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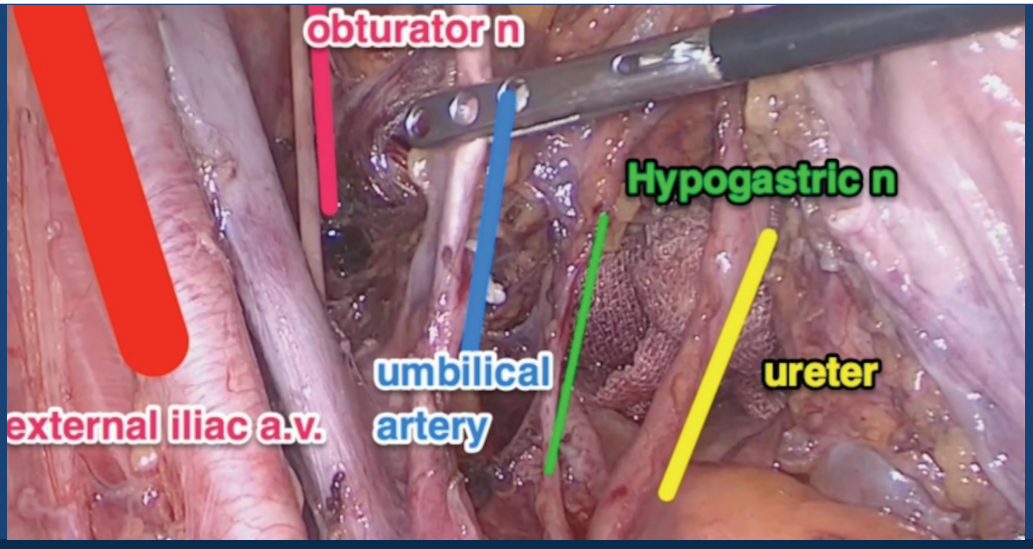

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Aims and Scope

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.

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PRISMA for preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/),

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

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All manuscripts should be accompanied by an abstract. A structured abstract is required with original articles and it should include the following subheadings: Objective, Material and Methods, Results and Conclusion. A structured abstract is not required with review articles. The abstract should be limited to 250 words for original articles and review articles.

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Editorial

Dear Colleagues,

I am delighted to introduce the third issue of the "Journal of the Turkish-German Gynecological Association (J Turk Ger Gynecol Assoc)" in the publishing year of 2021. This issue is consisted of eight articles and three reviews that we hope you will read with interest.

Sacrocolpopexy has become the gold standard treatment for pelvic organ prolapse. Rectopexy is indicated when posterior compartment prolapse is identified. Sacrocolpopexy with concomitant rectopexy is becoming increasingly more common for patients with multicompartmental disease. You will read an interesting study determining whether ventral mesh rectopexy at the time of sacrocolpopexy reduces the rate of future posterior wall prolapse.

Granulocyte-macrophage CSF (GM-CSF) is a multi-functional cytokine and is synthesized in the epithelial cells of the female reproductive tract, which is essential for modulating stress response genes, heat shock proteins, and apoptosis. You will read an article investigating the effects of adding GM-CSF to culture medium on embryological data and reproductive outcomes in patients with previous early embryonic developmental arrest.

Sentinel lymph node (SLN) mapping has been proposed as a less invasive technique used for assessment of lymph nodes in gynecologic cancers. You will get the occasion to read the current situation of SLN mapping in gynecologic cancers.

Dear Esteemed Readers,

Clarivate Analytics recently introduced its new metric, the Journal Indicator. By normalizing for different research fields and their widely varying rates of publication and citation, the Journal Citation Indicator (JCI) provides a single journal-level metric that can be easily interpreted and compared across disciplines. The Journal Citation Indicator can be calculated for all journals in the Web of Science Core Collection - including those that do not have a Journal Impact Factor (JIF) - and published in the 2021 JCR in June. In light of this information, we are delighted to state that J Turk Ger Gynecol Assoc's JCI is 0.37. It ranks 94th out of 122 indexed journals in SCI-E and ESCI in obstetrics & gynecology. In the upcoming period, our aim is to increase the journal's JCI rank.

Another innovation we would like to announce in this issue was to ensure that our articles are managed via the PoolText system. PoolText is a technology company spun out of MIT and the first marketplace connecting journal editors and authors. In addition, PoolText includes new artificial intelligence integrations that will contribute to the publishing ecosystem. PoolText checks different features of the articles, such as plagiarism, redaction, reference currency and accuracy, and structural integrity of the articles, with an integrative approach.

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Sincerely,

Prof. Cihat Ünlü, M.D.
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President of TGGF

Identification and characterization of endometrial carcinoma with tumor markers HE4 and CA125 in serum and endometrial tissue samples

Tatiana Cuesta-Guardiola[1], Alicia Quiros Carretero[2], Javier Martinez-Martinez[3], Yolanda Cuñarro-López[4], Augusto Pereira-Sánchez[5], Alfonso Fernández-Corona[1], Juan Antonio de Leon-Luis[4]

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Abstract

Objective: Diagnosis of endometrial cancer (EC) is made by biopsy sampling with pathological analysis, but it is extremely important to make an accurate diagnosis in order to plan the specific treatment. We hypothesized that human epididymis protein 4 (HE4) in endometrial tissue and in serum could be beneficial for a more precise diagnosis.

Material and Methods: This prospective study compared patients with EC against non- EC, matched through several variables. The inclusion criteria were: females older than 18 years who accepted to participate; who had never undergone surgery for other oncological pathologies (ovarian, colon, cervical carcinoma or uterine sarcoma); none of them had received preoperative chemo- or radio-therapy; and no participant had any severe renal or liver pathology. All had pre-surgery blood sampling and then underwent hysterectomy. Histopathological assessment of endometrial samples was made by a pathologist who compared normal histopathological staining with HE4-antibody staining.

Results: In total there were 34 cases and 35 controls recruited. There was poor correlation between tissue HE4 in patients with and without carcinoma. However, serum HE4 was significant for the diagnosis of endometrial carcinoma (median EC: 123.1 U, median NE: 64.67 U, p=0.002), although the carbohydrate antigen 125 level was not significant (p=0.208).

Conclusion: The findings concerning the utility of HE4 contrast with earlier reports. However, the conclusions for serum measurements are positive and suggest that the tumor marker HE4 seems to be able to diagnose EC. (J Turk Ger Gynecol Assoc 2021; 22: 161-7)

Keywords: CA125, diagnosis, endometrial cancer, HE4, tissue

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Introduction

Human epididymis protein 4 (HE4), also known as acid protein (WFDC2), was first identified in the epithelial cells of the epididymal duct and plays a role in natural immunity and in sperm maturity (1-3). In 2001, the FDA approved HE4 as a serum tumor marker of ovarian cancer. Uterus, fallopian tubes and ovaries derive from the urogenital crest and, in turn, the first two arise from the paramesonephric tissue. Therefore, they have similar embryological properties suggesting that they could be related.

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Currently, only serum carbohydrate antigen 125 (CA125) is being used as a biomarker in endometrial cancer (EC), although serum HE4 has shown good results (4). Some positive results have already been shown in meta-analysis assessment of serum HE4 in relation to EC (5), though the sensitivity was not sufficient to make a firm recommendation for its routine use. To date, there are few publications investigating the utility of HE4 in tissue samples (6). The published studies show an association between HE4 concentration and worse prognosis or adverse clinicopathological variables (7-9).

The primary objective of this study was to identify and characterize HE4 in endometrial tissue samples obtained from patients diagnosed with EC. Moreover, comparison was made between samples of endometrial tissue from non-EC patients with tissue samples from EC patients. Similar comparison was made between serum levels of HE4 from EC and non-EC cancer groups and between tissue and serum levels. Finally, comparison was made between HE4 staining or serum HE4 and several prognostic variables.

Material and Methods

This prospective study was a case and control, nested in a hospital-based cohort. Initial comparison was made between EC patients (cases) and healthy patients (controls). Each sampling case was matched with one control selected from patients undergoing hysterectomy for non-oncological reasons.

The study was conducted at a tertiary hospital in Spain, during the period July 2017 to April 2018. All the targeted patients (in both arms) fulfilled the following criteria: diagnosed with EC; older than 18 years; and wanted to participate in the research study and signed consent forms voluntarily. Exclusion criteria were the following: patients who underwent surgery for other oncological pathologies, whether for ovarian, colon, cervical carcinoma or uterine sarcoma; and none of them had received pre-operative chemo- or radio-therapy. In addition, no participant had severe renal or liver pathology. The inclusion and exclusion criteria for the control patients were exactly the same, except none had a diagnosis of cancer.

The study was performed in compliance with the medical Declaration of Helsinki. Participation was voluntary and would not affect the standard of medical care the patients received in any way. All participants would be fully anonymized. This study was approved by the Leon Clinical Research Ethics Committee (approval number: 17104).

The recruitment of patients took place when the diagnosis of EC was made following a previous endometrial biopsy. Furthermore, controls were females undergoing elective hysterectomy for non-oncological reasons. Every patient who fulfilled the criteria, and from whom we were able to request the preoperative test was selected. There was ongoing recruitment from July 2017 to April 2018.

After accurate diagnosis, patients were contacted and the study was explained. If the patient understood, accepted and signed the informed consent, then they were included in the study and blood samples were obtained pre-operatively for analysis of CA125 and HE4. As usual, after surgery, the tissue samples were analyzed in the histopathology laboratory. Furthermore, histological assessment of HE4, Ki67 and p53 in endometrial tissue were carried out.

Variables used for matching patients were: parity, hypertension, obesity and diabetes. Data were collected from the medical record and by interview. Data items included: date of surgery; pre-operative images, tests and results; pre-operative staging according to FIGO guidelines; type of surgical procedure; and whether lymphadenectomy was performed. Pathological outcomes included: histological type; cell differentiation; size of tumor; myometrial invasion; vascular or lymphatic invasion; perineural or stromal invasion; invasion of other tissues; and final FIGO staging with node metastasis.

The main variable was the tissue HE4 (H-score determination). To analyze the H-score, tissue samples were routinely processed and paraffin embedded. Sections of 3 μm thick were produced and stained with hemotoxylin-eosin (H&E), and antibodies against HE4, Ki67 and some samples with p53. The calibration of the technique was designed according to the optimal result when the target tissue was human epididymis. The definitive dilution was 1:20.000, as it was necessary to modify it from the trading house, which was used at the beginning, to set it with the epididymis. Immunohistochemical staining of endometrial tissue sample was performed using recombinant rabbit monoclonal Anti-HE4 antibody [EPR16658] of Abcam® on a Ventana Benchmark IHC processor. Representative areas were chosen from H&E stained sections.

Immunohistochemistry results were semi-quantitatively assessed to assign an H-score to tumor samples. Cytoplasmic staining was graded for intensity (0-weak, 1-moderate and 2-strong) and the percentage of positive cells was scored as 0 (0-33%), 1 (34-66%) and 2 (67-100%).

A single scale, with scores 0-4, was obtained by multiplying the intensity and the percentage staining score, and a total score was calculated by grouping score 0 in total score 0, 1-2 in total score 1, and 3-4 in total score 2.

Serum HE4 was determined using HE4 enzyme immunometric assay using a monoclonal antibody. Measuring range was 15-900 ppmol/L.

Serum CA125 was identified by electrochemiluminescence immunoassay using two monoclonal antibodies. Measuring range was 0.6-5000 U/mL.

Immunohistochemistry results were assessed by a semi quantitative approach used to assign an H-score (Figure 1) to tumor samples. Cytoplasmic staining was graded for intensity (0-negative, 1-weak and 2-strong) and the percentage of positive cells were scored as 0 (0%), 1 (1-50%) and 2 (51-100%). Tissue Ki67 determination was carried out semi-quantitatively, as recommended in the "International Ki67 in Breast Cancer Working Group". The measure of Ki67 was conducted through the counting of stained cores in the studied area (200 cores) without taking into account the intensity of the immuno-staining and excluding the counting of cores of other cells. The cell proliferation index was established as the average of the values obtained in three different areas (including areas of more and less proliferation). Ki67 was analyzed as a continuous variable setting the cut-off point at 25% (10).

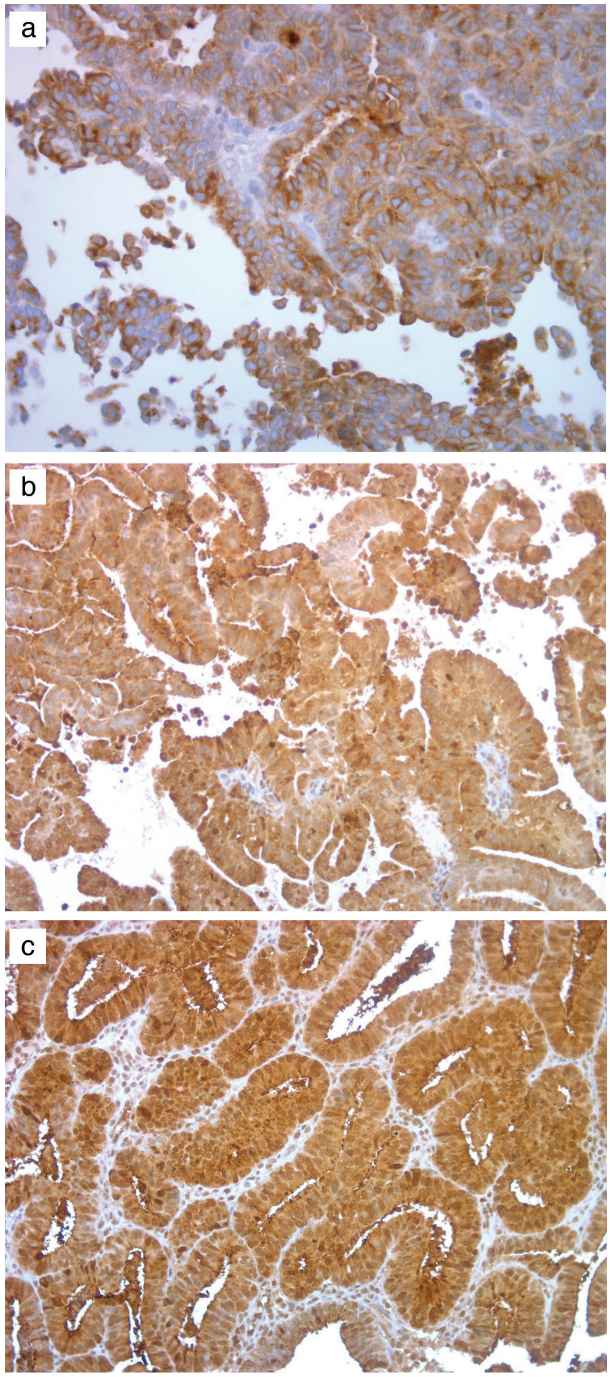

Figure 1. Representative immunohistochemical staining for HE4 in tissue microarrays of endometrial carcinoma: (a) H-score 0, endometrial endometrioid type adenocarcinoma FIGO G1; (b) H-score 1, endometrioid carcinoma FIGO G1; (c) H-score 2, FIGO G1 area of a G3 endometrioid carcinoma.

HE4: Human epididymis protein 4

Statistical analysis

Continuous variables were reported as mean ± standard deviation or median (25^{th}-75^{th} percentiles), according to the normality of their distribution, which was assessed by the Kolmogorov-Smirnov test. Categorical variables are reported as count (percentage). Comparisons of categorical variables between case-control groups were assessed using Fisher's exact test. For comparing continuous variables between groups, the Student's t-test was used if the samples were normally distributed or their variances were homogeneous; otherwise, the Mann-Whitney U test was used. Correlations between continuous variables were assessed using the Pearson (r) or the Spearman (ρ) rank correlation test. Possible biomarkers and H-score were compared using receiver operating characteristics curves and the corresponding area under the curve, whose differences were assessed using the DeLong test. Statistical analysis was performed using SPSS software, version 20 (IBM Inc., Armonk, NY, USA) and two-sided p-values $p<0.05$ were considered significant.

Results

There were 34 cases collected and 35 controls. Unfortunately, serum HE4 was not measured in all because recruitment was completed after preoperative analysis. So, in total pre-operative serum HE4 levels were available in 45 (65.2%) patients, 33 (94.3%) controls and 12 (35.3%) cases. Possible confounders were menopausal status (which was significantly higher in the cases group), the age at treatment (because patients with EC were older), and other variables (10) related to EC which, by definition would also be more frequent in the cases group.

Demographic features of both cases and controls are shown in Table 1, and the cohort of patients with EC treated, including the matching variables. Our sample consisted of 38% of all EC cases presenting during the study period. There were no differences between the participants with EC and the other EC cases who were not eligible for the study, suggesting that the risk of sample bias was low.

The expression of HE4 in endometrial tissue from patients with cancer was significantly weaker than in those without cancer (p=0.035). However, the difference between median serum HE4 levels in non-EC (64.67 U) compared with ECs (123.1 U) was statistically significant (p=0.002), although the CA125 level was not significant (p=0.208) as shown in Table 2. The comparison between modified H-score and different variables measured in pathological terms only shows statistical difference with a few variables related to the staining that are part of the staging itself. There was no difference among G1-G2-G3 cellular differentiation, with Ki67 or when comparing p53 (Table 2).

HE4 showed a considerably higher sensitivity compared with CA125 for detecting EC, 38.5% vs 7.7% and similar specificity of 84.8% compared with 90.9% for CA125 (Figure 2). However, this calculation was based on the reference ranges used in

Table 1. Demographic features of the study population

	Total cases during 2017/2018 (n=92)	Cases (n=35)	Controls (n=34)	p-value between C-C	p-value between total-cases
Age at treatment (years)	67.2±12.7	66.6±13.3	57.4±13.9	**0.006**	0.448
Parity	1.8±1.3	1.9±1.2	1.7±1.2	0.592	0.181
Menopause	85 (92.4%)	32 (91.4%)	20 (58.8%)	**0.002**	0.738
Hypertension	37 (40.2%)	12 (34.3%)	7 (20.6%)	0.282	0.241
Obesity	15 (16.3%)	8 (22.9%)	5 (14.7%)	0.540	0.153
Diabetes	18 (19.6%)	6 (17.1%)	2 (5.9%)	0.259	0.724
Other related to endometrial cancer	29 (31.5%)	9 (25.7%)	2 (5.9%)	**0.045**	0.250

Table 2. The association between HE expression in endometrial tissue and pathological parameters

		Cases	Controls		p-value
H-score					
0		5 (7.2%)	0		0.035
1		8 (11.6%)	5 (14.7%)		
2		22 (31.9%)	29 (42%)		
Serum HE4		123.1 (63.7-156.2)	62.05 (54.5-74.6)		0.002
Serum CA125		21.04±11.27	17.08±8.678		0.208
		Modified H-score			
		0	**1**	**2**	
Nuclear grade	G1	2 (5.7%)	2 (5.7%)	9 (25.7%)	0.729
	G2	1 (2.9%)	4 (11.4%)	9 (25.7%)	
	G3	2 (5.7%)	2 (5.7%)	4 (11.4%)	
Ki67	<25%	2 (6.1%)	2 (6.1%)	11 (33.3%)	0.586
	>25%	2 (6.1%)	5 (15.2%)	11 (33.3%)	
Staining macroscopic intensity	1	5 (7.2%)	0	0	<0.001
	2	0	8 (11.6%)	10 (14.5%)	
	3	0	5 (7.2%)	41 (59.4%)	
Cellular staining area	Cytoplasm	3 (4.3%)	2 (2.9%)	3 (4.3%)	0.005
	Cytoplasm + Nucleus	2 (2.9%)	11 (15.9%)	48 (69.6%)	
Staining tissue area	Apical	2 (2.9%)	1 (1.4%)	4 (5.8%)	0.124
	Diffuse	3 (4.3%)	12 (17.4%)	47 (68.1%)	
Staining	Homogeneous	2 (2.9%)	2 (2.9%)	51 (73.9%)	<0.001
	Heterogeneous	3 (4.3%)	11 (15.3%)	0	
p53	Normal	2 (5.7%)	6 (17.1%)	17 (48.6%)	0.267
	Aberrant	3 (8.6%)	2 (5.7%)	5 (14.3%)	

HE4: Human epididymis protein 4, CA125: Carbohydrate antigen 125

our laboratory which are 0-35 UI/mL for CA125 and for HE4, 0-70 pmol/L in postmenopausal women and 0-140 pmol/L in premenopausal ones.

The relationship between serum HE4 levels and the clinicopathological features of the EC patients are shown in Table 3. Higher serum HE4 levels were not significantly associated with any of the variables collected. However, serum CA125 was associated with being menopausal.

Discussion

EC is the most frequent malignant tumor of the female reproductive system in developed countries, although it is not the female reproductive cancer with the highest mortality rate (11). The mortality rate of EC has been reported to be approximately 20% in 5 years (12,13). In the USA, the estimated incidence of EC is 26.5 for every 100.000 per year, and accounts for 3.6% of all diagnosed cancer (12-15). Prognostic factors include histologic differentiation, deep myometrial invasion, non-endometrioid histologic subtype, lymphovascular invasion, lymph node status, cervical involvement, and the presence and extent of extra-uterine disease (11).

Localized EC treatment consists of hysterectomy and double adnexectomy. Moreover, the identification of those patients that should undergo a pelvic and/or para-aortic lymphadenectomy depends on several parameters (16-19).

Therefore, an endometrial biopsy and an abdomino-pelvic imaging technique (MRI as first election) are necessary for diagnosis and local extension prediction. Despite this, a significant proportion of EC cases require an extension of the planned surgery. For this reason, it was thought useful to investigate the role of HE4 as an efficient diagnostic test (20,21). However, the H-score did not have any significant result compared to any important variable studied. The only parameters with significance were pathological descriptions of the staining itself. This contrasts with the literature and some of the results are even negative, with the controls score more that the cases (14,17).

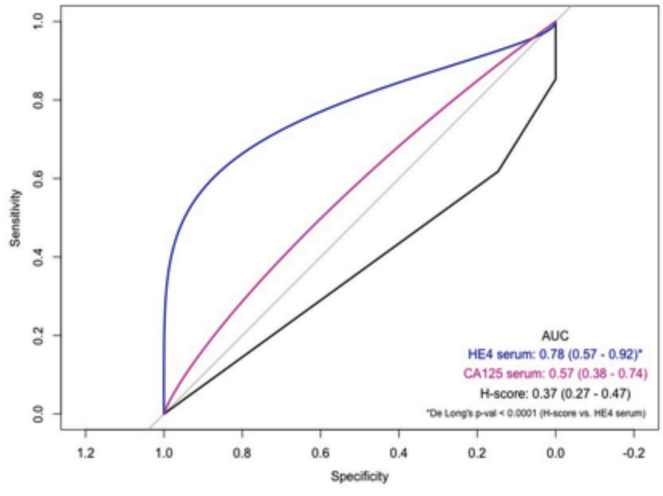

Figure 2. ROC curve: Modified H-score, serum HE4 and serum CA125

ROC: Receiver operating characteristics, HE4: Human epididymis protein 4, CA125: Carbohydrate antigen 125

Nevertheless, serum HE4 gave a positive result and we suggest that HE4 may represent a possible future biomarker for EC. HE4 is currently being studied by many research groups (18-25) and the outcomes are encouraging. There is still a requirement for additional studies of the role of serum HE4 in EC so that this can become a robust and useable biomarker. Moreover, we believe that HE4 may replace CA125 as a prospective and prognostic marker for EC because HE4 appears to have much greater specificity for EC while exhibiting a similar sensitivity to CA125.

The articles analyzed were homogeneous in terms of the number of patients, the H-score method and their general results. Furthermore, the prognosis with tissue HE4 was studied in the studies of Li et al. (8) and Deng et al. (9), but Bignotti et al. (7) only mentions serum HE4. The follow-up of patients in these three studies, as well as reported survival results are very unequal (survival of 14%, 18% and 33% respectively).

The sample of EC cases included in the study appear to be representative of our population of EC cases. However, it should be highlighted that the classical H-score, as described in the previous articles, was modified, so it was similar for basic criteria used but with fewer grades. This is because we were not able to distinguish more than 3 grades (modified H-score 0, 1 and 2), so, we designed a "modified H-score" which is the one described and used in our study.

Regarding bias, patients were interviewed by more than one researcher which will result in inconsistency in data acquisition. In addition, selection of cases was not randomized, as the incidence of EC was not high enough. As it is a case control design, there are several typical biases present including in selection and information, as well as not providing appropriate data for determining the incidence or prevalence of EC.

Study limitation

The most significant limitation of the study was that the sample size was too small. This was because our results were so discouraging for tissue HE4 in preliminary analysis, that recruitment was stopped before the expected recruitment number was obtained. Despite this, the results for measuring HE4 in serum as a biomarker for EC were encouraging.

Conclusion

The sensitivity and specificity of serum HE4 was not sufficient to recommend its adoption as a robust biomarker for EC.

Table 3. The relationship between serum HE4 and CA125 levels and the clinicopathological features

Variable	n	Serum HE4 median (Q1-Q3)	p-value	Serum CA125 ($\overline{X}$ ± SD)	p-value
Age (years)	45	67.3 (54.9-101.4) Rho= 0.247	0.102	19.1±9.9 r=-0.107	0.519
Diabetes					
Yes	6	71.4 (54.7-142.9)	0.616	19.0±13.4	0.991
No	39	64.9 (54.7-101.3)		19.1±95	
Hypertension					
Yes	14	70.6 (57.9-126.9)	0.384	19.3±11.9	0.924
No	31	64.4 (54.5-86.9)		19.0±8.9	
Menopause					
Yes	30	70.6 (55.9-111.8)	0.354	16.4±9.4	**0.029**
No	15	61.7 (54.7-82)		23.4±9.4	
Obesity					
Yes	8	66.6 (55.9-156.7)	0.449	20.0±7.8	0.783
No	37	67.3 (54.6-94.1)		18.9±10.4	
Parity					
Yes	40	69.2 (54.8-101.45)	0.448	18.6±9.5	0.373
No	5	58.8 (47.3-103.5)		23.3±13.3	
Other related to EC					
Yes	6	65.3 (57.2-121.6)	0.726	17.8±11.4	0.731
No	39	67.3 (54.7-101.3)		19.3±9.8	
Pelvic Lymphadenectomy					
Yes	5	101.3 (62.3-137.5)	0.181	15.6±0.5	0.402
No	40	64.7 (54.6-85.7)		19.6±10.3	
Paraaortic lymphadenectomy					
Yes	4	109 (67.6-147.3)	0.151	16.9±6.6	0.653
No	41	64.9 (54.6-84.5)		19.3±10.2	
Myometrial invasion >50%					
Yes	6	112.2 (55.1-156.0)	0.361	19.3±6.1	0.875
No	5	142.5 (93.1-210.3)		20.4±16.2	
Vascular, lymphatic or perineural invasion					
Yes	2	84.7 (51.4-)	0.182	12.5±4.1	0.182
No	6	132.8 (93.0-182.6)		18.4±10.6	
Adnexal affection					
Yes	1	-	0.333	-	0.660
No	11	138.2 (101.3-156.8)		20.1±11.0	
Lymph node status					
Affected	2	106.7 (56.3-)	1	20.2±7.5	0.958
Not affected	9	123.1 (84.8-156.2)		19.7±12.2	
Final FIGO stage					
I-II	6	132.8 (93.0-182.6)	0.286	18.4±10.6	0.490
III-IV	2	84.7 (51.4-)		12.5±4.1	
Second surgery					
Yes	1	-	0.397	-	0.891
No	44	66.1 (54.9-94.2)		19.1±10.0	

HE4: Human epididymis protein 4, CA125: Carbohydrate antigen 125, SD: Standard deviation, EC: Endometrial cancer

However, in our opinion it is essential to calculate a correct cut-off for EC and not to use the cut-off appropriate for ovarian cancer. This would allow correct comparison in positive and negative cases. In any case, it would be necessary to obtain data from larger studies in order to test the validity of our hypothesis.

