significantly associated with failed TOLAC.

The general higher age of motherhood (after 35 years) in recent times has been associated with a rise in pregnancy complications, such as preeclampsia, gestational diabetes, placental anomalies, and caesarean section (25). The role of maternal age as a predictor of the success of TOLAC is controversially discussed (26). Nevertheless, maternal age was regarded as an important parameter in our analysis. In an investigation of 335 women older than 40 years who had never delivered by the vaginal route, Levin et al. (27) registered successful TOLAC in 62.3%. In a subgroup analysis of women who underwent TOLAC, we found no difference between 128 women older than 40 years of age and those younger than 40 years.

The success of TOLAC was reported to be impaired by gestational diabetes and a high BMI, resulting in a high risk of fetal distress, labor arrest, and failed induction (26). However, Mei et al. (28) found no difference in TOLAC success rates stratified by obesity classes of BMI 30-34.9, 35-39.9, or more than 40 kg/m^2. Coleman et al. (29) noted that women with gestational diabetes were less likely to have a successful TOLAC than those without diabetes. In an analysis of 423 deliveries complicated by type I gestational diabetes versus 9,437 control deliveries, Marchiano et al. (30) observed similar success rates in both groups. We found no association between the success of TOLAC and gestational diabetes or severe obesity (BMI ≥35 kg/m^2). Regan et al. (31) compared the success of TOLAC between high-risk pregnancies (maternal BMI >30 or diabetes) versus low-risk patients, and observed similar rates of successful VBAC in the two groups.

We suspected that the apparently negative impact of gestational diabetes on the success of TOLAC was not due to the presence of diabetes itself, but due to fetal macrosomia and other differences in baseline characteristics. We examined the success of TOLAC in 156 women with fetal macrosomia (>4000 grams), and observed no significant difference in the outcome of TOLAC. Oboro et al. (32), on the other hand, reported a fetal weight in excess of 4000 grams as one of the most important factors underlying the failure of VBAC.

In a subgroup analysis, we found that an interpregnancy interval shorter than 12 months had no negative impact on the success of TOLAC. Similar data were reported in a study (33) on the success of TOLAC in 3,176 women with a short inter-delivery interval: a shorter interval than 12 months was no risk factor for maternal death, uterine rupture, or other major complications, but the risk of preterm delivery was higher in this group. In a large meta-analysis (26), an interpregnancy interval shorter that 24 months was not related to failed TOLAC. However, it should be noted that the meta-analysis included only one study (34) with an interpregnancy interval shorter that 18 months.

Although the induction or augmentation of labor is not contraindicated in patients undergoing TOLAC, the issue remains controversial among clinicians. The likelihood of uterine rupture is believed to be higher when oxytocin is used. A large study comprising 13,523 patients who underwent TOLAC showed an association between uterine rupture and the dose of oxytocin: a high rupture rate (2.07%) was registered for the highest dose (35). The rate of uterine rupture in our study (1.3%) was slightly higher than the range reported in the published literature (0.2-0.5 to 0.9%) (10,21). However, we found that the induction of labor with prostaglandin or the use of oxytocin was positively correlated with the success of TOLAC. We also observed no association between oxytocin and uterine rupture. The induction of labor with prostaglandin in women undergoing TOLAC was associated with higher rates of uterine rupture and perinatal morbidity compared to other types of labor induction (10). Thus, the use of prostaglandin in patients undergoing TOLAC requires further investigation.

Palatnik and Grobman (36) noted that the induction of labor at 39 gestational weeks might increase the chances of VBAC, but also those of uterine rupture, compared to expectant management. Our analysis revealed that a gestational age of 37-40 weeks was not associated with the success of TOLAC. A post-term pregnancy also did not affect the success of TOLAC, which is in line with a large study published by Ram et al. (37). In a cohort study, Hammoud et al. (38) noted lower rates of success and higher rates of uterine rupture in women who delivered at >41+0 weeks of gestation.

As a gestational age <37 weeks was an exclusion criterion in the majority of studies, we have a very limited body of published data concerning TOLAC before 37 weeks of gestation (36,37). We analyzed 186 patients who underwent TOLAC between gestational week 32 and 37, and noted a negative association between preterm deliveries and the success of TOLAC. Large studies will be needed to evaluate the outcome of preterm deliveries in patients undergoing TOLAC.

In our analysis, blood loss und uterine rupture were significantly greater in patients with failed TOLAC than in those with successful VBAC. Failed TOLAC was associated with poorer neonatal outcomes, thus parameters which may be associated to long-term neonatal outcome, such the rates of 5 minute Apgar scores below 6 (0.5% vs. 2.9%) or 7 (0.7% vs. 2.9%), were statistical significantly higher in this group and higher than the medial rate reported in the literature (less than 1%) (39). Furthermore, the incidence of postpartum acidosis (pH <7.1) was higher in the failed TOLAC group. Similar data were reported in previous studies (14,32). Neonatal complications, including respiratory distress syndrome, meconium, and retraction, were significantly higher in the failed group than in the successful VBAC group (40).

Study Limitations

The prime limitation of the present study is its retrospective design. Factors such as operator experience or physician preferences were not assessed, and might have accounted for the results. However, it may be very difficult to perform a prospective investigation in a large sample. A Cochrane review in an Australian population of women undergoing VBAC or ERCS highlighted the difficulty of randomization (41). Despite these limitations, we believe that the data obtained in the present study signify a valuable contribution to the current published literature.

Conclusion

In Germany, approximately two thirds of patients undergoing TOLAC are able to achieve a safe VBAC. A history of previous vaginal birth and the augmentation of labor with oxytocin are positively associated with the achievement of VBAC without major perinatal complications. TOLAC should be offered to all eligible women, and should not be discouraged in post-term pregnancies, older, or obese women. The use of data from the early 2000s might have been one reason for the diverse published reports concerning risk factors for TOLAC. Practical guidelines have changed significantly since that time. Further studies will be needed to evaluate the feasibility of TOLAC in preterm deliveries.

Ethical Committee Approval: *The study was in compliance with the Helsinki Declaration and was approved by the University of Luebeck Faculty of Medicine Ethics Committee (approval number: 19-285A).*

Informed Consent: *Written informed consent was obtained from all patients.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: G.G., I.A., K.A.E., A.R., L.P., M.K., S.B.; Concept: G.G., I.A., K.A.E., A.R., L.P., M.K., A.S.L., L.A., T.M., S.S., S.B.; Design: G.G., I.A., K.A.E., A.R., L.P., M.K., S.B.; Data Collection or Processing: G.G., I.A., K.A.E., M.K., S.B.; Analysis or Interpretation: G.G., I.A., K.A.E., A.R., L.P., M.K., A.S.L., L.A., T.M., S.S., S.B.; Literature Search: G.G., I.A., L.P., M.K., A.S.L., S.B.; Writing: G.G., I.A., K.A.E., A.R., L.P., M.K., A.S.L., L.A., T.M., S.S., S.B.*

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

Financial Disclosure: *The authors declared that this study received no financial support.*

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Transvaginal ultrasound evaluation of the pelvis and symptoms after laparoscopic partial cystectomy for bladder endometriosis

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Abstract

Objective: To evaluate transvaginal sonography (TVS) findings after laparoscopic partial cystectomy for bladder endometriosis and to correlate postsurgical ultrasound findings with symptoms.

Material and Methods: A retrospective study including women who underwent laparoscopic partial cystectomy for bladder endometriosis. Within 12 months after surgery, TVS examination was conducted in all patients to evaluate the bladder morphology, and the presence of any postsurgical sonographic findings of the pelvis. Painful symptoms were assessed using a visual analogue scale.

Results: A total of 40 women were included. At the follow-up visit, 25 patients were receiving medical treatment while 15 had declined post-surgical therapy and had tried to conceive. The presence of bladder deep-infiltrating endometriosis (DIE) was found in nine (22.5%), fibrotic thickening of the bladder wall was found in 15 (37.5%), and normal bladder morphology was observed in 16 (40%). There was a correlation between anterior adenomyosis and bladder DIE, and fibrotic thickening of the bladder. Patients with TVS signs of bladder DIE and anterior adenomyosis suffered more dysmenorrhea and dysuria than patients with normal bladder.

Conclusion: Post-operative TVS can detect the alteration of pelvis and could explain the causes of the persistence of symptoms. (J Turk Ger Gynecol Assoc 2022; 23: 145-53)

Keywords: Adenomyosis, bladder endometriosis, laparoscopy, ultrasound

Received: 26 May, 2022 **Accepted:** 19 July, 2022

Introduction

Bladder involvement is frequent in urinary tract endometriosis, occurring in 70-85% of cases (1,2) and has been reported in 20-53% of women with deep-infiltrating endometriosis (DIE) and in 0.3-12% of all women affected by endometriosis (3). The bladder base and bladder dome are frequently involved and, more rarely, the trigone (4,5).

Bladder DIE can cause dysuria, cramping, painful voiding, urinary frequency, recurrent urinary tract infections, dysmenorrhea, dyspareunia, urinary bleeding (hematuria) and, in severe cases, ureteral stenosis and occlusion (6,7). Nonetheless, 25-30% of women with bladder DIE are pain-free (8). The symptoms are generally caused by inflammatory factors irritating the bladder wall and by associations with other deep endometriotic lesions and adenomyosis.

The aim of treatment for bladder DIE is to resolve symptoms and improve fertility. Treatment can be expectant, medical, or surgical. Patients who respond to medical management can continue treatment until they achieve optimal quality of life and to reduce the risk of disease progression. Surgery should always be performed in patients with painful

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2022.2022-5-1

symptoms or infertility who do not respond to medical treatment (8).

Surgical techniques include shaving the superficial bladder lesions, cystoscopy approaches, and laparoscopic resection of the involved bladder wall (9). Laparoscopic resection of bladder endometriosis is associated with the best results in terms of long-term outcomes compared to other surgical techniques (10). Complications of this surgery include anastomotic stricture, anastomotic leak, utero-vaginal fistula, pelvic ureter injuries, and bladder hemorrhage (1). The complication rate is higher when the trigone or intramural ureter are involved. Surgery may also improve fertility, even when not removing all endometriotic lesions (11). Many studies have evaluated symptoms after partial bladder resection (12-15), but to date none has described bladder wall morphology. In fact, as with all surgical procedures, fibrotic tissues, adhesions, and residual disease can be expected after bladder DIE resection.

Transvaginal sonography (TVS) and magnetic resonance imaging are currently used as preoperative methods to diagnose bladder DIE, with the former being the first-line imaging modality (5,16,17). TVS can accurately evaluate bladder walls and the ureter pelvic segment (5,17). Furthermore, with dynamic TVS examination, it is possible to evaluate the presence of the "sliding sign" between the uterus and other organs of the pelvis, which can be a useful diagnostic tool for evaluating adhesions (18,19).

This retrospective study was performed with patients who had undergone laparoscopic partial bladder resection for DIE, and were subsequently evaluated by TVS for bladder features and ultrasound findings of the pelvis after surgery. Furthermore, the TVS findings were subsequently correlated to specific post-operative symptoms.

Material and Methods

This retrospective observational study included patients with bladder DIE who underwent laparoscopic partial cystectomy in order to remove the endometriotic lesion from 2014 to 2019 and had a TVS scan within 12 months after surgery (2015-2020).

The inclusion criteria were: premenopausal women with previous laparoscopic partial bladder resection for DIE and histological confirmation; TVS examination 12 months after surgery; and accurate history, symptoms, and surgical reports.

The exclusion criteria were: laparoscopic surgery for endometriosis without bladder resection; menopausal status; reproductive tract cancer; or absence of accurate history, symptoms, and surgical reports.

Ethical approval

All enrolled patients gave their informed consent before the TVS examination to allow the use of their data. The study was approved by Ethics Committee of University of Rome Tor Vergata (approval number: 194/21, date: 22.09.2021).

Clinical examination

Full patient histories and symptom evaluation, utilizing a visual analogue scale (VAS) system, were recorded before the scan. The complete medical, surgical, and obstetrical history included the patients' age, body mass index [(BMI) in kg/m^2], age at menarche, gravidity, parity (that is, the total number of all prior pregnancies, spontaneous pregnancy loss, and/or live births), and the mode of delivery were recorded. Previous uterine surgery (myomectomy or caesarean section) was recorded. Infertility was defined as no pregnancy after 12 months of unprotected intercourse. Patients were also asked about any medication that they were taking, including the use of analgesics for painful periods. Hormonal treatments were recorded according to type, for example, progestin, estroprogestin, and gonadotropin releasing hormone analogs, and mode of use (continuous or cyclic). The presence of the following post-surgical painful symptoms was evaluated before performing the follow-up scan: dysmenorrhea, dyspareunia, dysuria, dyschezia, recurrent cystitis, and hematuria. Symptom intensity was evaluated through the VAS system, using a 10 cm line with the extreme points 0 and 10 corresponding to "no pain" and "maximum pain", respectively. The presence of heavy menstrual bleeding (HMB) was also investigated. Patients were asked about the frequency and duration of menstrual periods and any episodes of intermenstrual bleeding. HMB was determined by subjective evaluation of the patient; this evaluation has been reported in the literature as reliable and comparable to the pictorial blood-loss analysis chart score (20,21).

Ultrasound examination

All TVS examinations and interpretation were performed by the same experienced sonographer, using a 4-9 MHz probe, with a three-dimensional (3D) facility (Voluson E6, GE Medical Systems, Zipf, Austria). Briefly, a conventional 2D ultrasound with greyscale and power Doppler for assessment of the pelvis was performed. Bladder was always evaluated with medium fullness and patients were therefore asked not to completely empty their bladder before the TVS scan as this made it possible to evaluate the structure of the walls and the presence of lesions or irregularities of the wall layers. The TVS probe was positioned in the anterior vaginal fornix and gently swung from side-to-side, visualizing the mucosa and muscularis for focal thickening and for hypoechoic linear or nodular lesions. Suspected bladder adhesions of the vesico-uterine pouch were evaluated by the presence or absence of the "sliding sign" between the uterus and the bladder.

In the TVS bladder assessment after surgery, lesions were described in terms of their size and location according to the following three zones (5,22):

1) The bladder base, which also included the trigone;

2) The bladder dome, which lay superior to the trigone/base and was intra-abdominal, with the demarcation point between the base and the dome of the bladder being the vesico-uterine pouch;

3) And the bladder anterior retro-peritoneal portion, which corresponded to the part of the bladder in the Retzius space.

The trigone was observed by TVS as lighting thicker bladder wall within 3 cm of the urethral opening, delimited by the two ureteral orifices laterally. The intramural and pelvic segment of the ureter was examined by moving the TVS probe from the midline toward the pelvic sidewall.

Bladder lesions after surgical resection of bladder endometriosis were defined as follow:

- Bladder endometriosis was defined as the presence of a nodule or a lesion that infiltrated the muscular layer of the bladder wall and did not include cases of adhesions or the presence of limited thickening or irregularities of the bladder internal wall (mucosa) or serosa. Bladder nodules or residual DIE appeared as hypo- or hyper-echoic linear or spherical thickening of the bladder wall, with or without cystic areas and regular/irregular margins, bulging towards the lumen, involving mostly the muscularis and serosa (Figure 1).
- Fibrotic thickening was identified as a hyperechoic bladder wall, which was thicker than the normal adjacent wall, without a well-defined lesion or nodule (Figure 2);
- Irregularities of the bladder wall were defined as small hypo- or hyper-echoic lines or interruptions or invaginations of the bladder wall (Figure 2).

The dimensions of the bladder DIE (measured in three orthogonal planes), as well as irregularities of the bladder wall, were recorded in addition to the distance between the lesion and the trigone and the meatus and intramural part of the ureter.

The uterus, myometrium, and endometrium were also scanned. The 2D examination was followed by the acquisition of the 3D volume of the uterus with and without power Doppler. This was important for evaluating the uterine cavity morphology and the myometrium to detect typical ultrasound signs of adenomyosis in the outer myometrium and the junctional zone, as previously described (23-25).

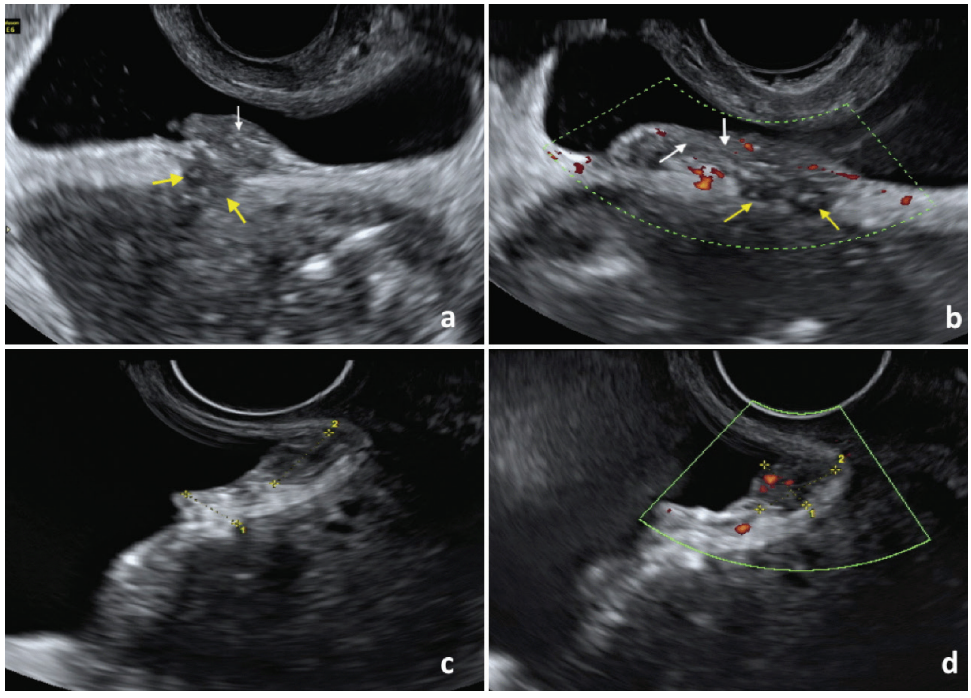

Figure 1. Residual endometriosis of the bladder wall after bladder resection for deep endometriosis TVS (view of the same case): a) 2D grey scale transverse view of the bladder; b) longitudinal view of the bladder lesion with power Doppler. Note the hypoechoic non-vascularized (white arrows) lesion interrupting the bladder wall and the surrounded hyperechoic fibrotic tissue (white arrows). In c) and d) another case of residual endometriosis in 2D grey scale c) and power Doppler view d). Note in c) the hypoechoic lesion the bladder wall attached to the uterus (yellow line 2) and the surrounded hyperechoic fibrotic scar tissue (yellow line 1). In d) the hypoechoic endometriotic lesion showed few vessels at power Doppler (yellow line 1 and 2) consistent with active endometriosis

TVS: Transvaginal sonography, 2D: Two-dimensional

Diagnosis of adenomyosis was made when at least two of the typical ultrasound features of the disease (intramyometrial cystic areas, hyperechoic intramyometrial islands, globally enlarged uterus, asymmetrically enlarged uterus, myometrial hypoechoic linear striations, irregular or infiltrated junctional zone) were observed. The location of the adenomyosis inside the uterus (anterior, posterior, lateral) was noted.

Furthermore, the uterus, and the bladder, and the suspected anterior adhesions between uterus and bladder were assessed by the absence of the "sliding sign".

The adnexa, pouch of Douglas, further pelvic organs (rectum, rectosigmoid junction, tubes) and other sites [parametria, rectovaginal septum (RVS), retro cervix, uterosacral ligaments (USL)] were examined to look for features of endometriosis according to a previously described ultrasound mapping system (5,16). The presence of DIE of the posterior pelvic compartment was recorded according to the specific sites, including the RVS, USL, vagina, torus, and rectosigmoid. Ovarian endometriosis and adnexal adhesions were accurately described.

All data were stored as 2D still images, 2D video-clips, and 3D volumes.

Surgical treatment

Laparoscopic partial resection of bladder endometriosis was performed in all patients by a multidisciplinary group, including gynecologist and urologist. The indication was the presence of bladder DIE lesions associated with painful symptoms that were unresponsive to medical therapy. The shaving technique and cystoscopy treatment were not considered due to the high risk of disease persistence. For all patients, the surgical and medical reports were analyzed to confirm bladder resection, to record other endometriotic lesions that had been removed, and to assess difficulties and complications during and after the surgery. Ureteral catheters were employed if the lesion involved the trigone or the nodule was <2 cm from the ureteral ostia. Only 21 of the patients needed a ureteral stent before surgery by cystoscopy performed by a urologist. The ureteral catheters were removed at the end of surgical procedure in all 21 patients. During the surgery the bladder nodule was then identified, and a bladder incision and radical excision of the disease was performed; the latter was carried out for 24 lesions of the bladder base, 14 lesions of the bladder dome, one retroperitoneal lesion of the Retzius space, and one lesion involving the trigone. In all cases, the bladder was sutured by a double-layered intracorporeal laparoscopic knot.

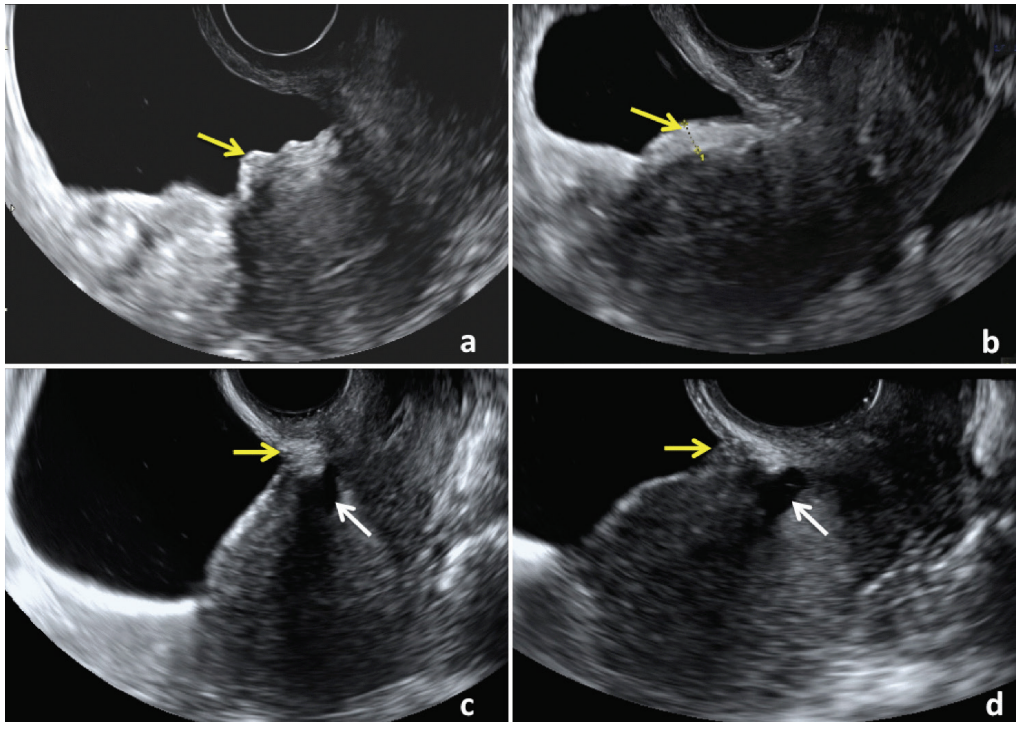

Figure 2. Four different cases of fibrosis and irregularities of the bladder wall after resection of an endometriotic nodule: a) small incision scar of the bladder wall (yellow arrow); b) hyperechoic thickening of the bladder wall (yellow arrow). Note the comparison of thickness with the normal adjacent wall (yellow line 2); c) residual fibrotic thickening of the bladder wall in patients with anterior adenomyosis (white arrow). Note the hyperechoic adenomyotic zone inside the myometrium and the asymmetric thickening of the uterine wall (orange arrow endometrial stripe); d) hypoechoic incision scar of the bladder wall (yellow arrow) adherent to the uterine adenomyosis (white arrow) with asymmetric thickening of the uterine wall (orange arrow endometrial stripe)

Concomitant pelvic endometriosis, such as endometrioma or posterior DIE, were removed, if indicated. Adenomyosis was treated with radiofrequency ablation in case of focal presentation, and no other surgical treatment was performed. No hysterectomy was performed. Cystography was performed before removing the Foley bladder catheter on day 7 after the surgery. There were no cases of ureteral endometriosis with ureteral obstruction and ureteral reimplantation. All included patients had stage III-IV endometriosis, according to the Revised American Society for Reproductive Medicine classification of endometriosis 1996. No major complications after surgery were recorded in any of the cases.

Statistical analysis

All patients enrolled in this study were divided into groups as follows: firstly, patients who received hormonal treatment after surgery and patients who did not receive hormonal treatment in order to attempt conception. The specific symptoms were calculated and analyzed. There was a statistical correlation only for dysmenorrhea and for HMB into two groups, so the subsequent statistical analysis was conducted on the total study population.

The prevalence of bladder features was divided into normal bladder, fibrotic thickening/irregularities of the bladder wall, and bladder DIE. The percentage of endometriotic pelvic findings were calculated. TVS findings were analyzed in the following manner:

- By comparing the presence of the TVS findings of pelvic endometriosis with TVS features of the bladder DIE, fibrotic thickening/irregularities of the bladder wall and normal bladder;
- Subsequently by correlating specific symptoms with the presence of TVS features of the bladder DIE, fibrotic thickening/irregularities of the bladder wall and normal bladder.

All continuous variables for population characteristics were expressed in terms of the mean ± standard deviation, while categorical variables were expressed in terms of frequency and percentage.

Characteristics were compared between groups using the chi-square test for categorical variables and the independent samples t-test or Mann-Whitney U test, as appropriate, for continuous data. Fisher's exact test was used to compare the prevalence. A $p<0.05$ was considered statistically significant.

Results

Fifty-three patients with laparoscopic partial bladder resection for endometriosis were initially identified. Seven patients were excluded because of incomplete history and symptoms reports, five were excluded for incomplete surgical report and one was excluded because the TVS examination occurred within 12 months of surgery. None of the included patients showed hydronephrosis before or after surgery. Therefore, a total of 40 women were included in this study.

Table 1 shows the characteristics and symptoms of all included patients. Mean age of the study patients was 36.4±5.0 years, most of the patients (55.0%) were nulliparous and 40.0% suffered from infertility. Twelve patients (30.0%) reported previous surgery (myomectomy or caesarean section). Painful symptomatology was reported after surgery; 19 patients (47.5%) had dysmenorrhea, 13 (32.5%) had dyspareunia, and 13 (32.5%) had dysuria.

The total study population was initially divided into the following two subgroups: patients receiving hormonal therapy and patients not receiving hormonal therapy. Following surgery, of the 40 patients included in the study, 25 received medical treatment: 13 patients were prescribed estrogen-progestin contraceptive pills (a cyclic regimen was offered to 10 patients and a continuous regimen was offered to three); and 12 patients were given continuous progestin treatment.

No differences in age, BMI and menarche were observed between the two groups (Table 1), except for parity, dysmenorrhea and HMB. Patients who did not receive hormonal therapy after surgery reported a higher percentage of dysmenorrhea and HMB. However, no other significant differences were found regarding symptoms when comparing those who did and did not receive medical therapy. Since the differences in these two groups were minimal, these two groups were considered together for further analysis.

The TVS endometriosis pelvic findings and the relationship with bladder DIE, bladder fibrotic thickening/irregularities of bladder wall and normal bladder are shown in Table 2. Bladder DIE was detected in nine patients (22.5%), ultrasound findings of bladder fibrotic thickening/irregularities were detected in 15 (37.5%), and normal bladder morphology was seen in 40.0%. Of note, there were 11 patients with bladder fibrotic thickening and four patients with irregularities of bladder wall, so we again considered these two-ultrasound features together.

In the groups with ultrasound findings of bladder DIE and fibrotic thickening, we observed a greater presence of anterior adenomyosis (respectively 77.8% and 80.0%) compared to patients with normal bladder walls (25.0%). Instead, for ultrasound findings of posterior DIE, that is DIE of RVS, USL, vagina, torus, and/or rectosigmoid, and endometrioma, we did not detect statistically significant differences.

In patients with negative "sliding sign" indicating probable anterior adhesions, we observed a statistically significant difference between bladder DIE (88.9%) and fibrotic thickening (86.7%) compared to normal bladder (18.7%). However, when considering patients with anterior negative "sliding sign" without ultrasound findings of adenomyosis, no statistically

significant differences were observed between the three groups.

The correlation between ultrasound bladder features and symptoms is shown in Table 3. Dysmenorrhea was mainly present in patients who had ultrasonographic findings of bladder DIE (77.8%), whereas it was less prevalent in patients with ultrasound findings of normal bladder morphology (31.2%) and fibrotic thickening (46.6%). Dysuria was statistically significant associated with the presence of bladder DIE (66.7%) compared to patients with ultrasound findings of normal bladder morphology (12.5%). No differences were observed for dyspareunia, HMB, recurrent cystitis and dyschezia regarding the bladder TVS findings.

Table 1. Patients' characteristics and referred symptoms

Patients characteristics	Total population (n=40)	Patients on hormonal therapy (n=25)	Patients not on hormonal therapy (n=15)
Age (years, mean ± SD)	36.4±5.0	37.6±4.8	35.1±4.0
BMI (mean ± SD)	23.5±2.8	23.1±3.5	22.1±4.5
Menarche (years, mean ± SD)	12.5±1.8	12.2±2.2	12.8±1.8
Nulliparity (n, %)	22 (55.0%)	9 (36.0%)*	13 (86.7%)*
Primiparity (n, %)	8 (20.0%)	6 (24.0%)	2 (13.3%)
Multiparity (n, %)	3 (7.5%)	3 (12.0%)	0 (0.0%)
Primary infertility (n, %)	16 (40.0%)	9 (36.0%)	7 (46.7%)
Previous uterine surgery (n, %)	12 (30.0%)	9 (36.0%)	3 (20%)
Symptoms after surgery			
Dysmenorrhea	19 (47.5%)	8 (32.0%)**	11 (73.3%)**
Dyspareunia	13 (32.5%)	8 (32.0%)	5 (33.3%)
Dysuria	13 (32.5%)	8 (32.0%)	5 (33.3%)
Recurrent cystitis	2 (5.0%)	2 (8.0%)	0 (0.0%)
Heavy menstrual bleeding	6 (15.0%)	1 (4.0%)***	5 (33.3%)***
Dyschezia	10 (25.0%)	6 (24.0%)	4 (26.7%)

Patients' characteristics and referred symptoms after surgery in the total study population and the following subgroups:
- Patients receiving medical therapy (continuous or cyclic hormonal therapy),
- Patients not receiving medical therapy (mostly because they are trying to conceive). *p<0.05 pts on hormonal treatment vs. not on hormonal therapy, **p<0.05 pts on hormonal treatment vs. not on hormonal therapy, ***p<0.05 pts on hormonal treatment vs. not on hormonal therapy, SD: Standard deviation

Table 2. Transvaginal ultrasound findings after partial bladder resection for DIE

TVS findings in patients (n, % of total)	Bladder DIE (n=9)	Fibrotic thickening/wall irregularities (n=15)	Normal bladder walls (n=16)
Adenomyosis 33 (82.5%)	9 (100%)	14 (93.3%)	10 (62.5%)
Anterior adenomyosis 23 (57.5%)	7 (77.8%)*	12 (80.0%)†	4 (25.0%)*†
Endometrioma 6 (15.0%)	1 (11.1%)	2(13.3%)	3 (18.7%)
Posterior DIE 22 (55.0%)	7 (77.8%)	5 (33.3%)	10 (62.5%)
Total adhesions 37 (92.5%)	9 (100.0%)	13 (86.7.%)	15 (93.7%)
Anterior negative sliding sign 24 (60.0%)	8 (88.9%)‡	13 (86.7%)§	3 (18.7%)‡§
Anterior negative sliding sign without adenomyosis, 12 (30.0%)	3 (33.3%)	2 (13.3%)	7 (43.7%)

TVS findings after laparoscopic bladder resection for deep-infiltrating endometriosis (DIE) in the total study population and correlation to TVS bladder features. *p<0.05 residual bladder DIE vs. normal bladder wall, †p<0.05 fibrotic thickening of the bladder wall vs. normal bladder wall, ‡p<0.05 residual bladder DIE vs. normal bladder wall, §p<0.05 fibrotic thickening of the bladder wall vs normal bladder wall, TVS: Transvaginal sonography, TVS: Transvaginal sonography

Table 3. Correlation between TVS bladder features and the specific symptoms in patients after laparoscopic bladder resection for DIE

Symptoms after surgery	Bladder ultrasound findings		
	Bladder DIE (n=9)	Fibrotic thickening/wall irregularities (n=15)	Normal bladder walls (n=16)
Dysmenorrhea 19 (47.5%)	7 (77.8%)*	7 (46.6%)	5 (31.2%)*
Dyspareunia 13 (32.5%)	3 (33.3%)	4 (26.6%)	6 (37.5%)
Dysuria 13 (32.5%)	6 (66.7%)**	5 (33.3%)	2(12.5%)**
Recurrent cystitis 2 (5.0%)	1 (11.1%)	1 (6.7%)	0 (0.0%)
HMB 6 (15.0%)	1 (11.1%)	1 (6.7%)	4 (25.0%)
Dyschezia 10 (25.0%)	2 (22.2%)	2 (13.3%)	6 (37.5%)

Correlation between TVS bladder features and the specific symptoms in patients after laparoscopic bladder resection for DIE. *p<0.05 residual bladder DIE vs normal bladder wall, **p<0.05 residual bladder DIE vs normal bladder wall, TVS: Transvaginal sonography, HMB: heavy menstrual bleeding

Discussion

This study described the TVS features of patients who were surgically treated with laparoscopic partial bladder resection for DIE. Many previous studies have described complications and symptoms following this type of surgery (26). The present study focused on the post-operative imaging of bladder features and pelvis. Laparoscopic partial bladder resection gave the best results in terms of long-term outcomes compared with other surgical techniques (10). The shaving technique and cystoscopy treatment were associated with fewer complications than laparoscopic resection but the results were often incomplete in terms of residual disease. A general improvement of pain after bladder resection has previously been observed, although many studies have reported a decrease in the severity of symptoms rather than total regression (9,15). In fact, dysuria has been reported in up to 70% of patients with bladder endometriosis (27), and a positive correlation was observed between severity and lesion diameter and so, after total removal or reduction in size, it is common to find fewer symptoms. Compared to surgical treatment for DIE of the pelvic posterior compartment (involving the USL, vagina, parametrium, inferior hypogastric plexus, and splanchnic nerves) (28,29), bladder surgery that does not involve the trigone has been reported to result in fewer complications and reduced risks of anatomical nerve damage (30). However, such as in the ultrasound evaluation after surgical treatment for bowel endometriosis (31), we observed some alterations of the pelvis after partial bladder resection for DIE.