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Ethics Committee Approval: *This study was approved by the Leon Clinical Research Ethics Committee (approval number: 17104).*

Informed Consent: *It was obtained.*

Peer-review: *Externally peer-reviewed.*

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Effect of double embryo transfer derived from autologous frozen oocytes on multiple pregnancy rates and presentation of success rates stratified by age at retrieval

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Abstract

Objective: To compare outcomes transferring one or two embryos in autologous frozen oocyte cycles.

Material and Methods: A retrospective cohort study conducted at an academic fertility center between January 2012 and December 2018. One-hundred and fourteen patients underwent frozen oocyte transfers; 67 single embryo transfer (SET) and 47 double embryo transfer (DET). No subjects had more than two embryos transferred. Data were analyzed using t-test and chi-squared testing. Multivariate logistic regression was used to control for confounding effects. Power analysis suggested an 82% power with alpha of 5% and effect size of 27%.

Results: Regarding the embryo stage, 72% were cleavage embryos and 28% were blastocyst embryos. Among those who had cleavage stage embryos, 48.8% underwent SET and 51.2% underwent DET. In the blastocyst embryos group these proportions were 84.4% and 15.6%, respectively. There were no difference in pregnancy rate for SET (40.3%) vs DET (36.2%) ($p=0.78$). Additionally, the live birth rate did not differ between SET and DET (28.4 vs 19.1%, respectively, $p=0.26$). The multivariate multilevel analysis provided adjusted odds ratios (95% confidence interval) of: 1.85 (0.46-7.44) for pregnancy; 0.497 (0.05-4.86) for clinical pregnancy; and 0.82 (0.11-6.29) for live birth when comparing SET and DET. Multiple pregnancy rates were significantly lower in the SET (0%), compared with DET group (44.4%) ($p<0.002$).

Conclusion: SET results in excellent live birth outcomes in autologous frozen oocyte cycles. However DET results in significantly increased rates of multiple pregnancies. This suggests that SET is a viable option in autologous frozen oocyte cycles. (J Turk Ger Gynecol Assoc 2021; 22: 168-73)

Keywords: Oocyte vitrification, single embryo transfer, autologus, multiple pregnancy rates

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Introduction

In the early days of in vitro fertilization (IVF) multiple embryos were often transferred in order to maximize the chances of a successful pregnancy but this number has gradually decreased over time to limit the complications associated with multiple pregnancy (1-3). The American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology (SART) developed guidelines to limit the number of embryos transferred in order to decrease the number of multiple gestations as a result of reproductive technologies (2). Keeping in mind that the ultimate goal of IVF is a healthy, singleton, live birth, recommendations are made regarding the number of embryos to be transferred based on particular characteristics, including patient age and stage of embryo being transferred. For women under the age of 35 years undergoing IVF, the transfer of a single embryo, regardless of embryo stage, is the generally accepted practice, though two embryos may be considered if the prognosis of pregnancy after the transfer is lower. For women between the ages of 35-37 years, a consideration should be made for a single-embryo transfer. For women between 38-40 years old, SART recommended not more than three

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cleavage-stage embryos or two blastocyst embryos should be transferred. For patients who have euploid embryos available after pre-implantation genetic testing for aneuploidy (PGT-A), the transfer of one embryo should be the practice norm. In the 41 to 42 year-old age group, there should be no more than four cleavage-stage embryos or three blastocysts transferred. Similarly, if euploid embryos are available, the norm should the transfer of a single blastocyst.

Significant efforts have been made to reduce the incidence of higher order births such as triplets and quadruplets, but twin pregnancy rates have not seen the same decline as a result of double embryo transfers (DET), which continues to be commonly practiced (4). It should be noted that the incidence of monozygotic twins in IVF pregnancies is in fact higher than the incidence in spontaneous pregnancies, a factor that is out of the provider's control (5).

A recent study by Freeman et al. (6) found that the transfer of a single vitrified-warmed blastocyst is an acceptable practice, given that it maintains live birth rates and decreases the rate of multiple pregnancies and its complications in patients younger than 38 years old. It was previously demonstrated that transferring more than one blastocyst in women 40 years of age and greater using autologous gametes, increases the multiple pregnancy rate without significantly changing the live birth rate (7,8). Cumulative live birth rates are maximized when transferring serial single blastocysts in women 40 years of age and greater (9). An advance in reproductive technologies was made with the introduction of vitrification. Vitrification is when the gamete or embryo undergoes dehydration followed by ultra-rapid cooling which causes minimal ice crystal formation and little if any damage. The development of vitrification allowed the expansion of the oocyte freezing industry. Until the introduction of vitrification, oocytes did not freeze well and usually did not survive once thawed or failed to be competent to develop into an embryo with high live birth potential. Women have increasingly used oocyte freezing to maintain fertility potential later in life. However, few centers have extensive knowledge of returning embryos from these frozen oocytes. As oocytes have previously demonstrated greater likelihood of suffering freezing damage than embryos, the role of single embryo transfer in this group has been minimally studied. Although we believe oocytes that have been vitrified provide excellent pregnancy potential, few large studies verify this. However, a study demonstrated that single embryo transfer (SET) after oocyte vitrification can lead to excellent pregnancy rates (6). This study failed to stratify for age of the patient at oocyte collection and did not include older women. The goal of our study was to compare SET with DET during autologous frozen oocyte cycles using live birth, pregnancy rates, and multiple pregnancy rates as our primary outcomes in women of all ages, stratified by age at oocyte collection.

Material and Methods

Study design

This is a retrospective cohort study conducted at a single academic fertility center between January 2012 and December 2018, including patients who underwent frozen embryo transfers. The patients were sub-divided into those undergoing SET and those undergoing DET. None of the patients received more than two embryo transfers during the time period of the study. This is the current limit based on government regulation in our jurisdiction. All included patients had a normal uterine cavity, no hydrosalpinx, and no thyroid or prolactin abnormalities.

McGill University Health Center Institutional Review Board (IRB) Ethics approval of this retrospective study was obtained (approval number: 2020- 5631).

The risks and benefits of SET vs DET were discussed between the clinical staff and the patients. The risks associated with multiple gestation pregnancy for both the mother and the fetus were also thoroughly discussed. Women under the age of 37 years at the time of embryo transfer must receive a single embryo while women 37 years of age or more could receive a maximum of two embryos. This permitted women who froze oocytes at age 30 and were now undergoing embryo transfer at the age of 37 years old or older to be eligible for the transfer of two embryos. Up until 2016 there was a limit of transfer for two embryos in women under 37 years of age. At the beginning of 2016 this limit was reduced to one embryo.

Exclusion criteria included untreated uterine leiomyoma, polyps, or hydrosalpinx, untreated thyroid or prolactin abnormalities, women who did not undergo transfer of embryos developed from oocyte vitrification and those who had experienced previous failed embryo transfers. All subjects were included only once.

Ovarian stimulation and egg retrieval

Women underwent gonadotropin stimulation with a combination of follicle stimulating hormone and human menopausal gonadotropin as part of a gonadotropin-releasing hormone agonist long protocol or antagonist cycle. These cycles have been previously reported in detail by our group (10).

A subcutaneous injection of either recombinant human chorionic gonadotropin (hCG) at a dose of 250 micrograms (Merck Serono, Canada) or 10000 IU menopausal hCG (Fresenius Kabi Canada Ltd, Merck Serono Canada, Ferring Canada, Canada) or buserelyn 1000 IU (0.1 mg) (Sanofi Aventis Canada, Laval, Qc) was given for final oocyte maturation.

Transvaginal ultrasound and rising serum estradiol (E2) levels were used to assess adequate follicular development, which was used to guide the timing of the hCG or leuprolide acetate injection.

Many of the cycles, particularly in the women 40 years of age or older, occurred in couples who refused donor sperm back up, had azoospermia and underwent testicular sperm aspiration (TESA) or microTESA, the day before oocyte collection per clinic protocol. These procedures failed to locate sperm and all oocytes were vitrified. The couple subsequently elected to use donor sperm and the oocytes were thawed and fertilized and transferred subsequently as fresh embryos obtained from frozen oocytes.

Vitrification and warming of oocytes

Vitrification and warming of mature oocytes were performed using a modification of the method described by Chian et al (11).

For vitrification, the oocytes were incubated in equilibration medium containing 7.5% (v/v) ethylene glycol (EG) and 7.5% (v/v) dimethyl sulfoxide (DMSO) for 15 minutes, then transferred to vitrification medium containing 15% (v/v) EG, 15% (v/v) DMSO, and 0.5 M trehalose for one minute. The oocytes were then loaded onto a CryoTop (Kitazato Biopharma, Japan) and were immediately plunged into liquid nitrogen for storage.

For warming, the CryoTop was directly inserted into medium containing 1.0 M trehalose for one minute at 37 °C. The oocytes were then transferred into diluent medium-I containing 0.5 M trehalose for three minutes and then into diluent medium-II containing 0.25 M trehalose for a further three minutes. Oocytes were washed twice in washing medium (three minutes each wash).

Fertilization, culture

Surviving oocytes were inseminated using intracytoplasmic sperm injection (ICSI) after completing the warming process. Oocytes were checked 18-20 hours post-ICSI for signs of fertilization. Embryos were cultured to the blastocyst stage in culture medium (Global total, Cooper surgical, USA). Embryo transfer was performed either on day 3 or day 5. All embryos underwent laser assisted hatching using a ZILOS-tk (Hamilton Thorne Instruments Biosciences, Beverly, MA, USA) device to create an opening of approximately 20 microns in the zona pellucida.

Endometrium preparation for Frozen embryo transfer

Women were treated with estradiol valerate, 2 mg orally three times daily, which was titrated up to 12 mg daily as a combination of vaginal and oral intake. When the endometrium reached at least 8 mm in maximum anterior-posterior diameter measured trans-vaginally using a Vuluson 8 machine (GE, USA), vaginal progesterone was started the day prior to oocyte thawing and continued until 12 weeks of pregnancy, if pregnant.

Statistical analysis

Statistics were analyzed using SPSS, version 23.0 (IBM Inc., Chicago, IL, USA). Continuous data was assessed for normalcy using the Kolmogorov-Smirnov test. Data is presented as mean ± standard deviation (SD) or percentage. Continuous data were analyzed using t-test and categorical data were analyzed using chi-squared testing. Non-parametric testing was used if indicated. A p-value <0.05 was considered to indicate statistical significance. Multivariate logistic regression was used to control for the confounding effects of female age and blastocyst or cleavage transfer. Power analysis suggested that with the current number of subjects, 82% power with alpha of 5% and effect size of 27% would be detectable.

Results

The study included 114 patients who underwent frozen embryo transfers. The patients were sub-divided into 67 (58.8%) patients who underwent SET and 47 (41.2%) who underwent DET. The mean age at the time of oocyte collection was 36±5 years old for the 67 patients undergoing SET and 39±5 years old for the 47 patients undergoing DET, which was significantly different ($p=0.001$). Women in the study varied with 29.8% being under the age of 35 years old, 26.3% between the age of 35-40 years old and 43.9% above the age of 40 years old at the time of oocyte collection (Table 1).

The mean (± SD) overall survival rate of the oocytes was 83.5% (±19.7), while the mean (± SD) overall survival of fertilized oocytes after ICSI was 70.1% (±22.4). In regard to the maturation stage at transfer, 72% were cleavage embryos and 28% were blastocyst embryos. Among those who had cleavage stage embryos, 48.8% underwent SET and 51.2% underwent DET. Among patients in the blastocyst embryos group, 84.4% underwent SET and 15.6% underwent DET (Table 1).

There were no statistically significant differences observed in pregnancy rates for SET (38.1%) vs DET (36.2%) ($p=0.78$) in all age groups (Table 2). Clinical pregnancy rates were similar between those undergoing SET (32.8%) and DET (21.3%) ($p=0.18$). In relation to live birth rates, there were no statistically significant differences between the patients who underwent SET (28.4%) and those who underwent DET (19.1%) ($p=0.26$).

Patients were divided into three different groups according to age: (<35, 35-40, >40; Table 2). In the <35 years old category, though there seems to be a relatively higher percentage of pregnancies (80% vs 48%), clinical pregnancies (80% vs 34%) and live births (60% vs 34%) in the DET group in comparison

to the SET group, none of these values were statistically significant. In the 35 to 40 years old category, pregnancy (20% vs 47%), clinical pregnancy (0 vs 47%) and live birth (0 vs 27%) outcomes were similar between DET and SET groups, respectively. Furthermore, in the >40 year-old category, we also failed to demonstrate a statistically significant difference in pregnancy (37% vs 26%), clinical pregnancy (22% vs 22%) and live birth rates (22% vs 22%) when patients who underwent DET as opposed to SET were compared (p=0.41, p=0.68, p=0.97), respectively.

The multivariate multilevel analysis provided an adjusted odds ratio (95% confidence interval) of 1.85 (0.46-7.44) for pregnancy, 0.497 (0.05-4.86) for clinical pregnancy, and 0.82 (0.11-6.29) for live birth when comparing SET and DET while controlling for age and stage of transfer, rendering the differences in outcomes between the two methods non-significant (Table 3).

Importantly, multiple gestation live birth rates were significantly lower in the SET (0%), compared with DET group (44.4%) (p=0.01), in spite of the possible masked role of lower embryo quality or less favorable patient history in the DET group.

Discussion

The comparison between SET and DET in autologous frozen oocyte cycles as measured by rates of pregnancy, clinical pregnancy and live birth rates showed no differences in outcomes in women <35, 35-40, and >40 years old. The use of DET, similar to other studies of frozen embryos, was again demonstrated to increase the risk of a multiple gestation (7-9). Our findings on the favourable use of SET while maintaining pregnancy success rates support previous literature that has addressed the same question (7-9). However, to the best of our

Table 1. Demographics and embryo characteristics of patients undergoing SET vs DET

		SET (n=67) (58.8%)	DET (n=47) (41.2%)	p-value (95% CI)
Age mean ± SD (median) at time of oocyte collection		36.45±5.2 (35)	39.64±5 (41)	0.001 (-5.08; -1.24)
Age at collection (stratified)	<35	29 (43.3%)	5 (10.6%)	-
	35-39	15 (22.4%)	15 (31.9%)	-
	> 40	23 (34.3%)	27 (57.4%)	-
Cleavage stage embryo Number of patients (% of cleavage transfers)		40 (48.8%)	42 (51.2%)	<0.001
Blastocyst stage embryo Number of patients (% of blastocyst transfers)		27 (84.4%)	5 (15.6%)	
Age distribution n (%)				
<35		34 (29.8)		
35-39		30 (26.3)		
>40		50 (43.9)		
SET: Single embryo transfer, DET: Double embryo transfer, CI: Confidence interval, SD: Standard deviation				

Table 2. Pregnancy outcomes stratified by age at oocyte collection

	Age	SET	DET	p-value
Pregnancy rate % (number of pregnancy/no of transfers)	All	38.1 (26/67)	36.2 (17/47)	0.78
	<35	48.3 (14/29)	80.0 (4/5)	0.32
	35-39	46.7 (7/15)	20.0 (3/15)	0.21
	>40	26.1 (6/23)	37.0 (10/27)	0.41
Clinical pregnancy rate % (number of clinical preg/no of transfers)	All	32.8 (22/67)	21.3 (10/47)	0.18
	<35	34.4 (10/29)	80.0 (4/5)	0.11
	35-39	46.7 (7/15)	0 (0/15)	0.03
	>40	21.7 (5/23)	22.2 (6/27)	0.68
Live birth rate % (live birth /no of transfers)	All	28.4 (19/67)	19.1 (9/47)	0.26
	<35	34.4 (10/29)	60.0 (3/5)	0.39
	35-39	26.7 (4/15)	0 (0/15)	0.22
	>40	21.7 (5/23)	22.2 (6/27)	0.97
Analysis was performed using chi-square test. SET: Single embryo transfer, DET: Double embryo transfer				

Table 3. Unadjusted and adjusted odds ratios for reproductive outcomes. Single ET versus double ET

Unadjusted			Adjusted	
	OR	95% CI	OR	95% CI
Pregnancy rate	1.50	0.42-5.32	1.85	0.46-7.44
Clinical pregnancy	0.44	0.05-3.98	0.497	0.05-4.86
Live birth rate	0.71	0.10-5.03	0.82	0.11-6.29

ET: Embryo transfer, OR: Odds ratio, CI: Confidence interval. The odds ratios are adjusted for age and stage of embryos

knowledge there is limited data on women above the age of 40 years old when using vitrified oocytes. In fact, little is known about how oocytes from women 40 years of age or greater vitrify and the resultant pregnancy and live birth rates from these oocytes. Interestingly our live birth rates obtained in this study of approximately 22% in women who vitrified oocytes at 40 years of age are similar to our outcomes in women using fresh autologous oocytes at 41 and 42 years of age, which were 20% in 2018, without the use of PGT-A (9). In a recent scientific impact paper published by the British Royal College of Obstetricians and Gynaecologists on elective egg freezing for non-medical reasons, the authors stated that "Success rates will be limited in women who are already in their mid-late 30s" (12). However; they could not cite a source for this statement. Our data suggests that success rates using autologous frozen oocytes remained similar to fresh IVF cycles when stratified for age although, they will be center specific. It should be noted that our success rates are preliminary and remain to be confirmed, given the relatively small number of subjects in our study. As such, we call on centers doing large numbers of cycles of elective oocyte cryo-preservation, particularly those in New York, to provide data stratified for age, so that success rates can be better established in this population.

Importantly, live birth rates after SET as a result of autologous oocyte vitrification was respectable at all age groups studied. However, bias must have existed in relation to allocation to SET or DET. Particularly, women with worse quality embryos or worse prognosis were more likely to receive DET in our study. Nevertheless, multiple gestation rates were high with DET in this group. Results suggest that SET is a viable and safe option after autologous oocyte vitrification, irrelevant of age.

Advanced maternal age (AMA) is generally well defined in the literature as maternal age above 35 years old. In Canada, the average age of mothers at first birth has been steadily increasing since the 1960s (13). Interestingly, Statistics Canada reported a shift in the age distribution of mothers who have a multiple birth (13). The proportion of women between the age of 35-39 years old who gave birth to twins increased from 9.8% to 23.1% in the span of 25 years and the proportion of those 40-44 years old increased from 1.0% to 5.6%. Simultaneously, there has been a decrease in the proportion of women who had twins and were in their late 20s, from 38% to 24.4%. The increased use of ART is the primary contributing factor to the increase in rates of twin births in older age groups. The results from our study suggest that SET is a viable alternative in women 40 years of age or greater when using vitrified oocytes. AMA is associated with fetal growth restriction, premature birth, neonatal intensive care unit admission, neonatal death, gestational diabetes, preeclampsia and stillbirth (14,15). The combination of the plethora of inherent risks that accompany AMA pregnancies with the well-established risks associated with higher order pregnancies could lead to an overall increase in regard to maternal and fetal co-morbidities and should be avoided.

A factor commonly discussed in the literature regarding embryos transferred in a cycle is the stage or quality of the embryo. In our study, we observed that the SET group had an almost equal distribution of cleavage and blastocyst transfers, while the DET group had a majority of embryos in the cleavage stage (89.4%) and fewer in the blastocyst stage (10.6%). Overall, the literature presents heterogeneous findings regarding whether there is a benefit to transfer at one stage versus the other. A Cochrane systematic review, published in 2016, that reported on 27 randomized controlled trials and a total of 4,031 women found moderate quality evidence for clinical pregnancy when employing fresh blastocyst stage transfer (16). However, there was no difference found in cumulative clinical pregnancy rates when both fresh and thawed cycles from a single egg collection procedure were used. A subsequent systematic review and meta-analysis of reproductive outcomes including clinical pregnancy, live birth, ongoing pregnancy, cumulative pregnancy, and miscarriage, was published in May of 2017 by Martins et al. (17), in which the group reported on 12 randomized controlled trials and 1200 women, and found no evidence for the superiority of blastocyst compared to cleavage stage transfers. Further studies and meta-analyses are needed to assess how transfer of thawed embryos at the cleavage stage versus the blastocyst stage compare in terms of desired outcomes. In our study, even when adjusting for the stage of the embryo at the time of transfer, our results showed no differences in pregnancy, clinical pregnancy and live birth rates in the SET vs DET group over all. Further and larger studies are needed to confirm our results.

Study limitation

One of the limitations of our study was the small population size and non-randomization of the patient population. Given the nature of the trial, the employment of a randomized controlled trial may be difficult to implement. While one method of treatment is not inferior to another, randomizing

patients to SET vs DET would put a group of patients at risk of a higher order pregnancy, which may not be a desired outcome of the woman or couple who are putting themselves through IVF treatment. In addition, we provide information on our outcomes of interest, including pregnancy, clinical pregnancy, live birth rates and multiple gestation live birth rates, but we did not further report on pregnancy complications observed or incidence of complications observed in the multiple gestation groups, which was unavailable at this time. The ages of some of the groups differed, although this was adjusted for using multivariate stepwise logistic regression analysis to control for confounding effects.

Conclusion

Vitrification of oocytes for women 35 years of age or older gives excellent pregnancy and live birth rates, similar to those seen with fresh autologous oocytes. This is even true for women of 40 years of age or greater. Multiple pregnancy rates are lowest with SET as opposed to DET, even after oocyte vitrification and even among women at least 40 years of age when the oocytes were vitrified. The limited use of DET, particularly in women over 35, may contribute to reduced rates of maternal and fetal co-morbidities by reducing the rate of multiple gestation live birth rates. Our study of 114 women does not support the use of DET in any age group, based on when oocytes were vitrified.

Ethics Committee Approval: *McGill University Health Center Institutional Review Board (IRB) Ethics approval of this retrospective study was obtained (approval number: 2020-5631).*

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Does ventral mesh rectopexy at the time of sacrocolpopexy prevent subsequent posterior wall prolapse?

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Abstract

Objective: To determine whether ventral mesh rectopexy at the time of sacrocolpopexy reduces the rate of future posterior wall prolapse.

Material and Methods: This was a retrospective cohort study of women with pelvic organ prolapse (POP) who underwent sacrocolpopexy or without concomitant rectopexy at a single community hospital from December 1, 2015 to June 30, 2019. Preoperative pelvic organ prolapse quantification (POP-Q) and urodynamic testing was used in evaluation of POP. Patients were followed for 12-weeks postoperatively and a 12-week postoperative POP-Q assessment was completed. The incidence of new or recurrent posterior prolapse was compared between cohorts.

Results: Women with POP (n=150) were recruited, of whom 41 (27.3%) underwent sacrocolpopexy while the remainder (n=109, 72.7%) did not receive rectopexy. Patient demographics did not statistically differ between cohorts. Post-surgical posterior wall prolapse was reduced in the robotic assisted sacrocolpopexy (RASC) + rectopexy group compared to RASC alone, however this did not reach statistical significance. There were no patients who underwent concomitant rectopexy and RASC that needed recurrent posterior wall prolapse surgery, compared to eight-percent of patients that underwent isolated RASC procedures.

Conclusion: Our findings suggest a reduction in the need for subsequent posterior wall surgery when rectopexy is performed at the time of sacrocolpopexy. In our study, no future surgery for POP was found in the concomitant sacrocolpopexy and rectopexy group, while a small proportion of the RASC only group required future POP surgery. Our study, however, was underpowered to elucidate a statistically significant difference between groups. Future larger studies are needed to confirm a reduced risk of posterior wall prolapse in patients who undergo concomitant RASC and rectopexy. (J Turk Ger Gynecol Assoc 2021; 22: 174-80)

Keywords: Pelvic organ prolapse, sacrocolpopexy, rectopexy, minimally invasive surgery, multicompartmental prolapse

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Introduction

Pelvic floor disorders are a common and costly healthcare concern. It is estimated that one in four adult women in the United States experience pelvic floor dysfunction, with prevalence increasing with age (1). Pelvic organ prolapse (POP) occurs when the muscles that hold the pelvic organs (e.g. uterus, bladder, and or rectum) weaken and these organs are displaced from their normal anatomical position, typically resulting in protrusion of the anterior or posterior vaginal wall

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into the vagina. By the age of 60 years, more than one-third of women will have one or more pelvic floor disorders (2). The lifetime risk of undergoing pelvic floor surgery is estimated to be 11%-20% (3,4). Furthermore, nearly 30% of patients who undergo surgical correction of POP will require reoperation for recurrent prolapse or incontinence (3).

There are several treatment modalities for POP, including expectant management, conservative approaches, and surgical correction. Conservative measures include pessary and pelvic floor physical therapy, which has been shown to be effective for early stages of POP (5). In patients with more severe POP, which may be assessed with tools such as the preoperative pelvic organ prolapse quantification (POP-Q) with more severe POP being indicated with a staging of POP-Q 3 or 4, pessaries and surgical options have been shown to have similar outcomes (6,7). Surgical options can be obliterative or reconstructive in nature. Obliterative surgery corrects prolapse by removing portions of the vagina, narrowing part of the vagina, or closing off the vagina entirely (8). Conversely, reconstructive surgery aims to restore normal anatomy through either the use of natural structures, such as ligaments, or by creating pelvic organ support with interposition of synthetic mesh (9). Of the surgical options, minimally invasive sacrocolpopexy has become the "gold standard" in POP repair (10-13).

When compared to vaginal procedures for POP, sacrocolpopexy has been shown to be more efficacious, resulting in decreased risk of recurrent prolapse and the need for repeat surgery, decreased risk of postoperative stress urinary incontinence, and decreased risk of sexual side effects (e.g. dyspareunia) compared to vaginal approaches (12,13). Furthermore, studies have shown that apical support reduces anterior vaginal wall prolapse (14-16), owing to the effectiveness of sacrocolpopexy. As a result, minimally invasive sacrocolpopexy has become the treatment of choice for advanced stage POP. Unfortunately, even after repair with sacrocolpopexy, recurrence of POP has been reported in up to 23.2% of patients, with risk increasing with greater presurgical clinical severity and stage (17).

Multicompartmental prolapse complicates the treatment of POP. It is estimated that 10-55% of patients with POP have two or more concomitant pelvic floor disorders (18,19). The organs involved can include any combination of the bladder and anterior vagina, the posterior vagina and rectum, the uterus, the vaginal cuff in patients status post hysterectomy, the perineum, and partial- or full-thickness rectum. Traditionally, rectal prolapse and vaginal prolapse have been regarded as separate entities and treated by disparate surgical procedures (20,21). Minimally invasive ventral mesh rectopexy has become increasingly used in the treatment of posterior compartment defects (22-24). Recently, there has been a shift from a compartmentalized approach toward concomitant procedures for correction of multicompartmental prolapse. Accordingly, ventral mesh rectopexy at the time of sacrocolpopexy is increasingly performed to treat rectal prolapse (20,21). Currently, however, there is a paucity of research examining the effects of rectopexy at the time of sacrocolpopexy and subsequent posterior vaginal wall prolapse.

To date, no study has investigated ventral mesh rectopexy at the time of sacrocolpopexy for the prevention of future posterior vaginal wall prolapse. The aim of this study was to determine whether ventral mesh rectopexy, completed at the time of sacrocolpopexy for apical vaginal prolapse, can reduce future posterior wall prolapse recurrence.

Material and Methods

This is a retrospective cohort study of women aged 18-years or older who underwent minimally invasive robotic-assisted sacrocolpopexy (RASC) at an urban community teaching hospital from December 1, 2015 to June 30, 2019 (ICD 10 CPT 57425). Patients were stratified depending on if they underwent ventral mesh rectopexy at the time of sacrocolpopexy or if they only had a sacrocolpopexy procedure. Each patient had medical clearance by a primary medical provider prior to surgery. Each patient underwent a preoperative presurgical POP-Q interactive assessment (25). Each POP-Q was performed by one board-certified female pelvic medicine and reconstructive surgeon.

Urodynamic testing was performed for each patient to determine the presence of occult stress incontinence or detrusor overactivity. If the patient was determined to have occult stress urinary incontinence, a tension-free vaginal tape (TVT) was performed at the time of the procedure. Patients with posterior wall prolapse on POP-Q or fecal incontinence underwent magnetic resonance (MR) defecography. Based on the results of the POP-Q and MR defecography or if the patient had rectal prolapse (internal, partial, complete) they underwent ventral wall rectopexy at the time of RASC. Each ventral mesh rectopexy was performed by a single, board-certified, colorectal surgeon. If the patient had not previously undergone a hysterectomy, a robotic assisted total hysterectomy was performed. A Y-shaped lightweight polypropylene mesh (Vertessa® Lite, Caldara Medical, Augora Hills, CA, USA) was used for the sacrocolpopexy. A 0- delayed-absorbable polydioxanone suture (PDS) in a running fashion was used for the anterior and posterior arms of the vaginal mesh. The vaginal cuff (where applicable) was closed with 0-PDS suture in a running fashion. The mesh was affixed to the sacral promontory with a 0-braided polyester (TiCron, Covidien, Minneapolis, MN, USA), nonabsorbable suture via two simple interrupted sutures.

Patient demographics, medical comorbidities, past medical history, surgical history, obstetric history and social habits were recorded for each patient undergoing POP repair. Operative complications, postoperative complications, and hospital length of stay were analyzed. The study was approved and deemed exempt by the Ascension St. John Institutional Review Board (approval number: #1477635). However, because this was a retrospective study, informed consent was not obtained. Success of rectopexy with concomitant RASC was determined by the absence of posterior vaginal wall prolapse in subsequent postoperative visits. Patients were followed for a total of 12 weeks postoperatively. A POP-Q assessment was performed at each patient's 12-week postoperative evaluation. Failure was defined as posterior vaginal wall prolapse recurrence using the POP-Q interactive assessment tool, defined as Ap or Bp greater than point 0 - beyond the hymenal ring, or the need for future surgery for posterior vaginal wall prolapse. Point C was considered only in the context of Ap or Bp, owing to the fact that for deviation of point C, Bp would be expected to be affected in posterior wall prolapse.

Statistical analysis

Data were tested for normality and homogeneity of variance. Normally distributed data was reported as mean ± standard deviation. Non-normal data was reported as median + interquartile range. A paired t-test was used to compare preoperatively and postoperative POP-Q values. Univariate analyses were conducted with Student's t-test, the chi-squared test, and analysis of variance (ANOVA). Multivariate analyses were done using logistic regression. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS Software v. 25.0 (IBM, Armonk, NY, USA). There is a paucity of literature examining prevention of posterior wall prolapse recurrence by performing rectopexy at time of sacrocolpopexy. Accordingly, our power analysis was limited to estimations based on observations at our institution. For our power analysis, we estimated that the posterior wall recurrence without rectopexy was approximately 25% and two-percent with concomitant rectopexy. Using this criterion, it was estimated that 141 subjects in each group would be necessary to detect a difference at a power of 80% and $\alpha=0.05$.

Results

In total, 150 women were retrospectively reviewed. Of these 41 (27.3%) underwent RASC with concomitant rectopexy while the remaining 109 (72.7%) underwent only RASC (Figure 1). Baseline demographic variables, including race, body mass index, parity, diabetes mellitus and smoking were not statistically different between groups (Table 1). Patients undergoing RASC alone had greater proportion of advanced stage POP compared to patients undergoing RASC + Rectopexy, (76% stage 3, 23% stage 4 vs 68% stage 3, 5% stage 4, $p<0.0001$) (Table 2). Furthermore, preoperative POP-Q scores were significantly different for points Aa (1.3±1.6 vs 2.7±1.0, $p<0.0001$), Ba (1.8±2.2 vs 4.5±2.3, $p<0.0001$), C (-2.1±4.5 vs 2.6±4.6, $p<0.0001$), Pb (3.1±1.0 vs 2.5±1.0, $p=0.002$), Ap (0.0±1.8 vs -1.2±1.7 cm, $p=0.001$), and Bp (0.3±2.2 vs -1.2±1.4 cm, $p<0.0001$) for patients undergoing RASC and rectopexy comparted with RASC alone. Patients undergoing RASC alone had greater anterior prolapse but less posterior compartment prolapse compared to patients who underwent concomitant RASC and rectopexy (Table 2). Concurrent surgeries were similar between groups with the exception of bilateral-salpingoophorectomy, which was completed more often in the RASC group, ($p=0.014$; Table 3).

Overall there was a low complication rate. Within the sacrocolpopexy alone cohort there was one umbilical port hernia, one rectal injury, and two cystotomies at the time of TVT placement. In the RASC and rectopexy group, there was one mesenteric bleed with hemoperitoneum that was controlled with a hemostatic agent (Table 4). Congruent with these results, hospital length of stay (in days) did not statistically differ between RASC and RASC + rectopexy (1.1±0.5 vs 1.3±0.7, $p=0.071$). In the combined cohort, postoperative POP-Q scores were significantly improved for Aa, Ab, C, Gh, Ap, and Bp ($p<0.0001$ for each) (Supplemental Table 1). When comparing postoperative POP-Q scores, point C was the only score that significantly differed, with RASC alone having slightly higher point C (-9.3±1.5 cm vs -8.4±3.3 cm, $p=0.036$) (Table 5).

Recurrence and/or subsequent postsurgical posterior compartment prolapse were found in 10% of the RASC and 3% of the RASC + Rectopexy cohort ($p=0.181$). Eight-percent of patients in the RASC cohort had to undergo subsequent posterior repair, while no patients in the RASC + rectopexy cohort needed repeat surgery ($p=0.114$) (Table 6).

Discussion

There is a paucity of research examining the recurrence or subsequent posterior wall prolapse following concomitant sacrocolpopexy and rectopexy compared to sacrocolpopexy alone. The aim of this study was to determine if concomitant ventral mesh rectopexy during minimally-invasive RASC reduced the rate of subsequent posterior vaginal wall prolapse. To our knowledge, this is the first study to investigate this matter. The Colpopexy and Urinary Reduction Effort trial did investigate the impact of POP surgery on posterior compartment symptoms; however, posterior colporraphy rather than ventral mesh rectopexy was performed at the discretion of the surgeon

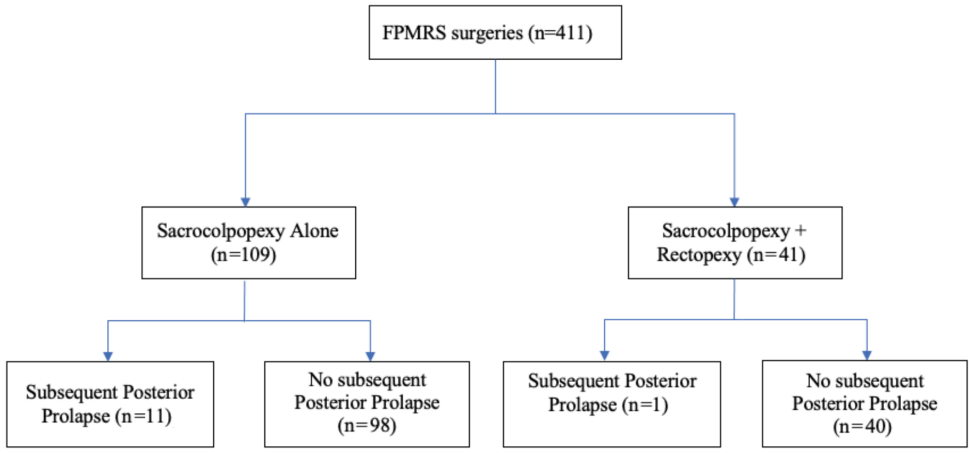

Figure 1. Study cohorts

FPMRS: Female Pelvic Medicine and Reconstructive Surgery

Table 1. Patient demographics and medical comorbidities

Characteristic	RASC	RASC + Rectopexy	p
	n=109	n=41	
Age (years)	63.3±8.4	62.8±12.9	0.792
Race			
White/Caucasian	88 (82)	33 (83)	1.00
Black/African American	20 (18)	7 (17)	
BMI	28.5±5.0	27.5±4.8	0.264
Parity	2.9±1.4	2.8±1.4	0.693
Diabetes mellitus	11 (10)	5 (12)	0.768
Smoking	8 (7)	7 (17)	0.122

All data reported as mean ± standard deviation or n (%).
RASC: Robotic Assisted Laparoscopic Sacrocolpopexy, BMI: Body mass index in kg/m^2

(26). Ultimately, the authors found that the anorectal symptoms improved in both the posterior colporraphy group and those without posterior repair at the time of POP surgery (26).

In our study, preoperative and postoperative POP-Q measurements predictably improved following surgical intervention, regardless of cohort. Our findings are consistent with a meta-analysis by Hudson et al. (27), who observed an overall anatomical success rate of 98.6% (27). However, the mean follow-up from this meta-analysis was 26.9±17.3 months as compared to our study that used a relatively short 12-week follow-up period.

Patients in the RASC alone group had greater anterior prolapse and significantly greater stage of prolapse compared to RASC and rectopexy (Table 2). This could have contributed to the increased risk of future posterior wall prolapse found in the RASC group. However, we do not believe that this would entirely account for our findings. Patients in the concomitant

Table 2. Preoperative pelvic organ prolapse quantification and prolapse stage

POP-Q	RASC	RASC + Rectopexy	p
	n=109	n=41	
Aa	2.7±1.0	1.3±1.6	**<0.0001**
Ba	4.5±2.3	1.8±2.2	**<0.0001**
C	2.6±4.6	-2.1±4.5	**<0.0001**
Gh	5.1±1.5	4.7±1.4	0.197
Pb	2.5±1.0	3.1±1.0	**0.002**
TVL	9.6±1.4	9.1±1.9	0.113
Ap	-1.2±1.7	0.0±1.8	**0.001**
Bp	-1.2±1.4	0.3±2.2	**<0.0001**
D	-4.6±2.3	-5.3±1.1	0.236
Stage			
Stage 2	1 (1)	11 (27)	**<0.0001**
Stage 3	83 (76)	28 (68)	
Stage 4	25 (23)	2 (5)	

All data reported as mean ± standard deviation or n (%).
RASC: Robotic Assisted Laparoscopic Sacrocolpopexy, POP-Q: Postoperative pelvic organ prolapse quantification

Table 3. Concomitant surgeries at time of sacrocolpopexy

Surgery	RASC	RASC + Rectopexy	p
	n=109	n=41	
Hysterectomy	55 (51)	14 (34)	0.098
Bilateral salpingectomy	22 (20)	8 (20)	1.000
BSO	35 (32)	5 (12)	**0.014**
Tension free vaginal tape	88 (81)	34 (83)	0.819

All data reported as mean ± standard deviation or n (%).
RASC: Robotic Assisted Laparoscopic Sacrocolpopexy, BSO: Bilateral salphigo-oopherectomy

RASC and Rectopexy cohort, however, had significantly greater posterior compartment defects as indicated by their POP-Q assessment. These findings are consistent with anatomical defects which would lead to the necessity of posterior repair.

Multicompartmental prolapse is evident in approximately 10-55% of patients with POP (18,19). Recently, there has been a shift from disparate, compartmentalized procedures toward concomitant procedures for correction of multicompartmental prolapse. Increasingly, ventral mesh rectopexy is being performed at the time of sacrocolpopexy for rectal prolapse (20,21). Our study evaluated ventral mesh rectopexy at the time of sacrocolpopexy and subsequent incidence of posterior wall vaginal prolapse. Our findings show a trend in reducing subsequent posterior wall prolapse when ventral mesh rectopexy was performed at the time of sacrocolpopexy. Patients who underwent ventral mesh rectopexy at the time of RASC showed better posterior wall integrity on the POP-Q assessment and also required less posterior wall corrective surgery compared to patients who underwent sacrocolpopexy alone. This reduction in subsequent posterior prolapse, however, was not statistically significant, secondary to inadequate sample size. Our study lacked power to discern a statistically significant difference and an appropriately powered study is required to confirm or refute this trend.

Table 4. Operative/postoperative complications and length of hospital stay

Complication	RASC	RASC + Rectopexy	p
	n=109	n=41	
Bowel/mesenteric injury	1 (0.9)	1 (2.4)	0.473
Port site hernia	1 (0.9)	0 (0)	1.000
Length of hospital stay (days)	1.1±0.5	1.3±0.7	0.071

All data reported as mean ± standard deviation or n (%), we did not run statistical analysis on complications given the low complication rates in each cohort.
RASC: Robotic Assisted Laparoscopic Sacrocolpopexy

Supplemental Table 1. Pre/postoperative POP-Q values for combined cohorts

POP-Q	Preoperative	Postoperative	p
Aa	2.3±1.3	-2.7±0.5	**<0.0001**
Ba	3.7±2.6	-2.7±0.6	**<0.0001**
C	1.4±4.9	-9.0±2.1	**<0.0001**
Gh	5.0±1.5	3.4±2.2	**<0.0001**
Pb	2.7±1.0	2.7±0.7	0.775
TVL	9.4±1.6	9.5±1.1	0.725
Ap	-0.8±1.8	-2.2±0.8	**<0.0001**
Bp	-0.8±1.8	-2.2±0.8	**<0.0001**

Data reported as mean ± standard deviation.
POP-Q: Postoperative pelvic organ prolapse quantification, TVL: Total Vaginal Length when in reference to POP-Q values

Study limitation

There are several limitations of this study. The retrospective nature of the study and the convenience sample are inherently prone to selection bias. However, this study provides an initial framework on which future prospective studies can be based. Furthermore, this study did not control for all medical comorbidities or confounding factors. Additionally, this study was underpowered to elucidate a statistical significance. However, we are currently conducting a study that addresses these issues and has an adequate sample size. Finally, subsequent posterior wall prolapse defined by POP-Q postoperatively was limited to 12 weeks of postoperative follow-up. It is possible that there could be posterior compartment prolapse past the follow-up

Table 5. Postoperative pelvic organ prolapse quantification and stage prolapse stage

POP-Q	RASC	RASC + Rectopexy	p
	n=109	n=41	
Aa	-2.7±0.5	-2.7±0.4	0.706
Ba	-2.7±0.6	-2.7±0.4	0.882
C	-9.3±1.5	-8.4±3.3	**0.036**
Gh	3.4±2.2	3.3±2.3	0.829
Pb	2.6±0.7	2.8±0.7	0.226
TVL	9.5±1.2	9.3±1.0	0.200
Ap	-2.1±0.8	-2.3±0.9	0.496
Bp	-2.2±0.7	-2.3±0.9	0.606
D	n/a	n/a	-
Stage	**n=103**	**n=40**	**-**
Stage 0	12 (12)	8 (20)	0.423
Stage 1	81 (78)	28 (70)	
Stage 2	10 (10)	4 (10)	

All data reported as mean ± standard deviation or n (%).
RASC: Robotic Assisted Laparoscopic Sacrocolpopexy, POP-Q: Postoperative pelvic organ prolapse quantification

Table 6. Outcomes and failure rates

Outcome	RASC	RASC + Rectopexy	p
	n=109	n=41	
Subsequent posterior repair	9 (8)	0 (0)	0.114
Failure (POP-Q definition)	11 (10)	1 (3)	0.181
Other reoperation	3 (3)	4 (10)	0.088

POP-Q: Postoperative Pelvic Organ Prolapse Quantification (POP-Q) Failure (POP-Q) defined as Ap or Bp greater than point 0 (beyond the hymenal ring).
RASC: Robotic Assisted Laparoscopic Sacrocolpopexy

period. However, we believe most failures would be evident in the three months postoperative timeframe.

Strengths of this study include its novelty. We believe that this is the first study to investigate the effect of concomitant rectopexy at the time of RASC and the recurrence of posterior wall prolapse. Furthermore, all the procedures were performed by one fellowship trained board certified surgeon in their respective fields, allowing consistency of technique and skill. In addition, all patients underwent standardized POP-Q evaluation both pre- and postoperatively.

Our study elucidated a trend towards fewer occurrences of posterior vaginal wall prolapse when ventral mesh rectopexy is performed at the time of sacrocolpopexy. Our study was underpowered, however, to prove a statistically significant difference.

Conclusion

It is possible that ventral mesh rectopexy provides important structural support of the posterior vaginal wall thereby reducing subsequent posterior prolapse. Overall there was a low complication rate, and surgical complications were not statistically different between groups. Additionally, hospital length of stay did not differ between groups. Patients at risk for posterior wall prolapse, for example POP-Q assessment demonstrating posterior wall defects, might benefit from concomitant ventral mesh rectopexy at the time of sacrocolpopexy for POP, without addition surgical complications or longer hospital stays. An adequately powered study is needed, however, to confirm these findings.

Ethics Committee Approval: *The study was approved and deemed exempt by the Ascension St. John Institutional Review Board (approval number: #1477635).*

Informed Consent: *This was a retrospective study, informed consent was not obtained.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: M.F.A.; Concept: M.F.A., C.R.; Design: M.F.A., C.R., K.H.H.; Data Collection or Processing: C.R.; Analysis or Interpretation: C.R., K.H.H.; Literature Search: K.R.M., M.G.B.J.; Writing: K.R.M., M.G.B.J.*

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

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The impact of using culture media containing granulocyte-macrophage colony-stimulating factor on live birth rates in patients with a history of embryonic developmental arrest in previous in vitro fertilization cycles

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Abstract

Objective: To investigate the effect of using culture media containing granulocyte-macrophage colony-stimulating factor (GM-CSF) on embryological data and reproductive outcomes in patients with early embryonic developmental arrest.

Material and Methods: Retrospective case-control study. A total of 39 patients, whose embryos were incubated with culture media containing GM-CSF due to embryonic developmental arrest in two previous in vitro fertilization (IVF) cycles in-between January 2016 and November 2017 at Hacettepe University IVF Center, were enrolled. Control group was generated among patients with first IVF attempts due to tubal factor in the same time period. All embryos in the control group were incubated with single step culture medium (without GM-CSF). For the control group selection, matching was done 1:2 ratio considering female age, body mass index, number of M-II oocyte retrieved, and number of embryo transferred (n=80).

Results: Demographic features and embryological data were comparable between two groups. Number of fertilized oocytes (2-pronuclear) was 3.7±2.0 in GM-CSF group and 3.9±2.5 in the control (p=0.576). Overall, number of embryos transferred (1.3±0.5 vs 1.3±0.5, respectively) and blastocyst transfer rate (67.6% vs 59.2%, respectively; p=0.401) were similar. For the reproductive outcomes, implantation rate (32.3% vs 33.1%, respectively; p=0.937), clinical pregnancy rate (33.3% vs 32.5%, respectively; p=0.770), and live birth rate (25.2% vs 26.2%, respectively; p=0.943) were similar.

Conclusion: Using GM-CSF-containing culture media in patients with two previous failed IVF attempts due to embryonic developmental arrest might rectify embryological data and reproductive outcomes. To make solid conclusion further randomized controlled trials are warranted. (J Turk Ger Gynecol Assoc 2021; 22: 181-6)

Keywords: GM-CSF, ICSI, recurrent IVF failure, embryonic developmental arrest

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Introduction

Up to 20% of in vitro fertilization (IVF) cycles are cancelled at the stage of follicular growth, oocyte retrieval, fertilization and cleavage stage before embryo transfer (1). Although optimal ovarian stimulation and successful fertilization steps have been completed, some embryos may stop cleaving at the 2- to 4-cell stage; this is termed “early embryonic developmental arrest” (2). The rate of early embryonic developmental arrest

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for human embryos in IVF cycles is ~10%, and 40% of patients experience at least one embryo arrest at the cleavage stage (2,3). The reasons for early embryonic developmental arrest are not fully understood (4,5). Chromosomal abnormalities are observed more often in arrested embryos than normal developed embryos (6). In addition, suboptimal culture conditions, reactive oxygen species (ROS), and inadequate oocyte maturation may also contribute to an increased risk of early embryonic developmental arrest.

Apoptosis is a mechanism for eliminating damaged cells in the human body. However, no biochemical, morphological or structural evidence of apoptosis has been detected in embryos before the 8-cell stage (7). Immature mitochondria in embryos in the 2-to 4-cell stages result in an absence of apoptosis and thus other mechanisms are induced to eliminate defective cells during this period of embryonic development (8,9). It is also known that early cleavage divisions are controlled maternally by transcription factors formed during oogenesis in vivo (10). Therefore, embryos, which are dependent on maternal infrastructure, become more vulnerable to environmental conditions such as ROS in vitro (11). During in vitro culture which negates the protective effect of the oviduct and reduces anti-oxidant defenses (12), lead to poor embryo quality and delayed embryonic development (5,13). Inappropriate expression of the genes encoding Adenosine Deaminase and glucose-6-phosphate dehydrogenase (14) in embryo metabolism and disabled correction of telomere length (15) at these early stages are other options for early embryonic development arrest. Embryo implantation requires embryo-endometrium synchronization, which is a multi-step process regulated by intracellular and intermolecular relations. These interactions are modulated by various cytokines and growth factors via autocrine, paracrine, and endocrine regulations (16). Deficiencies in some interleukins (IL) including IL-1, IL-4, IL-6, colony-stimulating factor-1 (CSF-1), granulocyte-macrophage CSF (GM-CSF), tumor necrosis factor-alpha (TNF-α), and TNF-b may result in poor embryonic growth, implantation, and pregnancy outcomes (17,18). GM-CSF is a multi-functional cytokine and is synthesized in the epithelial cells of the female reproductive tract, which is essential for modulating stress response genes, heat shock proteins, and apoptosis (19-21). Culture conditions for embryonic development are generally considered suboptimal. The supplementation of these factors may accelerate embryo development, increase blastulation rates, and decrease apoptosis. This study investigated the effects of adding GM-CSF to culture medium on embryological data and reproductive outcomes in patients with previous early embryonic developmental arrest.

Material and Methods

Patient selection

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical Committee approval was received from Hacettepe University Faculty of Medicine (approval number: 2019/19-02). A signed consent form was obtained from all patients.

For this study, the database of the IVF Centre of Hacettepe University Faculty of Medicine was scrutinized, for the period between January 2016 and November 2017. A total of 49 couples were identified whose embryos were treated with culture media containing GM-CSF (Embryogen®, Origio, Denmark) due to previous early embryonic development arrest (n=49). Inclusion criteria were as follows: 1) female <40 years old; 2) agonist and antagonist ovarian stimulation cycles; 3) Day 3 or 5 fresh embryo transfer cycles; 4) two consecutive IVF cycles with embryonic development arrest at the 2- to 4-cell cleavage stages. Embryonic development arrest was defined in couples with a history of ≥6 oocytes retrieval and ≥5 fertilized oocytes, but failure to reach embryo transfer during two consecutive IVF cycles. Exclusion criteria included male contributions necessitating surgical sperm retrieval. The inclusion and exclusion criteria narrowed the sample size of study group to 39 patients. To generate a control group from the patients that had undergone IVF treatment in the same time period (n=80) patients with tubal factor, female age (±1 year) and antral follicle (±2) were matched in a 1:2 ratio from the database. In the control group, all embryos were incubated in a single step culture medium with human albumin solution (Sage 1-Step, Origio®, Denmark).

The primary outcome of this study was investigating the live birth rate (LBR) in patients with a history of recurrent cycle cancelation due to embryonic development arrest after the enrichment of cultural media with GM-CSF. Secondary outcomes were embryo transfer rates on Day 5, implantation rates, and clinical pregnancy rates.

Ovarian stimulation

Recombinant follicle-stimulating hormone (r-FSH) (Gonal-F; MerckSerono GmbH, Kiel, Germany or Puregon; MSD, Haarlem, the Netherlands) or human menopausal gonadotropin (Merional; IBSA, Lamone, Switzerland, or Menopur, Menogon; Ferring Company, Kiel, Germany) were used solely or in combination for controlled ovarian stimulation. Starting dose varied between 175 and 200 IU. Pituitary suppression was maintained by a GnRH antagonist (Orgalutran; MSD or

Cetrotide; MerckSerono, addresses as above) or GnRH-agonist protocols, according to physician preference. Cycles were monitored by transvaginal ultrasound.

Laboratory procedure

Cumulus cell-oocyte complexes (COCs) were accumulated from aspirated follicular fluids under a stereomicroscope. Then COCs were washed twice with Flushing Medium with heparin (Origio) that contained 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid. At the end of the oocyte pick up, COCs were cultured in four-well dishes (Thermo Scientific™ Nunc™). Each well contained Fert solution (Origio) and was covered with liquid paraffin (Origio). The male patient semen sample was delivered to the laboratory on the day of Ovum Pick-up, and following liquefaction, the sperm sample was evaluated in terms of count, motility, and morphology. Subsequently, sperm sample was prepared using SupraSperm density gradient (Origio) with the "swim-up" technique. Prior to intracytoplasmic sperm injection (ICSI), COCs were removed from cumulus and corona cells using enzymatic (ICSI Cumulase, Origio) and mechanical digestion. After that, the oocytes were assessed in terms of nuclear maturation and all metaphase II (M-II) oocytes underwent ICSI under an inverted microscope with Hoffman optics at x200 magnification. These injected oocytes were cultured under two different conditions. One group (n=39) was cultured with 40 µl of EmbryoGen (Origio) media until Day 3, then with BlastGen (Origio) media up to day 5. The others (n=80) were cultured for 3 or 5 days with single step medium containing human albumin solution (Sage 1-Step, Origio). Oocytes were evaluated for fertilization after 16 to 18 hours. At 42-44 and 68-72 hours after ICSI, embryos were classified according to Hardarson et al. (22) morphological criteria. Embryo culture was extended to day 5, if there were at least three high quality embryos on day 3, according to the Gardner criteria (23). After culture with BlastGen or single step media, embryos were selected for transfer, were sampled with the Soft-Trans Embryo Transfer Catheter with 15-30 µl of media and transferred to the patient under ultrasound.

Statistical analysis

Mean and standard deviation, or median and 25^{th}-75^{th} percentiles (interquartile range), were used to describe continuous variables. Pearson chi-square test was used to identify differences between cycle and pregnancy outcomes between the groups. Groups were also compared with independent Samples t-test or Mann-Whitney U test. A two-sided p-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS, version 17.0 (IBM Inc., Chicago, IL, USA).

Results

The demographic characteristics and ovarian reserve tests were similar in the study and control groups (Table 1).

Assisted reproductive technologies cycles in the study and control groups

GnRH-antagonist protocol was administered to 23 (59%) of the 39 cycles in women in the study group and 55 (69%) of the 80 cycles in women in the control group (Table 2). Of the patients undergoing agonist protocols in the study group cycles, four (25%) were micro-dose flare-up and 12 (75%) protocols were luteal long leuprolide acetate. In the control group, 5 (20%) underwent micro-dose flare-up and 20 (80%) protocols were luteal long leuprolide acetate. On average, the duration of ovulation induction was 9.72 days and 9.70 days for the study and control groups, respectively (p=0.961). The median (25^{th}-75^{th} percentiles) total r-FSH dose was 2834 IU (1875-3375 IU) in the study group and 2576 IU (1600-3206 IU) in the control group (p=0.290).