Despite accurate standardization of the procedures, the reduction but also the persistence of painful symptoms after surgery for anterior DIE has often been reported. We evaluated the anatomical status of the pelvis at least one year after surgery using TVS mapping, particularly of bladder morphology, and correlated these ultrasound findings with post-surgical painful symptoms. These ultrasound findings could shed light on the possible mechanisms responsible for symptoms after surgery.

Table 1 shows both the patients' characteristics and their post-operative symptoms. Most important was the mean age (36.4±5.0) of the patients that showed how the moderate-severe endometriosis (stage III/IV) is more common in this age-range than in young girls in whom endometriosis occurs at earlier stages (32-34). Another important characteristic was the percentage of infertile women (40%) according to the literature (2). Regarding post-operative symptoms, the results agree with the data presented in the literature (2,15), in which most patients have reported some painful bladder symptomatology. In terms of dysuria, which was the major bladder symptom reported, we found a prevalence of 32.5% in our study population, 12.5% in cases with normal bladder morphology after surgery without any ultrasound signs of bladder DIE or fibrotic thickening, and 66.7% in those who had ultrasound signs of disease.

Persistent painful symptoms might be attributable to the presence of endometriotic disease, but also to anterior adenomyosis, which is associated with most cases of bladder DIE and fibrotic thickening. Furthermore, anterior negative "sliding sign" may be associated with previous bladder surgery and thus correlated to painful symptoms. However, in the presence of isolated anterior negative "sliding sign" without bladder DIE and anterior adenomyosis, symptoms seem to be less severe.

In fact, bladder DIE was most associated to anterior adenomyosis. This could be explained by the fact that even when treating the bladder disease, it was difficult to remove the lesion from the contextual anterior adenomyosis (35). By contrast, anterior adenomyosis could be the cause of the bladder DIE which remained in situ and thus constituted the main disease.

Some studies hypothesized that painful symptoms might be attributable to other causes, such as pelvic floor dysfunction, bladder pain syndrome, interstitial cystitis, and central sensitivity syndromes and could explain the persistence of pain in the patients without ultrasound sign of disease or adenomyosis (36,37). This is an important point because an accurate TVS evaluation could be useful to choose the appropriate therapeutic management in the presence of persistent symptoms. In fact, these symptoms could be related to endometriotic disease and adenomyosis requiring hormonal therapy. Otherwise, they could be related to other causes, such as central sensitivity syndrome, pelvic floor dysfunction, interstitial cystitis requiring a different diagnostic and therapeutic management (38).

Therefore, it is important to perform an accurate pre-surgical assessment of pelvic endometriosis/adenomyosis and to offer presurgical counselling to make patients aware of the possibility of residual bladder lesions in case of anterior adenomyosis. Symptoms persisting after bladder surgery could be due not only to residual disease but to the association with anterior adenomyosis and, in the absence of ultrasound sign of these pathologies, to the other causes listed above.

Study Limitation

A limitation of this study was the retrospective nature. Another limitation was the absence of pain score evaluation before surgery in all patients, as we know that all included patients were referred for painful symptoms that were unresponsive to medical therapy and therefore underwent surgery. Probably this information could be useful to quantify the improvement of the quality of life after the surgery, although this type of comparison data has been previously reported. A further limitation was the small sample, so there is a need for more studies, with larger samples. Finally, a possible critical issue is the definition of residual or recurrent bladder DIE. We cannot be sure if the lesions are residual or recurrent, but we described the presence of endometriotic lesions that infiltrated the muscular layer of the bladder wall.

Conclusion

This study showed that during the follow-up of patients who underwent partial bladder resection for DIE, symptoms sometimes remained and TVS pelvic anatomical findings were often abnormal. Post-operative TVS could be useful to choose the appropriate management for each patient.

Ethics Committee Approval: *The study was approved by Ethics Committee of University of Rome Tor Vergata (approval number: 194/21, date: 22.09.2021).*

Informed Consent: *All enrolled patients gave their informed consent before the TVS examination to allow the use of their data.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: F.G.M., E.Z., C.E.; Concept: F.G.M., E.Z., C.E.; Design: L.L., E.P.; Data Collection or Processing: C.R., A.S., T.S.; Analysis or Interpretation: L.L., E.P.; Literature Search: C.R., A.S., T.S.; Writing: F.G.M., E.Z., C.E.*

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

Financial Disclosure: *The authors declared that this study received no financial support.*

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Effects of stem cells and amniotic fluid on uterus and ovaries in a rat model of abdominal adhesions: a controlled study

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Abstract

Objective: This study aimed to compare the effects of human umbilical cord mesenchymal stem cells (hUCMSCs), amniotic fluid (AF), and a combination of both on the uterus and ovaries in a rat model of abdominal adhesions.

Material and Methods: This study was designed as a controlled study. Four groups, each consisting of six rats, were randomly formed. One group was designated as the control (CNT). hUCMSCs - applied (hUCSC), AF-applied (AMN), and a combination of both (hUCSC + AMN) were the experimental groups. All rats were given intraperitoneal talc powder to create adhesions. After 21 days, animals in experimental groups were further treated with hUCMSC, AF or a combination of these.

Results: There was a statistically significant difference in primordial follicle count, endometrial gland number, and endometrial blood vessel count ($p<0.05$). AMN provided the best results in the endometrial vessel and primordial follicle count. The average endometrial gland count in AMN and hUCSC + AMN was similarly higher than CNT and hUCSC alone.

Conclusion: There were significantly higher for counts for endometrial glands, endometrial blood vessels, and primordial follicles in the hUCSC, AMN and hUCSC + AMN groups compared to controls. Animals in the AMN group had the best result for endometrial vessel and highest primordial follicle count. (J Turk Ger Gynecol Assoc 2022; 23: 154-66)

Keywords: Amniotic fluid, case-control studies, infertility, ovaries, rats, stem cells, surgical adhesions, uterus

Received: 24 February, 2022 **Accepted:** 20 March, 2022

Introduction

Adhesion is one of the most common reactions observed between two tissues after abdominal surgery. It is reported as a common cause of morbidities, such as second surgery, infertility, ileus, pain, and intraoperative complications in further surgeries (1). It is reported that of the patients who undergo open abdominal or pelvic surgery, 79-90% develop adhesions (2-4). The ratio of relaparotomy due to adhesions varies between 5-20% (2). Laparoscopic surgery decreases the extent and severity of the formation of adhesions by approximately 50%, mainly at the incision line (3,5). However, even with the more widespread use of laparoscopic surgery, its overall surgical burden remains high (1).

There is no quantitative marker for the diagnosis of adhesions. As a result, the best evaluation is made via inspection. However, some objective techniques may be used to study the severity of adhesions, such as ultrasonography, computed tomography, and magnetic resonance imaging (6,7). Non-invasive techniques are better for aiding diagnosis, as laparoscopy may be a cause of adhesions (8).

Limiting and preventing adhesions would dramatically decrease potential complications, such as infertility, ileus, and pain (9). Many studies have investigated adhesion prophylaxis but the

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2022.2022-1-14

problem remains unsolved (10-15). According to Liakakos et al. (16), the primary aim should be minimalizing mechanical and energy-related trauma, such as avoiding powdered gloves in open surgical interventions, as minimal trauma allows for better vascularization in the postoperative period. Risberg (17) suggested that cleaning the necrotic debris with crystalloid solutions in the surgical site and using barrier gel for damaged and unprotected surfaces would decrease postoperative adhesions by decreasing the fibrosis and extracellular matrix accumulation. In a study by Canbaz et al. (18) in a rat model, gonadotropin-releasing hormone agonist therapy successfully reduced postoperative adhesion formation but was not superior to intraperitoneal Ringer's lactate solution. An increasing number of studies (19) have researched the adhesion prevention capability of various barrier agents (20-23), various combination gels (24-29), biomaterials (30), growth factor inhibitors (31,32), and stem cells (33-35), which is evidence of the importance of adhesion-related problems.

It is essential to understand the pathophysiological process of adhesion formation in order to find effective solutions, especially when using stem cells in regenerative treatments. After a surgery, inflammatory cells recruited for the healing process collect at the surgical site and the surrounding tissues via the vascular supply. Macrophages are dominant in the first 24 hours, followed by neutrophils. Peritoneal healing occurs in seven to ten days. Tissue surfaces are filled with highly regenerative promesothelial and mesothelial cells (36). When these cells arrive at the damaged tissue, they collect in the extracellular matrix. The extracellular matrix, formed by fibronectin, hyaluronic acid, and proteoglycans, gets replaced with permanent collagen and widespread fibrosis occurs (16). Therefore, primary repair through the mesothelial cells completes with scarring. Regenerative treatments mainly focus on cell therapies and biomaterials, such as umbilical cord and amniotic fluid stem cells (AFSCs) that are multipotent cells, and thus can differentiate into the tissue they are integrated into. This quality renders stem cells a candidate treatment to improve post-surgical tissue healing.

Many studies have demonstrated that the mesenchymal stromal cells of amniotic fluid (AF) have cytoprotective and regenerative effects (37-40). It has been demonstrated that AF mesenchymal stromal cells significantly reduced postsurgical intra-abdominal adhesions in a rat model (41). The epithelial cells of human AF are proposed as a novel stem cell candidate in the treatment of severe intra-abdominal adhesions in a second rat model study (42). Additionally, human umbilical cord mesenchymal stem cells (hUCMSCs) are also a significant source of regenerative potential, and have been described in many kinds of tissue, such as bone, wound, nerves and vessels (43-49). However, the effect of hUCMSCs on intra-abdominal adhesions is unknown. One of the important complications of intra-abdominal adhesions is infertility, and the effect of intra-abdominal adhesions on infertility needs further researching.

The primary aim of this study was to evaluate and compare the effects of hUCMSCs, AF, and a combination of both on the uterus and ovaries in a rat model with abdominal adhesions with a control group. The secondary aim of the study was to determine the penetration of hUCMSCs into the uterus and ovaries and the effects of the treatments on adhesion healing.

Material and Methods

The ethical approval of this study was authorized by Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine Animal Experiments Local Ethics Committee (approval number: 2017/37, date: 07.09.2017).

Selection and description of rats

Twenty-four 6-8 week-old, female Wistar-Albino rats, with an average weight of 350-400 g, were purchased from Acıbadem University ACU-DEHAM, following the Federation of European Laboratory Animal Science Associations guidelines and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The inclusion criterion was being healthy, and the exclusion criteria were being pregnant and having previous surgery. The rats were housed with a 24 °C room temperature, 12:12 hour day/night cycle, with adequate water and food supply.

Technical information

A hypothesis with its research questions was developed and are presented below:

Hypothesis: hUCMSCs, AF, and a combination of these are associated with a high number of follicles, endometrial glands, and endometrial blood vessels of the uterus and ovaries in a rat model with abdominal adhesions.

1. Is there a significant difference between the treatments in terms of the uterus (number of endometrial glands and endometrial blood vessels, macroscopic morphology)?
2. Is there a significant difference between the treatments in terms of the ovaries (primordial and preantral follicle counts)?

Therefore, the primary outcomes were the number of endometrial glands and endometrial blood vessels, macroscopic uterus morphology, primordial and preantral follicle counts. The secondary outcomes were the penetration of the hUCMSCs into the uterus and ovaries and adhesion healing.

Study design

This study was designed as a controlled study. Four groups, each consisting of six rats, were randomly formed. One group was designated as the control (CNT). hUCMSCs - applied (hUCSC), AF-applied (AMN), and a combination of both (hUCSC + AMN) were designated as the experimental groups. All rats were given intraperitoneal talc powder to create adhesions. After 21 days, animals in experimental groups were further treated with hUCMSC, AF or a combination of these. Two rats in the hUCSC group were given green fluorescent protein (GFP)-marked stem cells, and another two in the same group were treated with quantum dot (QD)-marked stem cells to evaluate deep penetration into gynaecologic tissues. The sample size and experimental interventions are shown in Table 1. After one week, the rats were sacrificed, and the abdominal walls were incised to evaluate the abdomen. Macroscopic evaluation of any adhesions (secondary outcome) was conducted according to the modified adhesion scoring scale defined by Canbaz et al. (18) (Table 2). Afterwards, the uterus, ovaries, and fallopian tubes were removed and then macroscopically and microscopically evaluated (primary and secondary outcomes). The researchers were not blinded to any data.

Table 1. Description of the sample size

Groups	Number	%
CNT	6	25
hUCSC	6	25
Non-marked	2	$8.\overline{3}$
GFP-marked	2	$8.\overline{3}$
QD- marked	2	$8.\overline{3}$
AMN	6	25
hUCSC + AMN	6	25
Total	24	100

CNT: Control group, hUCSC: Human umbilical cord mesenchymal stem cells (hUCMSCs)-applied group, GFP: Green fluorescent protein, QD: Quantum-dot, AMN: Amniotic fluid (AF)-applied group, hUCSC + AMN: Both hUCMSCs and AF-applied group)

Table 2. Modified adhesion scoring scale (18)

Degree of adhesion	Number of adhesion bands
0	No adhesion.
1	One adhesion band, no vessel, and easily separated.
2	Two thin adhesion bands, no vessel, and easily separated.
3	Three thin adhesion bands, no vessel, and easily separated.
4	More than three thin adhesion bands, easily separated with no vessel or diffuse adhesion bands with vessels.

Human umbilical cord mesenchymal stem cell preparation

The cells were obtained from the umbilical cord blood of an informed, healthy woman who underwent caesarean delivery. All steps were carried out with sterilised equipment. First, the umbilical cord was washed with normal saline [0.9% sodium chloride (NaCl)]. Afterwards, it was dissected, and the vasculature was removed using a scalpel to obtain Wharton's jelly. The Wharton's jelly was cut into pieces 5 mm in length, 5 mm in width, 3 mm in height and washed with 0.9% NaCl solution. The pieces were put on the base of T75 flasks so that each would contain 7-8 pieces. The flasks were gently turned upside down and put in incubators at 37 °C, 5% CO_2, and 7% O_2 for 45 minutes for the pieces to stick to the flasks. Afterwards, Dulbecco's modified eagle medium-low glucose (DMEM-LG) (Sigma-Aldrich, St. Louis, Missouri, USA) including 1% penicillin/streptomycin and 10% human serum was poured into the flasks. The flasks were put in incubators at 37 °C, 5% CO_2, and 7% O_2 for six days and the medium was changed on the sixth day. The cells were passaged after the confluency exceeded 70%. Then the cells were put in a solution containing Ringer's lactate and 1% human serum albumin. A total of 3.5 mL hUCMSCs divided into 500 µL ($1x10^7$ hUCMSCs) per animal was used.

Green fluorescent protein marking

The envelope pCMV-VSV-G [a gift from Bob Weinberg (Addgene, Watertown, Massachusetts, USA #8454. http://n2t.net/addgene:8454; RRID: Addgene_8454)] plasmid, the packaging psPAX2 [a gift from Didier Trono (Addgene #12260; http://n2t.net/addgene:12260; RRID: Addgene 12260)] plasmid, and the GFP-encoding pCDH-EF1-copGFP-T2A-Puro plasmid DNA was transformed into competent *E. coli* DH5α bacteria [NEB® 5-alpha competent *E. coli* (high efficiency)]. The endotoxin-free plasmids were amplified using the QIAfilter Plasmid Giga Kit (QIAGEN, Hilden, Germany), and quality control tests of the produced plasmid were performed in (blinded for review) with accredited protocols. HEK293T cells as host cells were cultured in 5-layer cell culture flasks in an incubator at 37 °C, 5% CO_2 (NEST) for 70% confluence the day before transfection under an inverted microscope. The isolated envelope, packaging, and pCDH-EF1-copGFP-T2A-Puro plasmids (1:1:2 ratio) were mixed with FuGENE HD (Promega, Madison, Wisconsin, USA) transfection reagent for lentivirus (LV) production in opti-MEM reduced serum media (Thermo Fisher Scientific, Waltham, Massachusetts, USA), including 1% penicillin/streptomycin. The packaged recombinant GFP-LV was harvested from the supernatant of the cell cultures 48 hours after transfection. The supernatant, including GFP-LV was filtered (0.45 µm) and concentrated 100x with the Lenti-X

concentrator (Takara Bio, Kusatsu, Shiga, Japan). Jurkat cell line (ATCC, Manassas, Virginia, Product Code: TIB-152™) was suspended as 10,000 cells in 100 µL of RPMI medium with glutamine HEPES with 10% foetal bovine serum (FBS), 1% penicillin/streptomycin, 1% non-essential amino acids, 1% sodium pyruvate, and 1% vitamins. The Jurkat cells in 100 µL of the medium were plated in 96-well plates from A to I. The wells were adjusted to have 10 µL, 3 µL, 1 µL, 0.3 µL, 0.1 µL, and 0.03 µL of the 100x-concentrated GFP-LV solutions in each 50 µL of the medium, respectively, and then 50 µL of virus dilution from each concentration was transferred to Jurkat cultured wells, the total volume was adjusted to 150 µL, and cells were incubated for 3-4 days. Flow cytometry was performed using MACSQuant flow cytometry (Miltenyi Biotec, Bergisch Gladbach, North Rhine-Westphalia, Germany) for GFP expression. Following the GFP-LV titer assay and other quality control tests, including sterility and purity analyses, the GFP-coding viruses were stored at -80 °C. Mesenchymal stem cells were infected with the GFP-coding LV (1-5 multiplicity of infection) expressing pCDH-EF1-copGFP-T2A-Puro. A flow cytometer confirmed stem cells synthesizing GFP at the end of the 4th day. Greater than 95% GFP positive stem cells were replicated in an incubator at 37 °C, 5% CO_2 up to $1x10^7$ cells by the antibiotic selected result, only GFP-labelled mesenchymal stem cells were obtained. A total of 1 mL GFP-marked UCSC, divided into 500 µL ($1x10^7$ umbilical cord stem cells) per animal were used.

Quantum dot marking

QD marking was utilised to be able to observe the depth of penetration of the hUCMSCs into gynaecologic tissues. A sample was taken from an unmarked cell (to use as control inflow). Qtracker™ 655 Cell Labelling Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) A and B components were gently mixed. For every $1x10^7$ cells, 10 µL A and B components were added into the tube. The mixture was incubated at 37 °C, 5% CO_2 for 5 minutes. Immediately after the incubation, 0.2 mL freshly prepared solution containing DMEM-LG, 10% FBS, and 1% penicillin was added and vortex mixed for 30 seconds. Cells were incubated at 37 °C, 5% CO_2 for 45-60 minutes in a tube. After the incubation, the cells were centrifuged for 10 minutes at 400 G and bathed in DMEM-LG, 10% FBS, and 1% penicillin medium. The batheing was repeated. A sample was taken from the medium to quantify the marked cells using flow cytometry. The marked and control cells were sent for quality control in terms of viability, sterility, and differentiability. A total of 1 mL QD-marked hUCMSCs divided into 500 µL ($1x10^7$ umbilical cord stem cells) per animal were used.

Amniotic fluid preparation

For the AF, the samples were derived from the amniotic sac of the same informed, healthy woman who underwent caesarean delivery. AF was put through 0.22 µm filters to decontaminate upon arrival. Afterwards, it was kept at -80 °C before gamma irradiation for decontamination. Lastly, the samples were liquefied for study use. A total of 24 mL of AF, 1 mL per animal, was used.

Intraperitoneal talc and treatment injection

The rats were anesthetized with xylazine (0.6 mg/kg/intraperitoneal) and ketamine (0.9 mg/kg/intraperitoneal) and fixed in a dorsosupine position. The abdominal walls were shaved and disinfected with povidone-iodine. The abdominal wall was incised with a vertical incision (Figure 1A), and sterile cannulas were placed into the abdominal cavities (Figure 1B). 1 cc talc powder was given intra-abdominally per rat to create adhesions. Afterwards, the cannulas were removed, and the peritoneum and the abdominal wall were closed with 2/0 rapid vicryl. The rats were given daily amoxicillin/clavulanic acid in the postoperative three days.

On the twenty first day of the study, the rats were injected with hUCMSCs or AF or both. After one week, on the twenty-eighth day, the rats were sacrificed and disected. The uterus, ovaries, fallopian tubes, and peritoneum were removed through an approximately 4 cm incision and placed in 10% formaldehyde solutions for histopathologic evaluations.

Statistical analysis

Normality of data was tested with the Shapiro-Wilk test. Non-parametric statistical methods were used for values with skewed distribution (non-normally distributed, Shapiro-Wilk $p>0.05$). Descriptive statistics are presented using mean and standard deviation for normally distributed variables and median (and minimum-maximum) for the non-normally distributed variables. For comparison of two normally distributed independent groups, the Student's t-test was used. Non-parametric statistical methods were used for values with skewed distribution. For comparison of two non-normally distributed independent groups, the Mann-Whitney U test was used. Statistical analysis was performed using IBM SPSS Statistics, version 24 (IBM, Armonk, New York, USA).

Results

The sample size for experimental groups is shown in Table 1. There was no incidence of intra-abdominal ascites, surgical site infection, nor animal death following the application of talc powder.

Microscopic evaluations of the gynaecological system

Uterus and ovaries (primary outcomes)

There was a statistically significant difference in terms of endometrial gland number, endometrial blood vessel count, and primordial follicle count distributions according to the groups (Kruskal-Wallis test, $p<0.05$) (Table 3).

According to the post-hoc pairwise comparison (Table 4), in terms of endometrial gland count, there was a significant difference between pairwise groups except for CNT versus hUCSC and AMN versus hUCSC + AMN (Table 4) (Mann-Whitney U test, $p<0.008$, Bonferroni correction). The average number of endometrial glands in AMN was higher than CNT and hUCSC and similar to hUCSC + AMN (Figure 2).

In terms of endometrial blood vessel count, there was a significant difference between pairwise groups except for CNT versus hUCSC, AMN versus hUCSC + AMN, and hUCSC versus hUCSC + AMN (Table 4) (Mann-Whitney U test, $p<0.008$, Bonferroni correction). The average blood vessel count in the AMN group was significantly higher than the others (Figure 3).

In terms of distribution of primordial follicle count, there was a significant difference between the pairwise groups, except for CNT versus hUCSC (Mann-Whitney U test $p<0.008$, Bonferroni correction). In addition, the average number of primordial follicles in AMN was found to be significantly higher than the other groups (Figure 4).

On histopathological semi-quantitative evaluation, in CNT the ovary germinal epithelium was observed as single-layered and cubical. Small-sized primordial follicles were lost in the oocytes. Additionally, atretic follicles (Figure 5A) and areas of vacuolation (Figure 5B) were present. The integrity of the uterine tissue was not preserved (Figure 6A) and the tunica albuginea layer was enlarged.

In hUCSC, the ovary germinal epithelium was observed as mildly proliferated. Dominant follicles were high in number. Antral follicles were surrounded with normal-sized preantral follicles (Figure 5C, D). Stromal vascularity and the number of endometrial glands were high. A high number of cells in the connective tissue and the superficial endometrium was

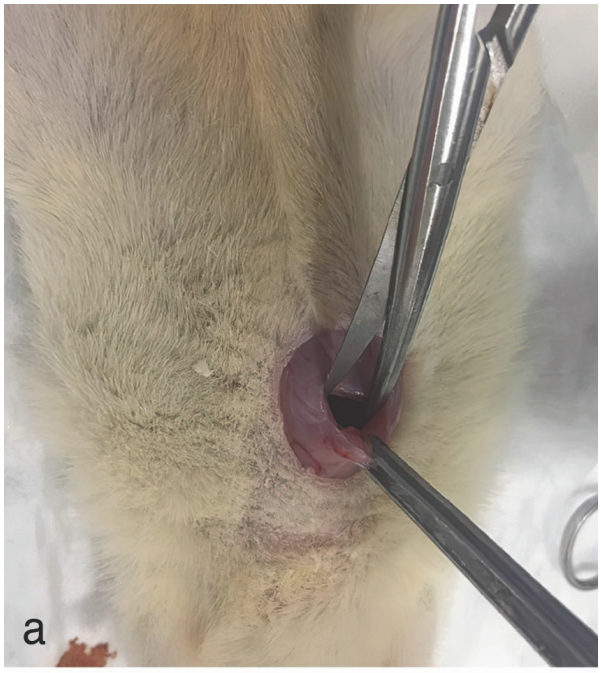

Figure 1. Images of the surgical procedure used. a) Vertical incision in the abdominal wall. b) Placement of a sterile cannula into the abdominal cavity for the application of talc powder

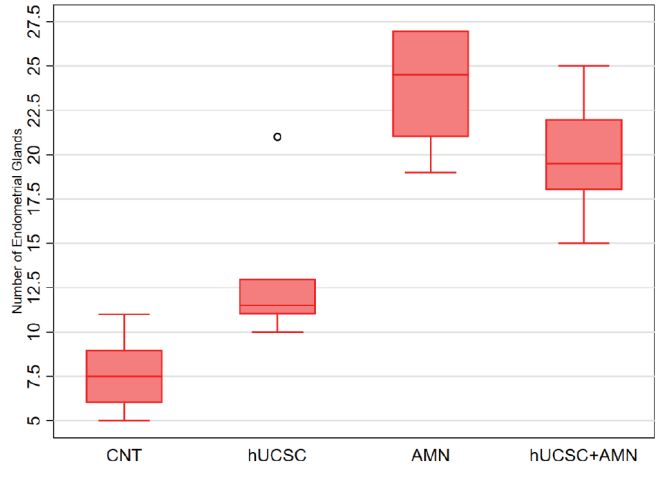

Figure 2. Box plot of the primordial follicle counts of the groups

CNT: Control group, hUCSC: Human umbilical cord mesenchymal stem cells (hUCMSCs)-applied group, AMN: Amniotic fluid (AF)-applied group, hUCSC + AMN: Both hUCMSCs and AF-applied group

present. Epithelial gland cells were proportionate to the surface epithelium, and the glandular lumen was filled with secretion. The integrity of the uterine tissue was preserved (Figure 6B).

In AMN, the ovary germinal epithelium was observed as proliferated. A high number of primordial follicles was present. A high number of corpus luteum was also present, suggesting ovulation (Figure 5E). Multiple endometrial glands and increased endometrial vascularity were present (Figure 5F). The endometrium showed general proliferation (Figure 6C).

In hUCSC + AMN, the ovary germinal epithelium was also observed as cubical-columnar cells, and the basal membrane

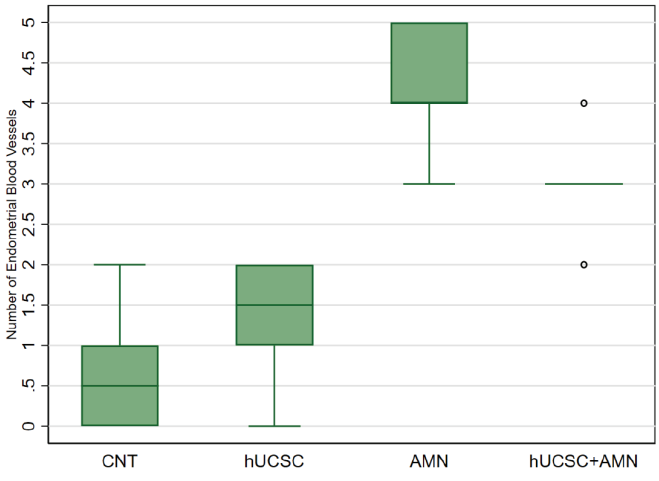

Figure 3. Box plot of the number of endometrial glands of the groups

CNT: Control group, hUCSC: Human umbilical cord mesenchymal stem cells (hUCMSCs)-applied group, AMN: Amniotic fluid (AF)-applied group, hUCSC + AMN: Both hUCMSCs and AF-applied group

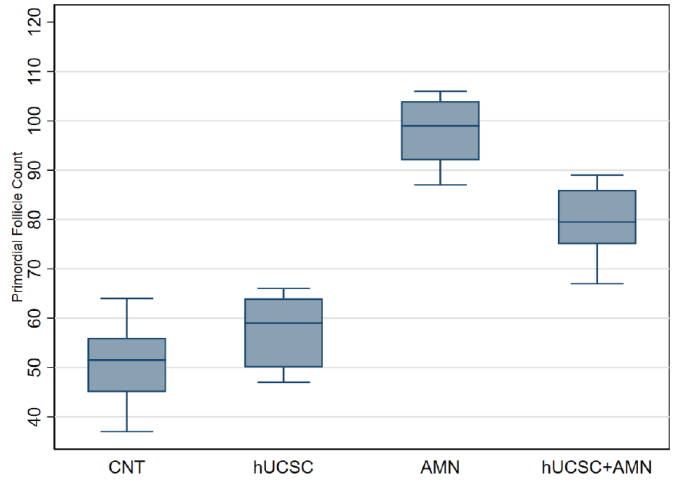

Figure 4. Box plot of the number of blood vessels of the groups

CNT: Control group, hUCSC: Human umbilical cord mesenchymal stem cells (hUCMSCs)-applied group, AMN: Amniotic fluid (AF)-applied group, hUCSC + AMN: Both hUCMSCs and AF-applied group

Table 3. Comparison of the groups in terms of uterus and ovarian histology

Groups	**CNT (n=6)**	**hUCSC (n=6)**	**AMN (n=6)**	**hUCSC + AMN (n=6)**	**p**
Parameters	**Mean ± SD Med. (min.-max.)**	**Mean ± SD Med. (min.-max.)**	**Mean ± SD Med. (min.-max.)**	**Mean ± SD Med. (min.-max.)**	
Primordial follicle count	50.8±9.3 51.5 (37-64)	57.5±7.9 59 (47-66)	97.8±7.6 99 (87-106)	79.3±8.16 79.5 (67-89)	**<0.001**
Preantral follicle count	3.67±1.9 3 (2-7)	4.5±3.0 4 (1-8)	7.2±1.7 8 (5-9)	5.8±0.9 5.5 (5-7)	0.055
Number of endometrial glands	7.67±2.2 7.5 (5-11)	13±4.05 11.5 (10-21)	23.8±3.25 24.5 (19-27)	19.8±3.4 11.8 (15-25)	**<0.001**
Number of endometrial blood vessels	0.67±0.8 0.5 (0-2)	1.33±0.8 1.5 (0-2)	4.17±0.7 4 (3-5)	3.0±0.6 3 (2-4)	**<0.001**

CNT: Control group, hUCSC: Human umbilical cord mesenchymal stem cells (hUCMSCs)-applied group, AMN: Amniotic fluid (AF)-applied group, hUCSC+AMN: Both hUCMSCs and AF-applied group, SD: Standard deviation, Med.: Median, min.: Minimum, max.: Maximum. Kruskal-Wallis test

Table 4. Post-hoc pairwise comparisons of the groups by Mann-Whitney U test.

Parameters	**CNT vs hUCSC**	**CNT vs AMN**	**CNT vs hUCSC + AMN**	**hUCSC vs AMN**	**hUCSC vs hUCSC + AMN**	**AMN vs hUCSC + AMN**
Primordial follicle count	0.310	**0.002**	**0.002**	**0.002**	**0.002**	**0.004**
Number of endometrial glands	0.009	**0.002**	**0.002**	**0.004**	0.026	0.093
Number of endometrial blood vessels	0.240	**0.002**	**0.002**	**0.002**	**0.004**	0.026

Values shown are p-values.
CNT: Control group, hUCSC: Human umbilical cord mesenchymal stem cells (hUCMSCs)-applied group, AMN: Amniotic fluid (AF)-applied group, hUCSC + AMN: Both hUCMSCs and AF-applied group. Mann-Whitney U test

was regular. Primordial and preantral follicles were present and intact. Additionally, several superovulated follicles were present (Figure 5G). Glandular, vascular, and connective tissue were proliferated (Figure 6D).

Penetration of human umbilical cord stem cells into uterus and ovaries (secondary outcome)

GFP and QD-marked areas were examined under a fluorescent microscope. None of the uteri gave signalling in both marked-hUCMSCs subgroups. However, the GFP-marked hUCMSCs were observed in the intrafollicular area and around oocytes (Figure 7A). In addition, a remarkable signal of QD-marked hUCMSCs in the interfollicular area and around oocytes was detected in terms of ovary penetration (Figure 7B).