Cycle and pregnancy outcomes

The mean COCs collected were 6.3±3.0 and 7.0±3.0 in the GM-CSF and control groups, respectively (p=0.292) (Table 2). The respective figure for mean fertilized oocytes (2-pronuclear) was 3.7±2.0 and 3.9±2.5 (p=0.576). Mean embryos transferred were 1.3±0.5 in both groups. Whereas embryo transfer on Day 5 was available in 67.6% of patients in the study group, 59.2% reached the blastocyst stage in the control group (p=0.401). Implantation (32.3% vs 33.1%, p=0.937), clinical pregnancy rates (33.3% vs 32.5%, p=0.770), and LBRs (25.2% vs 26.2%, p=0.943) were comparable between groups.

Discussion

According to the İstanbul consensus, embryos reach 7-9 cells by Day 3, with fragmentation under 15% and no multi-nucleation,

Table 1. Demographic characteristics of both groups

	GM-CSF group (n=39)	Control group (n=80)	p
Female age, (years)	32.8±4.1	31.7±5.3	0.219
AFC, (n)	12.2±8.3	12.9±8.0	0.412
AMH, (ng/mL)	2.38±1.6	2.30±1.6	0.800
BMI, (kg/m^2)	25.1±4.2	24.8±4.5	0.739
Duration of infertility, (months)	75.1±55.5	57.7±39.6	0.085

Data are expressed as means ± SD, p<0.05.
GM-CSF: Granulocyte-macrophage colony-stimulating factor, AFC: Antral follicle count, AMH: Anti-Müllerian hormone, BMI: Body mass index, SD: Standard deviation

Table 2. Comparison of cycle characteristics and pregnancy outcomes of both groups

	GM-CSF group (n=39)	Control group (n=80)	p
Antagonist protocol (n, %)	23/39 (59)	55/80 (69)	0.309
Total r-FSH dose (IU)	2834 (1875-3375)	2576 (1600-3206)	0.290
Cycle cancelation rate (n, %)	5/39 (12.8)	9/80 (11.2)	0.802
Number of retrieved oocytes	6.3±3.0	7.0±3.0	0.292
Number of M-II oocytes	4.9±2.6	5.6±3.5	0.263
Number of 2PN	3.7±2.0	3.9±2.5	0.576
Number of embryos transferred	1.3±0.5	1.3±0.5	0.707
Embryo transfer day (n, %)	-	-	0.416
Day 3	11/34 (32.4)	29/71 (40.8)	-
Day 5	23/34 (67.6)	42/71 (59.2)	-
Implantation rate, (%)	32.3±44.2	33.1±46.2	0.937
Clinical pregnancy rate, (n, %)	13/39 (33.3)	26/80 (32.5)	0.770
Miscarriage rate, (n, %)	3/13 (23.1)	5/26 (19.2)	0.997
Live birth rate, (%)	10/39 (25.6)	21/80 (26.2)	0.943

Data are expressed as means ± SD, $p<0.05$, median (min-max) and percentages.
GM-CSF: Granulocyte-macrophage colony-stimulating factor, r-FSH: Recombinant follicle-stimulating hormone, M-II: Metaphase II, SD: Standard deviation

and have cleaved during the preceding 24 hours are called "normal", embryos that have 6 or fewer cells on Day 3 (68±1 hour post insemination), but have cleaved during the former 24 hours are called "Slow", and embryos that have not cleaved within 24 hours are called "Arrested" (24). Approximately 40% of couples that undergo treatment exhibit at least one embryonic arrest per treatment cycle and 10-15% of all human embryos arrest at the early cleavage stage (2,3). Despite the sufficient number of oocytes and zygotes, embryonic development arrest is closely associated with assisted reproductive technology (ART) and may lead to recurrent treatment failure. The presence of arrested embryos may diminish the total number of available embryos and affect the treatment cycle outcome. This condition is destructive for the patients receiving treatment. The reasons for early embryonic developmental arrest are not fully understood (4,5). Chromosomal abnormalities, suboptimal culture conditions, ROS, apoptosis and inadequate oocyte maturation have all been shown to be a reason for early embryonic developmental arrest (6).

Suboptimal culture conditions are a possible cause of early embryonic development arrest (4,5). Culture medium and ingredients are crucial for embryo development and implantation. Therefore, various growth factors and cytokines have been examined by supplementing the culture medium to optimize embryo development. GM-CSF may be an ideal candidate for embryo development owing to its natural existence in the surface of the fallopian tubes and endometrial cells (19-21). GM-CSF initiates biological activity by binding to its receptor, which is a 18-22 kDa glycoprotein and is a cytokine/growth factor secreted by the uterine epithelium and oviducts under the influence of estrogens. GM-CSF acts as a principal mediator of recruitment and activation of neutrophils and macrophages during early pregnancy (19). GM-CSF also activates granulocytes, mononuclear phagocytes and dendritic cells, modulates their migration into tissues and activity in situ, and supports cytotoxicity, phagocytosis, antigen introduction, and cytokine discharge (25). GM-CSF is involved in embryonic development, growth and viability by effecting cell proliferation, blastocyst development, hatching and implantation (26). Some genetic combinations may result in the production of polymorphic *Killer immunoglobulin-like receptors (KIR)* genes from maternal decidual Natural Killer cells and defective *human leukocyte antigen-C (HLA-C)* genes from fetal trophoblasts. Incompatibility between KIR-HLA-C can cause abnormal placentation. GM-CSF also provides higher KIR-HLA-C coupling by increasing the migration of primary trophoblasts (27).

Culture media containing GM-CSF accelerates embryo development, increases early cleavage embryo counts that develop at the blastocyst stage, and increases viable inner mass cells with less apoptosis in cultured human embryos in vitro (28). Tevkin et al. (29) reported that using GM-CSF in the culture medium, to improve recurrent ART failure, increased, although not statistically significantly, the clinical pregnancy rates compared to the control group (39.1% vs 27.8%). The implantation rate after seven weeks of gestation (11.6% vs 20.4%, $p<0.05$) and clinical pregnancy rate after first trimester (9.1% vs 17.4%, $p<0.001$) were significantly higher in the GM-CSF group (29). They concluded that adding GM-CSF to the cultural medium improved implantation and enhanced the success of IVF/ICSI cycles (29).

Culture medium with GM-CSF was used in a randomized clinical trial to compare the effect of low (2 mg/mL) and high (5 mg/mL) human serum albumin (HSA) (28). Under the low HSA concentration, the ongoing implantation rate at first trimester for the GM-CSF group was significantly higher (23.5% vs 16.7%, $p=0.007$) than the control group, whereas the high HSA concentrations were similar between groups (22.4% vs

21.1%, p=0.53), respectively. Overall, LBRs were significantly higher in the GM-CSF group compared to the control group (28.9% vs 24.1%, p=0.03). Also, failed embryonic development in previous cycles in culture medium with high HSA, implantation, and clinical pregnancy rates were similar in the subsequent cycle with GM-CSF-supplemented culture medium compared to the control group (28). Also, in this study in terms of reproductive outcomes, implantation rates (32.3% vs 33.1% respectively; p=0.937), clinical pregnancy rate (33.3% vs 32.5%, respectively; p=0.770) and LBR (25.2% vs 26.2%, respectively; p=0.943) were the same.

The effect of culture media enriched with GM-CSF, heparin-binding epidermal growth factor-like factor, and leukemia inhibitory factor has been investigated in ICSI cycles and the ongoing pregnancy rates (30). Ongoing pregnancy rates were higher in the group using enriched culture media [106/224 (47%) vs 78/219 (36%); absolute rate difference (ARD): 12; 95% confidence interval (CI), 2.5-21]. Cumulative LBRs were also higher [132/224 (60%) vs 97/219 (44%); ARD: 12; 95% CI, 4-20], and pregnancy loss rates were lower [27/124 (22%) vs 37/103 (36%); ARD: -14; 95% CI, -26 to -2]. The authors argued that less biochemical stress occured in the enriched culture environment, so that less energy was consumed by embryo plasticity, and the reserve was then available for post-implantation development.

In a retrospective study using GM-CSF in culture medium in fresh transfer cycles, the cleavage rate and the blastocyst formation rate were found to be similar compared to the control group (31). However, the available embryo rate was significantly higher in the GM-CSF group. In the subgroup analysis, cleavage rate and blastocyst formation rate were found to be significantly higher in those over 38 years old.

In a review article evaluating the effects of GM-CSF in sub-fertile patients undergoing ART, the beneficial effects of cytokine supplementation have been observed in periods of embryonic development. However, the implantation rates and pregnancy rates were different among investigated studies. According to a Cochrane meta-analysis, there was no evidence that GM-CSF supplemented culture media had any superiority compared to standard media in terms of clinical outcomes (32). Furthermore, only one large, randomized, controlled trial ended with positive results in LBRs between the study and control groups (26). Similarly, in our study the number of M-II and fertilized oocytes were comparable between the groups.

Retrospective design and a small sample size were limitations of this study.

GM-CSF supplemented culture medium might be recommended to patients with a history of recurrent failed IVF attempts due to arrest in embryo development.

Conclusion

Using GM-CSF containing culture media in patients with two previous failed IVF attempts due to embryonic developmental arrest might rectify embryological data and reproductive outcomes. However, further studies are needed to confirm these findings and support the routine use of GM-CSF supplemented culture medium in IVF.

Ethics Committee Approval: *All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical Committee approval was received from Hacettepe University Faculty of Medicine (approval number: 2019/19-02).*

Informed Consent: *A signed consent form was obtained from all patients.*

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Assessing reference levels of nickel and chromium in cord blood, maternal blood and placenta specimens from Ankara, Turkey

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Abstract

Objective: Placenta is a temporary organ that connects the developing fetus and the mother. However, it cannot protect the embryo against chromium (Cr) and nickel (Ni) exposure. Quantification of Cr and Ni in biological and ecological subjects is challenging. Thus, the first goal of this study was to provide a validated Graphite Furnace Atomic Absorption Spectrometry (GFAAS) method to determine Cr and Ni in mother-newborn specimens. The second goal was to assess the reference Ni and Cr contents in cord blood, maternal blood, and placenta samples in a population from Ankara.

Material and Methods: Biological samples were collected from 100 healthy mother-newborn pairs. Metal levels were quantified by GFAAS. Method validation of this toxicological analysis was performed by the use of certified reference materials, and assessed through accuracy, precision, specificity, range, quantitation, and detection limits.

Results: Mean Cr levels of maternal blood, placentas, and cord blood were 0.337±0.222 μg/L, 0.221±0.160 μg/kg, 0.121±0.096 μg/L, respectively while mean Ni concentrations were 0.128±0.093 μg/L, 0.124±0.067 μg/kg, 0.099±0.067 μg/L, respectively. The method showed linearity with excellent correlation coefficients (r^2) for Cr (0.9994) and Ni (0.9999). Satisfactory recovery and coefficient of variation for Cr and Ni were 102.85% and 102.35%; 1.75% and 2.91%, respectively. Relative error did not exceed 3%, demonstrating the accuracy of the method. Control charts were drawn to assess inter-day stability. The predicted reference ranges for Cr and Ni concentrations in maternal blood, placenta and cord blood were: Cr 0.033-0.75 μg/L; 0.032-0.526 μg/kg; 0.031-0.309 μg/L and for Ni were 0.011-0.308 μg/L; 0.024-0.251 μg/kg; 0.066-0.209 μg/L, respectively.

Conclusion: The reported reference values of biological specimens in this paper will provide complementary aid to health professionals in terms of assessment of environmental and occupational exposure. (J Turk Ger Gynecol Assoc 2021; 22: 187-95)

Keywords: Placenta, cord blood, maternal blood, nickel, chromium, validation, reference range, GFAAS

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Introduction

Certain compounds of chromium (Cr) and nickel (Ni) are poisonous, particularly with increasing long-term exposure. For instance, nickel carbonyl or hexavalent chromium are categorized as carcinogens. Human exposure to Cr and Ni can arise via ingestion of polluted water or food, as well as inhalation or dermal contact, since these metals are applied in an elemental form in many industrial activities (1). There are also additional paths of exposure to Cr and Ni such as smoking or contact with coins, stainless steel and jewelry (2,3), particularly in the daily life of pregnant women.

Chromium-related ecological pollution has been increasing as a result of its greater worldwide industrial usage. Exposure to chromium can cause critical medical disorders, such as abnormal enzymatic activity, oxidation-reduction derangement and protein denaturation. In addition, asthma,

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back pain, dermatitis, cancer, chromosomal aberrations, chronic bronchitis, changes in hemoglobin, hypertension and metabolic syndrome have all been associated with chromium exposure (4,5). Chromium can cross the placenta (6). Previous animal studies have suggested that exposure to elevated chromium levels in the prenatal period harms implantation and embryonic growth (7). Furthermore, there is evidence that fetal resorption, intrauterine death, skeletal anomalies, decreased fetal weight, malformations and retarded fetal growth may be associated with chromium exposure (8). Most of the present information regarding the health effect of exposure to chromium depends on data obtained following occupational exposure. Nevertheless, several studies have suggested an increased risk of inborn abnormalities, reduced birth weight and DNA damage in neonates born into areas affected by chromium pollution (9,10).

Nickel also crosses the placenta and has been shown to impair fetal development in animal studies (11). Numerous investigations demonstrate that heavy metal contamination is a changeable risk issue in terms of perinatal results along with many congenital disorders (12). The relationship between cancer and Ni depends on industrial exposure and has been associated with different types of cancer including kidney, stomach, breast, and neck/head and nose malignancies (13). Additionally, exposure to high concentrations of Ni may lead to contact dermatitis, epigenetic changes, alteration in gene regulation and apoptosis induction (14). Also, exposure to Ni may cause developmental and reproductive toxicological effects, which include birth defects, abortion, fertility or subfertility (15,16). Moreover, embryonic progression, a declining proliferation of inner cell mass and trophoblast cells may be influenced by exposure to excess amounts of Ni (17). This evidence suggests that exposure to Ni is a critical problem, both for public health and environmental protection (18,19).

Since there is limited knowledge regarding the effect of Cr and Ni exposure in the population in terms of prenatal development, supplementary studies investigating placental transfer of these heavy metals are required (20). In addition, analysis of chromium and nickel in biological and ecological samples is not easy due to interferences in the matrix and possible low levels in specimens (21). Therefore, sensitive methods to quantify Cr and Ni in biological and environmental specimens has become a critical research topic for public health. Thus, an extremely sensitive and sophisticated analytical assay is necessary.

To the best of our knowledge, there is no study focusing on toxicological monitoring of Cr and Ni profiles in maternal blood, placenta and cord blood in the Turkish population. From this point of view, the overall objectives of this investigation were twofold. The first goal was to optimize and validate the Graphite Furnace Atomic Absorption Spectrometry (GFAAS) methods to quantify Cr and Ni concentrations at trace levels in biological samples. The second target was to provide toxicological monitoring of Cr and Ni profiles in maternal blood, cord blood and placenta samples in the Turkish population, thus providing a measure of possible environmental exposure, as well as providing reference values of chromium and nickel in these biological tissues.

Material and Methods

Study subjects

This scientific work was ethically authorized by the Research Ethics Committee of the Ankara University Faculty of Medicine (approval number: 33-730/July 11, 2011). Each volunteer provided written informed consent in line with the ethics as recognized in the Declaration of Helsinki (World Medical Association, Declaration of Helsinki, 1964).

All research samples were collected in Ankara, Turkey as the capital of Turkey experiences relatively light exposure to industrial pollution. Participants having no known industrial or environmental exposure to xenobiotics, including heavy metals, were included based on the sample selection criteria. Of 137 eligible participants, some were excluded due to improperly completed consent forms while several had a diagnosis of intrauterine growth retardation. Hence, 100 healthy mother-newborn pairs were recruited to the study. The final cohort consisted of mothers aged 19-41 years who had given birth at between 36-41 weeks gestation. Placenta, cord blood, and maternal blood samples were gathered at delivery by cesarean section or spontaneous labor. Blood specimens were collected into vacutainer blood tubes and stored at 4 °C, while placenta samples were kept at -20 °C until the day of analysis.

Standard solutions and reagents

Stock solutions of 1000 µg/mL Cr and Ni were purchased from SCP Science AA Standards (Canada). Nitric acid (HNO_3, 65% v:v) was procured from Merck (Darmstadt, Germany). The chemicals used for the laboratory work were at analytical reagent grade. High purity (99.999%) argon gas was bought from a local supplier (Vasak Gaz, Ankara, Turkey). With the resistivity of 18MΩ cm, ultrapure water (Merck Millipore Direct-Q8, Germany) was utilized to prepare the solutions for the experimental study. The certified reference material (CRM) NC SZC 73016-Chicken (NCS Testing Technology Co., Ltd., Haidian, Beijing, China) was used for validation of the method.

Sample preparation and procedure

To prepare calibration standards at concentrations of 0.5, 1.0, 5.0, 10.0, and 20.0 mg/L, a 1000-µg/mL of Cr and Ni stock solution were diluted with 10% (v:v) HNO_3. A relatively

high concentration of nitric acid was used in our calibration standards to simulate the acid content in the final digested biological samples. A previously described digestion protocol was followed (22-24) before starting the instrumental analysis. One milliliter volumes of blood samples and accurately weighed dry placenta samples (not exceeding 200 mg) were liquified with 5 mL of 65% (v/v) nitric acid in Teflon® microwave tubes. Digestion was carried out at 1600 W and 220 °C for 20 minutes by means of the microwave system Mars Xpress (CEM, Matthews, NC, USA). Then the liquified solutions were diluted in ultra-pure water to 10 mL in 15 mL polypropylene tubes. The samples were kept at 4 °C until the day of analysis.

Instrumentation

Cr and Ni levels in maternal blood, cord blood, and placenta samples were quantified using a Varian AA 240 GFAAS with Zeeman background correction (Varian Corp, Victoria, Australia). Boosted-discharge hollow cathode lamps (Agilent, USA) were utilized as the excitation source for Cr and Ni. The instrumental working parameters for the GFAAS system were shown in Table 1.

Table 1. Operating parameters of GFAAS method

Element - matrix	Cr-Blood/ Placenta	Ni-Blood/ Placenta
Instrument	Zeeman	Zeeman
Concentration unit	µg/L; µg/kg	µg/L; µg/kg
Instrument mode	Absorbance	Absorbance
Sampling	Auto-mix	Auto-mix
Calibration mode	Concentration	Concentration
Measurement mode	Peak height	Peak height
Replicates standard	3	3
Replicate sample	3	3
Expansion factor	1.0	1.0
Wavelength	357.9 nm	232.0 nm
Slit width	0.2 nm	0.2 nm
Gain	45%	75%
Current	15.0 mA	4.0 mA
Background	BC on	BC on
Standard 1	0.5 µg/L	0.5 µg/L
Standard 2	1.0 µg/L	1.0 µg/L
Standard 3	5.0 µg/L	5.0 µg/L
Standard 4	10.0 µg/L	10.0 µg/L
Standard 5	20.0 µg/L	20.0 µg/L
Reslope standard	Standard 3	Standard 3
Recalibration rate	50	50
Calibration algorithm	Linear	Linear

GFAAS: Graphite Furnace Atomic Absorption Spectrometry, Cr: Chromium, Ni: Nickel, BC: Background

Statistical analysis

The use of various statistical methods assessed the elemental quantifications in mother-newborn biological specimens. The Kolmogorov-Smirnov test was utilized for assessment of normality of data distribution while correlations between the parameters were examined through the Pearson's test. Statistical significances among mean values were evaluated employing the Student's t-test. Statistical test results were interpreted as mean ± standard deviation (SD) of the mean. Statistical significance was assumed when $p<0.05$. SPSS® software version 16.0 was used throughout the statistical analysis.

Results

Optimization

In order to achieve the best possible performance, this method was optimized in terms of digestion technique, appropriate wavelength for the placenta and blood matrix, calibration concentration range in keeping with the Cr and Ni concentration in real biological specimens, and approximating linearity as much as possible.

Absorbance was quantified as a function of Cr and Ni concentration at 357.9 nm, and 232.0 nm, respectively. The proposed methods show good linearity in the range of 0-20 µg/L for Cr and Ni. The correlation coefficients and equation of the calibration curves for Cr and Ni were respectively found to be r^2: 0.9994 Abs: 0.0384C+0.0044 and r^2: 0.9999 Abs: 0.0071C+0.0003, where Abs is integrated absorbance and C is the concentration in µg/L. Graphite furnace temperature programs for Cr and Ni are listed in Table 2.

Validation

In keeping with the validation guide ISO/IEC 17025 standard (25) method, validation of this toxicological assay was performed by use of CRM, which was resulted in calculation of the accuracy, precision, specificity, range, quantitation and detection limits. CRM was analyzed 11 times with triplicate measurements. The results were compared to the certified values to evaluate the accuracy, precision, and recovery of the method. The certified Cr content of the CRM was 590.00±11.00 µg/kg, while the measured value was 606.84±10.65 µg/kg with the successful percent recovery and coefficient of variation (CV) of 102.85% and 1.75%, respectively. Similarly, the certified Ni content of the CRM was 150.00±3.00 µg/kg, while the measured value was 153.53±4.47 µg/kg, with the successful percent recovery and CV of 102.35% and 2.91%, respectively. Relative error did not exceed 3%, indicating that the method was accurate. The validation study of this assay is summarized in Table 3.

The limit of detection (LOD) and lowest limit of quantification (LOQ) was computed utilizing the SD of the response and

the slope of the calibration curve, according to ICH guiding principle (26) (LOQ: 10σ/S, LOD: 3.3σ/S, where S is the slope of the calibration curve and σ is the SD of the response). GFAAS method for Cr and Ni analysis provided detection and quantification limits of 0.010 and 0.030 and 0.060 and 0.182, respectively.

Quality control

The control chart analysis offers an examination of the inter-day and intra-day stability of the instrument (27-29). In other words, control charts make available tracking the accuracy of routine analytical work (30). Therefore, a mixture solution containing Cr and Ni at a concentration of 100 µg/L was quantified by GFAAS assay once a day throughout two weeks, and the mean concentrations of Cr and Ni were quantified as 100.18±2.09 µg/L, and 100.05±2.21 µg/L, respectively. Next, warning limits were computed by the following formula: warning limits: $x_{mean} \pm 2\sigma$, while control limits were quantified from the formula: control limits: $x_{mean} \pm 3\sigma$. Thus, the lowest control limit, upper control limit, lowest warning limit and upper warning limit were calculated accordingly for Cr and Ni. The results of the control chart study for Cr and Ni are shown in Figure 1, 2, indicating that the inter-day stability of the instrument was acceptable.

Data analysis

The outcomes of this toxicological investigation were statistically analyzed with the SPSS, version 16.0 (IBM Inc., Armonk, NY, USA). Descriptive statistics for Cr and Ni analyses in maternal blood, placenta and cord blood are shown in Table 4. Mean Cr levels of maternal blood, placenta samples, and cord blood were 0.337±0.222 µg/L, 0.221±0.160 µg/kg, 0.121±0.096 µg/L, respectively. Similarly, mean Ni concentrations of these biological specimens were 0.128±0.093 µg/L 0.124±0.066 µg/kg, 0.099±0.067 µg/L, respectively. Hence, a statistically significant negative correlation was found between the maternal blood-Cr and cord blood-Cr levels ($r=-0.21$, $p<0.05$) while another negative correlation was determined between the placental nickel and maternal blood nickel concentrations ($r=-0.27$; $p<0.001$).

Table 2. Graphite furnace temperature programs

	Step	Temperature (°C)	Time (s)	Flow (L/min)	Signal collection		Reading	
Chromium	1	85	5.0	0.3	×	No	×	No
	2	95	40.0	0.3	×	No	×	No
	3	120	10.0	0.3	×	No	×	No
	4	900	5.0	0.3	×	No	×	No
	5	900	1.0	0.3	×	No	×	No
	6	900	2.0	0.3	×	No	×	No
	7	200	7.8	0.3	√	Yes	×	No
	8	200	2.0	0.0	√	Yes	√	Yes
	9	2550	1.1	0.0	√	Yes	√	Yes
	10	2550	2.0	0.0	√	Yes	√	Yes
	11	2550	2.0	0.3	√	Yes	×	No
Nickel	1	85	5.0	0.3	×	No	×	No
	2	95	40.0	0.3	×	No	×	No
	3	120	10.0	0.3	×	No	×	No
	4	800	5.0	0.3	×	No	×	No
	5	800	1.0	0.3	×	No	×	No
	6	800	2.0	0.0	×	No	√	Yes
	7	2400	0.8	0.0	√	Yes	√	Yes
	8	2400	2.0	0.0	√	Yes	√	Yes
	9	2400	2.0	0.3	×	No	√	Yes

Table 3. Analysis of certified reference material (NCSZC3016)

	n	Certified value[a] (µg/kg)	Measured Value[a] (µg/kg)	CV%[b]	RE%[c]	R%[d]
Cr	11	590.00±11.00	606.84±10.65	1.75	2.85	102.85
Ni	11	150.00±3.00	153.53±4.47	2.91	2.35	102.35

[a]: Values are expressed as mean ± standard deviation, [b]: Coefficient of variation, [c]: Relative error, [d]: Recovery, Cr: Chromium, Ni: Nickel, CV: Coefficient of variation

Discussion

Human toxicological biomonitoring is a unique technique to screen public health in the event of chemical exposure. Thus, an explanation of toxicological monitoring data can be utilized for health risk assessment in the presence of chemical exposure (31,32). Measurement and description of toxic substances in biological specimens of healthy normal mothers and newborns provides impartial information of general population exposure (33). The prenatal period is critical since chemical exposure to toxins may result in biological alteration (34). Estimating the reference values in biological specimens provides complementary data for health professionals in terms of assessment of environmental and occupational exposure. Therefore, toxicological monitoring of Ni and Cr before and during pregnancy has become important because of the health effects on embryos, birth defects, growth retardation and neurodevelopmental disorders.

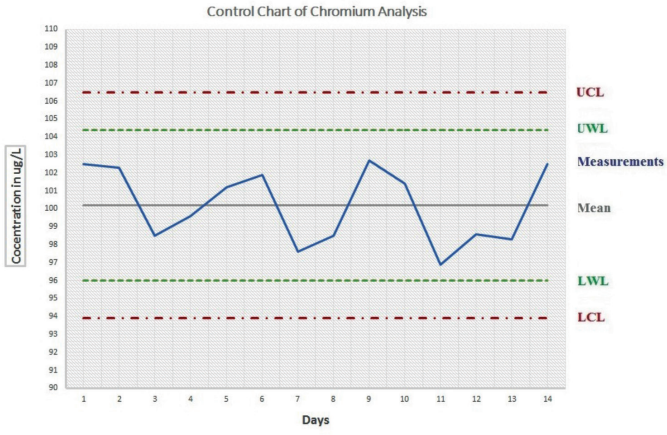

Figure 1. Control Charts of Chromium by GFAAS. The concentration of chromium is presented in ppb while UCL, UWL, LWL and LCL stand for upper control limit, upper warning limit, lowest warning limit and lowest control limit, respectively. The blue line represents the stability in the chromium concentrations quantified among days

GFAAS: Graphite Furnace Atomic Absorption Spectrometry, UCL: Upper control limit, UWL: Upper warning limit, LWL: Lowest warning limit, LCL: Lowest control limit

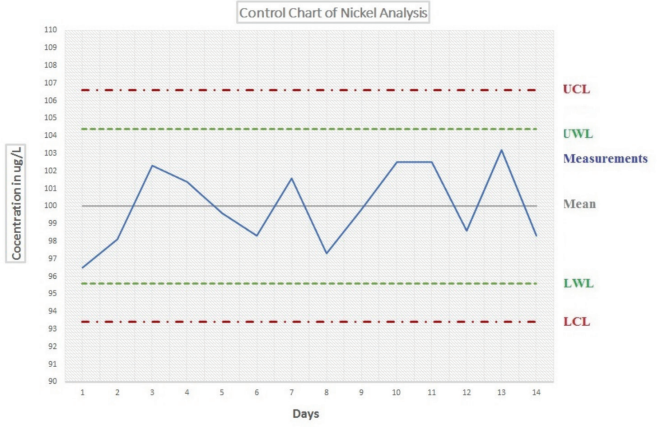

Figure 2. Control charts of nickel by GFAAS. The concentration of nickel is presented in ppb while UCL, UWL, LWL and LCL stand for upper control limit, upper warning limit, lowest warning limit and lowest control limit, respectively. The blue line represents the stability in the nickel concentrations quantified among days

GFAAS: Graphite Furnace Atomic Absorption Spectrometry, UCL: Upper control limit, UWL: Upper warning limit, LWL: Lowest warning limit, LCL: Lowest control limit

As can be seen in Table 4, mean concentrations of chromium levels in maternal blood, placenta samples and cord blood were significantly higher than nickel levels in this cohort ($p<0.05$). This finding is consistent with previous research in which blood Cr and Ni levels were studied (35). On the contrary, there are also studies reporting Ni levels are higher than Cr in biological specimens (Table 5). This may be due to the characteristics of the exposure source. Goullé et al.

Table 4. Descriptive statistics of Cr and Ni levels in biological specimens

	n		Cr[a]	Ni[a]	p value[b]
Maternal blood	100	Minimum	0.033	0.011	0.03
		Maximum	3.077	0.800	
		Mean[c]	0.337±0.222	0.128±0.093	
		5% Trimmed mean	0.306	0.122	
		Reference range	0.033-0.750	0.011-0.308	
Placenta	100	Minimum	0.032	0.024	0.04
		Maximum	0.914	0.683	
		Mean[c]	0.221±0.160	0.124±0.066	
		5% Trimmed mean	0.206	0.119	
		Reference range	0.032-0.526	0.024-0.251	
Cord blood	100	Minimum	0.031	0.066	0.04
		Maximum	1.202	0.588	
		Mean[c]	0.121±0.096	0.099±0.067	
		5% Trimmed mean	0.117	0.105	
		Reference range	0.031-0.309	0.066-0.209	

[a]: Values are given µg/L for blood samples while µg/kg for the placenta, [b]: p-values reported here indicate those mean concentrations of chromium levels in biological specimens were found significantly higher than nickel levels in these biological specimens since it is <0.05, [c]: Mean values are expressed as mean ± standard deviation, Cr: Chromium, Ni: Nickel

(36) reported reference Cr and Ni levels for blood samples of 99 healthy children as 0.49-1.86 μg/L and 0.68-2.62 μg/L, respectively. Therefore, Cr and Ni contents in biological specimens from our present study were comparatively lower than the formerly reported reference ranges, indicating that our results seem to be at safe levels. Based on previous papers (37,38), our reference values estimated in Table 4 were computed by means of the 5% trimmed mean ± 2σ, so as to lessen the impact of the skew in all paths. Since the mean -2σ value emerged as continuously inferior to the minimum value of the experimental measurements, this low-end value was involved in the reference range. Consequently, the predicted reference ranges for Cr and Ni content in maternal blood, placenta and cord blood were as follows: Cr 0.033-0.75 μg/L; 0.032-0.526 μg/kg; 0.031-0.309 μg/L and Ni 0.011-0.308 μg/L; 0.024-0.251 μg/kg; 0.066-0.209 μg/L, respectively.

Further comparison of Cr and Ni content in various matrices among the previous reports and our present study are summarized in Table 5. Zhang et al. (2) investigated the relationship between Ni exposure and the occurrence of congenital heart defects. The outcome proposed that the frequency of congenital heart defects is conceivably linked with Ni exposure (2). Novak et al. (18) showed that women with metal-on-metal implants and their children have higher cobalt and Cr levels than controls, indicating that the placenta is to some degree permeable to metal ion transport. Manduca et al. (39) researched the effect of war on metal levels in maternal hair. Their results showed that war in Gaza, as environmental exposure, elevated the metal levels including Cr and Ni levels in maternal hair. Pan et al. (10) investigated the relationship between ecological Cr exposure and premature labor in the general population. Their results indicated a potential association between the risk of delivering preterm infants and elevated exposure to Cr throughout the pregnancy (10). Callan et al. (40) performed a study highlighting maternal exposure to metals, including Cr and Ni levels in maternal blood. In Spain, Bocca et al. (41) predicted the gestational exposure to essential and toxic metals by determining their levels in maternal blood, cord blood and maternal urine. Their study suggested

Table 5. Comparison of chromium and nickel contents in various matrices among previous studies and present report

Country	Tissue	Metal	Value [a]	References
USA	Maternal serum (implant group)	Cr	1.870	(2)
USA	Infant serum	Cr	0.288	(2)
USA	Maternal serum (implant group)	Ni	0.136	(2)
USA	Infant serum	Ni	0.304	(2)
China	Maternal urine	Cr	1.01 [b]	(10)
China	Placenta (case group)	Ni	178	(18)
China	Placenta (control)	Ni	148	(18)
Palestine	Maternal hair (military attack)	Cr	2930	(39)
Palestine	Maternal hair (military attack)	Ni	2760	(39)
Australia	Maternal urine (non-smoking)	Cr	0.53	(40)
Australia	Maternal urine (non-smoking)	Ni	4.7	(40)
Australia	Maternal blood (non-smoking)	Cr	3.15	(40)
Australia	Maternal blood (non-smoking)	Ni	11.7	(40)
Spain	Cord blood	Cr	0.6	(41)
Spain	Maternal blood	Cr	0.5	(41)
China	Maternal blood	Cr	6.36	(42)
China	Cord blood	Cr	12.6	(42)
China	Maternal blood	Ni	14.5	(42)
China	Cord blood	Ni	6.1	(42)
China	Placenta (e-waste recycling town)	Cr	234.31 [b]	(43)
China	Placenta (control)	Cr	228.40 [b]	(43)
China	Placenta (e-waste recycling town)	Ni	7.64 [b]	(43)
China	Placenta (control)	Ni	14.30 [b]	(43)
China	Maternal blood	Cr	0.98	(44)
China	Maternal blood	Ni	1.81	(44)

Table 5. Continued

Country	Tissue	Metal	Value [a]	References
UK	Maternal blood	Cr	1.28	(45)
UK	Umbilical cord blood	Cr	0.378	(45)
UK	Maternal blood (control)	Cr	0.199	(45)
China	Maternal urine	Cr	2.69 [b]	(46)
USA	Placenta	Ni	111.0	(47)
Turkey	Maternal blood	Cr	0.337	Present study
Turkey	Cord blood	Cr	0.121	Present study
Turkey	Placenta	Cr	0.221	Present study
Turkey	Maternal blood	Ni	0.128	Present study
Turkey	Cord blood	Ni	0.099	Present study
Turkey	Placenta	Ni	0.124	Present study

[a]: Values are given μg/L for blood and urine samples while μg/kg for placenta and hair, [b]: Median, Cr: Chromium, Ni: Nickel, USA: United States of America, UK: United Kingdom

that metabolic and physiological variations throughout pregnancy changed the content of essential and toxic metals (41). Li et al. (42) determined the impact of heavy metals including Cr and Ni exposure during pregnancy in Beijing, China. The authors stated that there was neither a significant relationship between birth length/weight and toxic metal nor a possible issue in terms of neonatal developmental toxicity (42).

As was shown in the statistical analysis, this study highlighted a statistically significant negative correlation between maternal blood-Cr and cord blood-Cr levels ($r=-0.21$, $p<0.05$). Also, another negative correlation was determined between the placental Ni and maternal blood Ni concentrations ($r=-0.27$; $p<0.001$). These findings suggest that the placenta, between the maternal and fetal circulation, can be utilized as a biological indicator for exposure to metals during pregnancy (43).

Study limitation

Our research has some limitations. These include the relatively low number of participants and the small catchment area of the sample population as specimens were only obtained in Ankara, Turkey. In order to define global reference values for these heavy metals in these biological tissues, carefully selected and much larger sample populations would be required. As is demonstrated by the widespread neoteric studies mentioned here, numerous investigators are performing novel and innovative designs in the field of toxicological monitoring of metal levels in human biological materials, including maternal blood, cord blood and placenta. There is thus hope that advances will be forthcoming in the prevention of potential birth defects caused by prenatal exposure to chemicals.

Conclusion

This study showed that quantification and identification of Cr and Ni in biological samples of mother-newborn pairs can be used as evidence of neonatal exposure. The results also reiterate that the placenta is not a perfect preservative against metal ion transport, although the placenta appears as a regulating organ. The measured concentration of Cr and Ni were relatively low compared to other reference ranges and this suggested that exposure to these metals poses little threat negative for the mothers and the newborns in our cohort. Besides, this GFAAS method offers excellent versatility for clinical research laboratories, since the validation was appropriate in terms of ISO 17025 certification. Last but not least, the reported reference values of Cr and Ni in the biological specimens through this paper will provide complementary aid to health professionals in terms of assessment of environmental and occupational exposure.

Ethics Committee Approval: *This scientific work was ethically authorized by the Research Ethics Committee of the Ankara University Faculty of Medicine (approval number: 33-730/July 11, 2011).*

Informed Consent: *Each volunteer provided written informed consent in line with the ethics as recognized in the Declaration of Helsinki (World Medical Association, Declaration of Helsinki, 1964).*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - B.Y., E.A., T.S.; Design - T.S.; Data Collection or Processing - B.Y., E.A.; Analysis or Interpretation - B.Y., T.S.; Literature Search - B.Y., T.S.; Writing - B.Y.*

Conflict of Interest: *All authors declare no conflict of interest.*

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Clinical and radiologic characteristics of symptomatic pregnant women with COVID-19 pneumonia

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Abstract

Objective: To describe the radiological features, diagnostic accuracy and features of imaging studies and their relation with clinical course of Coronavirus disease-2019 (COVID-19) pneumonia in pregnant women.

Material and Methods: The clinical, laboratory and radiological features of symptomatic pregnant women suspected of COVID-19 were retrospectively reviewed. Chest radiography (CXR) and chest computed tomography (CT) findings of COVID-19 in pregnant women were identified.

Results: Fifty-five of eighty-one pregnant women were included in the final analysis. The most common admission symptoms were dry cough (45.4%), fever (29.1%) and dyspnea (34.5%). Radiological imaging studies were performed in 34 (61.8%) patients. Fourteen (66.7%) of the laboratory-confirmed COVID-19 patients had parenchymal abnormalities on CXR, and most common abnormalities were airspace opacities (61.9%) and prominent bronchovascular shadows (28.6%). Seventeen (85.0%) of the patients had parenchymal abnormalities consistent with COVID-19 on their chest CT. Chest CT most commonly showed bilateral (88.2%), multilobe (100%) involvement; peripheral and central distribution (70.6%); patchy-shape (94.1%) and ground-glass opacity (94.1%). The sensitivity of CXR and chest CT was calculated as 66.7% and 83.3%, respectively. Preterm birth rate was 41.2% (n=7/17). Five (9.1%) of the 55 pregnant women admitted to the intensive care unit, three of those developed acute respiratory distress syndrome and one died.

Conclusion: This study describes the main radiological features of symptomatic pregnant women infected with COVID-19. The refusal rate among pregnant women for the imaging modalities involving ionizing radiation was high but these had high sensitivity for COVID-19 diagnosis. The preterm birth and cesarean section rates were observed as remarkably increased. (J Turk Ger Gynecol Assoc 2021; 22: 196-205)

Keywords: COVID-19, pregnancy, computed tomography, chest radiography, radiology

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Introduction

Pregnancy is known to cause significant anatomical and physiological changes in respiratory function and these changes can increase susceptibility to respiratory tract infections and can quickly lead to respiratory failure (1). In addition, the modulation of the immune system during pregnancy leaves pregnant women vulnerable to viral infections, which may lead to even more severe symptoms (2). Previous studies have also shown that severe acute respiratory syndrome (SARS) and middle east respiratory syndrome infections were associated with serious maternal disease, maternal deaths, and spontaneous abortions (3-5). Pregnant women are at increased risk for severe illness from Coronavirus disease-2019 (COVID-19) compared to non-

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pregnant people (6,7). Additionally, there may be an increased risk of adverse pregnancy outcomes, such as preterm birth, among pregnant women with COVID-19 (8,9). Moreover, the high refusal rate of imaging modalities involving ionizing radiation during pregnancy and the risks to fetuses present as a limiting factor in the diagnosis and treatment of pregnant women, making the diagnostic process more difficult when compared to the non-pregnant population (10). Therefore, pregnant women constitute a vulnerable group that requires special attention in the diagnosis and treatment of COVID-19.

The most commonly used reference standard in the diagnosis of COVID-19 is the reverse transcription polymerase chain reaction (RT-PCR) test. However, due to the technical limitations of the test and its relatively high false negative rate, radiological imaging including chest CT and chest radiography (CXR) play an important role in the diagnosis and evaluation of pregnant women suspected with COVID-19 infection (11,12). Chest CT has been reported to be more sensitive than other modalities in the diagnosis of COVID-19 pneumonia (13).

Robust data concerning radiological and clinical features of pregnant women with COVID-19 pneumonia is scarce in the literature. This study aimed to identify the demographic characteristics and evaluate the clinical, laboratory, and radiological findings of symptomatic pregnant women with COVID-19 pneumonia.

Material and Methods

Patient population and study design

This retrospective study was conducted at two tertiary health care centers dedicated to treating severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pandemic between March 15th and September 1th, 2020. Symptomatic pregnant women suspected of COVID-19 infection were included in the study. Patients who were tested for SARS-CoV-2 infection for universal screening purposes were not included in the study (14). Patients who refused chest CT or CXR with negative PCR test result were excluded. Those patients were managed according to the national guidelines (15). Pregnant women with COVID-19 infection confirmed either by PCR-testing or imaging studies were included to the final analysis (Figure 1). The disease was classified as mild, moderate and severe according to its clinical severity (15).

The demographic characteristics, clinical signs and symptoms, and laboratory results of the patients were obtained from the patients' electronic health records. Clinical symptoms, including fever (≥37.3 °C), cough, dyspnea, sore throat, and fatigue, were assessed in terms of COVID-19. RT-PCR tests were performed using combined swab samples taken from the oropharynx and the nasopharynx of the patients, were used to confirm SARS-CoV-2 infection. The radiological examinations (CT and/or CXR) of the pregnant women were re-evaluated,

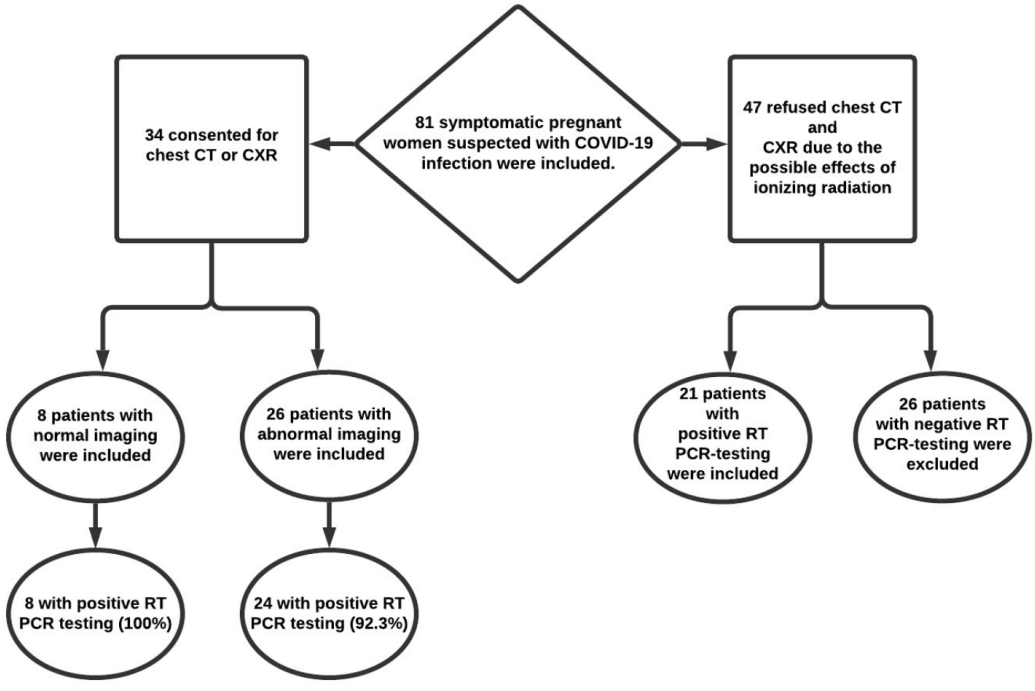

Figure 1. Flowchart of the study

CT: Computed tomography, CXR: Chest radiography, COVID-19: Coronavirus disease-2019, RT-PCR: Reverse transcription polymerase chain reaction

independent from and blinded to the initial report. The study was approved by the Ethical Committee of the University of Health Sciences Turkey, Kartal Koşuyolu Training and Research Hospital (approval number: 2020.4/07-312) and the national health authorities.

Radiological imaging

Low-dose imaging protocol was followed. Radiological imaging criteria were met if at least two of the following symptoms were present: a fever above 38.3 °C, a respiratory rate of 22 breaths/minute and above, saturation of peripheral oxygen (SpO_2) below 93%, or severe dyspnea. The possible effects of radiation exposure on the fetus were explained in detail and written consent was obtained from each patient. The choice of imaging method (CXR and/or CT) was decided together with the patient, considering diagnostic performance of imaging methods, the pregnancy trimester and the clinical condition of the patient.

CXR was performed using a digital X-ray machine (DRGEM Radiography System, South Korea). The CXR parameters were as follows: 75-110 kVp, 4-8 mAs, and detector size 35x43 cm with grid. During the examination, the abdomen and pelvis were protected with a lead sheath. The effective dose for CXR images did not exceed 0.07 mSv (millisieverts).

Chest CT was performed in all patients using a 16 or 128-slice CT scanner (Optima 520 CT, GE company or Ingenuity Core 128, Philips Healthcare). CT images were obtained with the patient in the supine position at full inspiration and without contrast medium. For the pregnant participants, 80 kV tube voltage, 50 mAs automatic tube current modulation, 5 mm slice thickness, 5 mm slice interval, a noise index of 16, 36.0 DFOV, and 512 x 512 matrix were used. The thyroid, abdomen, and pelvis were protected by a lead sheath. The dose-length product was 25-100 mGy.

Image analysis

The reconstructed images were transmitted to the workstation and picture archiving and communication systems for multiplanar reconstruction post-processing. The chest radiographs and chest CT images of the cases were evaluated by three radiologists, blinded to RT-PCR results, at the radiology workstation. In cases where the three radiologists evaluated differently, the result was reached by consensus.

The CXR findings were classified as typical, indeterminate, atypical, and negative for COVID-19 (16). For statistical evaluation, typical and indeterminate groups were considered COVID-19 positive, and atypical and negative groups were accepted as COVID-19 negative. An example of chest CXR findings is shown in Figure 2.

CT findings were categorized as non-COVID-19, indeterminate COVID-19, probable COVID-19, and classic COVID-19 according to the COVID-19 infection version 2 of the British Society of Thoracic Imaging (17). For statistical evaluation, non-COVID-19 cases were categorized as CT negative group, while indeterminate COVID-19, probable COVID-19 and classic COVID-19 cases were categorized as CT positive group.

The distribution in the lung, shape, location, appearance, and size of the largest lesion were recorded. In addition, vascular enlargement, intralobular/interlobular septal thickening, air bronchogram, subpleural curvilinear lines, parenchyma findings such as bronchial wall thickening, fibrous bands, halo sign, and reversed halo sign were noted. Extrapulmonary findings such as pleural effusion, pleural thickening, and enlarged lymph nodes were also included. In the chest CT positive cases, CT severity index according to the degree of lesion distribution was calculated, as described previously (18).

Statistical analysis

Descriptive analyses were performed for the characteristics of the patients. The normally distributed continuous random variables were expressed as the mean ± standard deviation

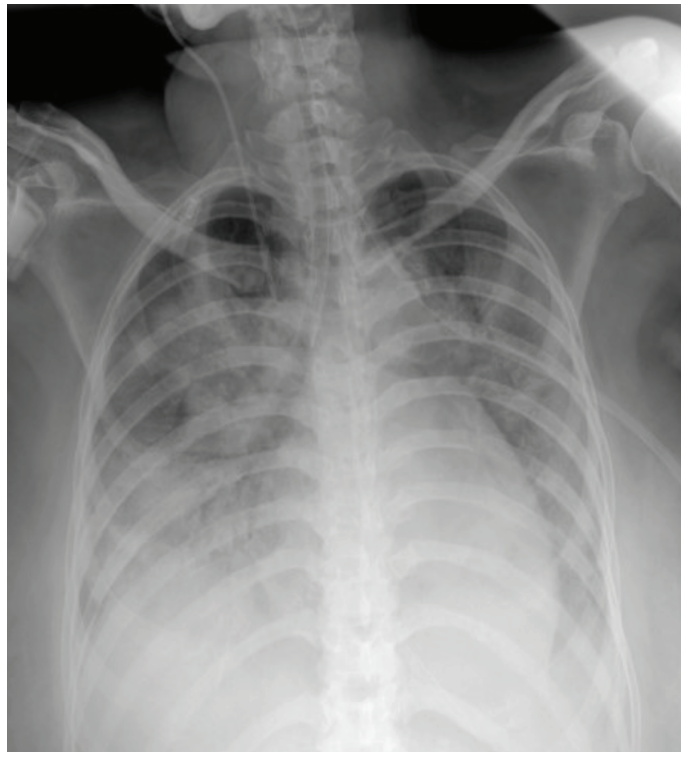

Figure 2. A 35-year-old, laboratory-confirmed COVID-19, pregnant woman with 25 weeks of pregnancy presented with fever and dyspnea. The patient developed hypoxic respiratory failure and was admitted to intensive care unit. Anteroposterior chest radiograph shows an ARDS pattern with ill-defined alveolar consolidation bilaterally in the predominantly lower zones

COVID-19: Coronavirus disease-2019, ARDS: Acute respiratory distress syndrome

and categorical variables are expressed as percentages. Fisher's exact test was used to compare the severity of the disease between trimesters and the One-Way ANOVA test was used to compare the severity of pneumonia involvement (CT severity index). The sensitivity of CXR and chest CT was calculated for the diagnosis of COVID-19 disease, using RT-PCR as reference. SPSS 17.0 (IBM Inc., Armonk, NY, USA) was used for the statistical analyses.

Results

Eighty-one pregnant women suspected with COVID-19 infection were enrolled. Forty-seven patients (58.0%) refused chest CT and CXR due to the risks of receiving ionizing radiation when pregnant. Out of these, 26 patients who had negative RT-PCR-testing were excluded. Fifty-five pregnant women with COVID-19 infection confirmed either with RT-PCR-testing or imaging studies were included in the final analysis. Pregnant women were confirmed as having COVID-19 by RT-PCR alone (n=29), imaging studies alone (n=2), and both (n=24). Radiological imaging studies were performed in 34 (61.8%) patients. The positivity rate in PCR-testing among patients with abnormal imaging was 92.3% (n=24/26).

Demographic, clinical and laboratory characteristics of pregnant women are given in Table 1. The first, second, and third trimester distribution of pregnant women at the time of presentation were n=8 (14.6%), n=24 (43.6%), and n=23 (41.8%), respectively. The patients' clinical condition was mild in eight (100%) pregnant women in the first trimester. In the second trimester, 20 (83.3%) cases were mild, 3 (12.5%) cases were moderate, and 1 (4.2%) case was severe, in the third trimester 18 (78.3%) cases were mild, 2 (8.7%) cases were moderate, and 3 (13.0%) cases were severe. Although mild and severe cases were more frequent in the third trimester, the difference between trimesters was not statistically significant (p=0.672). CT severity index of pregnant women in first, second, and third trimesters was 3.7, 7.5, and 6.3, respectively. Although pneumonia involvement (CT severity index) was higher in the second and third trimester, the difference between trimesters did not reach a statistically significant level (p=0.697). During the study period, 12 cases gave birth by cesarean (C/S), and five cases had a normal spontaneous vaginal delivery, for a total of 17 cases delivered (seven preterm, 10 term), and one case of missed abortus at seven weeks. The remaining 37 were still pregnant during the study.

Five of 55 patients (9.1%) were admitted to the intensive care unit (ICU). One patient and the fetus died in her 22nd weeks of gestation due to acute respiratory distress syndrome (ARDS). CXR detail of that patient was common airspace opacities more prominent in the lower lobes, compatible with ARDS (Figure 2).

Repeated RT-PCR testing was performed in nine of 34 (26.5%) patients whose first RT-PCR test was negative. Second RT-PCR test increased the COVID-19 positivity rate from 58.0% (47/81) to 65.4% (53/81). There was no vertical transmission.

The radiological classification of imaging studies is summarized in Table 2. Twenty-one (61.8%) of 34 patients underwent CXR and 20 of them underwent low-dose chest CT. In seven patients,

Table 1. Demographic, clinical and laboratory characteristics of pregnant women at presentation (n=55)

Patient demographics		
Age mean ± SD (range)		29.7±6.4 (19-53)
RT-PCR positivity n (%)		53/81 (65.4%)
Presenting findings n (%)		
Fever (>37.3 °C)		16 (29.1%)
Dry cough		25 (45.4%)
Dyspnea		19 (34.5%)
Fatigue		11 (20.0%)
Sore throat		17 (30.9%)
Anosmia		5 (9.1%)
Suspicious contact		26 (47.3%)
Laboratory tests		
WBC	Low	1 (1.8%)
	Normal	43 (78.2%)
	High	11 (20.0%)
Lymphocyte	Low	23 (41.8%)
	Normal	32 (58.2%)
	High	0 (0%)
CRP (n=52)	Normal	18 (34.6%)
	High	34 (65.4%)
LDH (n=43)	Normal	26 (60.5%)
	High	17 (39.5%)

SD: Standard deviation, RT-PCR: Reverse transcription polymerase chain reaction, WBC: White blood cell count, CRP: C-reactive protein, LDH: Lactate dehydrogenase

Table 2. Radiologic imaging of patients (n=34)

Chest radiography classification (n=21)	
Negative	6 (28.6%)
Atypical	1 (4.7%)
Indeterminate	9 (42.9%)
Typical	5 (23.8%)
Chest CT classification (n=20)	
Normal	3 (15.0%)
Non-COVID-19	0 (0.0%)
Indeterminate COVID-19	2 (10.0%)
Probable COVID-19	1 (5.0%)
Classic COVID-19	14 (70.0%)

CT: Computed tomography, COVID-19: Coronavirus disease-2019

both CXR and chest CT were performed. The sensitivity of CXR and chest CT was calculated as 66.7% [95% confidence interval (CI) 43.0 to 85.4%] and 83.3% (95% CI 58.6 to 96.4%), respectively, using RT-PCR as reference. Fourteen of the 21 COVID-19 patients (66.7%) had parenchymal abnormalities in CXR. Twelve had bilateral and one had unilateral airspace opacities (consolidation or ground-glass opacity) while the last had prominent bronchovascular shadows only. The distribution of the airspace opacities was central and peripheral in five cases, central in four cases, and peripheral in five cases. Prominent bronchovascular shadows were observed in six cases, five of which were bilateral. The radiological findings of abnormal chest CT were given in Table 3.

Discussion

This study defined the clinical presentation, laboratory and radiological features of symptomatic pregnant women diagnosed with SARS-CoV-2 infection either by RT-PCR testing or imaging studies.

Imaging features of COVID-19 infection in pregnant women, as in the non-pregnant population, are predominantly peripheral and bilateral patchy ground glass opacities with or without consolidation (12,19-21). In this study, the radiological features were commonly seen in both central and peripheral lung tissues. This difference might be related to the phase of the disease or the diseases might progress rapidly in pregnant women (20). The other imaging features of COVID-19 were similar with previous studies (12,20,21).