Macroscopic evaluations

Uterus (primary outcome)

hUCSC, AMN, and hUCSC + AMN increased the ovarian volume, fallopian tubes, uterus serosa, and peripheral vascularity, with the AMN having the greatest increase when all experimental groups were compared with CNT.

Table 5. Macroscopic adhesion scoring of the groups

Degree of adhesion/groups	0	1	2	3	4
CNT					+
hUCSC		+			
AMN			+		
hUCSC + AMN			+		

CNT: Control group, hUCSC: Human umbilical cord mesenchymal stem cells (hUCMSCs)-applied group, AMN: Amniotic fluid (AF)-applied group, hUCSC+AMN: Both hUCMSCs and AF-applied group

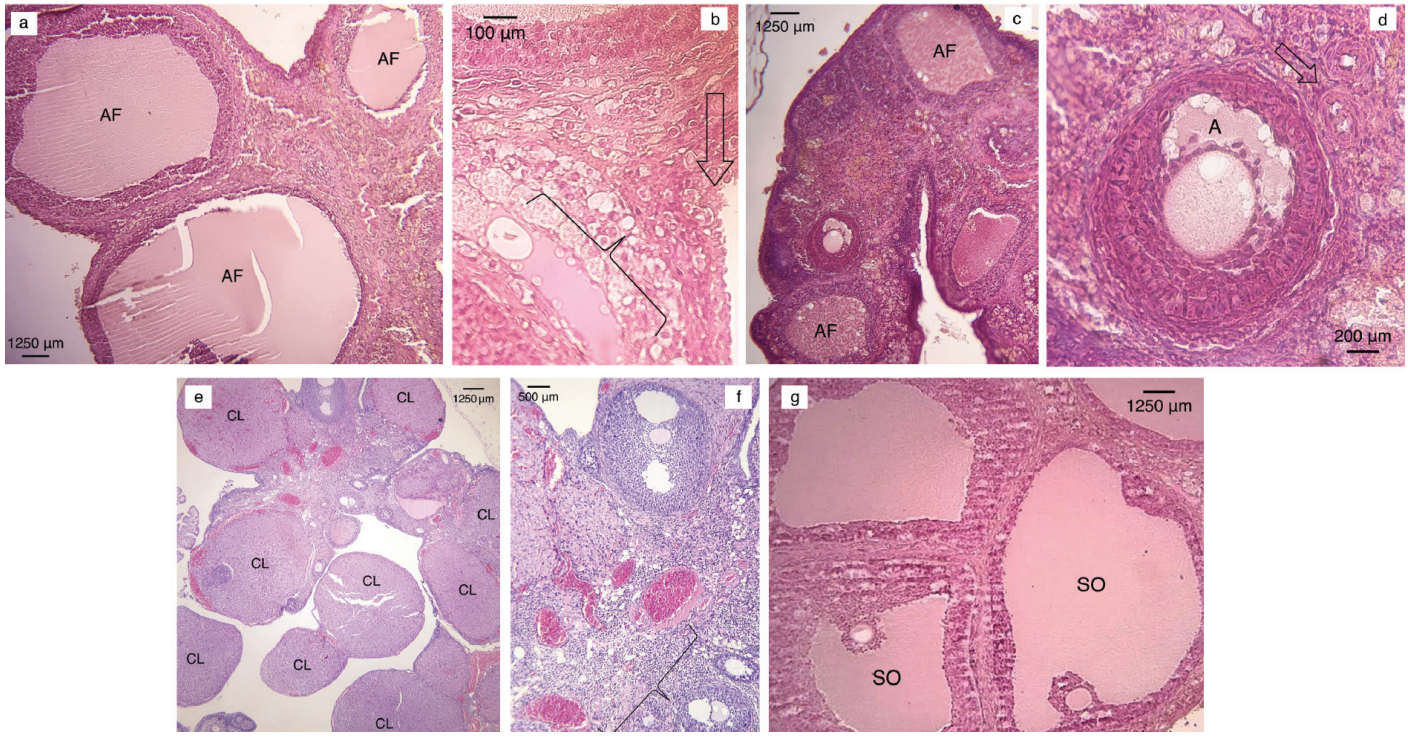

Figure 5. Photomicrographs of the ovaries of the groups. (haematoxylin & eosin). a) Different stages of degenerated atretic follicles (AF) are evident in the CNT group animal samples (x40 magnification). b) Vacuolation in the interfollicular area (parenthesis) is evident in the CNT group samples in the interstitial area (arrow) (x400 magnification). c) The human umbilical mesenchymal cord stem cells (hUCMSCs)-applied group shows healthy antral follicles (AF) surrounded by a preantral follicle (x40 magnification). d) The hUCMSCs-applied group demonstrating intact zona pellucida and antrum a) and oocytes and primordial follicles (arrow) (x200 magnification). e) The amniotic fluid (AF)-applied group exhibited superovulation and multiple corpus luteum (CL) (x40 magnification). f) The AF-applied group displayed multiple endometrial blood vessels (parenthesis) (x100 magnification). g) Both hUCMSCs and AF-applied groups showed superovulated follicles (SO) (x40 magnification)

Abdominal adhesions (secondary outcome)

Macroscopic examination revealed that the talc powder caused a high degree of intra-abdominal adhesions. However, all groups were internally uniform in abdominal adhesions (Table 5) and defined according to the modified adhesion scoring scale (Table 2).

In CNT, dense adhesions between bowels, uterus, peritoneum, ovaries and residual foci of talc powder collections and the highest number of adhesions were present (Figures 8A). The least number of adhesions was observed in hUCSC (Figures 8B). AMN (Figure 8C) and hUCSC + AMN had a moderate and similar number of adhesions (Figures 8D).

Discussion

The current study evaluated the effect of hUCMSCs, AF, and a combination of both on uterus and ovaries in a rat model of abdominal adhesions. In terms of the primary outcome results regarding endometrial gland number, AMN was better than CNT and hUCSC and similar to hUCSC + AMN. In addition, AMN was better than all groups in terms of endometrial blood vessel count, primordial follicle count, and macroscopic uterus morphology (Table 3, 4). In terms of secondary outcomes, the hUCSCs penetrated the ovaries, but not the uterus. Furthermore, in terms of adhesion healing, hUCSC produced better results than groups without hUCSCs and even out-performed the combined group of hUCSC + AMN (Table 5).

Studies to prevent intra-abdominal adhesions after surgery have been ongoing since the beginning of the last century (10-35). Considering the significant postoperative complications caused by adhesions, such as second surgery, ileus, pain, intraoperative complications in further surgeries, and infertility, initiating an effective treatment is clinically available (1).

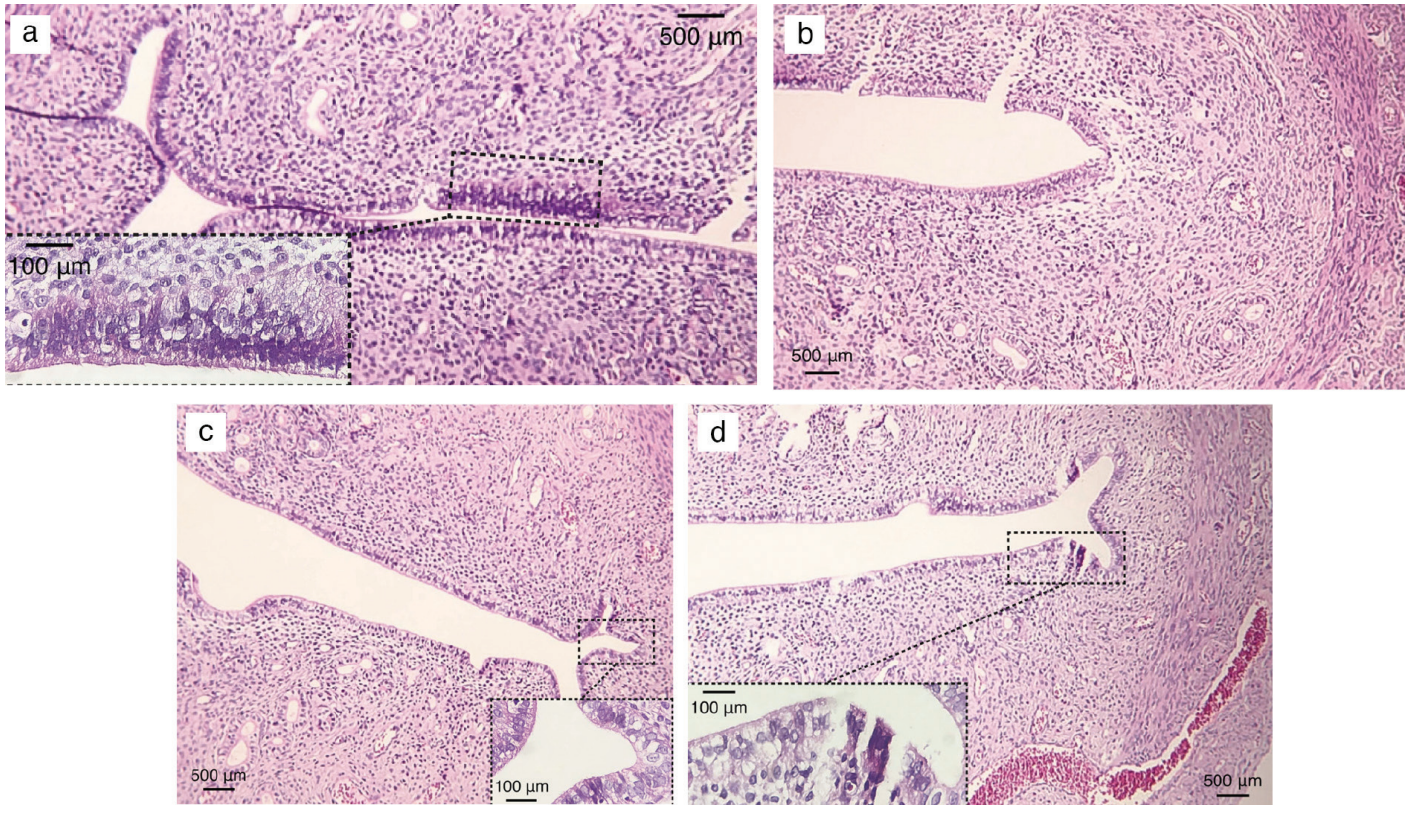

Figure 6. Photomicrographs of uterine tissue and endometrium of the groups (haematoxylin & eosin). a) The control group exhibited hyperplasia, metaplasia, and vacuolation of the epithelial lining of the endometrium, shedding of the epithelium and perimetrium, narrow uterine lumen, and decrease in myometrial smooth muscle cells (main image, x100 magnification), and connective tissue loss in the endometrium (lower-left corner image, x400 magnification). b) The human umbilical cord mesenchymal stem cells (hUCMSCs)-applied group displayed endometrial proliferation, extensive myometrium, and blood vessel increase in the stratum vasculare (x100 magnification). c) The amniotic fluid (AF)-applied group showed narrow uterine lumen (main image, x100 magnification) due to increase and enlargement of the uterine glands, and their movement towards the surface of the endometrium (lower-right corner image, x400 magnification). d) Both hUCMSCs and AF-applied groups demonstrated connective tissue proliferation in the uterine wall with the formation of newly formed blood capillaries, growth in myometrium and increased collagen (main image, x100 magnification), and irregularity in glandular structures (lower-left corner image, x400 magnification)

Surgical techniques and principles of surgery are the initial strategies in the prevention of adhesions. Laparoscopic surgery was significantly more successful in preventing adhesions when compared to open surgery (3,5). However, the overall surgical load of adhesions is still high (1). In addition, infertility is a significant and costly complication of intra-abdominal adhesions (50-53). Therefore, prevention and successful treatment of intra-abdominal adhesions could conceivably decrease infertility.

In the current century regenerative therapy has become one of the most important emerging treatment methods for adhesions. The regenerative characteristics of hUCMSCs and AF play a significant role in treating and preventing adhesions. Additionally, several animal studies utilising AFSCs have been published (33-35,41). However, adult stem cells have a limited capacity for regeneration, and foetal stem cells may be ethically unacceptable. However, umbilical cord, placenta, and AF are biological waste that may have a great regenerative and cytoprotective potential with minimum ethical issues (54). Additionally, in the current study, hUCMSCs were also used for intraperitoneal application, and no adverse effects on the experimental rat gynaecological system were observed.

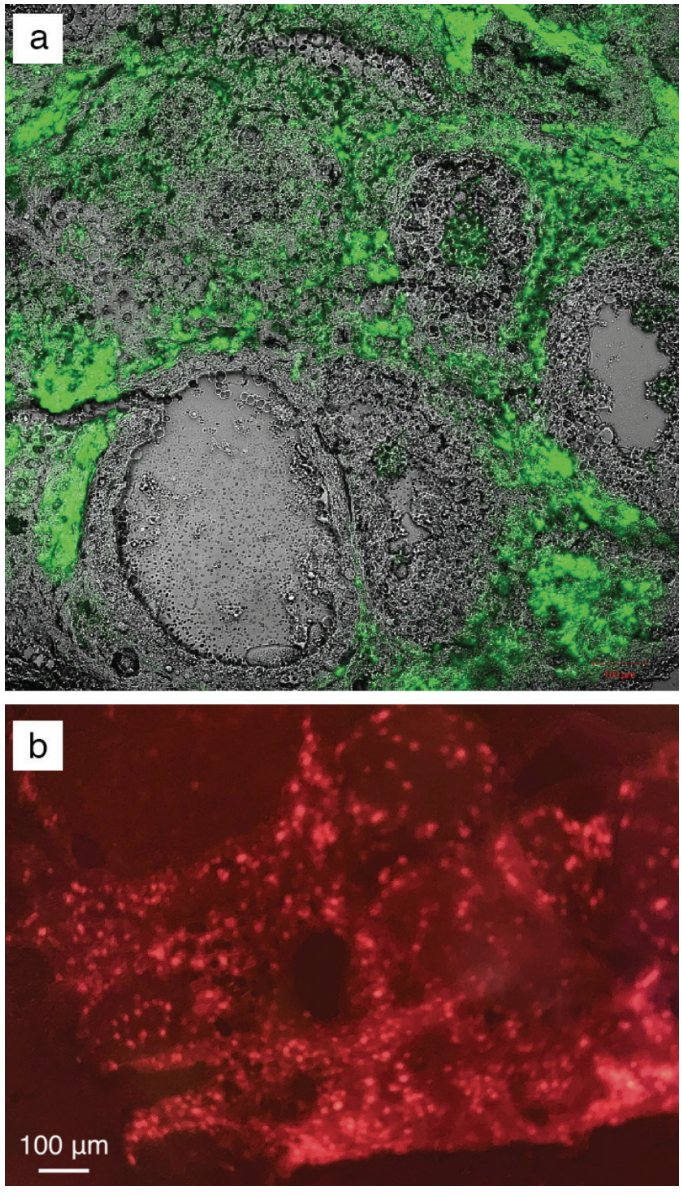

Figure 7. Fluorescent microscopy images of the marked-human umbilical cord stem cell applied subgroups. a) Increased signalling of green fluorescent proteins. b) Increased signalling of the quantum dot.

Bollini et al. (38) described the positive in vivo effects of cells in various organs by paracrine effect, and they claimed that these effects were performed by secretomes possessed by these cells' membranes. The regenerative potential of the secretomes produced by the stem cells is vital in maintaining microvascular structure healing and increasing glandular structures (55). In the current study, hUCMSCs were observed to positively affect both the uterus and the ovaries histologically and morphologically. Similar to the current study's results regarding positive regenerative effects of hUCMSCs on the uterus, Kuramoto et al. (56) found out that hUCMSCs also improve uterine incision repair in rats. hUCMSCs sheets were used and showed significantly smaller fibrotic-to-normal myometrium ratios.

Additionally, Tang et al. (57), in their rat model with intrauterine adhesions, reported that hUCMSCs transplantation significantly increased the number of endometrial glands, decreased fibrosis, and improved the proliferation of endometrial cells. The low immunogenic properties of hUCMSCs make them suitable options for repairing endometrial damage (58). Moreover, similar to the current study's results regarding the effect of hUCMSCs on ovaries, Zhu et al. (59) showed regenerative effects of hUCMSCs on ovaries with an ovarian injury model in rats with an intraperitoneal injection of cyclophosphamide. The levels of sex hormones, oestrous cycle, and reproductive potential of the treated rats were recovered to some extent, and some transplanted rats even recovered fertility. Other studies also showed the positive effects of hUCMSCs on premature ovarian failure and ovarian dysfunction in a rat model (60-64). In the current study, hUCSC was better than all groups in terms of intra-abdominal adhesions. It could be because increased angiogenesis enables the delivery of more anti-inflammatory mediators (36,55).

AF contains many types of cells, including mesenchymal stem cells. Therefore, it is used as a proven resource for regenerative treatments (65-71). The stem cells in the AF do not possess oncogenic properties, and they could be used even in immunosuppressed rats (72).

Regenerative effects of the AF on the uterus and ovaries were observed in the current study. The current study showed that AMN was better than all groups in terms of endometrial blood vessels and primordial follicle counts. In terms of endometrial glands, it was better than CNT and hUCSC and similar to hUCSC + AMN. Positive effects of the AF on the uterus could be explained by its promotion of oestrogen receptor expression in the endometrium and uterine microenvironment regulation. Bai et al. (42) highlighted this in their study and also mentioned inhibition of fibrosis progression, promotion of proliferation, and angiogenesis in a rat model. A similar study showed that human amniotic mesenchymal cells facilitated endometrial regeneration after injury (73). Additionally, AF was observed to restore chemotherapy-induced damage to ovarian morphology (64). In the current study, AMN and hUCSC + AMN were equally effective and better than the other groups for healing of adhesions. This could be attributed to the high angiogenetic potential of AF. Hypothetically, the combination of two highly regenerative biomaterials could synergistically increase their effects. hUCSC + AMN provided positive results on the uterus and ovaries. However, it was not better than hUCSC alone nor AMN alone in all parameters. Lastly, it had an equal effect on endometrial glands and adhesion healing compared to AMN alone.

This study evaluated and compared the different effects of highly potent biomaterials such as hUCMSCs and AF on the uterus and ovaries of rats in a model of talc-induced abdominal adhesion. The increase in uterus connective tissue, endometrial glands, and improved vasculature; and increase in the number of primordial and preantral follicles in ovaries

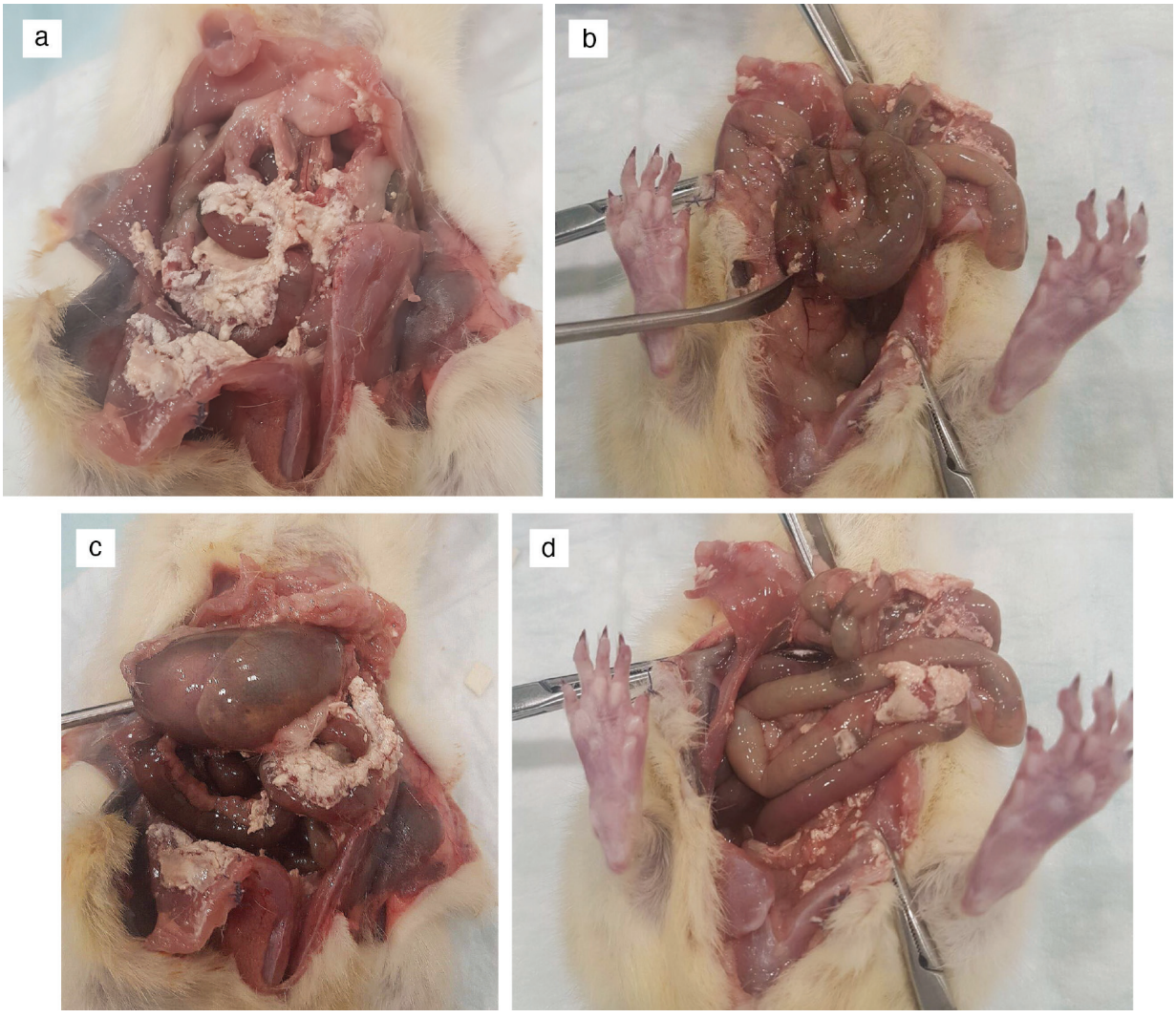

Figure 8. Exposed abdomens of the groups. a) The control group exhibited the highest number of intra-abdominal adhesions. b) The human umbilical cord mesenchymal stem cell (hUCMSCs)-applied group displayed the lowest number of intra-abdominal adhesions. c) The amniotic fluid (AF)-applied group showed a moderate number of intra-abdominal adhesions. d) Combined hUCMSCs and AF-applied group also demonstrated a moderate number of intra-abdominal adhesions.

support the regenerative effects of hUCMSCs and AF on the gynaecological system. However, further studies with infertile or ovarian dysfunctional rats must be conducted to investigate the effect of biomaterials on infertility more fully.

Study Limitations

The limitations of this study are, first, the limited number of rats. Secondly, only one single adhesion agent was used. Studies with larger sample sizes with control groups researching the effects of various surgical traumas induced by suturing, electrosurgery, and abrasion on the uterus and ovaries must be conducted to further explore the regenerative effects of biomaterials.

Conclusion

The results suggest that AF, hUCMSCs, and a combination of both have a significant positive effect on the gynaecological system of experimental animals in a model of abdominal adhesion. Compared to control animals, all groups except showed significantly better results regarding the number of endometrial glands, endometrial blood vessels, and primordial follicles. AMN had the best results in the endometrial vessel and primordial follicle count and equal results with hUCSC + AMN in the endometrial glands. None of the experimental groups had any significant effect on the number of preantral follicles compared to controls.

Ethical Committee Approval: *The ethical approval of this study was authorized by Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine Animal Experiments Local Ethics Committee (approval number: 2017/37, date: 07.09.2017).*

Informed Consent: *Patient approval has not been obtained as it is performed on animals.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: E.G.A., G.T.; Concept: E.G.A., G.T.; Design: E.G.A., G.T.; Data Collection or Processing: E.G.A., G.T.; Analysis or Interpretation: E.G.A., G.T.; Literature Search: E.G.A., G.T.; Writing: E.G.A., G.T.*

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

Financial Disclosure: *The authors declared that this study received no financial support.*

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Investigation of the effects of trophectoderm morphology on obstetric outcomes in fifth day blastocyst transfer in patients undergoing in-vitro-fertilization

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Abstract

Objective: Trophectoderm (TE) cells are the first differentiating cells in embryo development and have epithelial features. TE cells, which associate with implantation of the blastocyst into the uterine endometrium, contribute to the formation of the placenta. Inner cells mass (ICM) together with TE cells are used for determining embryo quality. The aim of this study was to investigate the role of TE and ICM cells on pregnancy outcome in 5th day blastocyst transferred in-vitro-fertilization (IVF) pregnancy.

Material and Methods: This was a retrospective study using data from all patients who applied for blastocyst transfer IVF between January 2015 and March 2019 at the Reproductive Endocrinology and Infertility Center of Akdeniz University Faculty of Medicine, Department of Obstetrics and Gynecology. ALPHA İstanbul consensus evaluation system was used for grading of the blastocyst. The embryo quality, expansion, ICM and TE morphology of the 5th day transferred blastocyst was assessed, together with abortion rate, live birth rate, pregnancy complications, and pregnancy outcomes.

Results: There was a significantly increased risk of preeclampsia (PE) (7.8% vs 1.1%; p=0.041), preterm delivery (PD) (36% vs 17.7%; p=0.037), and antenatal bleeding rates (13.6% vs 5%; p=0.021) in TE-C compared to the TE-A + TE-B blastocysts. Furthermore, a higher rate of obstetric complications was observed in ICM-C compared to ICM-A and B (p=0.003). There was a significant correlation between TE morphology and implantation success, ongoing pregnancy rate, and abortion incidence.

Conclusion: These results suggest that TE cell morphology is related to implantation success and pregnancy outcomes, especially in terms of the risk of abortion, PE, PD, and antenatal bleeding. It may be advisable to counsel women concerning possible poor obstetric outcome due to poor ICM quality. Future prospective and controlled studies are needed to clarify this association. (J Turk Ger Gynecol Assoc 2022; 23: 167-76)

Keywords: Trophectoderm, inner cell mass, pregnancy complications

Received: 05 January, 2022 **Accepted:** 10 May, 2022

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2022.2021-10-8

Introduction

Obstetric complications continue to be one of the leading causes of maternal and perinatal mortality and morbidity. Preeclampsia (PE), for example, is responsible for more than 10% of all maternal deaths worldwide (1), and is still a risk factor for perinatal mortality (2). Similarly, multiple pregnancies, fetal growth restriction (FGR), and prematurity are other obstetrics complications associated with perinatal mortality and morbidity (3).

In-vitro-fertilization (IVF) is increasingly used as a therapy for infertility. Although the majority of IVF pregnancies have a good perinatal outcome, several studies have found that IVF pregnancies have a greater prevalence of PE, FGR, preterm delivery (PD), and other obstetric issues compared to normal pregnancy (4). However, it is still unclear if these additional complications in IVF pregnancies are related to the cause of infertility or associated with IVF procedures (5).

Blastocele expansion, organization of inner cell mass (ICM) and trophectoderm (TE) cells are commonly used to assess blastocyst quality (6). There is very limited data investigating the effect of blastocyst quality on the prognosis and outcome of IVF pregnancies (7-12). Although blastocyst morphology has been shown to be closely associated with implantation success (13-16), there is a scarcity of evidence on the impact of blastocyst morphology on obstetric outcome (12,16,17). Therefore, this study was conducted to investigate whether there was a relationship between blastocyst morphology and obstetric outcome in IVF pregnancies.

Material and Methods

This study was conducted at Akdeniz University Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology IVF Center. After Akdeniz University Faculty of Medicine Local Ethical Committee approval (approval number: 1164, date: 11.12.2019), and obtaining informed consent, the files of patients treated at the IVF Center between 01.01.2015-31.03.2019 were reviewed retrospectively. All patients had 5th day blastocyst transfer. Demographic characteristics of the patients, etiology of infertility, the number of previous IVF treatments and controlled ovarian hyperstimulation (COH) protocols were noted. Additionally, the quality information of the embryos was reviewed from an electronic database. Information related to pregnancy, pregnancy weeks at delivery, delivery type, weights and gender of the newborns, and obstetric complications were obtained from Akdeniz University Medical School Hospital's electronic database and from the patients by phone or interview.

Blastocysts obtained during IVF were examined using the ALPHA İstanbul consensus evaluation system (18). This system includes separating the blastocyst from the zona pellucida - hatching (grade 1-4), the size of ICM and arrangement (grade A-C), and the number of TE cell and arrangement (grade A-C). In the blastocyst examination procedure, ICM-A is composed of a large number of tightly packed polygonal cells, ICM-B is composed of a small number of easily distinguished, loosely assembled cells, and ICM-C consists of a small number of hardly distinguishable cells. TE-A is of good quality and consists of a large number of continuous cells, TE-B is of medium quality and cells are loosely arranged, with a small number of cells, TE-C is of poor quality and has been reported to consist of a small number of cells. In all cases, one of the best-quality embryos was transferred, and if there were other good quality embryos among the developing embryos, they were frozen and stored. In contrast, for both fresh and frozen embryo transfers (ET), non-viable embryos (development has been arrested for at least 24 hours, or in which all the cells have degenerated or lysed) were not transferred (18). For frozen ETs, viability below 80% is considered as "not survived" and those embryos were not transferred (19).

Gestational age at delivery, weight and gender of the newborns and any complications, including abortus, PE, eclampsia, FGR, oligohydramnios, polyhydramnios, PD, gestational diabetes mellitus and antenatal hemorrhage, were recorded through the hospital registry system and analyzed.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS), version 20.0, was used for statistical analysis (IBM Inc., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation, and categorical variables as numbers and percentages. When the number of independent groups was two, the comparison between groups was investigated by Student's t-test, and the difference between more than two groups was compared with ANOVA. Mann-Whitney U test, Kruskal-Wallis analyses, Pearson chi-square and Fisher's exact tests were used as needed.

The possible relations between obstetric complications and ICM, TE and blastocyst expansion were analyzed by using each grade of these parameters.

Results

A total of 291 patients, who had ET at the blastocyst stage on the 5th day, were included in the study. The mean age of the patients was 32.0±4.5 years. The numbers of patients who had frozen ET and fresh ET, in case of having no frozen ET, were 25 (8.8%) and 266 (91.2%), respectively. Fresh ET was only performed in cases when no frozen ET was available. The mean duration of infertility was 5.0±3.7 years. The rate of biochemical pregnancy was 13%, while abortion in the first 12 weeks was 11.2%, and

abortion between 12-20 weeks was 4%. It was found that 71.8% of the women delivered after 20 weeks, and the live birth rate was 71.4%. Thirty percent of pregnancies were found to have at least one obstetric complication.

The patients were evaluated by comparing grades A, B and C in the ICM and TE parameters, and the degree of expansion under the headings of early blastocyst and blastocyst groups. The ICM-A, B, C groups included 126, 81 and 39 patients and the TE-A, B, C groups consisted of 106, 74 and 66 patients, respectively. Transfers of embryos were performed in the early blastocyst stage in 73 patients and in the blastocyst stage in 217 patients.

The rate of biochemical pregnancy in ICM-A, B, and C groups was 7.1%, 18.5%, and 20.5%, respectively (Table 1). While there was no significant difference between ICM group B and C, less frequent biochemical pregnancy was seen in group A (p=0.019 for both). Abortion rates in first 12 weeks and between 12-20 weeks was similar among ICM-A, B, and C groups (p=0.734). Delivery incidence over 20 weeks was 76.2%, 66.7% and 61.5% in the ICM-A, B, and C groups, respectively (p=0.132). Birth weights of the newborns were not significantly different between the ICM-A, B and C groups (Table 1).

Comparison of pregnancy outcome according to TE grade showed that biochemical pregnancy frequency was strongly related to TE grade and was 7 (6.6%), 11 (14.9%), and 14 (21.2%), in the groups of TE-A, B, and C respectively (Table 2). Abortion rates in the first 12 weeks and 12-20 weeks, delivery frequency beyond 20 weeks, and live birth rates were not significantly different among the TE-A, B, and C groups (Table 2).

The frequencies of biochemical pregnancy, abortion in the first 12 weeks, abortion between 12 and 20 weeks, birth beyond 20 weeks, and the live births rates were not significantly different between the early blastocyst and the blastocyst stages groups (Table 3). Delivery weeks and weight of the newborns were also similar between the early blastocyst and the blastocyst groups (Table 3).

The incidence of obstetric complications in the ICM-A, B, and C groups was significantly different at 39 (46.4%), 24 (49%), and 19 (86.4%), respectively (Table 4). After Bonferroni correction, while there was no significant difference between groups A and B, but a higher rate of obstetric complications was found in group C (p=0.003).

At least one obstetric complication was seen in 29 (41.4%) pregnancies in the TE-A, 22 (48.9%) in the TE-B, and 31 (77.5%) in the TE-C groups. While the complication frequency was similar in TE-A and B, the TE-C group had significantly higher complication rate when compared to TE-A and B (p<0.001). Non-complicated pregnancy frequency was found similar in TE-A and B. However, TE-C group has significantly lower non-complicated pregnancy rate (Table 5).

Early blastocyst and blastocyst groups were similar in terms of obstetric complication rates and there was no significant difference among groups (p=0.586).