Three of every five pregnant women did not give consent for imaging studies involving ionizing radiation in this study. To the best of our knowledge, this finding was not previously reported. Royal College of Obstetricians and Gynecologists guidelines state that maternal health is more important than fetal health in pregnant patients (2). Therefore, radiological examination can be performed in pregnant women while exposure to ionizing radiation should be as low as reasonably achievable. In routine chest CT, the radiation dose is approximately 4-7 mGy, and the radiation dose of a CXR or low-dose chest CT is far below the accepted limit for a fetus (22). Radiation exposure of less than 100 mGy in utero after implantation has no proven deterministic effect on the fetus. However, stochastic effects of cancer induction are known to exist, albeit slightly, and increase in proportion to dose (23). The use of radiological examinations in the diagnosis of COVID-19 pneumonia in pregnant patients requires special attention due to the risk of fetal teratogenicity caused by radiation exposure. Lung ultrasound may provide a good solution for patients who refuse chest CT or CRX (14,24).

The diagnostic performance of CXR in detecting COVID-19 pneumonia is lower than that of CT, and the sensitivity was reported to be 33-69% in studies involving few non-pregnant cases (25). Chest CT sensitivity was reported to be as high as 94% in a meta-analysis (26). Similarly, the sensitivity of CXR in detecting COVID-19 pneumonia was found to be 66.7% in this study, whereas chest CT sensitivity was 86.6%. The authors postulate that CXR can be used as the initial radiological examination for symptomatic pregnant patients with COVID-19 considering its relatively lower radiation dose and moderate sensitivity. However, a normal CXR cannot rule out COVID-19. In this study, ARDS development was detected in the CXR in one case and in the chest CT in two cases and, one of these cases involved concomitant pneumothorax and pneumomediastinum (Figure 3). This severe patient was treated in the ICU and required mechanical ventilation. Although spontaneous pneumomediastinum is a rare complication of COVID-19, the mechanism of pneumomediastinum is not clear (27). These individual cases have highlighted the importance of radiologic imaging, not only in diagnosing COVID-19 pneumonia, but also in detecting accompanying complications of the disease.

The most common admission symptoms of the patients included in the study were dry cough, fever, and dyspnea, which were similar with those in the non-pregnant population. Laboratory findings showed a normal leukocyte count with lymphocytopenia, and increased C-reactive protein and lactate dehydrogenase concentrations, which were similar to the findings in non-pregnant population (28,29).

In our study group, six of the nine pregnant women whose first RT-PCR test was negative, had a positive result on the second RT-PCR test. Although the RT-PCR test is accepted as a reference in the diagnosis of COVID-19, the sensitivity of the test is low. The positivity rate of the first test is 60-71%, and the positivity rate increases with subsequent tests (30). Thus, a diagnosis of COVID-19 should not be ruled out in pregnant patients with a single negative RT-PCR test result. Considering the method used to obtain the sample, and low sensitivity due to technical reasons, repetition of the test should not be avoided in cases where the first test is negative if clinical, laboratory, or radiological findings are consistent.

It is shown that COVID-19 in pregnancy was associated with maternal morbidity and preterm birth and required a high rate (8%) of intensive care admission (6,9). Similarly, in this study 54.3% of all births were performed with cesarean section and the preterm birth rate was 58.8%. In addition, 9.1% of the pregnant patients included in the study were admitted to the ICU, three of whom developed ARDS and one of them died. Some of the studies conducted at the beginning of the pandemic claim that the course of COVID-19 during pregnancy is not different to non-pregnant cases (31,32). Contrary to these studies, our preliminary results suggest that clinical course of the COVID-19 in pregnancy seems more severe, similar to more recent studies (7,33). Similar to our study results, the

Table 3. Case-based chest CT findings of pregnant women with COVID-19

Case	1	2	3	4	5	6	7	8
RT-PCR	+	+	+	+	+	+	+	+
CT classification Classic COVID-19 Probable COVID-19 Indeterminate COVID-19 Non- COVID-19	Classic	Classic	Classic	Classic	Classic	Classic	Classic	Classic
Lung involment Bilateral (B/L) Right Left	B/L	B/L	B/L	B/L	B/L	B/L	B/L	B/L
Number of lobes involved (1-5)	5	5	5	5	4	5	5	5
Max diameter of lession (cm)	2.1	5.5	3.5	6.7	4.7	3.7	6.6	2.2
CT severty index Mild (≤5) Moderate (6-11) Severe (≥12)	Severe (18)	Moderate (7)	Severe (12)	Moderate (6)	Moderate (6)	Moderate (10)	Moderate (7)	Moderate (7)
Distrubition of lesions Peripheral (P) Central (C) Peripheral and central (P&C)	P&C	P&C	P&C	P&C	P&C	P&C	P&C	P&C
Shape of lesions Patchy Diffuse	Patchy	Patchy	Patchy	Patchy	Patchy	Diffuse	Patchy	Patchy
Appeareance of lesions Ground glass opacity (GGO) Consolidation (C) GGO and consolidation (GGO&C)	GGO&C	GGO&C	GGO	GGO&C	GGO&C	GGO&C	GGO&C	GGO
CT Paranchimal findings								
Vasculer enlargement	+	-	+	-	-	+	-	-
Intralobular reticular density	+	-	-	-	+	+	-	-
Bronchial wall thickening	+	-	-	-	-	-	+	-
Subpleural curvilineer lines	+	-	-	-	-	-	+	-
Air bronchogram	-	-	-	+	-	+	-	-
Fibrous band	+	-	-	+	+	-	-	-
Subpleural sparing	-	+	+	-	-	+	+	-
Halo sign	-	-	-	-	-	+	-	-
Reversed halo sign	-	-	+	-	-	-	-	-
Extrapulmonary manifesation								
Pleural thickening	-	-	-	-	-	-	-	-
Lymphadenopaty	-	-	-	-	-	-	-	-
Pleural effusion	-	-	-	-	-	+	-	-
Pneumomediastenum and Pneumothorax	-	-	-	-	-	-	-	-

CT: Computed tomography, RT-PCR: Reverse transcription polymerase chain reaction, COVID-19: Coronavirus disease-2019

Table 3. Continued

Case	9	10	11	12	13	14	15	16	17
RT-PCR	+	+	+	+	+	+	+	-	-
CT classification Classic COVID-19 Probable COVID-19 Indeterminate COVID-19 Non- COVID-19	Classic	Classic	Indeter-minate	Classic	Classic	Classic	Classic	Indeter-minate	Probable
Lung involment Bilateral (B/L) Right Left	B/L	Right	B/L	B/L	B/L	B/L	B/L	B/L	Right
Number of lobes involved (1-5)	5	2	2	4	5	3	5	2	2
Max diameter of lession (cm)	2.3	5.1	4.7	3.4	8	4.3	9.2	3.1	1.0
CT severty index Mild (≤5) Moderate (6-11) Severe (≥12)	Mild (5)	Mild (4)	Mild (2)	Mild (4)	Modarete (6)	Mild (3)	Severe (22)	Mild (2)	Mild (2)
Distrubition of lesions Peripheral (P) Central (C) Peripheral and central (P&C)	P&C	P	P	P	P&C	P	P&C	P	P&C
Shape of lesions Patchy Diffuse	Patchy	Patchy	Patchy	Patchy	Patchy	Patchy	Diffuse	Patchy	Patchy
Appeareance of lesions Ground glass opacity (GGO) Consolidation (C) GGO and consolidation (GGO&C)	GGO	GGO	C	GGO&C	GGO	GGO	GGO&C	GGO	GGO&C
CT Paranchimal findings									
Vasculer enlargement	-	+	-	+	+	+	-	-	-
Intralobular reticular density	-	-	-	-	+	-	+	-	-
Bronchial wall thickening	-	-	-	-	-	-	-	-	-
Subpleural curvilineer lines	-	-	-	-	-	-	-	-	-
Air bronchogram	-	-	+	-	-	-	+	-	-
Fibrous band	-	-	+	-	+	-	+	-	-
Subpleural sparing	-	-	-	+	+	-	-	-	-
Halo sign	-	-	-	-	-	-	-	-	-
Reversed halo sign	-	-	-	-	-	-	-	-	-
Extrapulmonary manifesation									
Pleural thickening	-	+	+	+	-	-	-	-	-
Lymphadenopaty	-	-	-	-	-	-	-	-	-
Pleural effusion	-	-	-	-	-	-	-	-	-
Pneumomediastenum and Pneumothorax	-	-	-	-	-	-	+	-	-

CT: Computed tomography, RT-PCR: Reverse transcription polymerase chain reaction, COVID-19: Coronavirus disease-2019

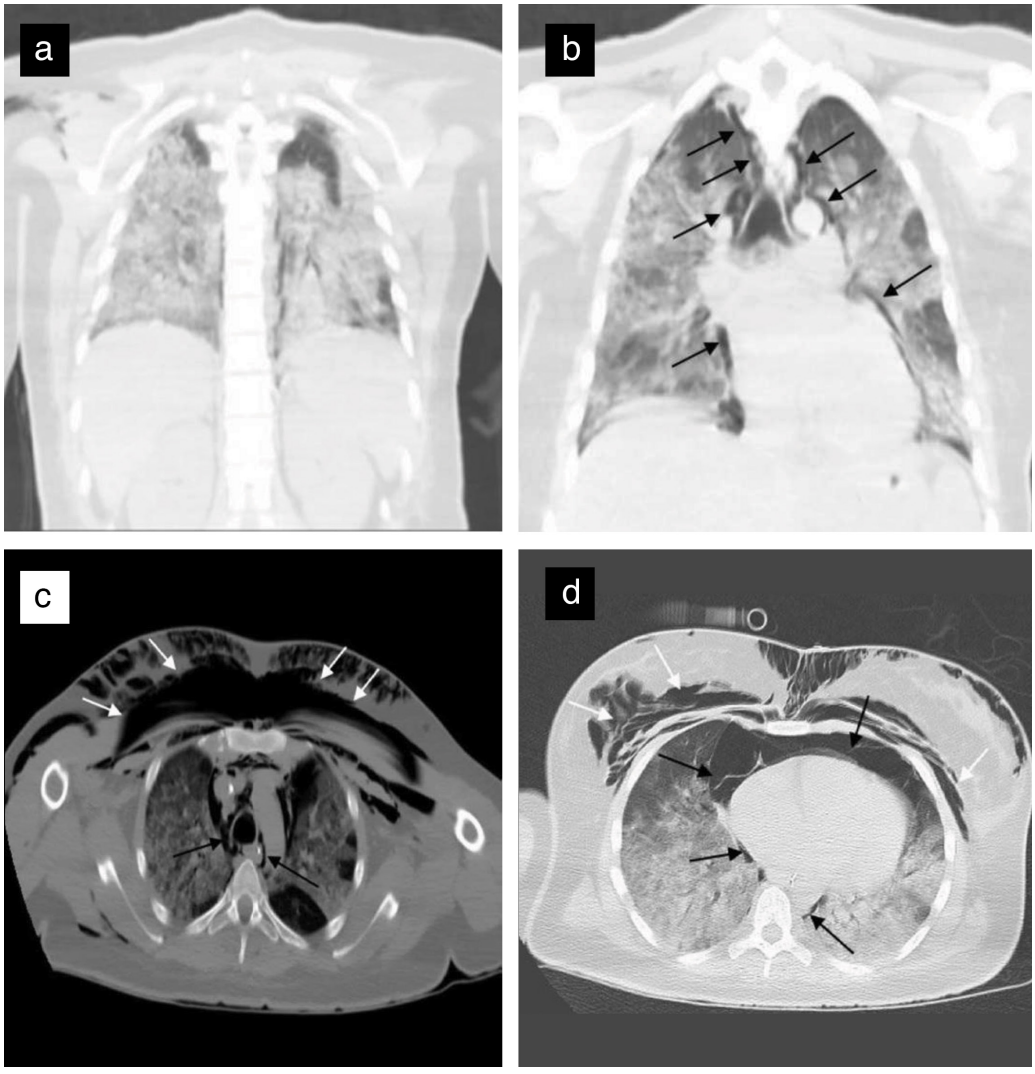

Figure 3. CT images obtained 2 hours after emergency cesarean section due to fetal distress of a 25-year-old woman with COVID-19. Coronal images (a,b) show bilateral diffuse and multiple patchy ground-glass opacities with partial consolidation. CT severity index is 22 and classified as severe. All CT images (a-d) show air in the mediastinum which outlines mediastinal organs (black arrows). Also, axial CT images (c,d) show extensive subcutaneous emphysema (white arrows) in the anterior chest wall

CT: Computed tomography, COVID-19: Coronavirus disease-2019

trimester of pregnancy has been shown to affect the clinical severity of COVID-19 (34). However, in our study, although there is a percentage difference between trimesters, the reason for not having a statistically significant relationship may be the relatively small number of patients. RT-PCR test positivity was not observed in any of the delivered fetuses, which supports the notion that the disease has no vertical transmission (35,36).

Study limitation

The limitations of our study are the absence of multiple RT-PCR tests in some pregnant women and the relatively low number of patients included in the study.

Conclusion

This study describes the main radiological features of symptomatic pregnant women infected with COVID-19. The refusal rate among pregnant women for the imaging modalities involving ionizing radiation was high but both CXR and chest CT had high sensitivity for COVID-19 diagnosis. The preterm birth and cesarean section rates were observed as remarkably increased.

Ethics Committee Approval: *The study was approved by the Ethical Committee of the University of Health Sciences Turkey, Kartal Koşuyolu Training and Research Hospital (approval number: 2020.4/07-312) and the national health authorities.*

Informed Consent: *It was obtained.*

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The effect of diagnostic hysteroscopy performed before fresh and frozen-thawed embryo transfer in IVF cycles on reproductive outcomes

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Abstract

Objective: Hysteroscopy is frequently performed in infertile women and thought to improve pregnancy rates. The data obtained from studies investigating the effect of hysteroscopy in in-vitro fertilization (IVF) cycles is variable. We aimed to evaluate the effect of hysteroscopy on pregnancy outcomes of fresh and frozen-thawed embryo transfers (FET) performed during IVF cycles.

Material and Methods: The data of the 765 patients, who had IVF treatment between January 2015 and July 2017 in an infertility center, were retrospectively analyzed. Of those, 586 (76.6%) patients underwent fresh embryo transfer, while 179 (23.4%) patients underwent FET. Hysteroscopy performed by a single experienced surgeon was scheduled two months before transfer. Hysteroscopy was performed in 101/586 (17.2%) in those undergoing fresh embryo transfer and 44/179 (24.6%) patients in the FET group. Pregnancy outcomes of the groups were compared respectively within their own group.

Results: The mean age was similar in patients in the fresh and FET groups (p=0.365, respectively). There was no difference in the number of transferred embryos between the groups (p=0.218). In the fresh embryo group there were 246 pregnancies, of which 44 had undergone diagnostic hysteroscopy while 202 had not, (p=0.516) and 79 pregnancies in the FET group, of which 20 had undergone diagnostic hysteroscopy while 59 had not (p=0.711). There was no statistical difference according to pregnancy rate between the groups (p=0.538).

Conclusion: Performing diagnostic hysteroscopy before fresh or FET does not improve the pregnancy rates.(J Turk Ger Gynecol Assoc 2021; 22: 206-11)

Keywords: Diagnostic hysteroscopy, in-vitro fertilization, frozen-thawed embryo transfer, fresh embryo transfer, uterine cavity, pregnancy rate

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Introduction

In-vitro fertilization (IVF) has given hope to infertile couples in the 20th century. However, live birth occurs in only one third of IVF and intracytoplasmic sperm injection (ICSI) cycles (1). This low success rate is thought to be due to the failure of implantation. The exact reason underlying this implantation failure is not understood, and may depend on uterine cavity factors, embryo quality or a combination of these (2,3). An abnormal uterine finding such as polyps, uterine leiomyoma, and adhesions are present in approximately 50% of infertile women (4,5). Abnormalities of the uterine cavity may lead to implantation failure, in turn resulting in reduced chance of successful pregnancy outcome. Hysterosalpingogram (HSG),

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saline infusion sonohysterography (SIS) or hysteroscopy are alternative methods to evaluate the uterine cavity. The false positive rate and the false negative rate for HSG are 15.6% and 35.4%, respectively. Additionally; HSG offers no chance of management for the abnormalities of the uterine cavity. To obtain enhanced endometrial visualization, saline fluid is introduced into the uterine cavity transcervically during transvaginal ultrasound examination. Although this method is feasible and highly sensitive and specific when used for detection of endometrial abnormalities (97.3% and 95.8%, respectively), SIS, like HSG, does not provide a possibility for management of the abnormalities of the uterine cavity (6). Hysteroscopy is a more powerful technique to evaluate the uterine cavity and apply treatment simultaneously (7,8). This makes hysteroscopy the most useful test for assessing the uterine cavity. The evaluation of uterine cavity with hysteroscopy is especially valuable in women with prior IVF failures (9).

Studies evaluating the usefulness of hysteroscopy in IVF cycles have produced conflicting results. The TROPHY study, a multicenter, randomized controlled trial reported that, especially for women with recurrent, unsuccessful implantation following IVF, hysteroscopy had no effect on the live birth rate (LBR) (10). However, this contrasts with the results of a prior systematic review suggesting that routine hysteroscopy improved the LBR for women with recurrent unsuccessful IVF cycles (11). Comparing the performance of hysteroscopy with no hysteroscopy prior to any (first or subsequent) IVF/ICSI attempt in infertile aymptomatic patients, there was very limited evidence suggesting that hysteroscopy was useful to increase LBR (12). Similar to the results of the TROPHY study, another multicentre, randomised controlled trial published in the same year, the INSIGHT trial, reported that LBR in infertile women with a normal uterine cavity on transvaginal ultrasound has not been improved by applying routine diagnostic hysteroscopy prior to the first IVF treatment (13). Pabuçcu et al. (14) also found that hysteroscopy did not make a statistically significant difference regarding implantation, pregnancy rate and LBR in infertile women having a history of recurrent implantation failure.

Another important issue that has been investigated is that of endometrial scratching during hysteroscopy before IVF treatment. Endometrial scratching results in superficial injury of uterine cavity, which is thought to enhance the receptivity of the uterus for the embryo (15). Subsequent studies have reported that endometrial damage did not increase pregnancy rates (16).

In this study, pregnancy outcomes of IVF cycles were evaluated when the embryo transfer was either fresh or frozen-thawed embryo that were applied with or without hysteroscopy to evaluate the pre-IVF uterine cavity.

Material and Methods

Participants

The data of 768 patients who underwent IVF cycles between January 2015 and July 2017 in a private infertility center was evaluated retrospectively. For this study, ethical approval was taken from the Ethical Committee of Acıbadem Mehmet Ali Aydınlar University. All patients who participated in this study gave written informed consent for this study. Women aged 18-45 years with primary infertility due to tubal factor, male factor or unexplained were selected for the study. Patients who had infertility with known uterine factors and recurrent miscarriage were excluded from the study. Transvaginal ultrasound of the endometrial cavity, HSG, and SIS were performed and patients having any pathology were also excluded from the study. Only women with no pathology detected by hysteroscopy were included in the study.

Fresh embryo transfer was used in 589 of these patients while frozen-thawed embryo transfer (FET) was performed in 179 patients. Each of these two groups was separated into two new groups, depending on if the subjects had undergone diagnostic hysteroscopy or not. Hysteroscopy was performed two months prior to IVF in women with suspected structural lesions in the uterine cavity before the embryo transfer by a single experienced surgeon. A total of 101 (17.2%) of the patients in the group of fresh embryo transfer and 44 (24.6%) in the FET group underwent hysteroscopy.

Procedure

Hysteroscopy was performed during the early proliferative phase in the outpatient clinic without anesthesia using by a 1.9 mm Karl Storz hysteroscope with a 30° view. Saline distension medium was used. A paracervical block was applied in the patients with intolerance. Patients were hospitalized only for 15-60 min and no complications were experienced.

Controlled ovarian stimulation (COH) was achieved using human menopausal gonadotropin (Merional 75 IU, IBSA Institut, Switzerland) with the adjusted dose based on the individual response and human chorionic gonadotropin (hCG) at a dose of 10,000 IU in the fresh embryo transfer group. COH was begun at the time of menses as antagonist protocol. An antagonist was administered when the follicles became greater than 14 mm in largest diameter and daily injections of the antagonist were continued until hCG administration. After oocyte retrieval and fertilization, embryo transfers were performed on day three. Frozen-thawed embryo protocol was used in the other group. For patients with thin endometrium, hormone treatment was offered to prepare the endometrium (2 mg micronized estradiol tablet). After the endometrial thickness in each patient became greater than 8 mm, progesterone therapy (Progestan 200 mg;

Koçak, Tekirdağ, Turkey) three times a day via vaginal pathway or daily progesterone gel (Crinone 8%, Merck Serono, Italy) was added to treatment at the 14th day of cycles. Upon completion of endometrial preparation, the transfer of a day 3 embryo (cleavage stage) was performed on the third day. Although the number of embryos transferred changed depending on a number of factors, such as maternal age, the number of oocytes retrieved and availability of embryos for cryopreservation, no more than two embryos was transferred in our population. While selecting the subjects for different groups, an attempt was made to keep the number of transferred embryos alike.

Beta hCG values were determined from blood samples of the patients after 11 days from embryo transfer and results over 10 mIU/mL were accepted as pregnancy.

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (IBM Inc., Chicago, IL, USA) version 16.0. Difference in mean values and characteristics between groups was analyzed with Independent samples t-test and chi-square test. Means were presented with standard deviation. A $p<0.05$ was accepted as statistically significant.

Results

The total cohort numbered 765 women, of whom fresh embryo transfer was done in 586 (76.6%) while FET was performed in 179 (23.4%). In the fresh embryo transfer group, diagnostic hysteroscopy was performed in 101 (17.2%) and in the FET group this number was 44 (24.6%). No pathological findings were found in any of the subjects during the hysteroscopic procedure, so no treatment was required. The clinicodemographic characteristics of the participants are presented in Table 1. The mean age of the patients was comparable between the patients in fresh and FET groups ($p=0.365$). There was no statistical difference in transferred embryo numbers in fresh and FET groups ($p=0.218$). The quality of the embryos was statistically similar in both groups ($p=0.177$).

In the fresh embryo group there were 246 pregnancies, of which 44 had undergone diagnostic hysteroscopy while 202 had not, ($p=0.516$) and 79 pregnancies in the FET group, of which 20 had undergone diagnostic hysteroscopy while 59 had not ($p=0.711$). There was no statistical difference in take-home baby rates between the groups in which hysteroscopy was performed and was not performed ($p=0.513$) (Table 2). Table 3 presents the comparison of the patients, grouped by age. There was a significant difference between the patients regarding take-home baby rates, pregnancy results and obstetric outcomes ($p<0.001$, $p=0.001$ and $p=0.001$, respectively) when age was taken into account.

Discussion

Considering that the success rate of IVF and ICSI treatments is 25-30% and the most common reason for low success rate is implantation failure, the evaluation of the endometrial cavity prior to IVF procedures is undoubtedly very important (3). Hysteroscopy has been accepted as the "gold standard" test to evaluate the uterine cavity. Hysteroscopy also provides an opportunity for simultaneous treatment of any pathology detected during the procedure. Intrauterine lesions such as polyps, submucous myomas, and adhesions may be a significant factor resulting in implantation failure. Transvaginal ultrasound, SIS, and HSG may be insufficient to see small

Table 1. Demographic and clinical characteristics of the patients

Characteristics	Mean ± SD or number (%)
Age (years)	31.2±6.0
Age group (years)	
<20	20 (2.6)
21-25	112 (14.6)
26-30	249 (32.4)
31-35	192 (25)
36-40	139 (18.1)
>40	56 (7.3)
Hysteroscopy	
Not performed	623 (81.1)
Performed	145 (18.9)
Type of embryo transfer	
Fresh	589 (76.7)
Frozen-thawed	179 (23.3)
Hysteroscopy regarding type of embryo transfer	
Fresh embryo transfer	
Not performed	488 (63.5)
Performed	101 (13.2)
Frozen-thawed embryo transfer	
Not performed	135 (17.6)
Performed	44 (5.7)
Pregnancy result	
Negative	444 (57.8)
Positive	324 (42.2)
Pregnancy outcome	
Biochemical	51 (15.7)
Abortus	48 (14.8)
Live birth	216 (66.7)
Preterm birth	9 (2.8)
Take-home baby rate	28.6%

SD: Standard deviation

lesions in the uterine cavity (17). Unsuspected intrauterine abnormalities have been diagnosed using hysteroscopy in an asymptomatic IVF population with a prevalence of as high as 50% (18). Assuming that performing hysteroscopy before IVF treatment can improve reproductive outcomes, studies of this issue have produced conflicting results and high-quality studies are lacking (19).

A meta-analysis reported by Pundir et al. (20) proved that LBR increased following hysteroscopy in women scheduled for a first IVF cycle (risk ratio: 1.30, 95% confidence interval:1.00-1.67;

Table 2. Comparison of the patients based on different hysteroscopy and embryo transfer groups

Characteristics	Group 1 (n=488) (fresh, non-hysteroscopy)	Group 2 (n=101) (fresh, hysteroscopy)	Group 3 (n=135) (frozen-thawed, non-hysteroscopy)	Group 4 (n=44) (frozen-thawed, hysteroscopy)	p
Age (years)	31.4±6.2	31.5±5.7	30.8±5.5	29.9±5.2	0.365
Age group (years)	-	-	-	-	0.132
<20	16 (3.3)	0	2 (1.5)	2 (4.5)	-
21-25	74 (15.2)	14 (13.9)	20 (14.8)	4 (9.1)	-
26-30	147 (30.1)	36 (35.6)	47 (34.8)	19 (43.2)	-
31-35	120 (24.6)	21 (20.8)	38 (28.1)	13 (29.5)	-
36-40	86 (17.6)	24 (23.8)	23 (17)	6 (13.6)	-
>40	45 (9.2)	6 (5.9)	5 (3.7)	0	-
Pregnancy result	-	-	-	-	0.960
Negative	285 (58.4)	58 (57.4)	77 (57)	24 (54.5)	-
Positive	203 (41.6)	43 (42.6)	58 (43)	20 (45.5)	-
Pregnancy outcome	-	-	-	-	0.402
No pregnancy	285 (58.4)	58 (57.4)	77 (57)	24 (54.5)	-
Biochemical	34 (7)	8 (7.9)	6 (4.4)	3 (6.8)	-
Abortus	26 (5.3)	11 (10.9)	7 (5.2)	4 (9.1)	-
Live birth	135 (27.7)	24 (23.8)	45 (33.3)	12 (27.3)	-
Preterm birth	8 (1.6)	0	0	1 (2.3)	-
Take-home baby rate (%)	28.1	24.8	33.3	29.5	0.513

Table 3. Comparison of the patients based on different age groups

Characteristics	<20 years (n=20)	21-25 years (n=112)	26-30 years (n=249)	31-35 years (n=192)	36-40 years (n=139)	>40 years (n=56)	p
Hysteroscopy	-	-	-	-	-	-	0.256
Not performed	18 (90)	94 (83.9)	194 (77.9)	158 (82.3)	109 (78.4)	50 (89.3)	-
Performed	2 (10)	18 (16.1)	55 (22.1)	34 (17.7)	30 (21.6)	6 (10.7)	-
Type of embryo transfer	-	-	-	-	-	-	0.080
Fresh	16 (80)	88 (78.6)	183 (73.5)	141 (73.4)	110 (79.1)	51 (91.1)	-
Frozen-thawed	4 (20)	24 (21.4)	66 (26.5)	51 (26.6)	29 (20.9)	5 (8.9)	-
Pregnancy result	-	-	-	-	-	-	**0.001**
Negative	13 (65)	56 (50)	136 (54.6)	103 (53.6)	90 (64.7)	46 (82.1)	-
Positive	7 (35)	56 (50)	113 (45.4)	89 (46.4)	49 (35.3)	10 (17.9)	-
Pregnancy outcome	-	-	-	-	-	-	**0.001**
No pregnancy	13 (65)	56 (50)	136 (54.6)	103 (53.6)	90 (64.7)	46 (82.1)	-
Biochemical	1 (5)	2 (1.8)	20 (8)	13 (6.8)	11 (7.9)	4 (7.1)	-
Abortus	1 (5)	6 (5.4)	21 (8.4)	10 (5.2)	10 (7.2)	0	-
Live birth	4 (20)	47 (42)	68 (27.3)	63 (32.8)	28 (20.1)	6 (10.7)	-
Preterm birth	1 (5)	1 (0.9)	4 (1.6)	3 (1.6)	0	0	-
Take-home baby rate (%)	20	42	28.5	33.9	20.1	8.9	**<0.001**

p=0.05). The results of this meta-analysis are controversial because most studies included in this meta-analysis were non-randomized studies (20). However, another randomized controlled trial investigating LBR in a similar study population showed enhanced pregnancy rates of up to 70% following hysteroscopy (21).

By contrast with the findings of these studies, Smit et al. (13) suggested that routine hysteroscopy prior to IVF or ICSI treatments have no effect on fertility outcomes in infertile women with normal uterine cavity on transvaginal ultrasound at their multicenter randomized controlled trial. In this study, a limitation of this study was that hysteroscopy was performed by different gynecologists in several clinics because it is known that diagnostic accuracy of hysteroscopy may change depending on the operator (22). Moreover, the TROPHY trial, another randomized controlled trial evaluating the effect of hysteroscopy on LBR in women having more than two failed IVF cycles showed that hysteroscopy had no impact on LBR (10).

The reasons for conflicting results of the studies about the utility of hysteroscopy before IVF or ICSI cycles include methodological weakness and lack of quality. A recently published meta-analysis from the Cochrane database confirms this opinion. Kamath et al. (23) investigated the feasibility of routine hysteroscopy in sub-fertile women undergoing evaluation for infertility and in sub-fertile women scheduled for intrauterine insemination or IVF in this meta-analysis. After reviewing 11 publications, they concluded that there was no publication having strong evidence to support hysteroscopy as a screening method in sub-fertile women with a normal basic fertility work-up for increasing live birth and clinical pregnancy rates (23).

The important issue that has been suggested about the use and benefit of hysteroscopy before IVF is that endometrial scratching was reported to improve reproductive success rates. Although there are contradictory studies demonstrating that hysteroscopy is useful or not in this regard, a recently published randomized controlled trial and a systematic review showed that endometrial scratching does not increase pregnancy rates, and therefore larger studies with high levels of evidence are needed before they can be used in daily practice (24,25).

In this study, we retrospectively assessed the pregnancy outcomes of IVF cycles applied either by fresh or FET transfers that were performed with or without hysteroscopy to evaluate the uterine cavity prior to IVF. It was demonstrated that diagnostic hysteroscopy did not improve pregnancy rate in women who underwent fresh or FET embryo transfer. There were some limitations and strengths to our study. One of the most important strengths of our study was that all the hysteroscopies were performed by a single, experienced surgeon. Hence the evaluation of the uterine cavity was consistent and should reduce once source of variability in this study. The retrospective design of the study is the limitation of our study.

Conclusion

This study has shown that performing diagnostic hysteroscopy before fresh or FET does not improve the pregnancy rates in this cohort. However, randomized-controlled prospective trials are necessary to further understand the feasibility of performing hysteroscopy before IVF or ICSI cycles.

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The effect of placental angiogenic and anti-angiogenic factors on pregnancy outcome in patients with early onset preeclampsia

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Abstract

Objective: The aim was to evaluate the possible effects of anti-angiogenic factors including soluble endoglin (sEng), placental growth factor (Pgf), and soluble fms-like tyrosine kinase 1 (sFlt-1) in both normotensive pregnant patients and preeclampsia (PE) patients.

Material and Methods: The study was carried out at the Departments of Gynecology and Obstetrics and Biochemistry of Yozgat Bozok University Training and Research Hospital. Eighteen women with PE who were pregnant for at least 20 weeks comprised the study group. The control group consisted of 33 pregnant women with no complications and with similar demographic features. In the study, laboratory parameters, demographic characteristics, sEng, sFlt-1, and Pgf levels, delivery type, APGAR scores of the infants, and birthweight were determined and a comparison was made between the groups.

Results: It was found that the sEng level was significantly lower in the PE group compared to the control group ($p<0.05$). In addition, the Pgf, birthweight, and 1st and 5th-minute APGAR scores were significantly lower in the PE group compared to the control group ($p<0.05$).

Conclusion: The decrease in Pgf may have an effect on the pathogenesis of PE and can be utilized for the determination of PE. (J Turk Ger Gynecol Assoc 2021; 22: 212-6)

Keywords: Preeclampsia, soluble fms-like tyrosine kinase 1, soluble endoglin, placental growth factor

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Introduction

Preeclampsia (PE), which can result in maternal and fetal mortality, is a major complication of pregnancy although its etiology remains unclear (1). PE is characterized by a sudden onset of hypertension and end organ damage in a previously normotensive patient (2). PE complicates 2-8% of pregnancies and if early diagnosis is not established, fatal perinatal and maternal complications may occur. Globally, PE leads to more than fifty thousand maternal deaths a year (3). Therefore, accurate detection of PE is critical and can requires close monitoring of patients with PE.

The balance between angiogenic and anti-angiogenic factors is as important as placental vasculature for a healthy placenta. The pathophysiology of PE might be as follows: An inadequate invasion of syncytiotrophoblasts into spiral arteries in the maternal placental bed leads to the impairment of fetal perfusion and, consequently, ischemia-reperfusion attacks in the placental bed. Then, the release of anti-angiogenic factors into the maternal circulation occurs due to lack of blood supply. Finally, maternal systemic endothelial function deteriorates and systems such as hematologic, neurologic, cardiac, pulmonary, renal, and hepatic tissues become involved (4).

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Despite considerable advances in the management of patients with PE, early prediction of these cases remains a challenge. Assessment of the levels of angiogenic factors, such as placental growth factor (Pgf) or anti-angiogenic factors, including soluble fms-like tyrosine kinase-1 (sFlt-1), and soluble endoglin (sEng) could be useful (5,6). However, measurement of these biochemical parameters was reported as unable to aid in diagnosis of PE in a study designed by the National Institute for Health and Care Excellence (7). The present study aimed to detect the early onset PE by measuring levels of Pgf, sEng, and sFlt-1 levels.

Material and Methods

The research was carried out at the Gynecology and Obstetrics Department and Biochemistry Laboratory of Yozgat Bozok University Faculty of Medicine, Turkey. The subjects were recruited from patients referred to our clinic between January 2018 and July 2019. Approval was provided from the Ethical Committee of Yozgat Bozok University Faculty of Medicine. Informed consent was taken from all patients. The present study was funded by Yozgat Bozok University Project Coordination Application and Research Center (6602c-TF/19-247).

To detect an effect size of 0.92 at alpha error of 0.05 and statistical power of 0.95, 50 participants would be required for our study. The clinical studies of the present research included 51 women. The participants were divided into study groups and one control group. Eighteen of these participants comprised the study group by meeting the early onset preclinical criteria (8). In accordance with the literature, the diagnosis of PE was made with the presence of hypertension (systolic blood pressure ≥140 mmHg/diastolic blood pressure ≥90 mmHg) emerging after 20 gestational weeks, accompanied by at least one of the following findings: new-onset proteinuria; renal dysfunction; increased transaminases; arthritis, thrombocytopenia; visual impairment; changes in mental status; epigastric tenderness; fetal growth retardation; and umbilical artery disorder. After the diagnosis of PE, all patients were hospitalized and started on corticosteroid (betamethasone 12 mg/day for two days). Both groups were compared with maternal age, gravidity, parity, gestational week, systolic/diastolic blood pressure, protein levels in spot urine samples, protein levels in 24-hour urine, leucocyte count, hemoglobin level, platelet counts and creatinine, urea, liver function markers [aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH)], sEng, sFlt-1, and Pgf levels. In addition, neonatal parameters such as delivery route, first and fifth minute APGAR score, and neonatal birth weight were compared.

Patients having a pregnancy between 20 and 29 weeks and 6 days were included in the study. The gestational week was calculated according to their last menstrual period or by using ultrasonography. For those in the outpatient clinics, the blood pressure values of the subjects were determined using an adult-type blood pressure monitor. The blood pressure values of the patients in the inpatient clinics were measured using a patient monitor. Following a minimum of 12-hour fasting, a 5 mL venous blood sample was taken at 08.00 a.m. from all the patients for laboratory measurements. In the study group, the proteinuria values were determined in a 24-hour urine protein test.

Fasting blood taken from the patients with PE before the administration of any medication and from normotensive pregnant women was centrifuged within 30 minutes and stored at -80 ºC until analysis. sEng, sFlt1 and Pgf levels were assessed with commercial Elisa kits (Quantikine R&D Systems Europe, United Kingdom) and analyzed in accordance with standard protocols. The study was conducted at the Gynecology and Obstetrics Department and Biochemistry Laboratory of Yozgat Bozok University Faculty of Medicine, Turkey.

Exclusion criteria included a preexisting diagnosis of eclampsia or the presence of HELLP syndrome, multiple pregnancies, presence of malignancy, or psychological disorders. Also, those with a history of preeclampsia, metabolic and hormonal diseases (type 1 and type 2 diabetes mellitus and thyroid diseases), and chronic diseases (gestational hypertension, renal, and hepatic diseases) were not included in the study.

Statistical analysis

The statistical evaluation of the study was carried out using SPSS, version 17.00 (SPSS Inc., Chicago, IL, USA). Continuous variables were examined for normality of distribution using either Kolmogorov-Smirnov or Shapiro-Wilk's test. Data was compared using the Mann-Whitney U test. For categorical variables, the chi-square test was used while the independent sample t-test was utilized for continuous variables showing normal distribution. The receiver operating characteristic (ROC) test was performed to determine the threshold value of the data that can have an effect on preeclampsia. The significance level was set as p-value <0.05.

Results

The number of women who participated in the study was 51. The study group included 18 patients with early onset PE while the control group included 33 patients with no PE diagnosis. All patients in the study group had severe PE and fetal growth restriction was found in five of these (data not shown). The age, gravidity, and parity values of the participants were analyzed and the groups were similar (Table 1). The mean values of arterial blood pressures of the control group were: systolic 105.93 (±10.42) mmHg and diastolic 65.31 (±8.31) mmHg. In the PE group these values were 150.27 (±12.65) and 93.33

(±9.07) mmHg for systolic and diastolic pressures, respectively. The difference was statistically significant (p<0.05).

AST, LDH, and urea levels were significantly higher in the PE group compared to the control group (p<0.05). The mean levels of sEng and Pgf were both significantly lower in the PE group than in the control group (Table 2). sFlt-1 tended to be present at higher concentration in the PE group compared to controls, but this was not significant.

Neonatal parameters, such as delivery route, one and five minute APGAR scores, and neonatal birth weight were shown in Table 3. ROC curves for Pgf, sEng, and sFlt-1 were shown in Figure 1A, B, C. The area under the curve (AUC) for Pgf was 0.983 and the cut-off value was 314.97 pg/mL (sensitivity 93.9%, specificity 94.4%). The AUC for sEng was 0.70 with a cut-off value of 6.87 ng/mL (sensitivity 61.1%, specificity 63.6%). Similarly, the AUC for sFlt-1 was 0.754 with a cut-off value of 754.3 ng/mL (sensitivity 88.9%, specificity 66.7%) (Table 4).

Table 1. Demographic characteristics of the patients

	Study group (n=18)	Control group (n=33)	p-value
Age (year)	30.5±3.68	26.65±5.14	0.790
Gravidity	2.94±1.66	2.40±2.15	0.075
Parity	1.47±1.17	0.71±0.72	0.014
Systolic blood pressure (mmHg)	150.27±12.65	105.93±10.42	<0.001
Diastolic blood pressure (mmHg)	93.33±9.07	65.31±8.31	<0.001
Values are given as mean ± standard deviation			

Table 2. Biochemical parameters of the patients

	PE group (n=18)	Control group (n=33)	p-value
AST (µ/L)	35.66±33.09	16.71±6.31	**<0.001***
ALT (iU/L)	28.88±21.49	15.78±7.65	**0.006***
Urea (mg/dL)	8.88±3.72	6.65±1.87	**0.001***
Creatinine (µmol/L)	0.60±0.09	0.53±0.04	**0.006***
LDH (iU/L)	262.25±108.44	176.07±28.33	**<0.001***
WBC (µ/L)	10,616±3,494	9,981±2,263	0.801
Platelet (/µL)	225,555±77,555	225,781±49,922	0.801
Hb (g/dL)	11.90±1.29	12.00±1.20	0.801
sEng (ng/mL)	5.54±0.68	7.30±0.67	**<0.001***
sFlt-1 (ng/mL)	803.34±52.03	743.71±141.48	**0.044***
Pgf (pg/mL)	87.85±18.96	486.33±102.29	**<0.001***
Values are means ± standard deviation. AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, WBC: White blood cell count, Hb: Hemoglobin concentration, sEng: Soluble endoglin, sFlt-1: Soluble fms-like tyrosine kinase-1, Pgf: Placental growth factor			

Discussion

In this clinical study, the relationship between angiogenic and anti-angiogenic factors, including sEng, Pgf, and SFlt-1, and the outcome of early-onset PE was investigated. Our study has shown that Pgf levels were significantly lower in patients with early-onset PE. However, sFlt-1 levels were similarly distributed in the two groups. Paradoxically, sEng levels were found to be significantly higher in the control group compared to the PE group. Previous studies reported that higher sEng levels in PE group than control group (6-8).

PE is a systemic disease that begins after the 20th week of pregnancy and progresses with hypertension and proteinuria. PE is a leading cause of both maternal-fetal morbidity and mortality but the etiology of the disease is still a dilemma. Both maternal and fetal/placental factors might be responsible for the pathogenesis of PE. Redman devised the two-stage model hypothesis for PE (9). The first of these (stage 1) is the preclinical stage, associated with inadequate placentation, while the second (stage 2) is the clinical staging associated with the maternal syndrome. However, Roberts and Redman (10) claimed that the two-stage model hypothesis was not valid for all PE cases. Afterwards, Palei et al. (11) presented another theory and suggested that defective placentation causes the formation of vasoactive substances (sFlt-1 and sEng) by creating ischemia-reperfusion attacks in the placental bed. Elevation of these anti-angiogenic agents could cause harmful effects in endothelial cells. Therefore, we hypothesized that early detection of these parameters could be useful for early diagnosis of PE.

Angiogenic factors, such as Pgf, and anti-angiogenic factors, such as sFlt-1 and sEng should work in synchrony. It is assumed that maternal Pgf levels decrease, while maternal sFlt-1 and sEng levels increase before clinical preeclampsia is evident (12). In our study, Pgf was found to be low, in accordance with the literature, while sEng were also low, which is in contrast to previous reports. sFlt-1 tended to be higher in the PE group but this was not significant when compared to the control group. This result may be related to the small number of patients and

Table 3. Perinatal outcome of the subjects

	Study group (n=18)	Control group (n=33)	p-value
Mode of delivery			
C/S (%)	28.88±21.49	15.78±7.65	0.006
1 min APGAR	6.48±1.72	6.95±1.87	0.140
5 min APGAR	7.10±2.09	8.03±1.74	0.790
Neonatal weight (g)	2786,56±1058.44	2954.27±1128.33	0.450
C/S: Cesarean section			

Table 4. Cut off, sensitivity and specifity of the Pgf, sEng, and sFlt-1					
Parameters	**AUC[μ]**	**Cut-off**	**Sensitivity (%)**	**Specifity (%)**	**95% CI**
Pgf	0.983	314.97	93.9	94.4	0.955-1.000
sEng	0.700	6.87	61.1	63.6	0.535-0.836
sFlt-1	0.754	754.30	88.9	66.7	0.621-0.887
sEng: Soluble endoglin, sFlt-1: SolublE fms-like tyrosine kinase, Pgf: Placental growth factor, CI: Confidence interval, AUC[μ]: Area under the curve					

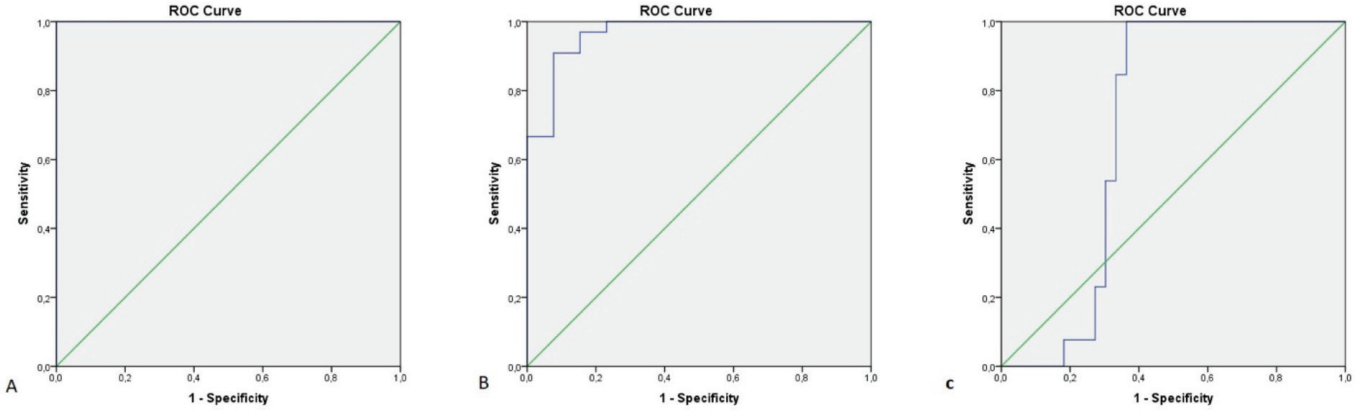

Figure 1. A) ROC curve of Pgf. B) ROC curve of sEng. C) ROC curve of sFlt-1

sEng: Soluble endoglin, sFlt-1: Soluble fms-like tyrosine kinase, Pgf: Placental growth factor, ROC: Receiver operating characteristic

the weeks of gestation. In a study comparing late preeclampsia patients with healthy controls, both sEng and sFlt1 levels were remarkably high in patients with late-onset preeclampsia. However, only sEng may be a useful tool in the determination of the severity of preeclampsia.

Two large studies have been conducted on the measurement of angiogenic and anti-angiogenic factors in PE. One of them was the PARROT trial (13). Pgf alone was evaluated in the PARROT trial. Measurement of Pgf levels was found to be a useful tool to reduce severe maternal complications. However, fetal/neonatal adverse outcomes remained the same. Another large trial was the INSPIRE trial (14) which concluded that these markers alone do not have sufficient use for predicting preeclampsia. Therefore, the sFlt-1/Pgf ratio was measured in the INSPIRE trial. However, it was shown that maternal, fetal, or neonatal outcomes remain still a challenge.

Despite all the previous studies reporting that Pgf, sFlt-1, and sEng levels were important for healthy placentation, there is still debate about their predictive value. Therefore, the present study planned to predict early-onset preeclampsia with these parameters.

Study limitation

The limitations of our study were small patient numbers and heterogeneity of the population.

Conclusion

This study showed that there was a strong association between Pgf and early-onset PE. However, sFlt-1 and sEng were found to be poor in determining early onset preeclampsia.

Ethics Committee Approval: *The study protocol was reviewed and approved by the Ethical Committee of Yozgat Bozok University Faculty of Medicine.*

Informed Consent: *An ethical consent was obtained from the patients.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices - M.K., E.S.Y., M.E.K.; Concept - E.S.Y., D.A.K.; Design - M.K., M.D.Ç.; Data Collection or Processing - T.P., M.D.Ç.; Analysis or Interpretation - E.B., T.O.; Literature Search - M.K., D.A.K.; Writing - M.K., E.B., T.O.*

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

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Changes in anthropometric and blood 25-hydroxyvitamin D measurements in antenatal vitamin supplemented gestational diabetes mellitus patients: a systematic review and meta-analysis of randomized controlled trials

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Abstract

Objective: Gestation weight (GW), body mass index (BMI), and blood 25-hydroxyvitamin D [25(OH)D] level during pregnancy are important determinants of the gestational outcomes. This study aimed to study how these parameters vary between antenatal vitamin D recipients and non-recipients in gestational diabetes mellitus (GDM) patients.

Material and Methods: The randomized controlled trials comparing these outcomes between vitamin D recipient and non-recipient GDM patients were searched in electronic databases (PubMed, Embase, and Scopus). The reviewed studies' data were abstracted and critically appraised using the Cochrane tool. The estimation of the weighted mean difference for GW and BMI and standardized mean difference (SMD) for 25(OH)D levels occurred by juxtaposing the interventions meta-analytically (random-effect model). The statistical inconsistency was determined by Chi^2 and I^2 method. The statistical significance was estimated at $p<0.05$ and 95% confidence interval (CI).

Results: Eleven eligible trials (all Iran-based, except one), sourcing data from about 875 GDM patients, were reviewed. Overall, the risk of bias was low, except for selection and performance bias. On random-effect model meta-analysis, the 25(OH)D levels of the GDM patients favored the vitamin D recipients when compared to non-vitamin D (SMD 1.97, 95% CI: 1.06-2.88, $p<0.001$; I^2 96.2%, p of Chi^2 <0.001) and placebo (SMD 1.86, 95% CI: 0.95-2.77, $p<0.001$; I^2 95.3%, p of Chi^2 <0.001) recipients, respectively. On meta-regression, sample size was a predictor of the observed heterogeneity. For GW and BMI the interventions did not differ statistically significantly.

Conclusion: In GDM patients, antenatal use of vitamin D aids in the rise of blood 25(OH)D levels. However, vitamin D supplementation did not affect change in GW or BMI. (J Turk Ger Gynecol Assoc 2021; 22: 217-34)

Keywords: Gestational diabetes, vitamin D, dietary supplement

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Introduction

Gestational diabetes mellitus (GDM) refers to any degree of glucose intolerance that develops or is identified initially during gestation (1). Its global prevalence is about 7-10% (2-5). GDM diagnosis is made using glucose challenge tests between 24-28 weeks of gestation (1). Initial GDM management encompasses diet and exercise therapy, but if these fail to achieve glycemic control, physicians start insulin therapy (1).

GDM is a crucial health burden since it can affect both the GDM patient and her neonate. Gestational weight (GW)

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and body mass index (BMI) in pregnancy are two important anthropometric determinants of GDM-related outcomes. Studies in GDM patients suggest that an excessive GW accumulation increases the risk of maternal complications, such as increased likelihood of cesarean delivery, large for gestational age, and gestational hypertension and fetal problems, such as macrosomia, large for gestational age, hypoglycemia in newborns, and poor APGAR score (6-11). It remains unclear if the Institute of Medicine's guideline (2009) for recommended GW gain for respective BMI categories can be applied to the GDM subpopulation or not (8,12). However, studies on overweight and obese GDM patients found that gaining GW less than that recommended for their respective BMI categories resulted in favorable obstetric and neonatal outcomes (8,13,14). Maintaining an optimum weight before and during pregnancy, therefore, can decrease the complications of pregnancy (9). Nevertheless, it remains unclear if any antenatal intervention in GDM patients may be beneficial in achieving an acceptable GW and BMI.

In this respect, vitamin D has emerged as a potentially useful agent that has attracted attention. In GDM patients, various clinical trials (15-18) have tested the maternal health effect of antenatal vitamin D supplementation, and due to the different relationships between GDM and vitamin D status in the body, such testing appears pertinent. For instance, inadequate vitamin D levels in the body are associated with an increased risk of developing GDM (19-23). Vitamin D deficiency (<20 ng/mL) has a nearly fourfold increased risk of GDM development than women with sufficient vitamin D level (>30 ng/mL) after adjusting for the age of the mother, race, ethnicity, and family history of type 2 diabetes among first-degree family members (24). Moreover, studies showed a decreased GDM prevalence in prenatal vitamin D recipients (25,26). Given this evidence it is important to understand how vitamin D supplementation in GDM mothers can affect GW and their BMI. Additionally, as the fetus entirely depends on maternal 25-hydroxyvitamin D [25(OH)D] levels, maternal levels in GDM mothers after vitamin D supplementation also requires evaluation (27).

Intervention description

The inactive forms of the fat-soluble vitamin D are D2 (ergocalciferol) and D3 (cholecalciferol) (28,29). Both forms are available from diet and supplements and vitamin D3 is also produced in the skin on exposure to sunlight (28,29). On hydroxylation of pre-vitamin D in the liver, the main circulating form, 25(OH)D, of vitamin D is produced (27). In blood, 25(OH) D is either present in the bound form (to albumin) or free form (27). For its physiologic role, it is converted to the active form, calcitriol 1,25-dihydroxyvitamin D (28,30). The physiological effect of Vitamin D in pregnancy is mediated via calcitriol's action on the vitamin D receptors in uteroplacental tissue (28,30). Compared to calcitriol, which has a half-life of 4-6 hours (27), the relatively longer half-life of 25(OH)D of between two and three weeks (31) makes the latter an ideal marker for vitamin D status (32).

In GDM patients, contemporary trials have supplemented vitamin D at various dosages. For oral preparations, while some trials used it at a dose of 50,000 IU, two to three weeks apart for three to eight weeks (33-36), other trials used it twice daily at 200-500 IU for six to sixteen weeks (17,37). One trial used a single intramuscular injection of vitamin D at a dose of 300,000 IU (38). Furthermore, while few trials used the vitamin as a single supplement (33,37,38), others co-supplemented it with various micronutrients, including zinc, magnesium, and calcium (17,34).

What this review adds?

In contemporary medicine, several clinical trials have tested the changes in GW, BMI, and plasma 25(OH)D level in GDM mothers, after antenatal vitamin D supplementation (15,34-36). Recent reviews have studied the effect of antenatal vitamin D supplementation on certain maternal complications such as cesarean section rate, pre-eclampsia, preterm delivery, macrosomia, and polyhydramnios and/or on neonatal complications including hyperbilirubinemia, hypoglycemia, and hospitalization (39-41). However, to the best of our knowledge, there is no systematic review and meta-analysis that studied how maternal GW, BMI, and 25(OH)D levels change in the blood on vitamin D supplementation in GDM patients. Therefore, this study explores this under-reviewed area of modern medicine by a systematic literature search, critical appraisal, and meta-analysis.

Aims

This study compared the GW, BMI, and 25(OH)D levels among vitamin D supplemented and not-supplemented GDM patients.

Material and Methods

This systematic review is registered in the PROSPERO database (CRD42020149613) and has a pre-published protocol (42,43). This report adheres to the PRISMA 2009 reporting guideline (Supplement Table 1) (44).

Inclusion criteria

1. Study design: Parallel arm randomized controlled trials of any number of intervention arms.

2. Population: Pregnant females of any age were eligible, irrespective of their pre-pregnancy BMI and 25(OH)D levels.

They must be diagnosed with GDM during their concurrent pregnancy.

3. Intervention arm: The treatment arm/s should have received vitamin D as a sole or co-supplement.

4. Comparator arm: The comparator arm/s may have received a placebo or any other supplement except vitamin D. Comparator arm/s not receiving any intervention were also eligible.

5. Outcomes: The trials must report the GW (kg), BMI (kg/m^2), and 25(OH)D (in ng/mL or mmol/L) in the above GDM patients before and after receiving these interventions and before childbirth.