Table 1. Pregnancy results among ICM groups

	ICM-A, n (%)	ICM-B, n (%)	ICM-C, n (%)	p
Biochemical pregnancy (%)	9 (7.1)[a]	15 (18.5)	8 (20.5)	0.019
Abortion (first 12 weeks)	17 (13.5)	8 (9.9)	5 (12.8)	0.734
Abortion between 12-20 weeks	4 (3.2)	4 (4.9)	2 (5.1)	0.768
Delivery beyond 20 weeks (%)	96 (76.2)	54 (66.7)	24 (61.5)	0.132
Live birth rates (%)	95 (75.4)	54 (66.7)	24 (61.5)	0.172
Birth weight (grams)	3068±782	2909±722	2958±617	0.463
Delivery weeks (days)	261±23	256±27	258±17	0.503
ICM: Inner cells mass. ICM-A, B, C groups included 126, 81 and 39 patients, respectively. [a]: ICM-C vs B and A				

Table 2. Pregnancy results between TE groups

	TE-A, n (%)	TE-B, n (%)	TE-C, n (%)	p
Biochemical pregnancy (%)	7 (6.6)[a]	11 (14.9)[a]	14 (21.2)[a]	0.018
Abortion (first 12 weeks)	13 (12.3)	12 (16.2)	5 (7.6)	0.296
Abortion between 12-20 weeks	4 (3.8)	2 (2.7)	4 (6.1)	0.592
Delivery beyond 20 weeks (%)	82 (77.4)	49 (66.2)	43 (65.2)	0.137
Live birth rates (%)	81 (76.4)	49 (66.2)	43 (65.2)	0.189
Birth weight (grams)	3073±826	2979±679	2902±649	0.488
Delivery weeks (days)	260±25	259±24	256±21	0.726
TE: Trophectoderm. TE grade A, B, C groups included 106, 74 and 66 patients, respectively. [a]: (p<0.018), TE grade A vs B, A vs C and B vs C				

Obstetric complications did not significantly different between TE grade A and B (with Bonferroni correction). However, a higher obstetric complication rate was observed in the TE grade C compared to TE grade A and B. For further analysis, ICM grade A and B were grouped together (high quality group) and compared to grade C (poor quality group) for all further comparisons.

Demographic characteristics of the patients, etiology of infertility, COH protocols and ET (fresh/frozen) information was presented in Table 6. The comparison was made between high-quality embryos versus poor quality groups. Paternal age was significantly higher in the ICM poor quality group (p=0.047), body mass indexes (BMI) were significantly lower in the ICM poor quality group (p=0.041) and maternal age was significantly higher in the TE poor quality group (p=0.005) (Table 6). The other demographic characteristics were similar between the ICM and TE high- and poor-quality groups.

ICM and TE groups were compared according to the type of delivery, live birth rates, mean delivery week, gender and weight of the newborns. Both groups were similar in terms of these parameters and there was no statistically significant difference between the two groups (Table 7).

High and poor quality ICM groups were similar in biochemical pregnancy, abortus in the first 12 weeks and abortus between 12-20 weeks of pregnancy and live births frequency. In the high- and poor-quality groups by TE, only the biochemical pregnancy rate was significantly higher in the poor-quality TE group when compared to high quality group (p=0.021, Table 8).

Comparison of preterm birth and low birth weight infants according to ICM and TE groups showed that only the rate of preterm birth before 37 weeks was significantly higher in ICM and TE poor quality groups when compared to high quality group (p=0.049 and p=0.02, respectively, Table 9).

Pregnancy complication rates in high and poor quality ICM groups was found to be similar. The TE poor quality group had an increased rate of PE (p=0.041), antenatal bleeding (p=0.021) and PD (p=0.037, Table 10).

The findings from multivariate regression analysis for obstetric complications in the ICM groups are presented in Table 11. Analyses showed that the transfer of poor quality ICM grade C embryos still had predictive value in the development of obstetric complications and poor-quality ET increased the risk of obstetric complications by 6.6 times. Paternal age and BMI (Table 6), which had statistically significant differences between high and low quality ICM groups, lost their significance in multiple logistic regression analysis.

In Table 12, the findings from multivariate regression analysis of factors possibly affecting obstetric complications in the TE group is presented. This showed that transfer of poor-quality TE embryos still carried predictive value in the development of obstetric complications and transfers of poor-quality TE embryos increased the risk of obstetric complications 4.3 times. However, maternal age (Table 6), which had a

Table 3. Pregnancy results in the early blastocyst and the blastocyst groups

	Early blastocyst, n (%)	Blastocyst, n (%)	p
Biochemical pregnancy (%)	13 (17.8)	25 (11.5)	0.168
Abortion (first 12 weeks)	9 (12.3)	28 (12.9)	0.899
Abortion between 12-20 weeks	2 (2.7)	10 (4.6)	0.488
Delivery beyond 20 weeks (%)	49 (67.1)	154 (71.0)	0.535
Live birth rates (%)	49 (67.1)	153 (70.5)	0.586
Birth weight (grams)	2924±754	3001±754	0.569
Delivery weeks (days)	257±27	258±24	0.740
The numbers of patients in the early blastocyst stage and in the blastocyst stage were 73 and 217, respectively			

Table 4. Obstetric complications in ICM groups

	ICM-A, n (%)	ICM-B, n (%)	ICM-C, n (%)	p
Pregnancy with OC	39 (46.4)	24 (49.0)	19 (86.4)[a]	0.003
Pregnancy without OC	45 (53.6)	25 (51.0)	3 (13.6)	
ICM: Inner cells mass, OC: Obstetric complication, [a]: p=0.003, ICM C vs A and C vs B				

Table 5. Obstetric complications in TE groups

	TE-A, n (%)	TE-B, n (%)	TE-C, n (%)	p
Pregnancy with OC	29 (41.4)	22 (48.9)	31 (77.5)	0.001*
Pregnancy without OC	41 (58.6)	23 (51.1)	9 (22.5)	
OC: Obstetric complication, TE: Trophectoderm, *: p=0.001, TE C vs A and TE C vs B				

statistically significant difference between high- and low-quality TE groups, lost its significance after multiple logistic regression analysis.

Discussion

Since TE mediates the implantation of the embryo and contributes to placental formation, we aimed to investigate whether TE quality was associated with pregnancy outcome and placental complications in IVF pregnancies. There are some studies investigating the effect of expansion degree, ICM and TE quality on implantation rate and pregnancy outcomes. Thompson et al. (20) reported that TE morphology and the degree of blastocyst expansion were associated with clinical pregnancy and live birth rates in their 3,510 IVF pregnancies.

Table 6. Demographic characteristics, infertility etiology, protocol and ET information of ICM and TE groups in high- and low-quality embryos

	ICM- A + B, n (%)	ICM-C, n (%)	p	TE-A + B, n (%)	TE-C, n (%)	p
Maternal age	31.7±4.6	33.2±4.1	0.064	31±4.6	33±4.2	0.005
Paternal age	34.6±5.4	36.5±4.4	0.047	34.5±5.5	35.8±4.8	0.093
Body mass index (kg/m^2)	25±4.8	23±6.4	0.041	25.6±4.9	24.2±5.6	0.104
Infertility duration (years)	5±3.6	4.8±4.4	0.869	4.9±3.6	5.1±4.0	0.709
Primary infertility (%)	144 (69.6)	27 (69.2)	0.967	125 (69.4)	46 (69.7)	0.970
Secondary infertility (%)	48 (23.2)	6 (15.4)	0.280	40 (22.2)	14 (21.2)	0.865
Unexplained infertility etiology (%)	76 (36.7)	12 (30.8)	0.477	64 (35.6)	24 (36.4)	0.907
Male factor (%)	34 (16.4)	4 (10.3)	0.328	31 (17.2)	7 (10.6)	0.203
Tubal factor (%)	15 (7.2)	4 (10.3)	0.518	14 (7.8)	5 (7.6)	0.958
Anovulatory (%)	1 (0.5)	0	1.000	1 (0.6)	0	1.000
Decreased ovarian reserve (%)	59 (28.5)	11 (28.2)	0.970	50 (27.8)	20 (30.3)	0.697
Endometriosis	3 (1.4)	1 (2.6)	0.501	1 (0.6)	3 (4.5)	0.060
Protocol (%), GnRH antagonist	159 (76.8)	28 (71.8)	0.501	139 (77.2)	48 (72.7)	0.464
Long GnRH agonist	15 (7.2)	1 (2.6)	0.277	12 (6.7)	4 (6.1)	0.864
Estradiol priming	5 (2.4)	2 (5.1)	0.307	3 (1.7)	4 (6.1)	0.086
Fresh ET (%)	171 (82.6)	31 (79.5)	0.641	146 (81.1)	56 (84.8)	0.498
Frozen ET (%)	21 (10.1)	3 (7.7)	0.776	19 (10.6)	5 (7.6)	0.485
ICM: Inner cells mass, TE: Trophectoderm, GnRH: Gonadotrophin-releasing hormone, ET: Embryo transfers						

Table 7. Pregnancy results of patients giving live birth

	ICM, A + B, n (%)	ICM-C, n (%)	p	TE, A + B, n (%)	TE-C, n (%)	p
Type of birth						
Normal delivery	12 (8.8)	2 (9.1)	1,000	10 (9.2)	5 (11.7)	0.907
Cesarian delivery	120 (91.2)	20 (90.9)		107 (90.8)	38 (88.3)	
Gender of live born						
Female	67 (49.6)	12 (52.2)	0.822	61 (52.1)	19 (44.1)	0.364
Male	68 (50.4)	10 (47.8)		56 (47.9)	24 (56.9)	
Birth weeks (day)	259±24	258±17	0.822	259±24	256±21	0.437
Birth weight (grams)	3,009±762	2,958±617	0.760	3,036±770	2,902±649	0.320
ICM: Inner cells mass, TE: Trophectoderm						

Table 8. Abortion and live birth results according to ICM and TE groups

	ICM-A + B, n (%)	ICM-C, n (%)	p	TE-A + B, n (%)	TE-C, n (%)	p
Biochemical pregnancy (%)	24 (11.6)	8 (20.5)	0.129	18 (10)	14 (21.2)	0.021
Abortion (first 12 weeks) (%)	25 (12.1)	5 (12.8)	0.896	25 (13.9)	5 (7.6)	0.180
Abortion between 12-20 weeks (%)	8 (3.9)	2 (5.1)	0.714	6 (3.3)	4 (6.1)	0.337
Live birth rates (%)	150 (72.5)	24 (61.5)	0.169	131 (72.8)	43 (65.2)	0.244
ICM: Inner cells mass, TE: Trophectoderm						

They also reported that there was no relation between ICM quality and IVF success. We also found no relation between the degree of blastocyst expansion (early blastocyst and blastocyst stages) on pregnancy outcomes or obstetric complications.

In contrast, in their large study, Goto et al. (15) reported that blastocyst expansion was significantly correlated with ongoing pregnancy rate, but the TE and ICM qualities were not different between ongoing pregnancy or delivery rate. Although these authors classified blastocyst expansion of the embryos and compared the blastocyst with a 1-6 staging system and found blastocyst stage 1 and 2 had a higher pregnancy and live birth rate, they did not clearly state which parameters (expansion, ICM or TE) were more important for ongoing pregnancy and delivery rates (15).

Zaninovic et al. (21) showed that the expansion degree of the blastocyst (ICM morphology) was not related to the implantation rates. However, they found that an increased implantation rate in TE grade A when compared to grade B and C. Similar to the results of Zaninovic et al. (21) and Thompson et al. (20), our results also showed that blastocyst expansion and ICM morphology were not associated with successful implantation, but degree of TE was associated with implantation success. It

Table 9. Comparison of preterm birth and low birth weight infants according to ICM and TE groups

	ICM-A + B, n (%)	ICM-C, n (%)	p	TE-A + B, n (%)	TE-C, n (%)	p
Low birth weight baby <2500 g	16 (11.8)	4 (17.4)	0.136	12 (10.7)	8 (20.0)	0.051
Very low birth weight baby <1500 g	6 (4.5)	0	1,000	5 (4.8)	1 (3)	1,000
Preterm birth <37 weeks	30 (27.3)	10 (47.8)	0.049	26 (23.4)	20 (50)	0.002
Premature preterm birth <32 weeks	4 (7)	0	1,000	6 (6.6)	4 (4.8)	0.859
ICM: Inner cells mass, TE: Trophectoderm						

Table 10. Obstetric complication results of ICM and TE groups

	ICM-A + B, n (%)	ICM-C, n (%)	p	TE-A + B, n (%)	TE-C, n (%)	p
Preeclampsia	8 (3.9)	2 (5.1)	0.662	2 (1.1)	5 (7.8)	0.041
Gestational diabetes mellitus	15 (7.2)	5 (12.8)	0.332	12 (6.7)	8 (12.1)	0.165
Antenatal bleeding	14 (6.8)	4 (10.3)	0.442	9 (5.0)	9 (13.6)	0.021
Preterm delivery	34 (16.4)	10 (25.6)	0.168	32 (17.7)	24 (36)	0.037
Fetal growth restriction	8 (3.9)	4 (10.3)	0.089	8 (4.4)	4 (6.1)	0.739
Oligohydramnios	8 (3.9)	1 (2.6)	0.691	7 (3.9)	2 (3)	0.751
Polyhydramnios	2 (1.0)	0	0.538	2 (1.1)	0	0.390
Fetal anomaly/malformations/genetic diseases	13 (6.3)	2 (5.1)	0.783	8 (4.4)	7 (10.6)	0.127
ICM: Inner cells mass, TE: Trophectoderm						

Table 11. The investigation of the combined effects of all possible factors that may have an effect on obstetric complications in ICM group according to logistic regression analysis

Variable	B	S.E.	OR	95% CI	p
Paternal age	0.002	0.036	1,002	0.934-1,074	0.965
Body mass index	0.024	0.035	0.976	0.911-1,046	0.494
ICM (grade C)	1,891	1,432	6,626	1,823-24,091	0.004
B: Regression coefficient, SE: Standard error, OR: Odds ratio. Reference category: ICM grade A + B. ICM: Inner cells mass, CI: Confidence interval					

Table 12. Investigation of the combined effects of all possible factors that may have an effect on obstetric complications in TE group according to logistic regression analysis

Variable	B	S.E.	OR	%95 GA	p
Maternal age	-0.004	0.038	0.920	0.925-1,073	0.965
TE (grade C)	1,471	0.429	4,353	1,879-10,086	0.001
B: Regression coefficient, S.E.: Standard error, OR: Odds ratio, TE: Trophectoderm, CI: Confidence interval. Reference category: TE Grade A + B.					

appears that TE may be an important factor in implantation, as it plays an important role in the attachment of the blastocyst into the endometrium, trophoblastic development and uterine invasion. While the quality of TE may affect blastocyst implantation and survival, blastocyst expansion and ICM morphology may have less influence on implantation success (15,21).

Ahlström et al. (22) stated that the morphological grade of TE cells in patients who underwent IVF was the most important marker in predicting live birth, implantation, and abortion rates. In their study, 1117 fresh, day 5 embryos were transferred and they concluded that the TE cell parameter was more important than the expansion grade and ICM grade in the prediction of live birth, implantation, and abortion rates, in line with our findings. We showed that biochemical pregnancy losses in grade C for both ICM and TE were found to be significantly higher than grade A and B. On the other hand, there was no significant difference among the groups A, B, and C in terms of ICM and TE in terms of abortion (first 12 weeks), abortion less than 20 weeks (12-20 weeks), birth above 20 weeks, and live birth rates. The higher biochemical pregnancy losses in TE grade C embryos suggest that functional TE is more important for the development of pregnancy.

There are limited data analyzing the effect of ICM and TE on birth weight. Licciardi et al. (23) found that the birth weight of newborns was related to the ICM degree of the blastocyst. They found that for ICM, the more polygonal cells present in the fetus, the more division will occur and results in greater growth of the fetus. In our study, ICM grades were not related to newborn weights, and the weights of the newborns in grade A, B, and C were similar. Although there was an 86% increase in low birth rate in ICM grade C when compared to ICM grade A + B, this difference was not significant. This may be because TE contribute to development of the embryo by transporting substances that are needed for the development of the fetus and its metabolic activity.

Recently, Alfarawati et al. (24) examined human TE biopsy samples and reported that aneuploidy of TE cells was significantly associated with blastocyst morphology. Braude et al. (25) found that decreased TE grade from A to B and C was related to increased aneuploidy rates and they suggested that false gene expression was related to poor quality of TE and prevention of implantation of the embryo. We did not perform genome analysis of the transferred embryos or from the abortion materials. The finding of increasing biochemical pregnancy loses rates by decreasing TE grade, and increasing chromosomal abnormalities in early pregnancy losses need further research to identify the underlying mechanism.

Honnma et al. (26) investigated the effect of low-quality of blastocysts by performing multiple logistic regression analyses among blastocyst parameters. A significant relationship was found between TE morphology and pregnancy loss. In our study, ICM and TE groups were compared separately according to obstetric complication rates in grade A, B, and C, and obstetric complication rates were found to be close to each other in grade A and B but were much higher in grade C with a similar pattern emerging for TE grade. Therefore; grades A and B for both parameters were combined and a further comparison with grade C was made. When the abortion and live birth results were analyzed according to TE groups, it was observed that 21.2% of pregnancy loss grade C patients had biochemical pregnancy, 7.6% had an abortion, and 65.2% had a live birth, although there was no statistically significant difference. However, it was observed that the live birth rate in TE grade C (65.2%) was around 10% lower than for grade A + B combined. It is thought that the low number of cases may have affected this finding.

Hill et al. (27) found that implantation and live birth rate were related to TE quality but not ICM grade. However, we found that live birth rates were related to both TE quality and ICM grade. While their multiple logistic regression analysis showed that only patient age and TE grade were significantly associated with implantation and live birth, our analyses showed that only paternal age and BMI of the woman were related to ICM grade and only maternal age was related to TE grade. These differences may be due to differences in study sample sizes. The quality of embryos at the time of ET appears to have an effect on pregnancy outcome.

We also investigated the relationship between obstetric complications and the quality of TE. As TE initiates the placenta, obstetric complications due to abnormal placentation may occur in low-quality TE cases (28,29). The placenta is a complex, short-lived, and multi-functional organ that plays an important role in intrauterine development of the fetus. Any irregularity and insufficiency in placental development can lead to serious complications in both the mother and the growing fetus.

In our study, hypertensive diseases of pregnancy developed in 1.1% of the patients in the TE A + B group and 7.8% in the C group, and the difference was significant, while no statistical difference was found between ICM groups A + B vs C. Abnormal trophoblast invasion is thought to be related to the development of PE. It was suggested that the blastocyst needs healthy TE cells for implantation, which is a complex process that requires endometrial invasion in the early stages of embryonic development (30). Furthermore, the more quality of TE increases, the better implantation would be expected. Abnormal maturation and differentiation of the villous syncytiotrophoblast have been suggested to be related to barrier integrity of the placenta and therefore the increase in the release of necrotic, aponeurotic trophoblast fragments

contributes to the development of PE (30). Our results and recent literature suggest that the quality of TE may have a role in the development of PE.

The relationship between quality of blastocyst and the rate of antenatal bleeding were also investigated in our study. Although there was no relation between ICM grade and antenatal bleeding, it was found that TE grade C cases had a more than two-fold increased rate of antenatal bleeding risk when compared to TE A + B grades. Similar to our results, Bouillon et al. (31) also reported that antenatal hemorrhage was related to the quality of blastocyst in cases of IVF. It has been reported that the most common cause of antenatal bleeding is abortion during early pregnancy, and ablation of the placenta and placenta previa in late pregnancy. The common factor in the pathogenesis of these pathologies abnormal placentation (32). It was thought that poor quality of TE may cause insufficient trophoblastic invasion and negative effects on implantation, which may predispose to abortion or placental detachment. The fact that there is no significant difference in the ICM groups in terms of the rate of antenatal bleeding suggests that TE quality is more important for development of the placenta and its function than ICM grade.

Like the mechanisms that initiate normal birth, the pathogenesis of preterm labor is not fully understood. It is thought to be a complex, multifactorial event, in which genetic, pathophysiological, and environmental factors play a role in the pathogenesis of preterm birth. Infection, inflammation, uterine tension, and placental vascular disorders have all been suggested as possible factors for PD (33). In our study, while PD was not related to ICM quality we found PD rates were significantly increased in the TE C group when compared to TE A + B. Decidual hemorrhage has been reported to be related to PD. Maternal smoking, chronic hypertension, PE, and hereditary coagulopathies can cause decidual bleeding and each of these conditions may cause PD by damaging the uterine spiral arteries (34). In light of the recent literature and our results, we suggest that poor TE quality might be a risk factor for PD.

FGR is one of the major obstetric problems associated with impaired early placentation (35). Implantation site disorders appear to be both a cause and a consequence of hypoperfusion in the placental bed (36). This situation is consistent with the relationship between some placental angiogenic factors and hypertensive diseases of pregnancy. Huppertz (36) has suggested that the irregularity of the syncytiotrophoblast cells cause PE, while the irregularity of cytotrophoblast development can lead to FGR. The author stated that the impairment of cytotrophoblast differentiation may impair both villous and extravillous trophoblast expansion, resulting in failure of trophoblast invasion and incomplete transformation of uterine arteries, which are typical of FGR (36). In our study, FGR was detected in 3.9% of patients in the ICM A + B group and in 10.3% of the patients in the C group. Although FGR was found to be approximately 1.6 times more common in group C than the others, this difference did not reach statistical significance. Although the frequency of FGR increased approximately 38% in the patients in the TE A + B group compared to the patients in the grade C group, this difference was also not significant. Our findings support that the frequency of FGR may be affected by both ICM and TE grading. Once again, the reason for a lack of significant difference may be the low number of cases. Thus, there is a need for larger studies to clarify this issue.

There are no published studies comparing the relationship between obstetric complications and the quality of TE. Studies of this subject have generally been conducted in terms of implantation and live birth rates. The most important strength of our study is the investigation of the relationship between obstetric complications due to placenta and TE quality and it appears that TE quality is an important factor for developing obstetrics complications in IVF-ET cases. The low number of cases and the lack of aneuploidy screening in the blastocyst stage were accepted as limitations of the current study. Once again, larger studies will be necessary to provide more robust evidence concerning this relationship between blastocyst quality and obstetric outcomes.

Study Limitations

There are two main limitations in our study. Firstly, the inclusion of both fresh and frozen ETs in the statistical analyses was a limitation. Thus, we could not perform any statistical comparisons to elucidate the possible differences between fresh and frozen embryos. This limitation could be eliminated in further studies with adequate numbers of both fresh and frozen embryos. Secondly, it is well known that endometrial receptivity plays a very important role in implantation success (37). However, we only investigate the relationship between embryo quality and pregnancy outcomes in this study. In the future, more detailed investigations into the effects of embryo quality and endometrial thickness and vascularity on pregnancy outcomes should be conducted.

Conclusion

The scores of embryo evaluation may have value in assessing the prognosis of pregnancy during the ET process. This study showed that TE may have a greater impact on the development of pathologies of placental origin, especially PE. However, further studies are needed to clarify the relationship between TE quality and pregnancy prognosis in IVF pregnancies.

Acknowledgements: *This work was carried out as part of a PhD thesis by A. Hamidova presented to Akdeniz University Medical School.*

Ethics Committee Approval: *The study was approved by the Ethical Committee of the Akdeniz University Faculty of Medicine (approval number: 1164, date: 11.12.2019).*

Informed Consent: *It was obtained.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: Ş.O., E.K.D.; Concept: S.K., Ş.O., E.K.D., B.S.İ.; Design: S.K., Ş.O., E.K.D., B.S.İ.; Data Collection or Processing A.H., E.H.; Analysis or Interpretation: A.H., E.H.; Literature Search: S.K., Ş.O., E.K.D., B.S.İ., A.H., E.H.; Writing: E.H., B.S.İ., S.K.*

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

Financial Disclosure: *The authors declared that this study received no financial support.*

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The association between preterm delivery and postpartum bleeding in otherwise uncomplicated pregnancies

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Abstract

Objective: The primary aim was to investigate whether preterm delivery was an independent risk factor for blood or blood products transfusion in the intrapartum or postpartum period, considered as a proxy for severe obstetric bleeding.

Material and Methods: Throughout a 9-month-period, 216 uncomplicated singleton deliveries were included in a cross-sectional study after exclusion of severe maternal and fetal morbidity, such as chorioamnionitis, and use of medications including tocolytics. Maternal and neonatal data were evaluated and compared across preterm (between 24 0/7-36 6/7 weeks' gestation) and term (between 37 0/7-41 6/7 weeks' gestation) deliveries. Primary and secondary outcomes were requirement for blood or blood products transfusion until discharge and change in hemoglobin value and hematocrit from baseline to postpartum hour 6, respectively. Logistic regression models were constructed to evaluate the effect of preterm delivery on the primary outcome.

Results: There were 90 (41.7%) preterm deliveries with an overall cesarean section rate of 77.8%. Preterm delivery was not an independent risk factor for the primary outcome, when route of delivery, maternal body-mass index, antenatal steroid administration, and baseline (admission) platelet and leukocyte counts were controlled for [adjusted risk ratio, 2.46; 95% confidence interval (CI), 0.69-8.77; p=0.16]. Subgroup analysis, including cesarean deliveries, revealed a similar result (adjusted risk ratio, 1.65; 95% CI, 0.42-6.48; p=0.47). Secondary outcomes, including decrease in mean or percent values of hemoglobin and hematocrit measurements, were also similar across preterm and term groups, both after vaginal and cesarean delivery (for all comparisons, p>0.05).

Conclusion: Preterm delivery is not independently associated with increased requirement for blood transfusions or decreased hemoglobin and hematocrit values following otherwise uncomplicated vaginal or cesarean delivery of singletons. (J Turk Ger Gynecol Assoc 2022; 23: 177-83)

Keywords: Preterm delivery, postpartum bleeding, cesarean delivery, vaginal delivery

Received: 24 November, 2021 **Accepted:** 20 May, 2022

Introduction

Intrapartum bleeding and postpartum bleeding (PPB) are among most common causes of maternal mortality (1). Intrapartum bleeding is bleeding that occurs during delivery, particularly due to uterine rupture, placenta accreta spectrum and others, whereas PPB refers to hemorrhagic conditions that occur following delivery, such as uterine atony, genital tract injuries, and other causes. Commonly used proxies for PPB include requirement for blood transfusions, development of signs of hypovolemia, or postpartum hematocrit decrease of more than 10% (2). Maternal deaths associated with intrapartum bleeding and PPB can be prevented by providing appropriate medical measures. Therefore, it is important to determine the risk factors for intrapartum bleeding and PPB so that at-risk pregnant women can be delivered under proper supervision, preferably in tertiary settings (3).

The abstract was presented as an electronic poster at the 31st World Congress on Ultrasound in Obstetrics and Gynecology, Online, 15-17 October 2021.

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2022.2021-11-7

Some risk factors related to intrapartum bleeding and PPB have been identified in previous studies, with cesarean delivery emerging as the main risk factor, with higher morbidity compared to vaginal delivery (4,5). Multiple pregnancy and general anesthesia have been defined as main risk factors for PPB after cesarean delivery (6). Although some other risk factors have been defined (7), prediction of PPB is usually not possible (8).

Recently, an increase in the incidence of intrapartum and postpartum hemorrhage has been noted (9). Risk factors that may cause an increase in the frequency of hemorrhage are being investigated. Increases in postpartum hemorrhage have not been explained by the changing risk profile of women, such as cesarean delivery, women aged 35 years or older, post-term pregnancies, or large-for-gestational age infants (10).

Preterm delivery is commonly defined as delivery before 37 completed weeks of gestation. Maternal infection, adverse neonatal outcomes, and admission to the intensive care unit are more prevalent in early preterm deliveries (11). Considering the risk of PPB in early preterm cesarean deliveries, these risks can be expected to be higher with cesarean section regardless of the uterine incision type. Therefore, management of maternal complications after early preterm deliveries may be important. Recent data also indicate that relatively high preterm birth rates worldwide have not reduced, despite symptomatic treatment (12). It is not known whether this global preterm birth rate of about 11% contributes to an increased incidence of PPB. From a physiological perspective, preterm delivery can be hypothesized to be associated with increased uterine bleeding. The uterine lower segment is not fully formed, and sensitivity to oxytocin receptors may be relatively low in the preterm uterus (13).

Considering these features, we hypothesized that preterm delivery is a risk factor for an increased incidence of intrapartum bleeding and early PPB. To test this hypothesis, we designed a cross-sectional study to compare intrapartum bleeding and PPB in preterm and term pregnancies, stratified by vaginal and cesarean delivery, with the primary outcome defined as requirement for blood or blood products transfusion.

Material and Methods

The study protocol was approved by the Local Ethics Committee of the Süleyman Demirel University (approval number: 157, date: 22.05.2020), and informed consent was obtained from participants prior to inclusion. The study was performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2013. Deliveries between 24 0/7 to 41 6/7 weeks' gestation at a single tertiary obstetrics and gynecology unit throughout a 9-month-period (from 1 March 2020 to 30 November 2020) were included in a cross-sectional design study. Gestational age was calculated based on the last menstrual period (LMP) confirmed by the first trimester crown-rump length (CRL), and this had been corrected accordingly if LMP and dating with CRL diverged by more than two days.

Exclusion criteria included: 1) Multiple pregnancy; 2) preeclampsia and its complications including hemolysis, elevated liver enzyme levels, and low platelet levels syndrome; 3) maternal morbidity including thrombophilia, hepatic or renal disease; 4) third trimester bleeding, including a diagnosis of placenta previa or placental abruption; 5) sonographic diagnosis of leiomyoma >1 cm in diameter during pregnancy; 6) acetylsalicylic acid use within seven days before delivery; 7) anticoagulation use within 24 hours before delivery; 8) tocolysis within 12 hours of delivery; 9) complicated deliveries including instrumental vaginal delivery, cervical lacerations, and uterine rupture; 10) non-low transverse uterine incisions, including vertical incisions; and 11) requirement for maternal antibiotic treatment for suspected or confirmed chorioamnionitis.

Preterm labor was diagnosed when progressive cervical dilatation and/or effacement by cervical examination was accompanied by regular uterine contractions evident on external tocodynamometry. Nifedipine was the preferred agent for tocolysis in the study setting, with limited use of indomethacin in selected cases <30 weeks of gestation. Maximum tocolysis duration was 48 hours (acute tocolysis), aiming to delay delivery for fetal lung maturation with antenatal corticosteroids. Nifedipine was administered sublingually or orally using 10 mg capsules (nidicard 10 mg, Koçak İlaç, Tekirdağ, Turkey), 4 doses every 20 minutes initially, followed by 10 mg every 3-4 hours for a total duration of 24-48 hours. Indomethacin (endol 25 mg, Deva Holding, İstanbul) was administered orally as a 50 mg loading dose followed by 25 mg orally every 6 hours for up to 48 hours. Maintenance (>48 hours duration) or combined tocolytic treatment were not attempted.

Intramuscular maternal betamethasone (12 mg every 24 hours) administration was planned for presumptive preterm deliveries <37 weeks of gestation and for planned cesarean deliveries between 37 0/7 to 38 6/7 weeks gestation, depending on the attending physician's discretion. In certain circumstances with possible preterm delivery <32 weeks of gestation, a second betamethasone dose of 12 mg was administered earlier, that is after 8 to 23 hours of the first one.

All women were given 0.2 mg of intramuscular methylergonovine maleate (Metiler ampoule, Adeka, Samsun, Turkey) following delivery of the placenta after either vaginal or cesarean section. Depending on the discretion of the anesthesiologist, intravascular methylergonovine maleate diluted with normal saline solution to a volume of 5 mL was administered over 30-60 seconds during some of the cesarean

section operations only. As an institutional protocol, umbilical cord arterial blood was sampled immediately after birth by the neonatology team, particularly for pH measurements. All postpartum women were started on oxytocin (Synpitan fort, Deva Holding, İstanbul) infusion of 5 to 10 IU in 500 mL normal saline until transfer to inpatient care from the operating room or labor ward.

Postpartum follow-up included evaluation of vital signs, uterine tonus, postpartum vaginal bleeding, and early ambulation with physical examination focused on lower extremities and breasts. All women were encouraged to give exclusive breastfeeding through a baby friendly hospital initiative. At postpartum hour 6, maternal blood was sampled and sent for complete blood count. Lower extremity stockings were used for cesarean and at-risk vaginal deliveries for at least for 48 hours postpartum. Risk assessment for thromboembolic events was carried out in the postpartum period and pharmacological thromboprophylaxis with low-dose, low molecular weight heparin derivatives were initiated >6 hours and >12 hours after vaginal uncomplicated and cesarean delivery, respectively, to at-risk women.

Data retrieval was carried out by one of the researchers (Ü.K.T.). First, mode of delivery (vaginal or abdominal by cesarean section) and preterm or term delivery (defined as delivery between 24 0/7-36 6/7 and 37 0/7-41 6/7 weeks' gestation, respectively) were recorded. Demographic data and obstetric history (gravidity, parity, abortion, previous caesarean delivery, interval between pregnancies) were also retrieved. Maternal body mass index (BMI) at admission to the labor ward was calculated as the ratio of weight (kg) divided by the square of height (m^2). Intrapartum and labor characteristics included gestational age at delivery, route of delivery (vaginal/cesarean), type of anesthesia in the cesarean group, postpartum oxytocin use and cumulative dose, prepartum baseline (at admission to the labor ward) and postpartum (at hour 6) complete blood cell parameters, including hemoglobin value, hematocrit, platelet and leukocyte counts, and transfusion with blood or blood products. Beckman Coulter UniCel DxH 800 Coulter Cellular Analysis System was used to assess blood parameters with mean coefficient of variation of 0.74%, 1.78%, and 1.45% for hemoglobin measurement, platelet count, and leukocyte count, respectively. Birth weight, Apgar scores (at minutes 1, 5, and 10), and cord arterial pH value within 5 minutes after delivery were also recorded.

The primary outcome measure was requirement for blood or blood products transfusion in the intrapartum or postpartum period until discharge, used as a proxy for severe PPB. Secondary outcomes included change in hemoglobin value and hematocrit from baseline to postpartum hour 6.

Statistical analysis

Data were expressed as mean ± standard deviation for continuous data or frequencies (n) with percentages (%) for categorical data. Shapiro-Wilk test was used to test normality of data. Student's t-test or Mann-Whitney U test was used to compare continuous variables, whereas chi-square test and Fisher's exact test were used for comparisons of categorical data. Post-hoc power analyses were performed to evaluate the statistical power of univariate comparisons for the primary outcome measure. To reveal the independent effect of preterm delivery on the primary outcome (i.e., transfusion requirement), logistic regression with a backward stepwise selection approach was used, controlling for parameters with significant differences at univariate comparisons. A p-value less than 0.05 was considered statistically significant in all analyses.

Results

Following exclusions, 216 women were available for analysis. Ninety (41.7%) and 126 (58.3%) of the included women had delivered in the preterm and term period, respectively. Cesarean section rate in the entire group was 77.8% (168/216). Since route of delivery is the main determinant of postpartum blood loss, data from vaginal and cesarean deliveries were evaluated separately. Table 1 shows comparisons of maternal demographic characteristics, and obstetric and neonatal data across preterm and term deliveries, stratified by mode of delivery. Mean maternal age, gravidity, parity, and interpregnancy interval were similar in preterm and term deliveries, irrespective of the route of delivery (Table 1). Mean BMI was higher in term vaginal compared to that of preterm deliveries (p=0.03, Table 1).

As expected, administration of antenatal corticosteroids was more frequent, and mean gestational age at delivery and birth weight were lower for preterm infants, both in vaginal and cesarean deliveries (Table 1). Use of regional anesthesia during cesarean delivery was more frequent (p=0.07) in term (51/97, 52.6%) compared to preterm deliveries (22/71, 31%), probably reflected in lower mean Apgar scores in preterm infants due to the effects of prematurity and general anesthesia (Table 1). No women who delivered vaginally were administered regional anesthesia.

Table 2 details the comparisons of outcome variables in preterm and term pregnancies, stratified by route of delivery. Admission leukocyte counts were higher before preterm deliveries, both by the vaginal (13.43±5.37 $x10^3/\mu L$ versus 10.16±2.88 $x10^3/\mu L$, p=0.009) and abdominal routes of delivery (11.27±4.05 $x10^3/\mu L$ versus 10.09±2.66 $x10^3/\mu L$, p=0.02). Women who delivered preterm had higher admission platelet counts (p=0.04) compared to women delivering at term (Table 1).

Table 1. Comparisons of maternal demographic characteristics, obstetric, and neonatal data across preterm and term deliveries concerning route of delivery

	Cesarean section			Vaginal delivery		
	Preterm (n=71)	**Term (n=97)**	**p**	**Preterm (n=19)**	**Term (n=29)**	**p**
Demographic data						
Maternal age (years)	33.6±6.8	32.2±7.1	0.2	31.6±7.5	31.6±5.1	0.9
Maternal body mass index (kg/m^2)	28.7±3.6	29.5±3.4	0.1	27.4±1.9	29.0±3.0	**0.03**
Gravidity	2.9±1.6	2.7±1.3	0.4	3.0±1.9	2.9±1.8	0.7
Parity	1.1±1.14	1.1±0.9	0.4	0.95±1.0	1.3±1.3	0.3
Previous cesarean section	30/71 (42.3%)	50/97 (51.5%)	0.2	2/19 (10.5%)	-	0.1
Interpregnancy interval (years)	6.7±3.6	6.3±3.1	0.4	5.1±3.7	5.1±2.3	1.0
Obstetric data						
Antenatal corticosteroids (complete course)	36/71 (50.7%)	6/97 (6.2%)	**<0.0001**	13/19 (68.4%)	-	**<0.0001**
Gestational age at delivery (days)	235.4±27.7	268.7±5.6	**<0.0001**	218.8±34.0	273.7±6.2	**<0.0001**
Postpartum oxytocin dose (IU)	5.25±1.18	5.29±0.09	0.8	4.05±1.61	4.14±0.58	0.7
Neonatal data						
Birth weight (g)	2221±842	3122±391	**<0.0001**	1798±995	3294±359	**<0.0001**
Cord blood pH	7.31±0.12	7.33±0.05	0.058	7.26±0.13	7.34±0.05	**0.02**
Apgar score at minute 1	7.50±1.04	7.86±0.47	**0.008**	7.60±0.84	7.83±0.57	0.3
Apgar score at minute 5	8.58±0.91	8.91±0.28	**0.003**	8.89±0.333	8.87±0.62	0.9
Apgar score at minute 10	9.67±0.81	9.95±0.27	**0.006**	10.0	10.0	1.0

Data are expressed as mean ± standard deviations or frequencies and percentages within parentheses. pH: Potential of hydrogen

Table 2. Comparisons of output variables for intrapartum and early postpartum bleeding across preterm and term deliveries concerning route of delivery

	Cesarean section			Vaginal delivery		
	Preterm (n=71)	**Term (n=97)**	**p**	**Preterm (n=19)**	**Term (n=29)**	**p**
Prepartum (admission)						
Hemoglobin (g/dL)	12.09±1.38	12.29±1.41	0.3	11.7±1.55	12.24±1.23	0.1
Hematocrit (%)	36.25±4.27	36.79±4.23	0.4	35.11±4.49	36.58±3.66	0.2
Platelet count (10^3/µL)	222.81±63.37	222.79±67.37	0.9	249.47±87.38	201.82±71.12	**0.04**
Postpartum (at hour 6)						
Hemoglobin (g/dL)	10.66±1.55	10.94±1.64	0.2	10.6±1.71	11.26±1.27	0.1
Hematocrit (%)	31.78±4.49	32.53±4.93	0.3	31.8±5.01	33.52±3.92	0.1
Platelet count (10^3/µL)	206.87±64.64	199.08±65.54	0.4	236.31±73.72	188.1±61.48	**0.01**
Change in blood parameters (postpartum minus prepartum)						
Change in mean hemoglobin (g/dL)	-1.43±1.20	-1.35±1.11	0.6	-1.1±1.15	-0.98±0.75	0.6
Percent change in hemoglobin (%)	-0.11±0.09	-0.11±0.08	0.6	-0.09±0.09	-0.08±0.0	0.6
Change in mean hematocrit	-4.47±3.59	-4.26±3.35	0.7	-3.30±3.56	-3.06±2.54	0.7
Percent change in hematocrit (%)	-0.12±0.09	-0.11±0.09	0.7	-0.09±0.1	-0.08±0.07	0.7
Change in mean platelet count (10^3/µL)	-15.94±28.83	-23.84±28.1	0.07	-13.15±36.34	-13.72±23.21	0.9
Percent change in platelet count (%)	-0.07±0.13	-0.10±0.12	0.08	-0.03±0.13	-0.05±0.10	0.6
Decrease >10% in hematocrit and/or >3 g/dL in hemoglobin	3/71 (4.2%)	3/97 (3.1%)	0.6	1/19 (5.3%)	-	0.3
Requirement for transfusion with blood or blood products	6/71 (8.5%)	4/97 (4.1%)	0.3	1/19 (5.3%)	-	0.3

Data are expressed as mean ± standard deviations or frequencies and percentages within parentheses

The primary outcome measure (requirement for transfusion with blood or blood products) was similar across the preterm and term groups (Table 1). Secondary outcomes, such as decrease in either mean or percent values of hemoglobin and hematocrit measurements following delivery did not significantly differ between the groups (Table 2). Change in platelet counts followed a similar pattern with no significant differences following preterm or term delivery (Table 2).

Post-hoc power analysis revealed 23.1% and 28.0% power in cesarean and vaginal deliveries, respectively, for detecting a significant difference of the primary outcome across preterm and term deliveries at an alpha value of 0.05.

A logistic regression model that included route of delivery, maternal BMI, antenatal steroid administration, and baseline platelet and leukocyte counts as covariates, revealed that preterm delivery was not an independent risk factor (p=0.16) for the outcome variable [adjusted risk ratio, 2.46 and 95% confidence interval (CI), 0.69-8.77]. When cesarean deliveries were evaluated separately (n=168) in an additional logistic regression model with similar parameters to control for the use of regional anesthesia, comparable results were obtained with no significant effect of preterm delivery (p=0.47) on the primary outcome with an adjusted risk ratio of 1.65 (95% CI, 0.42-6.48).

Discussion

The present study showed that, after the effects of possible main risk factors that may cause intrapartum and postpartum hemorrhage were excluded or controlled for, preterm delivery was not a risk factor for increased intrapartum bleeding and early PPB, when requirement for transfusion with blood or blood products until discharge was taken as the proxy. This result persisted when cofactors, such as maternal BMI, antenatal steroid administration, and type of anesthesia were included in regression models. Our data also revealed no significant differences between preterm and term deliveries considering change in hemoglobin and hematocrit values following vaginal or cesarean deliveries, when analyzed separately.

In a population-based cohort study that included over 8.5 million deliveries in the United States, advanced (>35 years) maternal age, multiple pregnancy, leiomyoma, preeclampsia, chorioamnionitis, placenta previa or abruption, cervical laceration, uterine rupture, instrumental vaginal delivery, and cesarean delivery were significant risk factors for PPB (7). In this analysis (7), preterm delivery was not reported as a risk factor, although data on augmentation of labor, type of analgesia or anesthesia, and BMI were lacking. Another study from Tibet with a smaller number of participants (n=4796) revealed similar risk factors, including advanced maternal age, cesarean section, macrosomia, and presence of neonatal asphyxia (14). Gestational age was stratified as <37, 37-40, and >40 weeks of gestation, revealing similar percentages across PPB and non-PPB groups. Interestingly, previous (but not present) preterm birth was found to be associated with a 2.6-fold increased risk of PPB in a logistic regression model. This was explained by possible coexistence of pregnancy complications with preterm deliveries that may lead to endometrial damage and PPB in subsequent pregnancies (14).

Some studies evaluated vaginal and cesarean deliveries, similar to our design. In a study that used regression models for all deliveries and a second one restricted to vaginal deliveries, preterm birth was not associated with PPB (15). A recent case-control study (16), aiming to identify risk factors for relaparotomy due to intra-abdominal hemorrhage following cesarean deliveries, found a significantly higher rate of preterm delivery <37 weeks of gestation among cases. However, this association disappeared in multivariate analysis, leaving other conditions, such as urgent cesarean delivery and surgical difficulties, as independent factors rather than the gestational age (16). Our results generally support these previous data. Therefore, rather than the timing of delivery, other factors seem to be independently associated with PPB.

Mechanisms leading to preterm delivery, premature rupture of membranes, and cervical insufficiency may be associated with prior choriodecidual inflammation (17). Thus, preterm labor can theoretically be prevented with effective treatment of choriodecidual inflammation. Although the presence of maternal infection and findings of chorioamnionitis were excluded in our design, prepartum leukocyte counts in preterm deliveries were higher than that of term pregnancies. An elevated maternal leukocyte count was previously shown to identify patients with intrauterine infection and adverse perinatal outcomes in women with preterm labor and intact membranes (18).

Overall, these findings confirm the relationship between a subclinical intrauterine inflammation and preterm delivery. Mean platelet count was higher before and after preterm vaginal deliveries. This is in line with platelet counts decreasing throughout pregnancy, beginning in the first trimester (19). In a recent study evaluating the trajectories of platelet counts during pregnancy, a decline throughout pregnancy with the nadir occurring on postpartum day 1 was evident (20). Our data also support a relative decrement of platelet counts with advancing gestation. The reason this change was not significant in cesarean deliveries is probably due to earlier mean gestational age at delivery (and therefore timing of blood sampling for platelet count) in the vaginal delivery group (219 versus 235 days).

Cesarean delivery is associated with increased rate of maternal complications including PPB, venous thromboembolism,

amniotic fluid embolism, other surgical morbidities, and anesthesia complications, compared to vaginal delivery (21). There are some conflicting data, however, whether cesarean delivery independently increases the risk of PPB. Although some studies (14,22) found previous cesarean section and emergency cesarean delivery as a risk factor for severe PPB, some epidemiological data found no direct association between cesarean delivery and PPB (23). In our study, none of the women that delivered vaginally at term required transfusions. However, we are not able to comment further on this issue, since our study did not primarily aim to detect differences across vaginal and cesarean deliveries.

Study Limitations

Limitations include a relatively small sample size following numerous exclusions to refine data. We were not able to stratify deliveries considering gestational age, such as early (<34 weeks) or late (34-37 weeks) preterm births due to the limited number of recruited preterm pregnancies. We also did not separately analyze scheduled and emergency cesarean sections. The primary and secondary endpoints provide short-term data, and long-term results, such as complications including placental retention or readmissions, were not evaluated. Similarly, the present analysis did not include postpartum factors, such as breastfeeding, as a covariate. We did not include birth weight, Apgar scores, and umbilical cord blood pH that are probable predictors of postpartum hemorrhage (considering macrosomia and perinatal asphyxia) in the logistic regression models to avoid severe multicollinearity.

Since our results were negative, the calculated post-hoc power was also relatively low, which should not directly be misinterpreted as the trial having inadequate power. Despite these limitations, the present design provides refined data from uncomplicated singleton pregnancies with rigorous exclusion criteria. Another strength was the cross-sectional recruitment of subjects during admission to hospital with longitudinal follow-up until discharge in an observational fashion. The preterm delivery and cesarean section rates were high due to the tertiary setting characteristics of the study site.

Conclusion

Preterm delivery was not an independent predictor of severe intrapartum bleeding and early PPB in uncomplicated pregnancies, when several confounders were excluded and controlled for. Therefore, clinicians can consider other risk factors for PPB in uncomplicated pregnancies, irrespective of gestational age at delivery.

Ethical Committee Approval: *The study protocol was approved by the Local Ethics Committee of the Süleyman Demirel University (approval number: 157, date: 22.05.2020).*

Informed Consent: *Informed consent was obtained.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: Ü.K.T., E.N.T., M.S.; Concept: Ü.K.T., E.N.T., M.S.; Design: Ü.K.T., E.N.T., M.S.; Data Collection or Processing: Ü.K.T., E.N.T., M.S.; Analysis or Interpretation: Ü.K.T., E.N.T., M.S.; Literature Search: Ü.K.T., E.N.T., M.S.; Writing: Ü.K.T., E.N.T., M.S.*

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

Financial Disclosure: *The authors declared that this study received no financial support.*

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The role of FSH to AMH ratio in poor prognosis patients undergoing ICSI cycle

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Abstract

Objective: The objective of this study was to estimate the number of oocyte retrieval and cycle cancellation using follicle stimulating hormone (FSH) to anti-Mullerian hormone (AMH) ratio in poor prognosis patients undergoing intracytoplasmic sperm injection treatment.

Material and Methods: This retrospective study including fresh cycles was conducted in Zekai Tahir Burak Women's Health Training and Research Hospital, between January 2015 and October 2018. Women aged between 24 and 44 years were recruited and the baseline serum hormone levels, FSH/AMH ratio, and the antral follicle count were recorded. Number of retrieved oocytes, metaphase-II oocytes, fertilised oocytes, and the number and grade of the embryos were also recorded.

Results: A total of 108 cycles, corresponding to 92 women with poor prognosis were eligible for analysis. The use of FSH/AMH ratio performed well in predicting retrieved oocyte count <5 with an area under the curve (AUC) of 0.82 [95% confidence interval (CI): 0.71-0.92]. A FSH/AMH ratio cut-off of 11.36 was set for the retrieval of <5 oocyte at oocyte pick-up (OPU) with 80% sensitivity and 87% specificity. The FSH/AMH cut-off value was 14.22 to differentiate cycle cancellation and no oocyte retrieval at OPU, with a sensitivity of 91% and a specificity of 44% (AUC of 0.71; 95% CI: 0.59-0.83). There was no correlation between FSH/AMH ratio and clinical pregnancy.

Conclusion: The assessment of this simple ratio at the beginning of the cycle may help clinicians better anticipate gonadotropin stimulation treatment and better counsel patients about cycle cancellation and the expected oocyte yield. (J Turk Ger Gynecol Assoc 2022; 23: 184-9)

Keywords: FSH to AMH ratio, cycle cancellation, ICSI, poor responder, oocyte retrieval

Received: 29 September, 2021 **Accepted:** 25 January, 2022

Introduction

The management of patients with poor ovarian response (POR) to exogenous gonadotropin stimulation is a challenging problem in in-vitro-fertilization (IVF) cycles. Since POR may be relevant to the decreased number of retrieved oocytes, together with extremely low pregnancy rates, and some patients cannot achieve oocyte pick-up (OPU) due to a cancelled cycle (1). Therefore, the prediction of ovarian response before treatment is fundamental for counselling patients including the management of expectations, especially about their chances of success. The incidence of poor response to ovarian stimulation is estimated to be 9-24%. Several tests have been postulated in an attempt to best assess POR in low prognosis patients (2,3). Currently, the markers most often used by physicians are the age, early follicular phase follicle stimulating hormone (FSH), estradiol (E_2), antral follicle count (AFC), and anti-Mullerian hormone (AMH) levels (4). Among these markers, FSH provides indirect assessment of ovarian reserve through suppression of hypophyseal production of FSH by ovarian E_2. The elevation

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2022.2021-9-19

of FSH at an early phase of the menstrual cycle indicates a decrease in secretion of ovarian hormones due to a failure in the ovarian follicular cohort (5). Although the specificity of basal FSH level >10 IU/L (10-20) is high (45-100%) when POR to ovarian stimulation is predicted, its sensitivity is low (11-86%) (6). Additionally, the intercycle and intracycle variability of basal FSH reduce its reliability (7).

Another predictive marker, AMH, is a glycoprotein that is a member of the transforming growth factor beta superfamily. AMH is secreted from the granulosa cells of preantral and antral follicles. AMH and AFC are currently used as the most reliable biomarkers for the estimation of ovarian reserve (8,9). AMH is reported to be as valid as AFC, but has primacy due to less interobserver variability (10). Many authors have reported that AMH concentrations simply reflect the total developing follicular cohort and POR to stimulation in ART cycles (11-13). Low AMH levels indicate a decrease in the number of selectable follicles and are correlated with decreased yield of oocytes, cycle cancellation, and low chances of achieving pregnancy in ART cycles (14,15). Therefore, FSH and AMH are, respectively, in positive and negative correlation with POR. There are already many studies showing the relationship between the use of variable derived markers, such as LH/FSH ratio, glucose-insulin ratio, and neutrophil-to-lymphocyte ratio (16-18). We hypothesised that the predictive effect of FSH and AMH can be used in the same way as a ratio. The aim of this retrospective study was to estimate the number of retrieved oocyte and cycle cancellations with FSH/AMH ratio in poor prognosis patients.

Material and Methods

This retrospective, monocentric study was conducted in Ankara Zekai Tahir Burak Women's Health Training and Research Hospital, between January 2015 and October 2018. The study protocol was approved by the Ankara Zekai Tahir Burak Women's Health Training and Research Hospital Institutional Ethics Committee (approval number: 9, date: 31.10.2018). All subjects gave informed consent for the utilization of their clinical data and were included as "low prognosis patients" in assisted reproductive technology according to the POSEIDON stratification (19). Only fresh IVF-intracytoplasmic sperm injection (IVF-ICSI) cycles were included. Patients who underwent frozen-thawed embryo transfer and with an element of oligo-azoospermia were excluded.

Women between 24 and 44 years were recruited, and baseline demographics and fertility characteristics were obtained from archive file records. Basal serum E_2, FSH levels, AFC and AMH levels were determined and FSH/AMH ratio was calculated. The serum levels of E_2 and FSH were measured with an electrochemiluminescence immunoassay (Roche, E170. ELECSYS, Mannheim, Germany) on Elecsys and Cobas immunoassay analysers. AMH values were determined with AMH Gen II enzyme-linked immunosorbent assay (Beckman Coulter, Brea, USA). The number of retrieved oocytes, metaphase II oocytes, fertilised oocytes, and number and grade of the embryos were also recorded. Controlled ovarian hyperstimulation was performed by either a gonadotrophin-releasing hormon (GnRH)-antagonist or microdose GnRH-agonist protocol. In the antagonist protocol, a daily GnRH antagonist dose of 0.25 mg was started based on a flexible protocol once a follicle reached ≥14 mm in diameter and continued up to the trigger day. Patients in the flare-up protocol were started on 50 μg of leuprolide acetate (Lucrin; Abbott, Turkey) subcutaneously twice daily on cycle day 1 and 2, and high dose gonadotropin was started on cycle day 3.

Human menopausal gonadotropin was used for controlled ovarian stimulation (Menagon; Ferring, İstanbul, Turkey) in different doses. Patients were monitored with serum E_2 and progesterone levels, and serial transvaginal ultrasonographic examinations. Ovulation was triggered with 250 mg recombinant-choriogonadotropin alpha (Ovitrelle; Merck-Serono, İstanbul, Turkey) when the leading follicle reached 18 mm in diameter or there were at least three follicles ≥17 mm in diameter. Oocyte retrieval was performed 36 hours later. Cycles were cancelled when the follicles persisted at <10 mm after 14 days of stimulation. OPU was performed even with the existence of a single dominant follicle. Luteal phase support was maintained by vaginal progesterone gel (Crinone 8% gel, Serono, İstanbul, Turkey). All eligible oocytes were fertilized by ICSI and embryos were cultured individually according to standard procedures. No more than two embryos were transferred. A serum pregnancy test was performed 14 days after embryo transfer. Clinical pregnancy was confirmed 10-14 days later by the presence of a gestational sac on trasvaginal ultrasound scan. Patients were designated as clinically pregnant, non-pregnant, cycle cancellation, no oocyte retrieved at OPU, and fertilization failure.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS), versdion 21 (SPSS Inc. Chicago, IL, USA) and the distribution of the groups was analyzed with one sample Kolmogorov-Smirnov test. Continuous variables were not normally distributed and expressed as median and range (minimum-maximum). Spearman rank R test was used for correlation analyses. All p-values were two-sided, and 5% was chosen to denote significance ($p<0.05$). Receiver operating characteristic (ROC) curves were generated for FSH/AMH ratio to predict outcomes. All the data were evaluated within 95% confidence interval (CI) in both directions. Non-parametric

Mann-Whitney U test was used for testing differences between groups that were based on FSH/AMH ratio.

Results

A total of 108 fresh IVF-ICSI cycles, corresponding to 92 women with poor prognosis were eligible for analysis. According to the Poseidon criteria categories, 8 (8.7%) were type 1, 8 (8.7%) were type 2, 40 (43.5%) were type 3 and 36 (39.1%) were type 4. Median (range) age and BMI were 35 (24-44) years and 24 (18-35) kg/m^2, respectively. Patient characteristics involving FSH/AMH ratio are presented in Table 1. Eighty-three patients with GnRH antagonist protocol and 25 patients with flare-up protocol were identified.

Embryo transfer was successfully carried out in 65 cycles and 18 clinical pregnancies were achieved. The pregnancy rate was 16.7% per initiated cycle and 27.7% per transfer cycle. Among patients whose cycles has no embryo transfer, there were eight patients with cancelled cycle, 20 patients with fertilization failure and 15 patients with no oocyte retrieved at OPU. Correlation analysis between FSH/AMH ratio and other parameters are presented in Table 2.

As a result, FSH/AMH ratio was moderately negatively correlated with the number of oocytes retrieved ($p<0.0001$, $r=-0.4$) and weakly positively correlated with cycle cancellation or no retrieval of oocyte at OPU ($p=0.002$, $r=0.3$) (Figure 1). The use of this ratio performed well with an AUC of 0.82 (95% CI: 0.71-0.92). A cut-off value of 11.36 was set for the retrieval of <5 oocytes at OPU with 80% sensitivity and 87% specificity. In addition, ROC curves were drawn separately for AMH, bFSH, and age to evaluate the prediction of oocyte yield less then 5. The AUC was below 0.5 for age and bFSH, whereas the AUC value for AMH was 0.80. A cut-off value of 1.2 AMH was predicted for the retrieval of less than 5 oocytes at OPU with 88% sensitivity and 40% specificity (Figure 2).

The optimal FSH/AMH cut-off value was 14.22 to predict the cycle cancellation or no retrieval of oocyte at OPU, with a sensitivity of 91% and a specificity of 44% (AUC of 0.71; 95% CI: 0.59-0.83) (Figure 3). There was no correlation between FSH/AMH ratio and clinical pregnancy.

Discussion

This is the first report to describe the prediction of POR to gonadotropin stimulation with the use of FSH/AMH ratio. We found that FSH/AMH ratio at a certain cut-off value of 11.36 may provide guidance for the estimation of the number of oocytes retrieved <5 with acceptable sensitivity and specificity (AUC 0.82 with 95% CI: 0.71-0.92, sensitivity 80% and specificity 87%). Although AMH alone had a predictive value with similar sensitivity for oocyte yield, it was not as specific as the FSH/AMH ratio. Furthermore, this study emphasized the significant role of this ratio at higher cut-off value to anticipate cancelled cycles and pointless OPU. A cut-off value of FSH/AMH ratio of >14.22 has been shown to be predictive of the cycle cancellation or no retrieval of oocyte at OPU (AUC 0.71, sensitivity 91%, specificity 44%). However, this ratio had low specificity and therefore clinical use may not be as valuable as the former pregnancy ratio.

POR was determined with reduced pregnacy rate during appropriate gonadotropin treatment (20). Advisable prediction of poor response could have clinical value because if the pregnancy chance is inconclusive, patients may want to avoid treatment. FSH, AFC, and AMH have all been used as markers for this purpose. Firstly, AMH inhibits primordial follicle recruitment and restrains follicle growth under the influence of FSH. Plasma AMH concentrations have been positively correlated with the size of the primordial follicle pool and AFC (4,21). Outstanding

Table 1. Clinical and laboratory findings of all patients

Parameters	Median (mean ± SD)
Age (year)	35 (33.97±4.5)
BMI (kg/m^2)	24 (25.23±4.4)
AMH (ng/mL)	0.59 (0.66±0.5)
Basal FSH (IU/L)	10 (10.47±4)
Basal E_2 (pg/mL)	36 (43.92±26.2)
FSH/AMH ratio	17.75 (55.02±136.62)
Antral follicle counts	5 (5.4±2.5)
Infertility duration (year)	4 (4.9±4)
Initial gonadotropine dose (IU)	300 (290.97±36.57)
Total gonadotropine dose (IU)	2700 (2707.59±804.73)
Peak E_2 (pg/mL)	815.5 (917.26±713.48)
Total stimulation day	9 (9.3±2.1)
Endometrial thickness (mm)	9 (8.8±2.6)
Patients with embryo transferred (n, %)	65 (60.2%)
Pregnant	18 (16.7%)
Non-pregnant	47 (43.5%)
Patients without embryo transferred (n, %)	43 (39.8%)
Fertilisation failure	20 (18.5%)
Cancelled cycle	8 (7.4%)
No oocyte retrieval at OPU*	15 (13.9%)
No. of total oocytes	3 (3.4±2.8)
No. of metaphase II oocytes	3 (3.2±2.4)
No. of fertilized oocytes	2 (2.4±1.7)
No. of embryo	2 (2.2±1.6)
The day of transferred embryo	3 (3±0.8)
Embryo quality (grade)	2 (1.8±0.6)

*OPU: Oocyte pick up, SD: Standard deviation, FSH: Follicle stimulating hormone, E_2: Estradiol, No.: Number, AMH: Anti-Mullerian hormone, BMI: Body mass index

Table 2. Correlation analysis between FSH/AMH ratio and other parameters based on ICSI cycles

Parameters	Correlation coefficient	p
Antral follicle count	-0.4	0.001
AMH (ng/mL)	-0.93	0.001
Basal FSH (IU/L)	0.52	0.001
Basal E_2 (pg/mL)	-0.36	0.001
Peak E_2 (pg/mL)	-0.19	0.04
Total stimulation day	0.005	0.9
Initial gonadotropine dose (IU)	0.33	0.001
Total gonadotropine dose (IU)	0.15	0.12
Endometrial thickness (mm)	-0.15	0.13
Clinical pregnancy	-0.06	0.5
No. of total oocytes	-0.4	0.001
No. of metaphase II oocytes	-0.28	0.01
No. of fertilized oocytes	-0.21	0.09
No. of embryo	-0.23	0.06
No. of transferred embryo	0.1	0.4
The day of transferred embryo	-0.1	0.4
Embryo quality	0.04	0.7
Cancelled cycle or no oocyte retrieval at OPU*	0.3	0.002

*OPU: Oocyte pick-up, FSH: Follicle stimulating hormone, AMH: Anti-Mullerian hormone, ICSI: Intracytoplasmic sperm injection, E_2: Estradiol, No.: Number

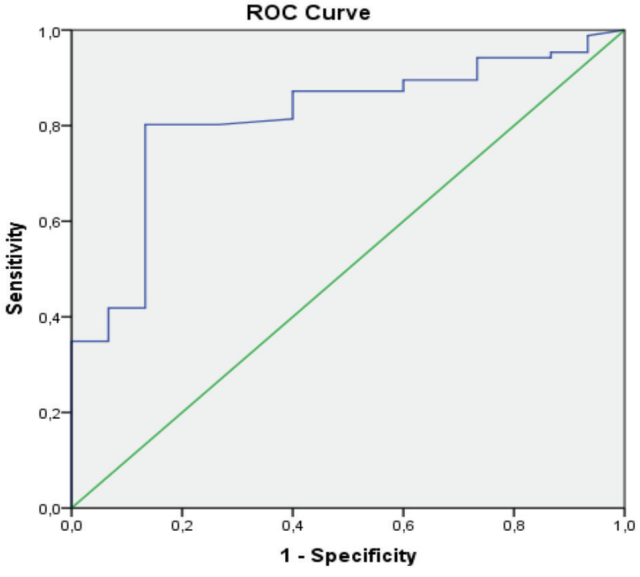

Figure 1. ROC curve for prediction of retrieved oocyte in all patients. ROC curve for FSH/AMH ratio (area below the curve 0.82; 95% confidence interval, 0.71-0.92) cut-off point, 11.36; sensitivity, 80%; specificity, 87%

ROC: Receiver operating characteristic, FSH: Follicle stimulating hormone, AMH: Anti-Mullerian hormone

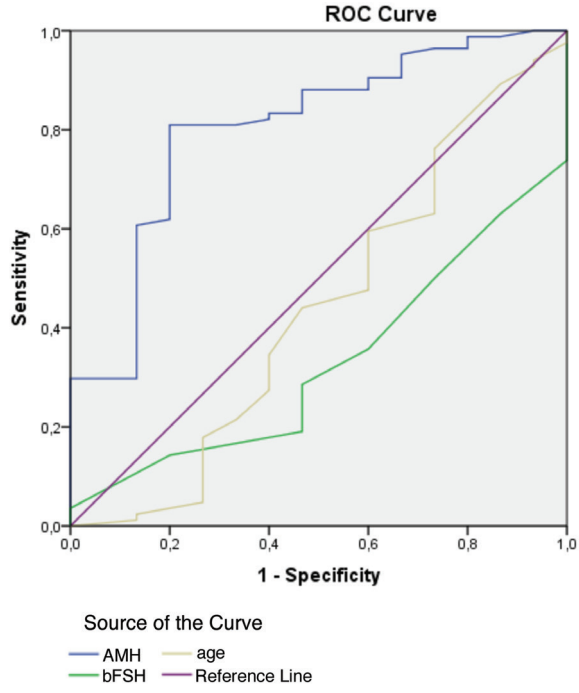

Figure 2. ROC curves of AMH, bFSH and age for prediction of retrieved oocyte in all patients. ROC curve for AMH (area below the curve 0.80; 95% confidence interval, 0.68-0.92) cut-off point, 1.2; sensitivity, 88%; specificity, 40%

ROC: Receiver operating characteristic, FSH: Follicle stimulating hormone, AMH: Anti-Mullerian hormone

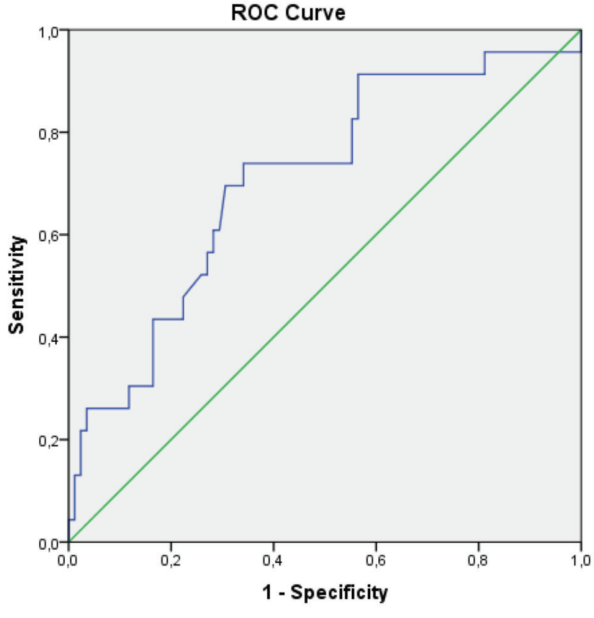

Figure 3. ROC curve for prediction of cancelled cycle and absence of oocyte after OPU. ROC curve for FSH/AMH ratio (area below the curve 0.71; 95% confidence interval, 0.59-0.83) cut-off point, 14.22; sensitivity, 91%; specificity, 44%

ROC: Receiver operating characteristic, OPU: Oocyte pick up, FSH: Follicle stimulating hormone, AMH: Anti-Mullerian hormone

correlation between AMH concentrations and the number of retrieved oocytes has been documented in previous studies (11,22). In one review including patients undergoing controlled ovarian stimulation, low AMH cut-off values (0.1-1.66 ng/mL) have been reported to have 44-97% sensitivity and 41-100% specificity to predict POR (23). In the present study, AMH at 1.2 cut-off value was predictive of oocyte yield with high sensitivity but low specificity. In a meta-analysis consisting of 28 studies, AMH was demonstrated as a decent predictor for POR, with an AUC of 0.78 (10). This dependence was substantially stronger than the associations reported with other ovarian reserve tests, including serum FSH and E_2 (24). However, AMH levels show interassay and intra-assay variability (9). In contrast, a more precise prediction with basal FSH levels rather than AFC has been reported in some patients (25). Secondly, FSH has been demonstrated to have a high specificity for prediction of POR but a low sensitivity. In our study, bFSH and age alone were not found as a predictive marker for oocyte yield. Lastly AFC, measured by transvaginal ultrasonography on the first days of menstrual cycle, quickly estimates and provides results for prediction of POR (26). However, AFC has limitation due to high interobserver and intracycle variability (21,27). Additionally, AFC can cause misjudgement of FSH-sensitive follicle count and oocytes retrieved because of atretic follicles with similar size (28,29). Therefore, each of these well-known methods has some advantages and disadvantages. We hypothesized that the logical combination of the first two tests in one parameter may provide a new assessment method in POR patients and this is supported by our findings.

There was a negative correlation between AFC and FSH/AMH ratio in the present study. This outcome favored the forementioned findings and the assessment of ovarian reserve condition in POR with this new ratio. A negative correlation between basal E_2 and FSH/AMH ratio was also found. However, the explanation of negative E_2 relevance can be troublesome because real E_2 levels may show reciprocal interference with FSH. High FSH levels can be easily masked by high E_2 levels. On the other hand, peak E_2 was negatively correlated with FSH/AMH ratio that favoured our other findings.

When the comparison was done based on the number of retrieved oocytes, there were no difference regarding the number of transferred embryos, the day of transferred embryo, and the total motile sperm count. So, this similarity in two groups favored our findings that were not affected by these variables. However, the day of transferred embryos was significantly higher in patients with FSH/AMH <11.36. This may indicate a possible relationship between this ratio and embryo quality which was not found when this correlation was tested statistically (p=0.7) Majumder et al. (22) demonstrated that serum AMH and AFC were significantly associated with the number of high-quality embryos and the number of embryos frozen. Some authors also found an association between AMH and the number of embryos (11,30), yet some did not (31,32). Unfortunately, neither AMH nor AFC independently predict pregnancy rates (33). Similarly, there was no correlation between FSH/AMH ratio and clinical pregnancy in our study. Due to the inclusion of fresh embryo transfer, a possible negative effect of gonadotropin on endometrial receptivity cannot be excluded. This could have prevented the reflection of our findings on clinical pregnancy.

Study Limitations

The retrospective design and small sample size were major limitations of our study. Furthermore, heterogeneous gonadotropin treatment protocols used, including flare-up and antagonist protocols, was also a limitation. Another limitation was that the clinical situation for frozen transfer patients was not known, since mostly fresh transfers are made in our clinic. To our knowledge this is the first study to suggest the utility of FSH/AMH ratio in IVF cycles. In addition, performing the study in a highly specific study group, that of poor responders who had been freshly transferred, was another strength of our study.

Conclusion

The FSH/AMH ratio can easily be calculated without bringing extra cost, since FSH and AMH are already evaluated in almost every infertile case. Assessment of this simple ratio at the beginning of the cycle may help clinicians better anticipate the gonadotropin-stimulation treatment and better counsel patients about cycle cancellation and expectations for the number of retrieved oocytes.

Ethical Committee Approval: *The study protocol was approved by the Ankara Zekai Tahir Burak Women's Health Training and Research Hospital Institutional Ethics Committee (approval number: 9, date: 31.10.2018).*

Informed Consent: *All subjects gave informed consent for the utilization of their clinical data.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: N.Y., H.C.G., Y.E.Ü., İ.K.; Concept: İ.G.; Design: İ.G., N.Y.; Data Collection or Processing: İ.G., E.A., M.U.C.; Analysis or Interpretation: İ.G., N.Y., İ.K.; Literature Search: N.Y.; Writing: İ.G., N.Y.*

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

Financial Disclosure: *The authors declared that this study received no financial support.*

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The association between protein levels in 24-hour urine samples and maternal and neonatal outcomes of pregnant women with preeclampsia

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Abstract

Objective: Hypertensive diseases of pregnancy are one of the leading causes of maternal and perinatal mortality worldwide. The aim of this study was to evaluate the association between protein levels in 24-hour urine samples and maternal and perinatal outcomes in preeclamptic patients.

Material and Methods: This retrospective cohort study was conducted with pregnant women who were diagnosed with preeclampsia (PE) and delivered in our clinic between 2010 and 2018. Patients were divided into those with a proteinuria value below 300 mg/24 h (non-proteinuria), proteinuria value between 300-2000 mg/24 h (mild proteinuria), proteinuria value between 2000-5000 mg/24 h (severe proteinuria) and proteinuria value >5000 mg/24 h (massive proteinuria) and were compared in terms of maternal and perinatal outcomes. Demographic characteristics (age, body mass index in kg/m^2, gravidity), PE-related clinical symptoms (epigastric pain, neurological and respiratory symptoms), laboratory findings (24 h protein level, lactate dehydrogenase, aspartate aminotransferase, platelet count and creatine levels) were recorded in all patients.

Results: A total of 1,379 patients meeting the study criteria were included. There were 315 (23%) patients in the non-proteinuria group, 704 (51%) in the mild proteinuria group, 234 (17%) patients in the severe group and 126 (9%) patients in the massive proteinuria group. The massive proteinuria group was found to have the highest rates of maternal and prenatal complications. The Apgar score, umbilical cord pH value, birth weight, gestational week at delivery, intrauterine growth restriction and intrauterine fetal death were significantly higher in the massive proteinuria group.

Conclusion: Our data showed that the degree of proteinuria appears to be associated with maternal, fetal and neonatal outcomes among women diagnosed with PE. Women with proteinuria of >5000 mg/24 hours had notably poorer natal outcomes. (J Turk Ger Gynecol Assoc 2022; 23: 190-8)

Keywords: Hypertensive diseases, preeclampsia, 24-hour urine protein, proteinuria, intrauterine growth restriction, perinatal mortality

Received: 16 May, 2022 **Accepted:** 19 July, 2022

Introduction

Preeclampsia (PE) is a pregnancy-specific health problem observed in 5-8% of all pregnancies, with potential serious maternal and perinatal outcomes (1). As a multisystemic disease, PE may also cause long term sequelae in the kidneys, liver, brain and coagulation system (2). PE is mainly characterised by new-onset hypertension in pregnancy accompanied by systemic signs and symptoms. There are also defined criteria for disease severity and they have been revised over the last decades. Until the early 2000s, proteinuria quantification was utilized to identify the disease severity and values above 2 g/24 h were used as the cut-off value for a decision to undertake emergency delivery (3,4). In 2013, proteinuria was removed from the main diagnostic criteria for the detection of PE, in accordance with the committee opinion of the American College of Obstetricians and Gynecologists (ACOG). Moreover, massive proteinuria (≥5 g/24 h) and

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2022.2022-4-3

fetal growth restriction (FGR), which were considered to be diagnostic criteria for severe PE, were also removed from the same classification (5). However, there is still no strong evidence concerning the possible effects of proteinuria on obstetric outcomes (4). Although many studies have demonstrated the relationship between massive proteinuria and adverse events in pregnancies affected by PE, poor obstetric outcomes have also been reported in pregnancies with hypertensive disorders without proteinuria (6,7).

Gestational week at the onset of PE is the most important marker to predict perinatal outcomes in PE, which is known to have a complex and unclear pathology. Perinatal outcomes are known to worsen in the presence of early onset PE and are mostly related to medically indicated preterm deliveries. When observed in early pregnancy, there is an average of 20-fold increased risk for adverse pregnancy outcomes compared to a term pregnancy (8). One fourth of preterm deliveries with medical indications are associated with PE (6). A study has shown that PE is responsible for a significant proportion of severe maternal complications seen at 30% (9,10). Furthermore, PE has been blamed for 14% of maternal deaths due to hypertensive disorders (10). Timing of delivery is crucial both for maternal and fetal well-being, but the unpredictable course of the disease makes this decision extremely challenging for the clinician.

Clarification of the severity of PE and prediction of possible complications in high-risk pregnant women can minimize undesired adverse feto-maternal outcomes. In our own practice, it was anecdotally evident that pregnancy outcomes were worse in patients with proteinuria. Considering that the diagnostic criteria of PE have been frequently reviewed in recent years, we decided that the clinical course of proteinuria and its correlation with feto-maternal outcomes should be reconsidered. Therefore, the aim of this study was to evaluate the association between different degrees of proteinuria, defined by a range of cut-off values, and maternal and perinatal outcomes in pregnant women with PE.

Material and Methods

This retrospective cohort study was conducted in a university hospital, which is a tertiary referral center both for obstetrics and neonatal care. Data regarding pregnant women who were diagnosed with PE, delivered in our hospital and whose 24-hour urine results were obtained from electronic records between the years of 2010 and 2018 were included for the study. The study was approved by the Atatürk University Faculty of Medicine Clinical Research Ethics Committee (approval number: B.30.2.ATA.0.01.00/368). The diagnosis of PE was defined as proteinuria and/or end-organ damage accompanying new onset systolic blood pressure (BP) of 140-159 mmHg, i.e. ≥140 mmHg, and/or diastolic BP ≥90 mmHg, measured on at least two occasions 4-hours apart in the left lateral decubitus position following the 20th gestational week in a woman, who had previously normal BP, according to the criteria of the ACOG (11). The presence of >300 mg/L protein in a 24-hour urine sample was considered as proteinuria. The group without proteinuria consisted of patients with at least one of the following disorders accompanying high BP: 1) renal failure (serum creatinine level >1.0 mg/dL or a doubling of creatinine concentration); 2) liver involvement (liver transaminases >40 IU/L and/or right upper quadrant or epigastric abdominal pain); 3) neurological complications (eclampsia, stroke, visual scotoma or severe headaches); and 4) hematological complications (thrombocytopenia ≤100000/μL) (12).

Those with multiple pregnancies, those with fetal anomalies, those with suspected hepatitis A, B, C or other infectious hepatitis, those with autoimmune hepatitis, those with chronic liver disease, those with kidney disease before or during pregnancy, those with gestational hypertension and those not examined for protein level using 24 h urine samples before delivery were excluded from the study.

Four groups were designated based on the degree of proteinuria in the 24 h urine collection samples. Patients with a proteinuria value <300 mg/24 h were designated non-proteinuria, those with a proteinuria value between 300-2000 mg/24 h were designated mild proteinuria, those with a value between 2000-5000 mg/24 h were designated severe proteinuria and those with a value >5000 mg/24 h were designated massive proteinuria. These groups were compared in terms of maternal and perinatal outcomes. Demographic characteristics (age, body mass index in kg/m^2, gravidity), PE-related clinical symptoms (epigastric pain, neurological and respiratory symptoms), laboratory findings [24 h protein level, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), creatine levels and platelet count] were recorded in all patients. Haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome (HELLP), eclampsia, placental abruption, oligohydramnios, premature membrane rupture, preterm delivery, mode of delivery and magnesium sulfate therapy were recorded as maternal complications. The Apgar score, presence of intrauterine growth restriction, fetal birth weight and pregnancy loss rate were examined and constituted neonatal complications. FGR was defined as an estimated fetal weight, calculated ultrasonographically, below the 10th percentile for gestational age (GA) (13). GA was calculated according to the last menstrual date confirmed by the earliest ultrasonographic findings. Patients with elevated liver function tests, prodromal symptoms of eclampsia and, preterm ruptures of membranes were delivered urgently.

Statistical analysis

The analyses were performed with SPSS, version 20 (IBM Inc., Armonk, NY, USA). The data were expressed as mean, standard deviation, median, minimum, maximum, percentage and number, as appropriate. The normal distribution of continuous variables was evaluated with the Shapiro-Wilk W-test when the sample size was <50, and the Kolmogorov-Smirnov test when the sample size was ≥50. In the comparisons between two independent groups, the independent samples t-test was used when the condition of normal distribution was met, and the Mann-Whitney U test was used when the condition was not met. For the comparison of continuous variables between more than two independent groups, the ANOVA test was used when the condition of normal distribution was met, and the Kruskal-Wallis test was used when the condition was not met. Bonferroni corrected z-test was used for multiple comparisons to compare multiple groups regarding a categorical variable. Following the ANOVA test, the Tukey post-hoc test was used for homogeneous variances, and the Tamhane's T^2 post-hoc test was used for non-homogeneous variances. Following the Kruskal-Wallis test, the Kruskal-Wallis One-Way ANOVA (k samples) test was used for post-hoc tests. In the comparison of two continuous variables, the Pearson correlation test was used when the condition of normal distribution was met, and the Spearman correlation test was used when the condition was not met. Receiver operating characteristic (ROC) curve analysis was used to determine whether the continuous variable could be used for diagnosis or not. In addition, the Youden index was used to determine cut-off values. The power of the new test to distinguish between patients and healthy individuals was determined by calculating the sensitivity, specificity, positive predictive value and negative predictive value for the validity of the diagnostic test results. A value of $p<0.05$ was considered statistically significant.

Results

A total of 1,379 patients who met the study inclusion criteria were included. Patients were divided into four groups based on degree of proteinuria. There were 315 (23%) patients with a proteinuria value below 300 mg/24 h (non-proteinuria; group1), 704 (51%) patients with a value between 300-2000 mg/24 h (mild proteinuria; group 2), 234 (17%) patients with a value between 2000-5000 mg/24 h (severe proteinuria; group 3) and 126 (9%) patients with a value >5000 mg/24 h (massive proteinuria; group 4). Demographic, laboratory and clinical data of the patients are shown in Table 1, 2. There was no significant difference between the groups in terms of age. However, gestational week at diagnosis, neurological symptoms (group 2 was significantly different from group 4; 0.03, 0.10 respectively), epigastric pain, HELLP syndrome (group 2 was significantly different from group 4; 0.05, 0.13 respectively), magnesium sulfate therapy (in group 1, group 2, group 3 and group 4; 0.07; 0.13; 0.38 and 0.83 respectively), preterm delivery (in group 1, group 2, group 3 and group 4; 0.44, 0.53, 0.78, 0.92 respectively) oligohydramnios [group 1 (0.19) was significantly different from group 4 (0.32); group 2 (0.17) was significantly different from group 3 (0.27); and group 4 (0.32)], cesarean delivery (group 1 (0.73) was different from group 2 (0.81) and group 3 (0.85)], LDH, AST, alanine aminotransferase (ALT) and creatinine (≥1.1 mg/dL) levels were significantly different between the groups ($p<0.05$) (Table 3).

Group 4 was found to have the highest rates of maternal and perinatal complications. Intrauterine growth restriction and intrauterine fetal death were significantly higher in this group. There was also a significant difference between the groups in terms of neonatal complications. The Apgar score, umbilical cord pH value, birth weight and gestational week at delivery were significantly higher in group 4. Systolic and diastolic BPs were significantly higher in group 4 ($p<0.05$). There was no significant difference between the groups in terms of premature membrane rupture. The rate of cesarean delivery was similarly

Table 1. Descriptive statistics for continuous variables of all patients

Variables	Mean ± SD	Median (minimum-maximum)
Age (year)	31±6	31 (16-58)
24-h urine protein level (mg/L of 24 h urine)	1694±2025	900 (3-9900)
AST (U/L)	36±69	22 (2-1251)
ALT (U/L)	29±67	13 (1-1045)
LDH (U/L)	316±168	282 (1-3051)
Creatinine (mg/dL)	0.83±9.3	0.5 (0.1-34)
Platelet count (μL)	222000±81000	219000 (8000-785000)
Systolic blood pressure (mmHg)	146±15	140 (100-220)
Diastolic blood pressure (mmHg)	91±10	90 (60-140)

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, SD: Standard deviation

high in all groups. Obstetric outcomes are shown in Table 3 and neonatal outcomes are shown in Table 4. As a result of multinomial regression analysis, creatinine, systolic BP and neurological symptom variables were found to be significant in the model (Table 5). Systolic BP (p=0.01) and creatinine values (p=0.001) were significantly higher in group 3 compared with group 1. Creatinine, systolic BP and epigastric pain levels were found to be significantly higher in group 4 compared with group 1 (p=0.002, p<0.001 and p=0.029, respectively).

ROC curve analysis revealed the area under the curve (AUC) for HELLP syndrome, giving a cut-off value, sensitivity and specificity of 1338,000, 51.2% and 65.2%. Similarly, for placental abruption these values were 3495,000, 29.1% and 84.4%, for magnesium sulfate they were 2324,500, 62.7% and 87.4%, for oligohydramnios 2250,000, 35.6% and 78.2%, for preterm delivery 1075,000, 53.4% and 76.2%, for vaginal delivery 1075,000, 32.4% and 56.5% and cesarean delivery were 402,500, 74.5% and 36.8% (Figure 1). ROC curve analysis demonstrated the AUC for neonatal mortality giving sensitivity, specificity and cut off values of 64.8%, 62.4% and 1185,000, respectively. Similarly, for FGR the cut-off value, sensitivity and specificity were 985,000, 53.4% and 57.3% (Figure 2).

Discussion

Discussions continue about the significance and clinical utility of a cut-off value of proteinuria, which is among the commonly used diagnostic criteria in PE (14). Although international guidelines recommend a cut-off value of 300 mg/24 h and greater for significant proteinuria, there are also studies that have reported only a weak association between these levels of proteinuria and maternal and perinatal outcomes (11). The findings of this retrospective study including 1,379 patients showed that AST, ALT, LDH, creatinine and BP levels increased with increasing proteinuria levels. Also, presence of HELLP syndrome, magnesium sulfate therapy requirement, preterm delivery and oligohydramnios were significantly more common in the group with massive proteinuria. Birth weight was higher, gestational week at delivery was later, intrauterine

Table 2. Descriptive statistics for discrete variables of patients

Variables		**Number of patients**	**%**
Primigravida		335	24
Multigravida		1045	76
24-h urine protein level	Group 1	315	23
	Group 2	704	51
	Group 3	234	17
	Group 4	126	9
Proteinuria in spot urine	0	614	45
	+1	328	24
	+2	214	16
	+3	199	15
	+4	16	1
Epigastric pain		99	7
Neurological symptoms		66	5
Eclampsia		15	1
HELLP syndrome		82	6
Placental abruption		55	4
Magnesium sulfate therapy		308	22
Oligohydramnios		281	20
Preterm delivery		812	59
Premature membrane rupture		129	9
Vaginal delivery		278	20
Caesarean delivery		1102	80
		Median	**Min.-max.**
Gravidity		3	1-8
Parity		2	0-6

HELLP: Elevated liver enzymes and low platelets, min.: Minimum, max.: Maximum

growth restriction more likely, umbilical cord pH higher and Apgar score lower in the massive proteinuria group. In addition, PE was diagnosed in the earlier weeks, and characteristics of severe PE were observed in the group with high 24 h proteinuria levels. As a result, these data demonstrated that there is an association between proteinuria and maternal, fetal and neonatal outcomes among pregnant women diagnosed with PE, which was especially strong in the subgroup with massive proteinuria. Early-onset PE is significantly associated with adverse maternal and neonatal outcomes. The rate of these adverse outcomes is significantly higher in early onset PE than late-onset PE. In the present study, the gestational week at diagnosis was significantly earlier in group 4 than in the other groups. However, severe PE may be associated with both early onset and high proteinuria levels. Today, the only effective treatment for PE is termination of pregnancy at the most appropriate time for both maternal and fetal well-being (15). Although urinary protein excretion increases significantly in normal pregnancy, protein excretion is considered abnormal when a value exceeding >300 mg/L in the 24 h urine sample (15). However, proteinuria is not present at initial admission with clinical symptoms in 10% of women with clinical and/or histological findings of PE and 20% of women with frank eclampsia (16). This may be due to the fact that multiple organ dysfunction, affecting the kidneys and liver, can occur without significant proteinuria, and the amount of proteinuria does not predict the severity of disease progression (4). Therefore, since 2014, the International Society for the Study of

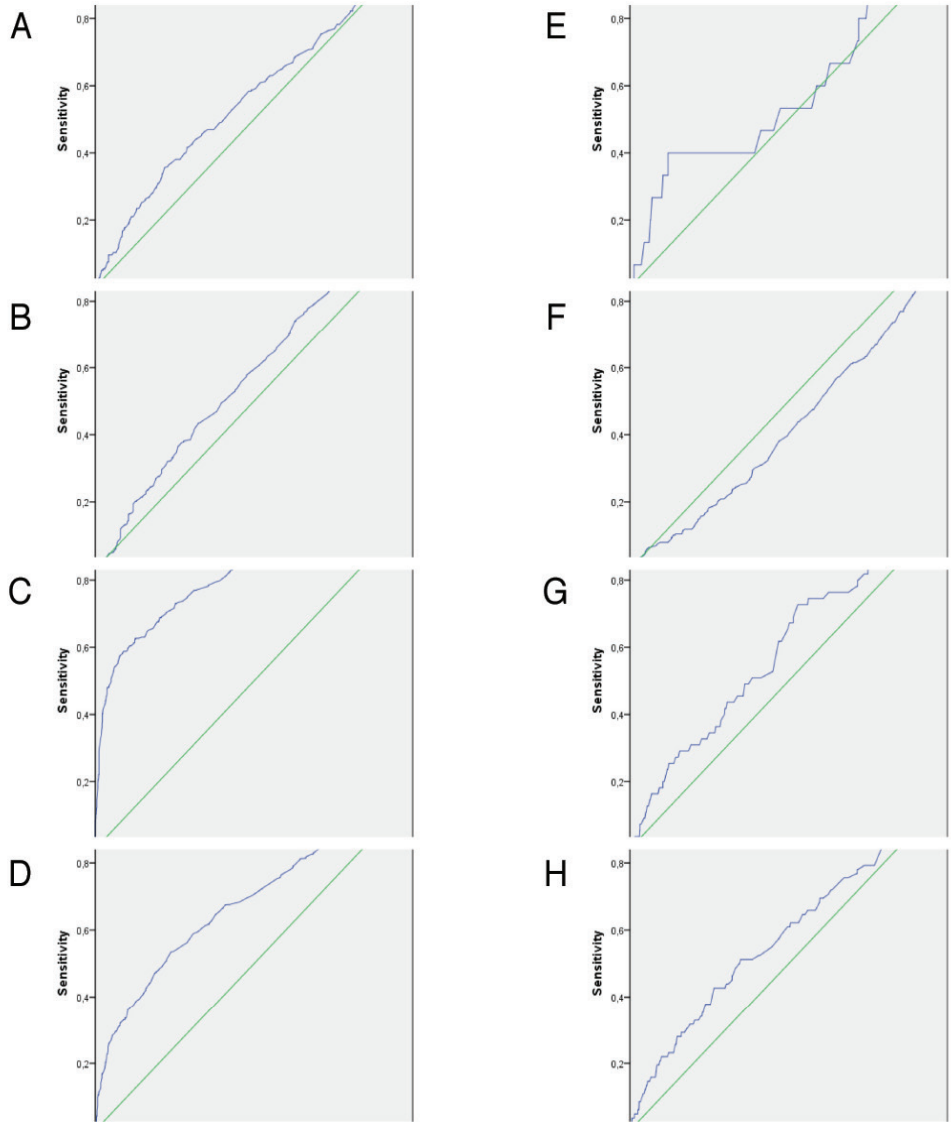

Figure 1. ROC curve analysis for maternal outcomes. A) oligohydramnios; B) caesarean delivery; C) magnesium sulfate therapy; D) preterm delivery; E) eclampsia; F) vaginal delivery; G) placental abruption; H) HELLP syndrome

ROC: Receiver operating characteristic, HELLP: Haemolysis, elevated liver enzymes and low platelets

Hypertension in Pregnancy and ACOG have not recommended the use of proteinuria as a criterion to diagnose PE (17,18). Although proteinuria is not recommended as a criterion in the diagnosis of PE, clinicians often use proteinuria levels in the decision-making process for delivery of PE cases (19). As many studies have shown that an increasing amount of proteinuria is associated with poor progression of the disease and poor perinatal outcomes (10,15,20), such as perinatal mortality (21) and preterm delivery (22). Guida et al. (3) reported that proteinuria, especially massive proteinuria, adversely affected fetal, maternal and neonatal outcomes, and poor maternal outcomes were also associated with the severity of proteinuria in their study (3). On the other hand, Thornton et al. (23) found the perinatal mortality rate significantly higher in the non-proteinuria group and did not detect any difference between the two groups in terms of maternal mortality.

Since this study was performed in a tertiary referral hospital, we found that almost all of the patients who were referred to our clinic with a pre-diagnosis of PE were examined for 24 h protein levels. No significant difference was observed among groups in terms of eclampsia and placental abruption, which significantly affect maternal mortality. Prematurity, an important cause of neonatal mortality, was observed to be high in the massive proteinuria group. We would like to draw attention to the correlation between prematurity and massive proteinuria in this study. Therefore, PE appears to be an isolated risk factor

Table 3. Obstetric outcomes between the groups

Variables		Group 1 0-300 24 h/mg (n=315)	Group 2 300-2000 24 h/mg (n=704)	Group 3 2000-5000 24 h/mg (n=234)	Group 4 ≥ 5000 24 h/mg (n=126)	p
Age (years)		32±6	31±6	31±6	31±6	0.222
BMI (kg/m²)		27.62±6.57	23.07±0.86	22.28±1.22	24.12±2.25	0.125
AST (U/L)		32±41	35±72	40±83	49±75	0.001
ALT (U/L)		27±46	27±67	33±82	41±77	0.001
LDH (U/L)		300±124	310±169	320±135	375±275	0.001
Creatinine (mg/dL)		0.55±0.15	0.57±0.24	2.13±22.76	0.64±0.29	0.001
Platelet (μL)		227,000±82,000	222,000±81,000	221,000±77,000	207,000±85,000	0.052
Systolic blood pressure (mmHg)		143±14	145±14	149±14	155±20	0.001
Diastolic blood pressure (mmHg)		90±10	90±9	93±9	95±11	0.001
Primigravida		78 (0.25)[a,b]	148 (0.21)[b]	66 (0.28)[a,b]	43 (0.34)[a]	0.005
Multigravida		238 (0.76)[a,b]	556 (0.79)[b]	168 (0.72)[a,b]	83 (0.66)[a]	0.005
Proteinuria in spot urine	0	177 (0.57)	318 (0.45)	83 (0.35)	36 (0.29)	-
	+1	81 (0.26)	184 (0.26)	40 (0.17)	23 (0.18)	-
	+2	32 (0.10)	121 (0.17)	45 (0.19)	16 (0.13)	-
	+3	21 (0.07)	69 (0.10)	65 (0.28)	44 (0.35)	-
	+4	1 (0.00)	8 (0.01)	1 (0.00)	6 (0.05)	-
Epigastric pain		17 (0.05)	45 (0.06)	22 (0.09)	15 (0.12)	0.044
Neurological symptoms		16 (0.05)[a,b]	23 (0.03)[b]	14 (0.06)[a,b]	13 (0.10)[a]	0.005
Eclampsia		2 (0.01)	7 (0.01)	2 (0.01)	4 (0.03)	0.060
HELLP syndrome		17 (0.05)[a,b]	34 (0.05)[b]	15 (0.06)[a,b]	16 (0.13)[a]	0.007
Placental abruption		9 (0.03)	27 (0.04)	10 (0.04)	9 (0.07)	0.220
Magnesium sulfate therapy		21 (0.07)[a]	93 (0.13)[b]	89 (0.38)[c]	105 (0.83)[d]	**<0.001**
Oligohydramnios		61 (0.19)[a,b]	117 (0.17)[b]	63 (0.27)[a,c]	40 (0.32)[c]	**<0.001**
Preterm delivery		138 (0.44)[a]	376 (0.53)[b]	182 (0.78)[c]	116 (0.92)[d]	**<0.001**
Premature membrane rupture		37 (0.12)	60 (0.09)	21 (0.09)	11 (0.09)	0.434
Vaginal delivery		86 (0.27)[a]	136 (0.19)[b]	34 (0.15)[b]	22 (0.17)[a,b]	**0.001**
Caesarean delivery		229 (0.73)[a]	569 (0.81)[b]	200 (0.85)[b]	104 (0.83)[a,b]	**0.001**
IUGR		58 (18%)[a]	159 (23%)[a,b]	66 (28%)[b]	39 (31%)[b]	0.008

[a,b,c,d]: Bonferroni correction was used for post-hoc tests after categorical comparisons; the letters symbolize the difference of the groups with each other. BMI: Body mass index (kg/m²), AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, HELLP: Haemolysis, elevated liver enzymes and low platelets, IUGR: Intrauterine growth restriction

for prematurity, as has been previously reported (24). There is a scarcity of evidence concerning how to identify the optimal delivery time in PE (25). However, it was shown, both in the present study and in the literature, that the decision to undertake preterm delivery prevented serious complications that might occur (3). Although the prematurity rate was high, no maternal mortality was observed in our study group. Furthermore, our cesarean delivery rates were found to be high in all groups due to the delivery protocol management in our clinic.

Newman et al. (4) detected no increased risk of maternal and neonatal morbidity, even in pregnancies with proteinuria >10 g/L in their study investigating the effects of massive proteinuria. They argued that massive proteinuria appeared to be a marker for early-onset PE and prognosis, and neonatal mortality was a

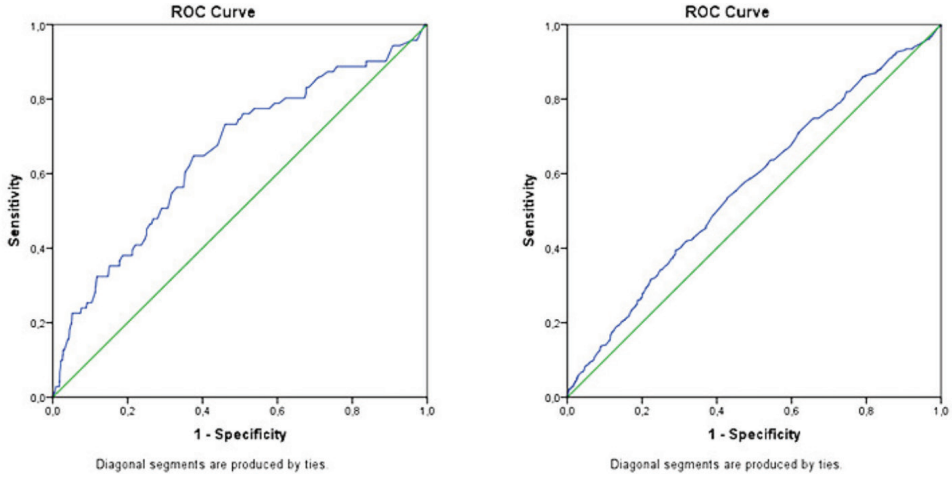

Figure 2. ROC curve analysis for perinatal outcomes. Neonatal death and IUGR

ROC: Receiver operating characteristic, IUGR: Intrauterine growth restriction

Table 4. Neonatal outcomes between the groups

Variables	Group 1 0-300 24 h/mg (n=315)	Group 2 300-2000 24 h/mg (n=704)	Group 3 2000-5000 24 h/mg (n=234)	Group 4 ≥ 5000 24 h/mg (n=126)	p
Apgar score	8.07±1.69	7.82±1.98	7.42±2.25	6.43±2.87	<0.001
Umbilical cord pH	7.30±0.07	7.30±0.07	7.30±0.08	7.29±0.07	0.511
Birth weight (g)	2626±805	2506±832	1957±858	1575±757	<0.001
Gestational week	36.1±3.3	35.4±3.4	32.7±4.1	30.7±3.9	<0.001

Table 5. Likelihood ratio tests

Effect	-2 log likelihood of reduced model	Chi-square	p
AST	3181,725	1,192	0.755
ALT	3182,411	1,877	0.598
Kreatinin	3199,974	19,441	**<0.001**
PLT	3183,960	3,426	0.330
SBP	3207,172	26,639	**0.001**
DBP	3182,492	1,959	0.581
Epigastric pain	3186,817	6,284	0.099
Neurological symptom	3190,410	9,877	0.020
Eclampsi	3182,043	1,510	0.680
Decolman	3180,812	0.279	0.964

The chi-square statistic is the difference in -2 log likelihoods between the final model and a reduced model. The reduced model is formed by omitting an effect from the final model. The null hypothesis is that all parameters of that effect are 0. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, PLT: Platelet count, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

consequence of prematurity, rather than massive proteinuria (4). Dong et al. (15) demonstrated that severe proteinuria was associated with the onset week of PE, incidence of FGR and earlier delivery than GA. Kim et al. (22) concluded that massive proteinuria might be associated with early-onset PE and preterm delivery in their retrospective study (22). Many studies have shown that maternal and perinatal outcomes deteriorate with increasing proteinuria levels, and PE severity is directly related to proteinuria severity (5). However, there are also studies showing the opposite. In a systematic review of selected studies with a proteinuria cut-off value of 5 g/L of 24 h urine collection, the authors argued that proteinuria should not be used for clinical decisions (15). Again, Schiff et al. (26) stated that there was no difference among pregnancies with a marked, minimal or no increase in proteinuria in terms of maternal or fetal outcomes in their retrospective study.

Despite all the advances in medicine, the treatment of PE, which is both difficult to manage and related to poor outcomes, is still unclear in obstetric practice. Currently, the most effective treatment for PE is to terminate pregnancy. However, there is no consensus on optimal timing for delivery (27). The desire to prevent maternal organ damage contrasts with the desire to avoid prematurity and related complications. Clinical conditions that may lead to prematurity, intrauterine growth restriction or even death may occur (4). Although PE *per se* is an isolated risk factor for prematurity, the amount of proteinuria is associated with the earlier occurrence of the disease. In our study, prematurity and intrauterine growth restriction were significantly more common in the massive proteinuria group compared to the other groups.

Study Limitations

This study has some limitations. The first is the retrospective nature of the study. In addition, serious losses were observed in the follow-up of the patients in the postpartum period because of the tertiary nature of the study center, and therefore, the duration or normalization of proteinuria and hypertension could not be monitored. However, the high number of patients, being the largest tertiary health center of the region, and application of the treatment protocol of our clinic by the same physician group are among the strengths of this study.

Conclusion

We want to highlight that proteinuria in pregnancies affected by PE and which can be easily evaluated in almost every laboratory, should not be ignored. We believe that proteinuria is a valuable marker in PE management. We advocate the use of assessing proteinuria when evaluating the severity of PE. We further believe that proteinuria should be taken into consideration until new factors are found that enable more reliable estimation of obstetric outcomes in all preeclamptic women with massive proteinuria. Although clear conclusions cannot be drawn from the current literature, massive proteinuria seems to be especially closely correlated with significant maternal and neonatal adverse events. As clinicians we suggest considering this situation as a secondary but a valuable variable throughout management of pregnant women with PE.

Ethical Committee Approval: *The study was approved by the Atatürk University Faculty of Medicine Clinical Research Ethics Committee (approval number: B.30.2.ATA.0.01.00/368).*

Informed Consent: *Retrospective study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: G.A.Y., E.P.T.Y.; Concept: G.A.Y., E.P.T.Y.; Design: G.A.Y., E.P.T.Y.; Data Collection or Processing G.A.Y., E.P.T.Y.; Analysis or Interpretation: G.A.Y., E.P.T.Y.; Literature Search: G.A.Y., E.P.T.Y.; Writing: G.A.Y., E.P.T.Y.*

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

Financial Disclosure: *The authors declared that this study received no financial support.*

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This article will be credited by TMA within the scope of CME/CPD.

Occupational and environmental mercury exposure and human reproductive health - a review

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Abstract

Mercury is a toxic heavy metal. Humans are exposed to mercury through several sources including environmental, occupational, contaminated food and water and from mercury-containing dental amalgam. Mercury exposure is known to harm the nervous system profoundly, and have a negative impact on digestive and immune systems, and other organs. To review and discuss the effect of mercury exposure through environmental or occupational routes on human reproduction, pregnancy, and its outcome. Published information about the potential toxic effects of mercury on human reproduction were collected and summarized. Literature was identified by systematic search using relevant keywords. Literature review revealed a number of negative impacts of mercury on human reproduction. These included effects on semen quality, including reduced sperm count, motility, and changes in morphology that may reduce fertility potential. There may also be an effect in changing reproductive hormone levels. Mercury exposure might also affect pregnancy but the data concerning mercury effects on female reproduction are limited except for some data about mercury exposure and poor pregnancy outcomes. Available data indicate that mercury exposure may have a toxicity effect on reproductive potential, especially in males. Prenatal mercury exposure may affect pregnancy or its outcome and this appears to be dependent upon dose, duration, and timing of exposure. Nutritional status of exposed individual might also influence the impact of mercury. (J Turk Ger Gynecol Assoc 2022; 23: 199-210)

Keywords: Mercury, reproduction, fertility potential, semen quality, pregnancy outcome, methyl mercury

Received: 24 March, 2022 **Accepted:** 08 July, 2022

Introduction

It is well known that mercury is a toxic heavy metal. It may be present in different forms, including elemental mercury, or as part of inorganic or organic compounds. Humans are exposed to mercury through both natural and synthetic sources. Sometimes they are subjected to higher quantities of exposure accidently or occupationally. Mercury poisoning might occur through breathing of mercury-contaminated air or consuming contaminated water or food, especially contaminated fish, or accidental exposure to mercury may occur through some occupations or in certain work processes. Accidental exposure may also occur when mercury-containing equipment is damaged. Mercury exposure can cause various health problems in human, and is known to affect child growth in pregnancy or in early life. Furthermore, mercury may also have a toxic impact on the nervous, digestive, and immune systems, and in organs including the lungs, kidneys, skin, and eyes. Mercury metal has been labelled by the WHO as one of the top ten key chemicals with potential public health concern (1).

One of the best known episodes of mercury poisoning occurred in Minamata bay and Niagata, Japan during 1950s, and methyl mercury poisoning happened in Iraq in the 1970s (2). Neurobehavioral deficiency and, in a few cases, clinical signs have been reported for both children and adults with respect to mercury exposure. There are some data on cytogenetic impairment, changes in immune function, and cardiovascular toxicity owing to mercury exposure (3).

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.

DOI: 10.4274/jtgga.galenos.2022.2022-2-6

Furthermore, the data on environmental mercury exposure and its influences on human health indicated that mercury has profound cellular, cardiovascular, hematological, renal, pulmonary, immunological, neurological, reproductive, endocrine, and embryonic toxicologic effects (4). Mutter et al. (5) reported that mercury leaching at low doses from dental amalgam used for tooth fillings may be absorbed by various body tissues, contributing to the mercury burden in humans. The debate continues about the consequences of this low-level chronic mercury exposure to the users of dental amalgam on their health or reproduction or impact of occupational mercury exposure to dental professionals while preparing/implanting dental amalgam.

The toxic potential of lead and cadmium on human reproduction has been reviewed (6,7). In this article, we endeavor to assess the effect of mercury exposure on human reproductive health, pregnancy, and its outcome from all of the most likely sources.

Material and Methods

A systematic review of available literature was carried out by searching within a number of databases, including PubMed, Google, PubChem, and Google scholar. These searches were conducted using a number of keywords or key terms, including which were: “mercury exposure and health”, “occupational mercury exposure”, “environmental mercury exposure” and “male human reporoduction”, “female human reproduction”, “pregnancy”, “pregnancy outcome”, “offspring development”, “puberty”, “reproductive hormones”, “reproductive toxic potential”, “erectile dysfunction”, “libido”, “semen quality”, “menstruation cycle”, and “fertility”. A further search was conducted into mercury exposure through dental amalgam and human reproductive health, consumption of fish/seafood with regards to mercury and reproduction, pregnancy or its outcome. More than five hundred articles were identified, and seventy-five relevant articles were incorporated in this review.

The article is divided into three sections: effects of mercury on male reproduction, effects of mercury on female reproduction and effects of mercury on pregnancy or its outcome. The data from the three sections is summarized in Tables 1 and 2 for better and quick appraisal. Some experimental information was also incorporated as and when necessary, either in the absence or scarcity of human data or to provide a better understanding of the mechanism behind reproductive toxicity of mercury. The collection of data and presentation of the information on mercury exposure and human reproduction is depicted in Figure 1.

Results and Discussion

Both acute and chronic mercury exposure can cause deleterious effects on human health during early life growth and development and there is no known safe dose of mercury exposure reported for human beings. Furthermore, prenatal, and postnatal mercury exposure may occur by different pathways, although bio-accumulation is reported to mostly occur through the aquatic food chain (8). Moreover, mercury exposure has been found to pose substantial health risks to certain occupational groups, such as goldminers and dental personnel, where there may be a greater chance of occupational exposure (9). As has been noted, mercury exposure negatively impacts on human reproductive health, by altering the reproductive as well as the endocrine systems of both sexes. However, the molecular mechanisms behind mercury-linked decline in fertility potential are unclear (10). Furthermore, mercury exposure could damage to Leydig cells, seminiferous tubules, testicular degeneration, and menstrual cycles disorders. Some studies reported spontaneous abortion (SAb) and adverse fertility outcome owing to work-related mercury exposure. They also stated a relationship between inhalation of mercury vapor and poorer reproductive outcome (11). Thus, mercury exposure has the potential to affect reproductive organs and various reproductive endpoints adversely including pregnancy or outcome.

Mercury Exposure and Male Reproductive Health

Numerous experimental studies are available on the effect of mercury on male reproduction. These studies show that mercury is a male reproductive poison, but studies on human male reproduction are few, with inconsistent findings (12). Based upon several published experimental studies, mercury negatively affects male reproductive potential as mercury exposure can deteriorate several male reproductive endpoints, such as sperm quality, motility, normal sperm morphology, testicular injury, sperm DNA damage, and fertility potential (13-17). A few clinical reports are available on the impact of mercury exposure on male reproduction, especially semen quality. One report examined the relationship between blood mercury levels and semen quality parameters in sub-fertile men (18). The sperm concentration, percentage of morphological normal sperm, percentage of normally motile sperm, curvilinear velocity, straight-line velocity, average path velocity, and amplitude of lateral head displacement was reduced in men with higher blood mercury levels, though the alteration was statistically insignificant. Mocevic et al. (12) also studied semen characteristics and reproductive hormones in association with environmental mercury exposure and did not

find any evidence of negative effects of mercury on studied biomarkers of male reproduction in men from Greenland and Europe.

Furthermore, an association between urinary metal levels and sperm DNA impairment was examined. Thirteen metals, arsenic, cadmium, cobalt, chromium, copper, iron, lead, manganese, nickel, molybdenum, selenium, mercury, and zinc were measured in urine samples, and sperm DNA injury was evaluated by comet assay. These authors found that urinary mercury and nickel were associated with elevation of tail length, and urinary manganese was associated with elevation of tail moment. This suggested that environmental exposure to mercury, nickel and manganese might be related to sperm DNA injury (19). Subsequently, Lu et al. (20) also confirmed an association between non-occupational exposure to mercury among reproductive-aged males and DNA methylation in the imprinting gene *H19* in sperm. Furthermore, *in utero* and adult exposure to EDCs is likely to be a modifier of male reproductive health. Mercury may be retained in testicular tissues and pituitary gland causing reduced testicular function, especially spermatogenesis (21). An association between methyl mercury (MeHg), and 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl (CB-153) exposure and sperm quality has been reported. Blood concentrations of MeHg were in the range of 0.11 to 16.59

Table 1. Mercury exposure and male reproduction, semen quality and male mediated reproductive outcome

Sl. No.	Exposure	Effects	Reference
Mercury and semen quality			
1	Blood Hg levels & semen quality in sub-fertile men	Sperm concentration, morphologically normal, motility, curvilinear & straight-line velocity, average path velocity, & amplitude of lateral head displacement, reduced insignificantly with higher blood Hg level	Leung et al. (18)
2	Semen quality, reproductive hormones & environmental Hg exposure	Environmental Hg exposure in Greenlandic & European men with median blood Hg level up to 10 ng mL (-1) not link with hostile effects on male reproductive biomarkers	Mocevic et al. (12)
3	Urinary metal (As, Cd, Cobalt, Cr, Cu, Fe, Pb, Mn, Hg, Ni, Zn, Molybdenum, Selenium) & sperm DNA damage	Urinary Hg & Ni linked with increasing trends of tail length, and Mn linked with tail moment. This advice that environmental exposure to Hg, Mn, & Ni related with sperm DNA damage	Zhou et al. (19)
4	Pre-conceptional Hg exposure & DNA methylation of imprinting genes H19, in human sperm DNA	Environmental Hg exposure related with altered DNA methylation at imprinting gene H19 in sperm, implicating in susceptibility of sperm for environmental insults	Lu et al. (20)
5	Examined an association amid MeHg, and 2, 2', 4, 4', 5, 5'-hexachloro biphenyl (CB-153) exposure, with reverence to sperm quality	No relationship amid MeHg & sperm quality parameters. Men with low MeHg & high CB-153 exposure had slightly higher DFI & fraction of Y-chromosome carrying sperms than men with lower level of these compounds	Rignell-Hydbom et al. (22)
6	Correlated semen quality with hair' Hg level	Hair' Hg level positively linked with sperm concentration, count, & progressive motility. These relations stronger in men with fish consumption. Semen volume & morphology non-significantly related to hair Hg levels	Mínguez-Alarcón et al. (24)
7	Predatory fish usage related with high blood Hg level & semen quality	Linked with lower sperm count & normal morphology. Predatory fish might be al risk factor for higher Hg level that might affect semen quality	Ai et al. (23)
8	Urinary Hg concentrations & semen quality	No positive link amid urinary Hg levels & semen quality, fertility index & quantity of dental amalgam fillings	Hanf et al. (26)
Male mediated reproductive outcome			
9	Male mediated elemental mercury exposure occupationally & reproductive outcomes	No associations demonstrated amid Hg exposure & declined fertility or higher malformations or serious childhood illnesses	Alcser et al. (29)
10	Spontaneous abortions among wives of workers exposed to Hg vapour occupationally	An elevation of SAbs with elevation of Hg in fathers' urine before pregnancy. At high levels above 50 μg/L the SAb risk become doubles	Cordier et al. (30)
11	Reviewed data on parental exposure to metals & SAb	Paternal exposure to Pb or Hg might be related with the risk of SAb	Anttila and Sallmén (31)

MeHg- Methyl mercury; As-Arsenic; Mn- Manganese; Cd-Cadmium; Cr-Chromium; Cu-Copper, Fe-Iron; Pb-lead; Zn-Zinc; SAb-Spontaneous abortion; DFI- DNA fragmentation index

Table 2. Mercury exposure and Female reproduction

Sl. No.	Exposure	Effects	Reference
Mercury exposure and puberty, menstruation cycle and menopause			
1	Women (dentists & dental assistants) exposed to metallic mercury	A significant, risk amid hair' total mercury labels (TMLs) & reproductive failures. Relation with menstrual disorders was significant	Sikorski et al. (37)
2	Elemental mercury (used in gold mining) on menstrual cycle & miscarriages	Related with occurrence of irregular menstrual cycles but not related to miscarriage	Rodríguez-Villamizar et al. (38)
3	Reproductive risks in female workers exposed to low-level metallic mercury	Low-level long-term Hg exposure brought significantly more dysmenorrhoea, hypomenorrhea at above 0.06 mg/m^3 of Hg level, & below this, menstrual cycles, quantity, duration did not alter significantly. Rates of PTB, SAb, still birth, fetal death, & pregnancy snags in group exposed to 0.06-0.1 mg/m^3 of Hg & control was insignificant	Fu (41)
4	Female workers exposed to Hg vapor	Abdominal pain & dysmenorrhea more in Hg exposed workers	Yang et al. (42)
5	Meta-analysis on the reproductive effects of Hg exposure in female workers	Causes dysfunction of menstrual period, cycle, blood volume, dysmenorrhea & cause hostile outcomes, i.e., pregnancy-induced hypertension, stillbirth, LBW & birth defects	Pan et al. (43)
6	Prenatal Hg exposure & precocious puberty	Prenatal exposure to High doses of Hg related with precocious puberty. Highest risk in children with hostile birth outcomes whose mothers had high RBC-Hg level & cardio-metabolic conditions	Wang et al. (45)
7	Menopause & blood Hg level	Blood Hg was lower significantly in postmenopausal than premenopausal women	Yuk et al. (46)
Mercury exposure and pregnancy and outcome			
8	Mercury exposure in pregnancy	Pregnancy complications & developmental complications in infants	Solan and Lindow (73)
9	Prenatal Hg exposure & newborn anthropometric characteristics	Negative correlation amid blood Hg levels in 1st & 2nd trimesters & birth weight.	Vigeh et al. (47)
10	Assessed association amid exposure to Hg prenatally & birth weight, GST polymorphisms.	Mothers with GSTT1 null genotype, higher maternal blood Hg in late pregnancy linked with risk of LBW. Mothers with GSTM1 & GSTT1 null genotype, maternal & cord blood Hg levels were related with LBW	Lee et al. (48)
11	Prenatal maternal arsenic & Hg exposure & birth outcomes in artisanal & small-scale gold mining (ASGM) subjects.	In ASGM areas, risk of hostile birth outcome elevated with increasing total-As & total-Hg exposure. SAb, stillbirth & PTB significantly linked with elevated total-As, while elevated Hg significantly linked with stillbirth & congenital anomalies	Nyanza et al. (57)
Dental amalgam' mercury exposure and pregnancy outcome			
12	Reproductive history of dentists & dental assistants	No elevated rates of congenital abnormalities or SAb in children of men & women exposed to low v/s high dose of Hg in dental setting	Brodsky et al. (50)
13	Assessed links amid exposure to amalgam fillings & pregnancy outcome	No significant associations amid total teeth with amalgam fillings & early, late PTB, LBW, malformation, or stillbirth babies	Lygre et al. (51)
14	Effect of electro-magnetic fields on the release of Hg from dental amalgam	Dental amalgam fillings pregnant women should limit exposure to electromagnetic fields to prevent effects of Hg to fetuses	Mortazavi and Mortazavi. (52)
15	Dental workers exposed to mercury amalgam, acrylate compounds, solvents, disinfectants & miscarriage.	No strong or dose-response relation detected amid exposure to Hg amalgam, acrylate compounds, solvents, disinfectants & Miscarriage. A slightly more risk of miscarriage with these agents	Lindbohm et al. (53)
16	Exposures to Hg during amalgam preparation.	Women with more Hg exposure were less fertile. The fecundability (chance of conception at each menstrual cycle) of women those prepared 30 or more amalgams/ week have 63% chance of conception than control	Rowland et al. (54)

Table 2. Continued

Sl. No.	Exposure	Effects	Reference
17	Pregnant dental professionals	Suffered with higher odds of developing spontaneous abortion, pre-eclampsia, and babies smaller for gestational age and this may be connected to oxidative stress induced by mercury	El-Badry et al. (40)
Sea food/fish intake related mercury exposure and outcome			
18	Birth' anthropometry, placental wt. & gestational length & Hg	Prenatal Hg exposure by seafood may be related with lower placental & fetal growth	Murcia et al. (49)
19	Consumption of seafood in pregnancy	Hg exposure is undesirably related with birth weight. Seafood usage in pregnancy not to be avoided but find at what level Hg exposure might exceed the risk of seafood	Vejrup et al. (58)
20	Maternal seafood consumption	Maternal seafood intake linked with Hg level. No association amid Hg level & fetal growth, except negative relation with biparietal diameter	Drouillet-Pinard et al. (59)
21	MeHg level & fish consumption	Blood MeHg level significantly more in infertile than pregnant women & consistent with fish consumption	Lei et al. (60)
22	Mercury exposure level	No evidence of impairment with total Hg exposure if mother ate fish in pregnancy	Hibbeln et al. (61)
23	Pregnant women receive two messages with fish usage	Fish usages positively related with hair' Hg levels. Equated with women deliver at term, women who delivered prior to 35 weeks likely to have Hg hair at or more the 90th percentile (> or =0.55 microg/g)	Xue et al. (62)
24	Hg exposure & birth outcome	About 15.7% of subjects had PTB & 8.1% delivered LBW. Lower hair Hg exposure (lowest tertile < 2.34 μg/g), related with LBW while no link amid hair Hg & PTB	Baldewsingh et al. (44)
25	Total hair mercury (HHg) level as a pointer of fish usage & MeHg exposure	Birth weight considerably different among groups but not exhibit a consistent pattern with fish usage	Marques et al. (63)
26	Blood Hg in pregnant women on birth outcomes & compared with those who ate fish or not	No links amid maternal blood Hg & head circumference, birth weight, or crown-heel length & PTB. When compared into fish-eaters or not, no relations except a negative link with birthweight in non-fish-eaters. Moderate Hg level not linked with anthropometric variable, LBW or PTB risk. Fish usage may be protective	Taylor et al. (64)
27	Moderate fish intakes	Linked with improvements in metabolic health of children, while high maternal Hg exposure linked with hostile metabolic profile	Stratakis et al. (67)
28	Methyl mercury exposure from fish & sea mammals' consumption	Inorganic Hg in aquatic sediments methylated by microorganisms & stored in aquatic food. Fish users do not reveal hostile effects. Even, some tests show beneficial impacts	Clarkson and Strain (65)
29	Reviewed data on maternal exposure to MeHg & health of fetuses, neonates, children	Prenatal exposure to MeHg linked with LBW, & negative link with birth length. Hostile effect on anthropometric variables, cognitive or physical growth	Saavedra et al. (66)

LBW: Low birth weight, PTB: Pre-term birth, MeHg: Methyl mercury

μg/L and serum concentrations of CB-153 were in the range of 37 to 1,460 ng/g of lipid. No relationship was found between MeHg and any of the male-related endpoints examined. These authors noted that men with lower MeHg and higher CB-153 levels had slightly, but insignificantly, higher DNA fragmentation index and more Y-chromosome carrying sperms compared with men with lower levels of both compounds (22).

It has been reported that men consuming predatory fish had higher blood mercury concentration, which was related with poorer sperm count with normal morphology (23). Thus, consumption of predatory fish might be a crucial risk factor for deterioration of semen quality (23). In addition, Mínguez-Alarcón et al. (24) correlated semen quality with hair mercury concentration in males. They reported that the median hair mercury concentration was 0.72 ppm (range 0.03 to 8.01ppm) and 30% of their subjects had hair mercury concentrations >1 ppm. Hair mercury concentration was positively correlated with sperm concentration, count, and progressive motility.

Furthermore, men in the highest quartile of mercury hair concentration had 50, 46 and 31% more sperm concentration, count, and progressive motility, respectively, compared to men with the lowest mercury quartile. These relationships were stronger in men whose fish consumption was above the study population median level. Seminal volume and sperm morphology were not related to hair mercury levels. These data showed that occurrence of MeHg exposure through fish consumption may be a significant dietary source of mercury exposure, amongst other heavy metals, that may have an impact on semen quality. It was suggested that additional research is required to elucidate the complex relationship between Hg exposure through fish consumption, and impacts on male reproduction (24). Moreover, processed meat consumption had a negative impact on sperm morphology, while fish consumption was connected positively with sperm count and normal morphology. Likewise, consumption of fish in place of other meats, mostly processed red meats, might have a beneficial effect on semen quality. Thus, the role of nutrition (fish intake) might play some role in prevention of deterioration of semen quality, even though consumption of fish has been linked to mercury exposure in some studies (25). Mercury levels in urine and ejaculate were measured in the partners of women undergoing infertility treatment who were also assessed for the number of dental amalgam fillings. No positive association was identified in regard of partner urinary mercury *concentration* and *semen* quality. Similarly, no association was found between fertility index and the quantity of dental amalgam fillings (26).

Both the experimental and human studies with higher exposure to certain metals usually support a negative effect of some heavy metals, especially cadmium, mercury, lead, and arsenic, on human reproductive outcomes, while data on the impact of lower, environmentally-realistic exposure to these metals on male reproduction are limited. The effects of low-level exposure were strongest for lead, cadmium, and mercury, and to a lesser extent for arsenic (27). Further, it has been reported that moderate- to low-level lead exposure worsens certain reproductive parameters in humans, and cadmium exposure reduces serum testosterone levels and prostate function. Despite the negative effects of manganese, mercury, arsenic, and chromium on semen quality parameters, there is less evidence concerning the effect of these contaminants on serum hormone changes (28). Based upon the data available, there appears to be relatively good evidence of the adverse impact of mercury on human male reproduction, especially semen quality, but more research is required on

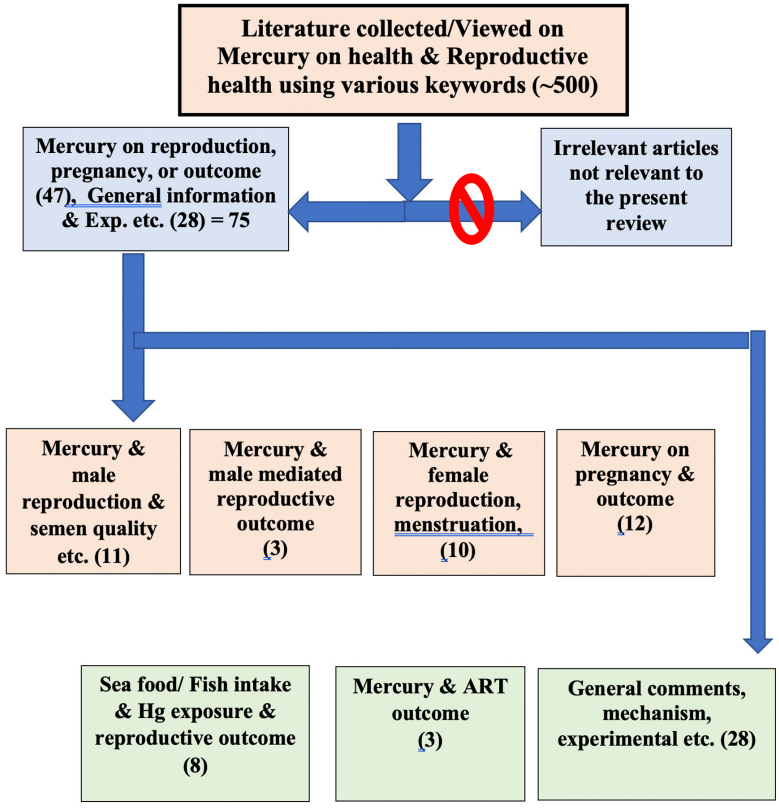

Figure 1. Flow diagram of literature collection on mercury and reproduction or outcome

ART: Assisted reproductive

the role of consumption of fish with regards to semen quality, despite the known association between fish consumption and increased retained mercury levels.

Mercury and Male-mediated Reproductive and Pregnancy Outcomes

There is relatively good evidence of the effects of mercury exposure on male reproductive function and reproductive organs. This may also impact male-mediated pregnancy outcome. The association between male workers exposed to elemental mercury and reproductive outcomes was investigated. All the study subjects worked for a minimum period of four months at a plant using elemental mercury. No links were reported between mercury exposure and diminished fertility, increased rates of major birth deformities or serious childhood illness in offspring but a limitation of this study was the lack of long-term recall of data concerning reproductive outcomes (29). Another study reported an association between male worker exposure to mercury vapor and rates of SAb in partners. There was a correlation between urinary mercury levels in the fathers' urine prior to pregnancy and SAb rate. Mercury concentrations at or above 50 μg/L of urine increased the risk of SAb two-fold (30). A review also stated that paternal exposure to lead or mercury might be related to the risk of SAb (31). More data are needed on the effect of mercury on human male reproduction or male mediated impact on pregnancy and outcome.

Impact of Mercury Exposure on Female Reproduction

Several studies are available concerning the adverse impact of mercury exposure on female reproductive health, on pregnancy and outcome in various animal species (32-36). Some clinical reports have been published about mercury exposure and female reproduction, as well as on pregnancy or outcome. A study in female dentists and dental assistants, who have a high risk of occupational exposure to mercury in dental amalgam, had significantly higher total hair mercury levels (TMLs). There was a notable, positive correlation between hair TMLs and the incidence of reproductive failure. Furthermore, there was a significant correlation between scalp hair TMLs and the manifestation of menstrual cycle illnesses (37). The effect of exposure to elemental mercury, used in gold mining, on menstrual cycle and miscarriages was evaluated among female inhabitants of gold mining areas in Colombia. A putative association was reported between elemental mercury exposure and the occurrence of menstrual cycles disorders, but no association was found with miscarriage (38). Similarly, the relationship between menstrual disorders in Tanzanian women involved in artisanal and small-scale gold mining (ASGM) was examined. This study reported that women workers exposed to mercury had a higher risk of menstrual disorders (39). Pregnant dental staff have been reported to be a greater risk of developing SAb and pre-eclampsia and giving birth to babies smaller for gestational age and it was suggested that this may be related to oxidative stress induced by mercury (40).

The reproductive risks of low-level exposure to metallic mercury in female workers was investigated and it was noted that longer duration of exposure caused dysmenorrhoea, and the incidence was dose dependent. At a mercury concentration over 0.06 mg/m^3, occurrence of hypomenorrhea significantly increased, while at a concentration <0.06 mg/m^3, menstrual cycles, flow quantity and length of flow did not alter significantly. Differences in occurrences of preterm delivery, SAb, fetal demise, still birth, and difficulties in pregnancy amid the group exposed to 0.06-0.1 mg/m^3 of mercury compared to the control group were not significant (41). Later, a retrospective study in female workers exposed to mercury vapor and non-exposed workers in food processing units showed the mercury level in the air of workplace was from 0.001-0.200 mg/m^3. The manifestation of abdominal pain and dysmenorrhea was significantly greater in the exposed workers (42). Furthermore, a meta-analysis showed that work-related mercury exposure could led to dysfunction in the menstrual period, menstrual cycle length, menstrual blood quantity, and dysmenorrhea among female workers and caused adverse reproductive outcomes, such as pregnancy-induced hypertension, stillbirth, low birth weights (LBW) and birth defects (43). A study assessed the association between mercury exposure, LBW (<2,500 g) and occurrence of preterm birth (PTB). About 15.7% of subjects delivered PTBs and 8.1% subjects delivered a LBW child. Lower mercury exposure, as in the group with the lowest tertile of hair mercury (<2.34 μg/g), was significantly related with LBW; this relationship was independent of maternal age, ethnicity, household income, and village location, while no relationship was found between hair mercury concentration and PTB (44).

In a very recent *in utero* mercury exposure study, prenatal exposure to high doses of mercury was related with an increased risk of precocious puberty, which was reinforced by concomitant maternal impaired cardiometabolic conditions and worse birth outcomes. The maximum risk of precocious puberty was noted amongst children who had poorer birth outcomes and their mothers had high erythrocyte mercury levels, together with impaired cardiometabolic conditions (45). Furthermore, a report indicated that mercury concentration in blood was significantly lower in postmenopausal women when compared to premenopausal women (46). These studies suggest that mercury exposure

related to menstruation dysfunction should be investigated further as there is uncertainty over the relationship between mercury exposure and the changes in age at menarche, puberty, and menopause.

Impact of Mercury Exposure on Pregnancy and Outcome

Some reports are available on maternal occupational/environmental exposure to mercury on pregnancy and outcome. Recently, a notable negative association between blood mercury concentration in the first and second trimesters and low birth weight (LBW) babies was observed (47). These authors suggested that pregnant and reproductive age women must avoid mercury exposure, even at low doses, as it has a potential severe negative effect on fetal development. Lee et al. (48) analyzed the relationship between prenatal mercury exposure birth weight and the influence of mercury exposure on *GST* polymorphisms. The geometric average concentration of mercury in the mothers' blood in late gestation and cord blood were 3.30 and 5.53 μg/L, respectively. For mothers with the *GSTT1* null genotype, a higher mercury level in maternal blood in late pregnancy was related with an elevated risk of LBW. For mothers having *GSTM1* and *GSTT1* null genotype, maternal and cord blood mercury was related to LBW. This suggested that mercury interaction with *GSTT1* and *GSTM1* polymorphisms might have some role in lowering birth weight (48). Furthermore, the effect of mercury exposure with neonatal auxology, placental weight, and gestational duration length in women exposed to mercury was assessed in terms of dietary seafood and it was found that prenatal mercury exposure may be associated with lower placental weight and poorer fetal progression (49).

A few reports on the effect of workplace mercury exposure while preparing and implanting of dental amalgam and pregnancy outcome are available. Brodsky et al. (50) collected the reproductive history of dental professionals, both male and female and including dentists and dental assistants._Their data showed no difference in the rates of SAb or congenital deformities in the children of both men and women when comparing those exposed to low versus high doses of mercury in a dental setting (50). In addition, the association between mercury exposure through dental amalgam fillings in pregnancy and birth outcome was investigated using logistic regression modeling, with variables including mother's age, body mass index, parity, education, smoking and alcohol intake during pregnancy. No significant relationship was found between the number of amalgam fillings and early or late preterm delivery, LBW, malformation, or stillbirth (51). There is some suggestion that exposure to electromagnetic fields may increase the discharge of mercury from dental amalgam and this led to the suggestion that pregnant women with amalgam fillings should avoid exposure to electromagnetic fields to minimize the toxic impact of mercury on their fetus (52). Furthermore, no robust correlation or constant dose-response association was found between exposure to chemical agents, including mercury amalgam, acrylate compounds, solvents and disinfectants, and the risk of miscarriage among dental workers. A slightly increased hazard of miscarriage was noted with acrylate compounds, solvents, mercury amalgam, and disinfectants (53). In addition, women with higher work-place mercury exposure were reported to be less fertile than non-exposed women. The fecundity, defined as the possibility of conception at each menstrual cycle, of women working in dental practice who prepared ≥30 amalgams per week and who had five or above poor mercury hygiene issues have 63% fecundity compared to unexposed women after controlling for covariates (54).

Inorganic mercury vapor exposure and reproductive outcomes were studied and a higher occurrence of contrary reproductive outcomes, particularly congenital anomalies, was reported amongst women who were exposed to inorganic mercury at or considerably lower than 0.6 mg/m^3, while no significant alterations in the miscarriage or stillbirth rates were observed between exposed and control groups (55). A higher miscarriages and stillbirth rate was observed in women who were exposed to five different heavy metals including mercury, lead, arsenic, chromium, and cadmium (56). Recently, a relationship between prenatal maternal mercury and arsenic exposure and adverse birth consequences was examined among ASGM subjects in Tanzania. In ASGM zones, the relative risk of poorer birth outcome was elevated with increasing total arsenic and total mercury exposure. Occurrence of SAb, stillbirth and PTB were significantly linked with elevated total arsenic levels, whereas elevated total mercury level was significantly related with stillbirth and congenital anomalies (57).

In addition, seafood consumption during pregnancy has been positively related with birth weight, while exposure to mercury was negatively linked with birth weight. Thus, seafood usage in pregnancy need not be avoided, but more data on the specific mercury exposure limits is needed to clarify at what level of mercury exposure poses adverse risk and reduce the advantages of seafood usage (58). However, maternal seafood consumption was linked with significantly higher levels of mercury. There was no relationship between mercury concentration and fetal growth, with the exception of a negative relationship with the biparietal diameter in offspring. A positive relationship was found between seafood consumption and fetal progress in women with higher BMIs, which remained after adjustment for mercury level. Nevertheless, seafood consumption was related to mercury contamination, but the contamination was

low. No consistent relationship was found between mercury level and fetal growth (59). Furthermore, blood MeHg level was significantly increased in infertile women compared to pregnant women and persistent with fish usage frequency. Compared to the reference blood MeHg concentration of <5.8 μg/L, the higher blood MeHg concentration (≥5.8 μg/L) found in infertile women was related to a 3.35 and 4.42-folds of risk associated with fish consumption of 1-2 meals/week or >3 meals/week, respectively (60). Moreover, there was no evidence of neonatal impairment related with mercury exposure due to consumption of fish during pregnancy and women should be confident that consuming fish during pregnancy is advantageous for their unborn child (61). These studies highlighted the positive role of fish consumption on reproductive outcome but that there is a risk of mercury exposure, depending on the source of dietary fish, which may impair fetal development. Hence, both risk and benefit should be considered with the consumption of fish in pregnancy.

Furthermore, pregnant mothers are usually given two different messages about the consumption of fish: 1) unsaturated fatty acids and protein consumption through fish are assumed to be advantageous; and 2) pollutants, such as MeHg, found in some fish might be hazardous. Fish intake was positively linked with hair mercury levels. Compared to women who delivered at term, women those delivered <35 weeks of gestation were more likely to have hair mercury levels ≥90th percentile (0.55 microg/g), after adjusting for maternal features and fish consumption (62). Furthermore, birth weights may be a good indicator of maternal health matters connected with nutrition and environmental pollutants. Hair mercury (HHg) level was examined as a marker of fish consumption and exposure to MeHg in mothers or newborns. Birth weight varied considerably among different groups and there was no consistent pattern with fish consumption nor HHg (63). The impact of blood mercury concentration in pregnant women on birth outcomes was evaluated and stratified by mothers who ate or did not eat fish. No significant relationship was found between maternal blood mercury and neonatal head circumference, birthweight, crown-heel length when analyzed by adjusted linear regression. Similarly, no increased odds of LBW or preterm delivery was observed. When this model was assessed by reference to the presence or absence of fish consumption, there was only a significant negative relationship for birthweight in non-fish-eaters. Moderate mercury level exposure during pregnancy was not connected with changes in neonatal anthropometry, or the odds of LBW or PTB and fish consumption might actually have a protective role on birthweight (64).

Clarkson and Strain (65) reported that a major source of human exposure to MeHg occurred usually from eating fish and sea mammals. Inorganic mercury is present in aquatic sediments, where it is methylated by microorganisms, and then enters the aquatic food chain. However, epidemiological studies among fish consumers from the Seychelles Islands have shown no negative effects. In contrast, the results of the few tests that were conducted in prenatally exposed offspring indicate advantageous outcomes (65). Very recently, the clinical consequences of MeHg exposure during pregnancy on fetuses, neonates, and child health were reviewed. It was reported that MeHg exposure during prenatal development was related with LBW, and one study described a negative relationship with birth length. The data are evidence of a clear negative effect of maternal MeHg exposure on anthropometric variables, cognitive or physical growth in children. Furthermore, mercury poisoning might sometimes be lessened by vital nutrients present in the maternal diet (66). A recent report indicated that moderate fish consumption, consistent with current health recommendations during pregnancy, was related to improvement in the metabolic health of offspring, while higher maternal mercury exposure was related with an unfavorable metabolic profile in children (67). Thus, an appropriate balance should be maintained in consumption of fish during pregnancy.

Finally, there are some data on the effects of mercury exposure and assisted reproductive outcome. The data on toxic agents in follicular fluid (FF) and in vitro fertilization outcomes indicated poorer IVF outcomes in couples exposed to certain reproductive toxins compared to couples not subjected to such exposure (68). HHg concentrations were not connected to ovarian stimulation outcomes (highest estradiol concentration, total or mature oocyte yields), embryo quality, fertilization frequency, clinical pregnancy, or live birth rate after IVF procedures (69). Very recently, it was reported that FF mercury level was related with a lower probability of biochemical pregnancy or live birth rate, and higher FF lead concentration was related to a lower probability of live birth. These data suggest that avoidance of exposure to mercury and lead might lead to improved IVF success rates (70). However, more data are required on this aspect. Hanna et al. (71) investigated methylation changes related to exposure to pollutants in women going through IVF and reported that DNA methylation was altered at several CpG sites related to exposure to mercury, lead and bisphenol A (BPA), offering candidates to be examined by utilizing a larger sample size.

The data on impact of mercury exposure on reproduction clearly suggests that mercury exposure, particularly exposure to MeHg, has deleterious effects on human reproduction, especially semen quality, menstruation, and pregnancy outcome. Additional data are needed on other reproductive endpoints to draw further inferences. A recent review based upon experimental data showed that testis and ovary are specifically sensitive to lead, cadmium, and mercury. In ovaries, toxic effects of lead, cadmium, or mercury diminished

follicular growth, led to the manifestation of follicular atresia, deterioration of the corpus luteum, and alterations in menstrual cycle. In testes, exposure to these heavy metals included changes in seminiferous tubules, testicular stroma, reduction of sperm count, viability and motility, and increased aberrant sperm morphology (72). Mercury exposure during pregnancy has been reported to be linked with pregnancy problems and impaired development in infants (73). Moreover, mercury exposure may have a substantial impact on several stages of reproduction, from prior to conception until maturation of organs and endocrine systems or even the healthy development of children (74). Tan et al. (75) reviewed the endocrine properties of mercury in humans and wildlife and reported five main mercury-related mechanisms within the endocrine system: 1) accumulation in the endocrine system; 2) precise cytotoxicity in endocrine tissues; 3) variations in hormone concentrations; 4) interactions with sex hormones; and 5) up-regulation or down-regulation of enzymes in the steroidogenesis pathway.

Conclusion

Based upon the data available, there is strong evidence that mercury exposure may have a deleterious impact on reproductive health of both sexes. Therefore, an adequate preventive strategy should be adopted to stop or significantly reduce mercury exposure for all populations. There is also a need for more data concerning various aspects of human reproduction and mercury exposure to substantiate the available data.

Acknowledgement

No financial assistance was received for the compilation of this review. One of the authors (SK) is thankful to various funding agencies i.e., Indian Council of Medical Research, Department of Science & Technology, and Department of Biotechnology, New Delhi, India for providing numerous *ad hoc* research grants on various aspect of reproductive health.

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept: S.K.; Design: S.K.; Literature Search: S.K., A.S., S.S.; Writing: S.K., A.S., S.S.*

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Open, laparoscopic and robotic myomectomies - comparison of outcomes

To the Editor,

We read the article entitled: "Comparison of perioperative outcomes among robot-assisted, conventional laparoscopic, and abdominal/open myomectomies" by Özbaşlı and Güngör (1) with a great deal of interest.

Myomectomy is the gold standard approach for women desiring fertility preservation. We would like to ask the authors a number of questions. These are:

In your cohort do you have any data regarding the pregnancy rates, spontaneous abortion rates, live birth rates and any comparison between the three groups?

What about the mode of deliveries and possible obstetric complications e.g. uterine rupture in such women?

Is there any experience with single-port approach in the minimal invasive groups? And how do you solve ergonomic issues?

Was there any difference found in conversion rates between laparoscopic and robotic groups? Have you identified any difference in blood loss based on the number of myomas excised in the different groups?

Since 2014, the Food and Drug Administration issued a warning against power morcellation to avoid tumor dissemination in the unexpected scenario of leiomyosarcomas (2). Are the authors using a power morcellator within an endoscopic bag?

As the incidence of unexpected leiomyosarcoma ranges from 1 in 225 to 1 in 580 (2), did you have any occult findings of leiomyosarcoma in your cohort?

Once again, we would like to thank the authors for their well-presented article.

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Received: 25 January, 2022 **Accepted:** 10 May, 2022

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2022.2022-1-7

Author's Response

Dear Editor,

We would like to thank the author of the letter commenting on our article, "Comparison of perioperative outcomes among robot-assisted, conventional laparoscopic, and abdominal/open myomectomies" and the opportunity to respond to their concerns.

The author of the letter asked about the data regarding pregnancy rates, abortion rates and possible obstetric complications. Although pregnancy rates and possible obstetric complications are some of the main concerns in reproductive age patients, in the discussion section of our study, it was stated that some of our limitations were retrospective design and the failure to compare long-term outcomes such as pregnancy rates (1,2). The long-term outcomes such as abortion rates, live birth rates and possible obstetric complications were not compared in our study. As the author pointed out and as it is stated in our study, more prospective, randomized trials with long-term outcomes such as pregnancy rates, possible obstetric complications are needed.

Although single port myomectomy is performed in our clinic, we excluded the cases with laparoscopic or robotic single port myomectomy in this study.

In Iavazzo et al.'s (3) meta-analysis, cases of conversion to open was higher in laparoscopy group than robotic group. But in our study, conversion to laparotomy was not observed either in the robotic myomectomy or laparoscopic myomectomy groups.

Although the number of myomas excised was significantly higher in the open myomectomy group, no significant difference was observed among groups regarding blood loss.

In Taylan et al.'s (4) review, the risk of unanticipated uterine sarcoma in patients undergoing a uterine morcellation was 0.22%. In this review, a retrospective study that included 40,000 patients the prevalence of cancer in women who underwent myomectomy with power morcellation was 0.09%. Although the overall risk of occult malignancy appears to be very low, as the author stated power morcellation should be used with caution especially in older patients. In regards to avoiding tumor dissemination, in most of our cases contained morcellation of myomas using a power morcellator within a tissue morcellation bag (MorSafe®) was performed. As it is stated in our study, pathology results were not significantly different among the groups and no leiomyosarcoma was detected among groups.

We hope this provides some clarification for the author of the letter.

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Intra-gestational agents for management of cesarean scar pregnancy: Is the long wait and stress worth it?

To the Editor,

Cesarean scar pregnancy (CSP) is a type of an intrauterine ectopic where the pregnancy implants at a deficient or an improperly healed area, specifically at a previous cesarean section scar. Thinning/absence of decidua at the scar site facilitates microinvasion of chorionic villi into the myometrium, bearing a great degree of similarity with placenta accreta spectrum (1). Although rare, the incidence of CSP is increasing with the increase in the number of cesarean sections worldwide (1). Incidence ranges from 1:1800 to 1:2216 of all pregnancies (2). At presentation, patients may be asymptomatic, have painless vaginal bleeding or may present with hemorrhage, which may either be external or internal (because of uterine rupture), with or without shock.

We describe a clinical series of six patients with CSP that underwent uterus sparing management by intra-gestation injection of methotrexate/potassium chloride solution (KCL) along with systemic methotrexate. Table 1 shows clinical and management details of patients with CSP.

In this series, one patient was asymptomatic, one presented with painless vaginal bleeding, three were referred to us with incomplete evacuation and one patient with bleeding for over two weeks after self-consumption of an over-the-counter pill for medical abortion. The mean period of gestation at diagnosis was 9 weeks and 3 days. Median beta-human chorionic gonadotropin (β-hCG) level at admission was 35390 IU/L. Diagnosis of CSP was made by us, using ultrasonographic criteria given by Timor-Tritsch et al. (3) in 2012. Two patients presented with live pregnancy at the scar site (Video 1, 2). In four patients, all of whom were referred due to an incomplete evacuation procedure/incomplete medical abortion, only adherent gestational tissue was visible embedded at the scar site. It appeared as a hetero-echoic mass in the lower uterine segment with increased vascularity. Interestingly, we found that in all but one of these patients it was easier to visualise and treat using a transabdominal approach as uteri were pulled up, acutely anteverted and stuck to the anterior abdominal wall.

The dose of methotrexate was calculated as 50 mg per kilogram of body surface area. Half of the dose was administered into the lesion, under transabdominal ultrasound guidance using a 20 G spinal needle and the other half was administered intravenously. In one patient with fetal cardiac activity, intracardiac KCL was administered followed by full dose methotrexate intravenously. Patients were discharged after 48 hours and kept on fortnightly follow up. We successfully managed all patients, with the exception of patient 6, who had to undergo emergency lifesaving hysterectomy due to heavy bleeding 23 days post procedure (Table 1). Figure 1a shows a hetero-echoic mass of adherent placental tissue at a previous scar site with vascularity (patient 3). Follow-up images after surgically-assisted medical management of Patient 3 at 1 month (Figure 1c), 2 months (Figure 1d) and complete resolution at 4 months (Figure 1e). Mean time for normalization of ß-hCG levels was 25 days. Mean time for disappearance of lesion on ultrasound was 96 days.

In CSP, securing a timely and a correct diagnosis is always challenging and there is also uncertainty and dilemma regarding the most suitable mode of management. The Society of Maternal and Fetal Medicine (1), recommends operative resection (laparoscopic/transvaginal approach), vacuum aspiration under ultrasound guidance or intra-gestational methotrexate. Uterine artery embolization may be considered as an adjunct to these management strategies to decrease bleeding. Expectant management of CSP is associated with a high risk of hysterectomy and hemorrhage due to morbidly adherent placenta. Hysterectomy of gravid uterus may be considered if the patient has completed her family and does not choose other management options.

It is imperative to counsel each patient in detail about the pros and cons of all management options available before choosing

Received: 21 January, 2022 **Accepted:** 10 May, 2022

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2022.2022-1-3

Table 1. Cesarean scar pregnancy: clinical and management details

Patient no	Age (in years)	Parity	Period of gestation at diagnosis	Diagnosis/referred with	ß-hCG at diagnosis (IU/L)	Procedure	Time for normalization of ß-hCG (days)	Time for disappearance of lesion on ultrasound (days)	Outcome
1	34	G3 P2L2 previous 2 LSCS	10 weeks	Live CSP (Video 1)	120000	Intracardiac KCL + i.v. methotrexate	28	180	Intermittent spotting for 3 months Complete resolution of CSP
2	26	G2 P1L1 previous 1 LSCS	9 weeks	Live CSP with painless vaginal bleeding (Video 2)	104000	Suction & evacuation followed by torrential bleeding; products adherent: bleeding controlled by tamponade by Foley tamponade Intralesional + i.v. methotrexate done next day	21	56	Uneventful
3	29	G2 P1L1 previous 1 LSCS	12 week 3 days	Referred in view of incomplete suction and evacuation procedure CSP was diagnosed by visualization of 4.9x4.9 cm adherent gestational tissue hetero-echoic mass was seen at the site of previous scar with profuse vascularity (Figure 1a, b).	55000	Intralesional + i.v methotrexate	21	120	Figure 1c, d, e are follow up images at 1, 2 and 4 months respectively Final outcome uneventful
4	27	G3 P1L1A1 previous 1 LSCS	9 week 5 days	Referred in view of incomplete suction and evacuation procedure	4466	Intralesional + i.v methotrexate	23	56	Uneventful
5	24	G3 P2L2 previous 2 LSCS	7 week 1 day	Referred in view of incomplete suction and evacuation procedure	15780	Intralesional + i.v methotrexate	32	68	Uneventful

Table 1. Continued

Patient no	Age (in years)	Parity	Period of gestation at diagnosis	Diagnosis/referred with	ß-hCG at diagnosis (IU/L)	Procedure	Time for normalization of ß-hCG (days)	Time for disappearance of lesion on ultrasound (days)	Outcome
6	32	G3 P2L2 previous 2 LSCS	8 weeks	Self-intake of MTP pill with bleeding per vaginum	15000	Intralesional + i.v methotrexate	Admitted in emergency, 23 days after with hemorrhagic shock. USG showed hypervascular ectopic mass at CSP site. Managed with resuscitation and immediate laparotomy. Atempt at conservative surgery failed despite preemptive bilateral uterine artery ligation, bed of scar pregnancy continued to bleed despite removal of all products and lifesaving hysterectomy had to be done. Patient subsequently developed sepsis and was in intensive care for 3 days. She recovered and was discharged in stable condition after 10 days of surgery		

i.v: Intravenous, G: Gravida, P: Parity, LSCS: Lower segment caesarean section, MTP: Medical termination of pregnancy, CSP: Cesarean scar pregnancy, USG: Ultrasonography, β-hCG: Beta-human chorionic gonadotropin

Figure 1. (a, b) Hetero-echoic mass of adherent placental tissue at previous scar site with vascularity [patient 3], (c-e) follow-up images after surgically assisted medical management of patient 3 at 1 month (c), 2 months (d)

this form of treatment. Torrential bleeding may also occur as a complication. However the stress and anxiety associated with this long term follow-up, which may last for weeks to months, may be acceptable if this minimally invasive method can preserve the uterus while avoiding a major surgical intervention.

Video 1. Live caesarean scar ectopic at 10 weeks period of gestation [patient 1]

https://www.doi.org/10.4274/jtgga.galenos.2022.2022-1-3.video1

Video 2. Live caesarean scar ectopic at 9 weeks period of gestation [patient 2]

https://www.doi.org/10.4274/jtgga.galenos.2022.2022-1-3.video2

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Serum neuregulin-4 levels in healthy and preeclamptic pregnancies: correspondence

To the Editor,

We would like to share ideas on the publication "Comparison of maternal serum neuregulin-4 (NRG-4) levels in healthy and preeclamptic pregnancies". Yakut et al. (1) noted that "No association was found between NRG-4 concentrations and preeclampsia (PE) patients, regardless of severity of PE, compared to healthy pregnancies. Future longitudinal studies are needed to confirm this lack of association in PE (1)". We agree that the maternal serum NRG-4 levels might or might not be associated with severity/existence of PE. Further studies are required. A longitudinal study might be helpful but it will be necessary to control confounding factors. Without controlling, any additional data might still be unreliable. The serum NRG-4 level may be affected by several factors. Some silent personal illnesses, such as liver disease and abnormal glucose metabolism, might affect the NRG-4 levels (2,3). Those background medical conditions should be recognized in interpreting the results.

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Received: 10 March, 2022 **Accepted:** 08 July, 2022

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2022.2022-3-4

Author's Response

Neuregulin-4 (NRG-4) is mainly produced by brown adipose tissue and plays a role as a signaling protein in cell-cell interactions (1). Studies have reported alterations in NRG-4 levels in lipogenesis, inflammatory processes, and energy metabolism (2,3). In the literature, diabetes mellitus (DM), non-alcoholic fatty liver disease, coronary artery disease, and obesity-related diseases have been shown to be associated with NRG-4 (4-10). In view of this information, our study evaluated the desired blood parameters (hepatitis panel, alanine transaminase, aspartate transaminase, biluribin level, gamma-glutamyl transferase, international normalized ratio, bilirubin level, glucose, etc.) to create a homogeneous study group, and an attempt was made to obtain as pure a group as possible. In addition, oral glucose tolerance tests are performed at 24-28 weeks of gestation and fasting glucose levels are checked in the first trimester. Pregnant women with a history of risk factors (e.g., macrosomic baby in history, gestational diabetes in previous pregnancy, morbid obesity) are screened for glucose metabolism disorders in the first trimester. We perform basal cardiac examinations in patients who describe symptoms of heart disease or who are found to be at risk for heart disease in their medical history (e.g., metabolic syndrome) and ask to be examined in the cardiology clinic, if necessary.

Therefore, exclusion criteria included all patients with chronic systemic disease, autoimmune disease, chronic drug use, multiple pregnancy, fetal congenital anomaly, and pregnancy complication, such as DM, chorioamnionitis, and premature preterm rupture of pregnancy. In addition to the assessments we made in our study to more clearly identify some silent personal diseases, advanced imaging techniques, large blood parameters for various diseases, or invasive procedures can be planned, and a more homogeneous study group with broader longitudinal studies can be formed. However, because we did not identify any additional findings that would be indicative during the baseline evaluation, our cases were not referred for additional investigations and invasive procedures.

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The Manchester procedure combined with laparoscopic sacrohysteropexy by retroperitoneal tunneling

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Abstract

This video will demonstrate a minimally invasive technique, in which the Manchester procedure was combined with laparoscopic sacrohysteropexy by retroperitoneal tunneling in patients with uterine prolapse and cervical elongation who wished to preserve the uterus. The principle steps and techniques to complete the operation are dictated in the video. The prolapse surgery was performed uneventfully, and the uterus was restored to its anatomical position. During the two years of follow-up, there were no complications from the prolapse or mesh-related events. No prolapse recurrence was observed. This technique facilitates uterine-sparing surgery, results in less bleeding and shorter operative time, and we believe that it may reduce the recurrence of prolapse due to the elongation of the cervix. (J Turk Ger Gynecol Assoc 2022; 23: 219-21)

Keywords: Cervical elongation, Manchester procedure, sacrohysteropexy

Received: 12 February, 2021 **Accepted:** 17 May, 2021

Introduction

Approximately half of advanced-stage uterine prolapse (46.1%) is accompanied by cervical elongation (1). There is an increased desire for uterine preservation in pelvic organ prolapse (POP) surgery, and cervical elongation can limit this possibility. Hysteropexy can result in less bleeding and take less time to complete, thus reducing anesthesia time. The risk of mesh erosion is five times lower in hysteropexy than in hysterectomy (2). However, the risk of reoperation is greater in hysteropexy (2). The technique described herein, which is a combination of two procedures, is recommended to the patient who desires to preserve the uterus, wants to minimize the risk of possible recurrence, and also has an active sexual life.

Materials and surgical technique

The 38-year-old patient was gravidity 3, parity 3 and had a grade-2 elongation of the uterine cervix (4) and stage-3 apical (uterine) prolapse. The Pelvic Organ Prolapse Quantification System (POP-Q) findings were as follows: Aa -1, Ba -2, C +2, D +2, Ap -2, Bp -3. The patient wished to preserve the uterus. (Supplementary Video S1).

Manchester procedure: The length of the cervix up to the sacrouterine ligament-uterus connection was measured as approximately 7-8 cm (Figure 1). A circular incision in the vagina was performed and ligated with bilateral cardinal ligaments (5). The extra-prolonged cervix was amputated (Figure 2), leaving 3-3.5 cm of the cervix intact. The vaginal mucosa was prepared in flap form, and the posterior vaginal mucosa was cut to the sacrouterine level.

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2021.2021.0029

Laparoscopic sacrohysteropexy: A 10 mm trocar was inserted into the abdomen by direct entry technique from the umbilicus, and a pneumoperitoneum was created. Two lateral ports were placed on bilateral lower quadrants, and one suprapubic trocar was placed in the same plane as one lateral trocar.

- Peritoneal incision: The location of the promontory was determined anatomically by laparoscopy, and an incision was performed on its peritoneum. The incision was enlarged caudally and distally. The loose connective tissue on the promontory was dissected and the anterior longitudinal ligament was uncovered.
- Sigmorectal colon suspension: The sigmorectal colon was suspended with a T-Lift device (Vectec™, France) for better visualization.
- Retroperitoneal tunneling: The remaining cervical tissue was held with a tenaculum. Tunneling was performed with ring forceps under the cervical tissue above the vaginal tissue retroperitoneally, from the point where the right sacrouterine ligament adheres the uterus to the right pelvic sidewall, under camera guidance. In order to avoid unintentional damage to adjacent anatomical structures, ring forceps with atraumatic and blunt ends were used. The vaginal mucosa was opened to the peritoneal incision, which was created earlier by laparoscopy extending to the cervix. The previously created peritoneal area reached the cervix and the vaginal mucosa was opened up to the incision previously prepared by laparoscopy.
- Preparation of the mesh: a 4x15 cm synthetic macroporous polypropylene mesh segment (Düzey Medikal, İstanbul, Turkey) was prepared and inserted through the umbilical trocar into the abdomen, and one side of the mesh was grasped by a vaginally-inserted forceps (Figure 3).
- Suturing of the mesh to the posterior cervix: Propilen® polypropylene synthetic, non-absorbable, monofilament, no: 1 suture (Doğsan, İstanbul, Turkey) was used.
- The Manchester procedure was completed with reattachment of the cardinal ligament, using Sturmdorf sutures.
- Tailoring the position of the uterus: The cervix was positioned at the -5, -6 level. Before fixing the mesh to the anterior longitudinal ligament, the cervix was confirmed to be at the -5, -6 level by hysterometry. After that measurement, the mesh was fixed to the ligament. We used five non-absorbable tackers to fix the mesh.
- Anchoring of the upper pole of the mesh to the anterior longitudinal ligament was accomplished with a 5 mm laparoscopic ProTack™ Fixation Device (Medtronic, Minneapolis, MN, USA).

The peritoneum was closed with a continuous running 2-0 absorbable suture line. The whole mesh was peritonized to avoid adhesion to or extrusion from the bowel. Reperitonization was performed with attention to the ureter. The T-Lift device was removed, and the ureter was visualized and inspected to ensure no damage had occurred, completing the process.

The procedure was completed successfully with a total operative time of 78 minutes and estimated blood loss of 180 mL. The uterine prolapse was corrected (postoperative POP-Q findings: Aa -1, Ba -3, C -5, TVL 7.5, Ap -2, Bp -3, D -7.5) (Figure 4). No intraoperative surgical complications were observed. On postoperative day 2, recovery had been uneventful and the patient was discharged from the hospital. During two years of follow-up, no recurrence, complications of mesh exposure, de novo stress urinary incontinence or bowel obstruction have occurred. The patient had reported that she had no plan for pregnancy before surgery.

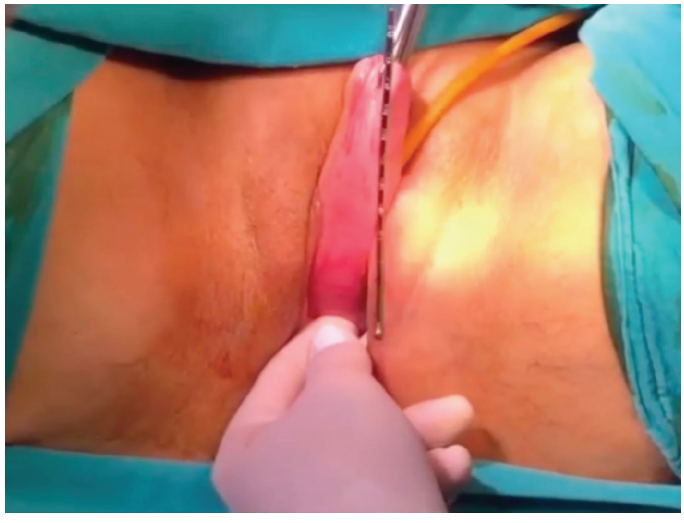

Figure 1. The measurement of the length of the cervix

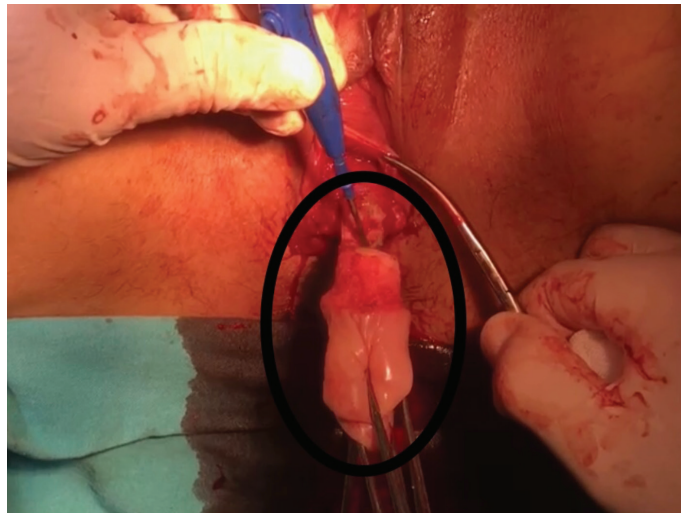

Figure 2. The view of elongated cervix at the time of incision

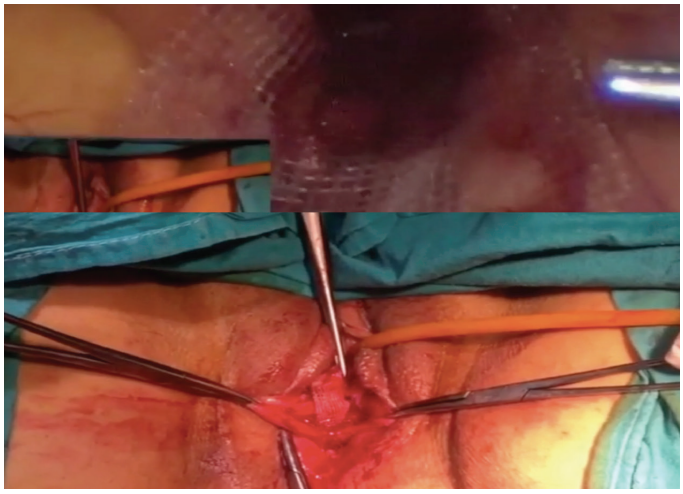

Figure 3. The appearance of the mesh by vaginal and abdominal route

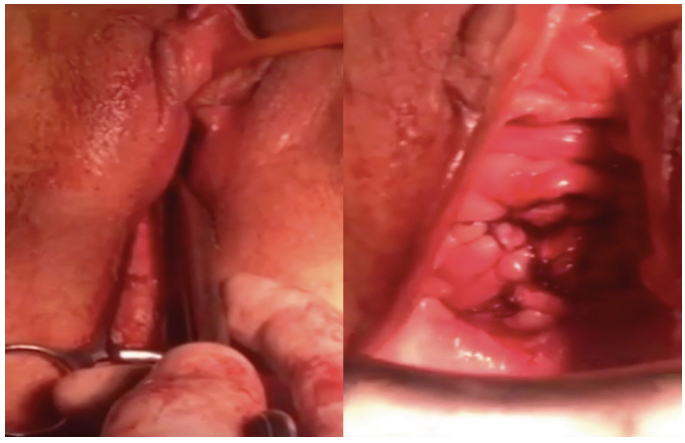

Figure 4. The depth of the cervix and the last view after operation

Supplementary Video S1. The Manchester procedure was combined with laparoscopic sacrohysteropexy

https://www.doi.org/10.4274/jtgga.galenos.2021.2021.0029.video1

Informed Consent: *Written informed consent was obtained from the patient for publication of this video article and any accompanying images*

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

Financial Disclosure: *The authors declared that this study received no financial support.*

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CONGRESS CALENDER

INTERNATIONAL MEETINGS

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

September 16-18, 2022	32nd World Congress on Ultrasound in Obstetrics and Gynecology, Venue not announced yet
September 30-October 02, 2022	International Gynecologic Cancer Society (IGCS) 2022, Meeting, New York, NY, United States
October 02-05, 2022	ESGE 31st Annual Congress, Lisbon, Portugal
October 22-26, 2020	American Society for Reproductive Medicine (ASRM) 78th Annual Meeting, Anaheim, CA, United States
October 26-29, 2022	18th World Congress on Menopause, Lisbon, Portugal
November 24-26, 2022	The 30th World Congress on Controversies in Obstetrics Gynecology & Infertility (COGI), Amsterdam, The Netherlands
November 24-26, 2022	New European Surgical Academy (NESA) Days-2022, London, UK
November 30-December 04, 2022	The 51st American Association of Gynecologic Laparoscopists (AAGL) Global Congress on Minimally Invasive Gynecologic Surgery (MIGS), Denver, CO, United States

CONGRESS CALENDER

NATIONAL MEETINGS

(for detailed International Meeting please go website:
http://www.kongre2022.com)

September 08-11, 2022	3. Uluslararası KKTC Obstetri ve Jinekoloji Kongresi, Girne, KKTC
September 22-25, 2022	4. Obstetrik ve Jinekoloji Tartışmalı Konular Kongresi, Antalya, Türkiye
September 23-25, 2022	Pelvik Taban ve Kozmetik Jinekoloji Kongresi, İstanbul, Türkiye
September 30-October 02, 2022	10. Ulusal Ürojinekoloji Kongresi, İstanbul, Türkiye
October 12-16, 2022	Türkiye Maternal Fetal Tıp ve Perinatoloji Derneği 13. Ulusal Kongresi, Antalya, Türkiye
November 02-06, 2022	IX. Üreme Tıbbı ve Cerrahisi Derneği Kongresi, Antalya, Türkiye
November 03-06, 2022	Uluslararası Jinekoloji ve Obstetri Kongresi, Muğla, Türkiye
November 10-13, 2022	10. Üreme Sağlığı ve İnfertilite Kongresi, TSRM 2022, Girne KKTC