We accepted the diagnosis and management of GDM and the dosage and regimen of interventions received by the participants in the respective treatment arms as per the trialists.

Exclusion criteria

1. Study design other than those described above, e.g., observational studies and crossover studies.
2. Participants with diabetes types besides GDM, like type 1 or type 2 diabetes.
3. Studies conducted on animals.
4. Editorials, abstracts from conference presentations (where a full published manuscript is not available), letters, or any other brief communications.

Database search

We searched the title and abstract of prospective trials matching the above eligibility criteria in PubMed, Embase, and Scopus databases, irrespective of the date and language of publication and geographical boundary. The following search terms were used "vitamin D" OR "calciferol" OR "vitamin D2" OR "ergocalciferol" OR "vitamin D3" OR "cholecalciferol" AND "GDM" OR "gestational diabetes" along with these MeSH terms- "Cholecalciferol", "ergocalciferols", and "diabetes, gestational". To identify the clinical trials in PubMed [(Clinical Trial) and (Randomized Controlled Trial)] and Embase [(controlled clinical trial) and (randomized controlled trial)], we used filters. In Scopus, instead of filters, the following search terms were used: "trial," "randomised," "randomized," and "controlled." The last date of the search was 17 September, 2020. Additionally, we reviewed the references of the papers included in this review.

We uploaded the retrieved citations (from database search) in the Rayyan systematic review software (45) and eliminated the duplicate articles. Successively, skimming of the remaining citations' titles and abstracts against the eligibility criteria commenced. Articles were read in full-text when it seemed to meet the inclusion criteria, or if the suitability for incorporation in this review was doubtful.

Data abstraction and risk of bias assessment

We extracted data about the study design, consent, ethics, registration number of the trial, participant features, interventions contrasted, and the outcomes of interest in a pre-piloted form. With the Cochrane collaboration tool, individual trial's risk of selection bias, performance bias, detection bias, attrition bias, reporting bias, and any other bias was determined, and each of these risk of bias (RoB) components was categorized as low, high, or unclear (46). To assess selection bias, the random allocation sequence generation method, and its concealment method from participants, were judged. The blinding mechanism of study participants and personnel and that of outcome assessors were used to evaluate the performance and detection bias, respectively. By evaluating missing outcome data, and its reason among the intervention arms, the risk of attrition bias, was evaluated. Any additional bias, besides the above, comprised the other bias type. For a visual presentation of the RoB, we prepared an RoB graph and an RoB summary using the Review Manager (RevMan) software (46,47).

The review authors independently performed study selection, data abstraction, and RoB assessment, and resolved any disagreement in an opinion by discourse.

Meta-analysis

The juxtaposed interventions' effect on each of the outcomes was contrasted by random-effects meta-analysis (using DerSimonian and Laird method) since we assumed clinical heterogeneity among the trials attributable to the different types of vitamin D co-supplements used in these. The use of endpoint means of the respective outcomes and their SDs ensued to conduct the meta-analysis. We estimated the meta-analytic effect sizes of GW and BMI in weighted mean differences (WMD) and that of 25(OH)D levels in standardized mean differences (SMD) due to the identical and non-identical types of measuring units used in the trials, respectively. A decrease in the summary effect of GW and BMI, and its increase in 25(OH)D levels, denoted a favorable meta-analytic finding. For any outcome, when multiple treatment arms tested an intervention, the post-intervention means and their SDs of those intervention groups were combined for meta-analysis (46). Outcome reported in the median were not considered for meta-analysis.

Heterogeneity and meta-regression

The statistical heterogeneity was determined by Chi^2 (statistically significant at $p<0.1$) and I^2 (categorized as low,

moderate, and high at I^2 values of 25, 50, and 75%, respectively) statistics (48). To account for any substantial heterogeneity, we performed univariate meta-regression by presence or absence of missing outcome data and sample size (categorized as <100 and ≥100). Using the predictor identified by meta-regression, we did a subgroup analysis to see how heterogeneity changed across the different categories of the predictor.

Publication bias and sensitivity analysis

The publication bias assessment incorporated visual inspection of funnel plots and Egger's test. For each outcome, a sensitivity analysis included iteration of the meta-analysis using a fixed-effect model and by dropping a trial each time.

Statistical analysis

Using random-effect and fixed-effect models, all outcomes were compared meta-analytically between vitamin D and placebo-receiving GDM patients.

We estimated the statistical significance of meta-analysis derived effect sizes at $p<0.05$ and 95% confidence interval (CI). Stata statistical software v16 (StataCorp, College Station, TX) was used for analysis.

Results

Scope of the review

The database search retrieved 271 citations. After eliminating the duplicates, 188 citations underwent skimming against the eligibility criteria. Out of the 22 articles needing full-text reading, 11 trials sourcing data from about 875 participants published between 2014-19, were included in this review (Figure 1) (15-18,33-35,37,49-51). All trials except the Chinese one (37) were Iran-based, and the average age of participants in the respective intervention arms was approximately 28-32 years. The intervention period of Iranian (16-18,33,49-51) and Chinese (37) trials were 6-8 and 16 weeks, respectively. In most trials (15-18,34,35,37,49-51), GDM was diagnosed primarily using the American Diabetes Association criteria (52,53). Insulin was not used during the intervention period, except in the trial by Yazdchi et al. (33). Eight trials (15,16,18,33,34,37,49,50) used the D3 form of the vitamin while this was not clear among the remaining trials (17,35,51). In most of the trials (81.8%), a co-supplement (e.g., calcium, magnesium, zinc, omega-3 fatty acid, evening primrose oil, probiotic) accompanied the vitamin D supplementation (15-18,34,35,37,50,51). The intervention was given between 24-28 weeks of gestation in nine trials (15-18,33-35,49,50), at 16 weeks of gestation in one trial (37), and in the remaining one, this was unclear (51). Table 1 depicts the salient features of the trials.

RoB assessment

In most studies, the allocation concealment component of the selection bias and performance bias was unclear (Table 2 and Figure 2). Otherwise, the RoB was low.

Meta-analysis findings

Eleven trials comparing GW (15,16,51,17,18,33-35,37,49,50), and 10 trials contrasting BMI (15-18,33-35,49-51) with one study (37) excluded as it did not report the follow up BMI, and 25(OH)D with one trial (33) excluded for reporting follow up value in median, were included in the meta-analytic juxtaposition between vitamin D recipients and its non-recipients.

The antenatal vitamin D use in GDM patients favored plasma 25(OH)D level attainment compared to its non-supplementation (random-effect model: SMD 1.97, 95% CI: 1.06-2.88, $p<0.001$; I^2 96.2%, p of Chi^2 <0.001).

The post-intervention GW (random-effect model: WMD 0.18, 95% CI, -1.10-1.47, $p=0.773$; I^2 0%, p of Chi^2 0.559) and BMI (random-effect model: WMD 0.27, 95% CI, -0.28-0.82, $p=0.331$; I^2 0%, p of Chi^2 0.838) were not statistically significantly different between the juxtaposed interventions (Figure 3).

Meta-regression and subgroup analysis

The univariate meta-regression suggested that sample size was a statistically significant predictor of the observed heterogeneity in the effect size of 25(OH)D level (Supplement Table 2). Upon subgroup analysis by the sample size, heterogeneity was moderate when sample size was ≥100, and the effect size increased (random-effect model: SMD 3.81, $p<0.001$; 95% CI, 3.03-4.59; I^2 72.5%) (Supplement Figure 1).

Publications bias

For 25(OH)D, a small study effect was suggested by the asymmetric funnel plots (Supplement Figure 2) and Egger's test ($p=0.005$). On trim-and-fill analysis, no additional study was imputed. Funnel plots for the rest of the outcomes were approximately symmetric.

Sensitivity analysis

On using a fixed-effect model meta-analysis, the summary estimate of 25(OH)D level, reduced slightly (SMD 1.74, 95% CI, 1.57-1.92, $p<0.001$). The fixed-effect meta-analysis results for the rest of the outcomes were identical to the preliminary analysis. The meta-analysis findings for all outcomes remained unchanged on dropping a study each time and repeating the meta-analysis.

Supplementary meta-analysis

Between vitamin D and placebo, ten trials (15-18,33-35,49-51) compared GW and BMI, and nine trials (15-18,34,35,49-

51) juxtaposed 25(OH)D levels, with one study (33) excluded because the study reported 25(OH)D values as medians. Vitamin D recipients achieved a favorable blood 25(OH) D level compared to the placebo recipients (random-effect model: SMD 1.86, 95% CI, 0.95-2.77, $p<0.001$; I^2, 95.36%, p of Chi^2 <0.001) (Figure 4). The effect size of 25(OH)D levels reduced slightly on using a fixed-effect meta-analysis model (SMD 1.45, 95% CI, 1.25-1.64). GW and BMI, when contrasted among the intervention arms, were not statistically significantly different. Since <10 studies were available for the 25(OH)D levels, we did not explore heterogeneity or assess the publication bias for it.

Discussion

Overall, 11 trials, mostly Iranian, tested the effect of antenatal vitamin D complementation (as a co-supplement primarily) on GW, BMI, and 25(OH)D in 875 GDM patients, were retrieved. The intervention favored a rise in blood 25(OH)D levels, and the sample size was the plausible predictor of the observed heterogeneity.

Evidence quality

Utilizing the GRADE Working Group's (2004) (54) approach of grading evidence quality we graded the evidence concerning the 25(OH)D level as of moderate-quality, due to the unclear RoB components and heterogeneity.

Comparison with what is known

As the context remains underexplored in contemporary literature, a direct juxtaposition of our findings to existing reviews is not possible. However, clinical trials studying the effect of vitamin D supplementation on 25(OH)D level in pregnant females with no glucose intolerance are available for a contrast. Two such trials found that vitamin D supplementation in the third trimester increased maternal plasma 25(OH) D levels compared to the control group (55,56). Another randomized trial found that vitamin D supplementation caused a statistically significantly greater increase in the 25(OH)D level than the placebo (57). Mirroring these trials' findings (55-57), we observed that vitamin D3 supplementation in GDM patients increased the maternal 25(OH)D level.

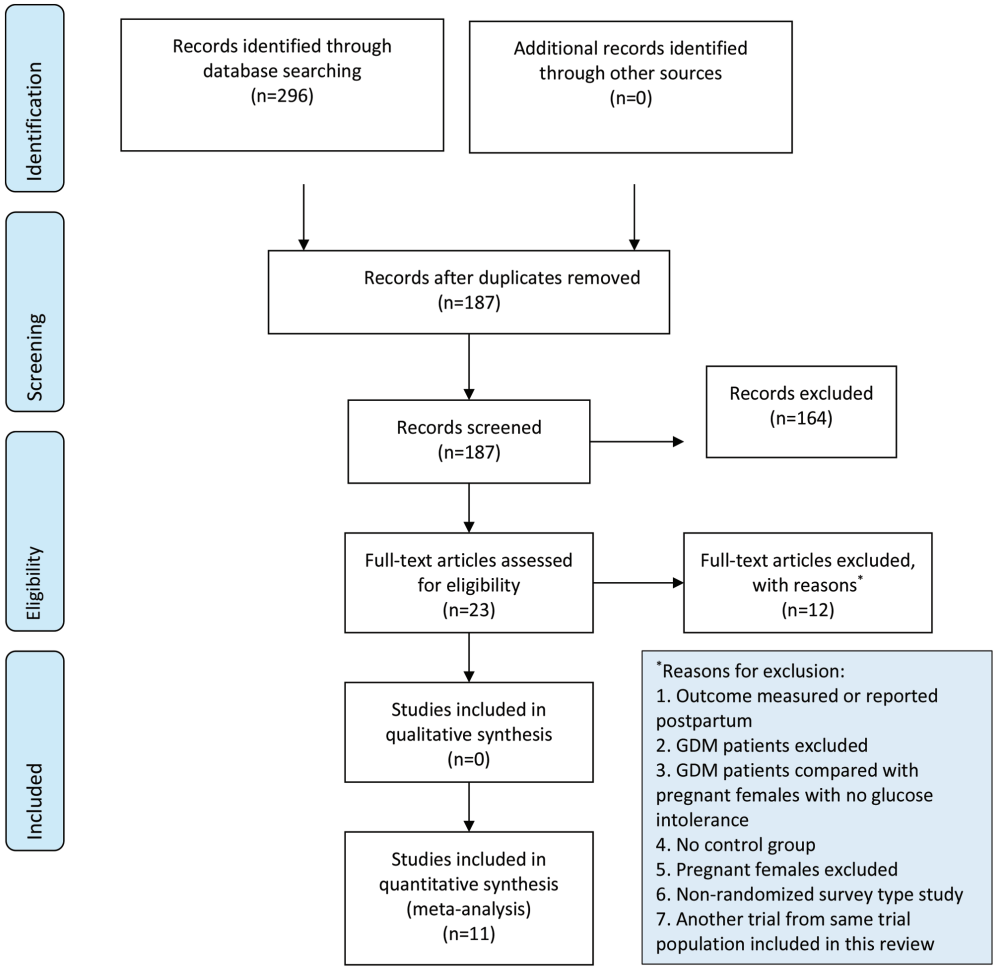


Figure 1. Study selection process [PRISMA flow chart (58)]

Table 1. Summary table

Trial	Design	Population	Intervention arms	Outcomes reported
Karamali et al. (16)	Randomized, placebo-controlled trial Blinding: double blinded Number of intervention arms: two Multi-center or single-center trial: multi-centric Study duration: six weeks Country where trial was conducted: Iran Ethical permission: obtained Consent from participants: obtained Information regarding funding: provided Clinical trial registration number: IRCT201407115623N23	Diagnosis: GDM (using ADA criteria) Number of participants randomized: 60 Calcium and vitamin D arm (n): 30 Placebo group (n): 30 Average age of Calcium and vitamin D arm: 28.7 (6.1) years Average age of placebo group: 31.6 (6.3) years Missing outcome data: 0 Baseline mean BMI (SD): Placebo group: 30.5 (4.5) kg/m^2; Calcium and vitamin D arm: 29.4 (4.7) kg/m^2 Baseline mean GW (SD): Placebo group: 78.1 (13.4) kg; Calcium and vitamin D arm: 73.7 (12.8) kg Baseline mean (SD) vitamin D levels: Placebo group: 20.8 (14.4) ng/mL; Calcium and vitamin D arm: 17.3 (10.9) ng/mL	Calcium and vitamin D arm: calcium carbonate 1000 mg/day (six weeks) and 50,000 IU D3 (at trial initiation and 21st day). Placebo arm. Intervention given between 24 and 28 weeks of pregnancy. Total vitamin D received in six weeks: 100,000 IU.	1. GW 2. BMI 3. 25(OH)D
Karamali et al. (17)	Randomizedplacebo-controlled trial Blinding: double blinded Number of intervention arms: two Multi-center or single-center trial: single-centric Study duration: six weeks Country where trial was conducted: Iran Ethical permission: obtained Consent from participants: not clear Information regarding funding: provided Clinical trial registration number: not available	Diagnosis: GDM (using ADA criteria) Number of participants randomized: 60 Magnesium, zinc, calcium and vitamin D supplements arm (n): 30 Placebo group (n): 30 Average age of magnesium, zinc, calcium and vitamin D supplements arm: 30 (4.5) years Average age of placebo group: 31.1 (4.2) years Missing outcome data: 0 Baseline mean BMI (SD): Placebo group: 27 (2.6) kg/m^2; Magnesium, zinc, calcium and vitamin D supplements arm: 27.4 (4.8) kg/m^2 Baseline mean GW (SD): Placebo group: 70.7 (7.2) kg; Magnesium, zinc, calcium and vitamin D supplements arm: 70.9 (12.8) kg Baseline mean (SD) vitamin D levels: Placebo group: 20.21 (10.73) ng/mL; Magnesium, zinc, calcium and vitamin D supplements arm: 18.96 (11.23) ng/mL	Magnesium, calcium, zinc and vitamin D arm: 100 mg magnesium, 400 mg calcium, 4 mg zinc, and 200 IU vitamin D 2x/d (six weeks). Placebo arm. Total vitamin D received in six weeks: 16,800 IU	1. GW 2. BMI 3. 25(OH)D
Asemi et al. (49)	Randomized, placebo-controlled trial Blinding: double blinded Number of intervention arms: two Multi-center or single-center trial: multi-centric Study duration: six weeks Country where trial was conducted: Iran Ethical permission: obtained Consent from participants: obtained Information regarding funding: provided Clinical trial registration number: IRCT201305115623N7	Diagnosis: GDM (using ADA criteria) Number of participants randomized: 50 Vitamin D arm (n): 25 Placebo group (n): 25 Average age of vitamin D arm: 31.1 (5.5) years Average age of placebo group: 30.8 (6.2) years Missing outcome data: 5 (three in vitamin D armand two in placebo arm); Causes of missingness: intra-uterine fetal death (n=1), placenta abruption (n=1), completed bed rest (n=1), insulin therapy (n=1), pre-eclampsia (n=1) Baseline mean BMI (SD): Placebo group: 30.5 (4.5) kg/m^2; Vitamin D arm: 30.7 (3.9) kg/m^2 Baseline mean GW (SD): Placebo group: 77.8 (12.9) kg; Vitamin D arm: 79.0 (9.7) kg Baseline mean (SD) vitamin D levels: Placebo group: 20.9 (14.3) ng/mL; Vitamin D arm: 18.9 (14.5) ng/mL	Vitamin D arm: 50,000 IU D3 (at trial initiation and 21st day). Placebo arm. Total vitamin D received in six weeks: 100,000 IU.	1. GW 2. BMI 3. 25(OH)D

Table 1. Continued

Trial	Design	Population	Intervention arms	Outcomes reported
Jamilian et al. (50)	Randomized, placebo-controlled trial Blinding: double blinded No. of treatment arms: two Single centered trial Study duration: six weeks Country where trial was conducted: Iran Ethical permission: obtained Consent from participants: obtained Information regarding fundingInformation regarding funding: provided Clinical trial registration number: IRCT201706075623N119	Diagnosis: GDM (using ADA criteria) Number of participants randomized: 90 Probiotic and vitamin D arm (n): 30 Probiotic arm (n): 30 Placebo group (n): 30 Average age of probiotic and vitamin D arm: 28.9 (6.1) years Average age of probiotic group: 31.2 (5.9) years Average age of placebo group: 29.9 (3.7) years Missing outcome data: 3; Causes of missingness: insulin therapy (n=1) and hospitalization (n=1) Baseline mean BMI (SD): Placebo group: 27.5 (3.3) kg/m^2; Probiotic and vitamin D arm: 27.8 (4.9) kg/m^2; Probiotic group: 26.4 (4.2) kg/m^2 Baseline mean GW (SD): Placebo group: 72.0 (7.7) kg; Probiotic and vitamin D arm: 71.9 (12.1) kg; Probiotic group: 70.0 (12.5) kg Baseline mean (SD) vitamin D levels: Placebo group: 14.3 (4.1) ng/mL; Probiotic and vitamin D arm: 13.4 (4.1) ng/mL; Probiotic group: 12.9 (3.2 ng/mL)	Probiotic and vitamin D arm: 50,000 IU D3 (every 2 weeks) and 8*10^9 CFU/g probiotic Probiotic arm: 8*10^9 CFU/g probiotic Placebo arm. Total vitamin D received in six weeks: 150,000 IU	1. GW 2. BMI 3. 25(OH)D
Jamilian et al. (18)	Randomized, placebo-controlled trial Blinding: double blinded No. of treatment arms: two Single centered trial Study duration: six weeks Country where trial was conducted: Iran Ethical permission: obtained Consent from participants: obtained Information regarding funding: provided Clinical trial registration number: IRCT201704225623N109	Diagnosis: GDM (using ADA criteria) Number of participants randomized: 60 Magnesium, zinc, calcium plus vitamin D arm (n): 30 Placebo group (n): 30 Average age of magnesium, zinc, calcium plus vitamin D arm: 27.7 (4.0) years Average age of placebo group: 29.1 (4.1) years Missing outcome data: 0 Baseline mean BMI (SD): Placebo group: 25.3 (2.5) kg/m^2; magnesium, zinc, calcium plus vitamin D arm: 25.8 (3.7) kg/m^2 Baseline mean GW (SD): Placebo group: 67.6 (6.1) kg; Magnesium, zinc, calcium plus vitamin D arm: 68.2 (9.4) kg Baseline mean (SD) vitamin D levels: Placebo group: 13.5±3.6 ng/mL; Magnesium, zinc, calcium plus vitamin D arm: 12.6±4.2 ng/mL	Magnesium, calcium, zinc, and vitamin D arm: 100 mg magnesium, 400 mg calcium, 4 mg zinc, and 200 IU D3: two times daily for six weeks. Placebo arm. Total vitamin D received in six weeks: 16800 IU	1. GW 2. BMI 3. 25(OH)D
Li and Xing (37)	Randomized, clinical trial Blinding: double blinded No of treatment arms: two Multricentric trial Study duration: 16 weeks Country where trial was conducted: China Ethical permission: obtained Consent from participants: obtained Information regarding funding: not clear Clinical trial registration number: not clear	Diagnosis: GDM (using ADA criteria) Number of participants randomized: 103 Yoghurt supplemented with vitamin D arm (n): 52 Plain yoghurt group (n): 51 Average age of yoghurt supplemented with vitamin D arm: 29.0±5.3 years Average age of plain yoghurt group: 28.3±4.1 years Missing outcome data: 6 [non-compliance (3) and personal reasons (3)] Baseline mean GW (SD): Plain yoghurt group 69.3±6.7) kg; Yoghurt supplemented with vitamin D arm: 67.9±7.1) kg Baseline mean (SD) vitamin D levels: Plain yoghurt group: 16.2 (3.4) ng/mL; Yoghurt supplemented with vitamin D arm: 16.8±4.6) ng/mL	Yoghurt and vitamin D arm: plain yoghurt and 500 IU D3 (twice daily for 16 weeks) Plain yoghurt arm: twice daily for 16 weeks. Total vitamin D received in six weeks: 112,000 IU	1. GW 2. 25(OH)D level

Table 1. Continued

Trial	Design	Population	Intervention arms	Outcomes reported
Razavi et al. (51)	Randomized clinical trial Blinding: double blinded No. of treatment arms: two Single centered trial (59) Study duration: six weeks. Country where trial was conducted: Iran Ethical permission: obtained Consent from participants: obtained Information regarding funding: provided Clinical trial registration number: IRCT201701305623N106	Diagnosis: GDM (using ADA criteria) Number of participants randomized: 120 Vitamin D arm (n): 30 Omega-3 arm (n): 30 Vitamin D and Omega-3 arm (n): 30 Placebo arm (n): 30 Average age of Vitamin D arm: 29.9±5.0 years Average age of Omega-3 arm: 29.7±3.6 years Average age of vitamin D and Omega-3 arm: 29.9±4.0 years Average age of placebo arm: 29.2±3.4 years Missing outcome data: 0 Baseline mean GW (SD): Vitamin D arm: 76.1±12.7 kg; Omega-3 arm: 74.3±5.8 kg; vitamin D and Omega-3 arm: 77.4±10.2 kg; Placebo arm: 75.1±7.7 kg Baseline mean (SD) BMI: Vitamin D arm: 29.2±5.0 kg/m^2; Omega-3 arm: 28.5±2.4 kg/m^2; vitamin D and Omega-3 arm: 29.5±3.8 kg/m^2; placebo arm: 28.8±3.4 kg/m^2 Baseline mean (SD) vitamin D levels: Vitamin D arm: 13.6±3.7 ng/mL; Omega-3 arm: 15.6±4.0 ng/mL; Vitamin D and Omega-3 arm: 14.2±2.9 ng/mL; placebo arm: 14.9±3.2 ng/mL	Vitamin D arm: 50,000 IU (two weekly) Omega-3 arm: 1,000 mg omega-3 fatty acids two times a day Vitamin D and Omega-3 arm: 50,000 IU Vitamin D (two weekly) and 1,000 mg omega-3 fatty acids: two times a day for six weeks. Placebo arm. Total vitamin D received in six weeks: 150,000 IU	1. GW 2. BMI 3. 25(OH)D
Yazdchi et al. (33)	Randomized controlled clinical trial Blinding: double blinded No of treatment arms: two Single centered trial Study duration: 8 weeks. Country where trial was conducted: Iran Ethical permission: obtained Consent from participants: obtained Information regarding funding: provided Clinical trial registration number: IRCT201306253140N11	Diagnosis: GDM (using International Association of Diabetes and Pregnancy Study Groups criteria) Number of participants randomized: 76 Vitamin D arm (n): 38 Placebo arm (n): 38 Average age of Vitamin D arm: 31.64±4.40 years Average age of placebo arm: 32.11±3.61 years Missing outcome data: 4 [severe preeclampsia (1), early childbirth (1), unwilling to continue (1), and hospitalization (1)] Baseline mean GW (SD): Vitamin D arm: 81.48±10.79 kg; Placebo arm: 81.09±9.80 kg Baseline mean (SD) BMI: Vitamin D arm: 31.51±3.74 kg/m^2; placebo arm: 31.47±3.71 kg/m^2 Vitamin D levels data was reported in median (25th and 75th percentiles) due to non-parametric distribution: Baseline: Vitamin D arm: 9.54 (6.12-15.94) ng/mL; placebo arm: 9.02 (7.29-14.70) ng/mL	Vitamin D arm: 50,000 IU D3 (two weekly) Placebo arm. Total vitamin D received in eight weeks: 200,000 IU	1. GW 2. BMI 3. 25(OH)D
Asemi et al. (34)	Randomized clinical trial Blinding: double blinded No. of treatment arms: two Multicentric trial Study duration: six weeks. Country where trial was conducted: Iran Ethical permission: obtained Consent from participants: obtained Information regarding funding: provided Clinical trial registration number: IRCT201311205623N11	Diagnosis: GDM (using ADA criteria) Number of participants randomized: 56 Vitamin D and calcium arm (n): 28 Placebo arm (n): 28 Average age of vitamin D and calcium arm: 28.7±6.0 years Average age of placebo arm: 30.8±6.6 years Missing outcome data: 5 Baseline mean (SD) GW: Vitamin D and calcium arm: 73.6±13.0 kg; placebo arm: 78.2±13.6 kg Baseline mean (SD) BMI: Vitamin D and calcium arm: 29.4±4.6 kg/m^2; placebo arm: 30.5±4.6 kg/m^2 Baseline mean (SD) 25(OH)D: Vitamin D and calcium arm: 43.11±28.17 nmol/L; placebo arm: 49.05±34.30 nmol/L	Vitamin D and calcium arm: 1,000 mg calcium carbonate (daily) and 50,000 U D3 (at trial initiation and on 21st day) Placebo arm. Total vitamin D received in six weeks: 100,000 IU	1. GW 2. BMI 3. 25(OH)D

Table 1. Continued

Trial	Design	Population	Intervention arms	Outcomes reported
Jamilian et al. (15)	Randomized placebo-controlled clinical trial Blinding: double blinded No of treatment arms: two Single centered trial Study duration: six weeks. Country where trial was conducted: Iran Ethical permission: obtained Consent from participants: obtained Information regarding funding: provided Clinical trial registration number: IRCT201509115623N52	Diagnosis: GDM (using ADA criteria) Number of participants randomized: 60 Vitamin D3 and EPO arm (n): 30 Placebo arm (n): 30 Average age of vitamin D3 and EPO arm: 28.4±6.2 years Average age of placebo arm: 29.6±4.3 years Missing outcome data: 6 (all withdrawn from the trial due to personal reasons) Baseline mean (SD) GW: Vitamin D3 and EPO arm: 71.5±10.8 kg; placebo arm: 72.3±8.5 kg Baseline mean (SD) BMI: Vitamin D3 and EPO arm: 27.0±4.2 kg/m^2; placebo arm: 27.6±3.5 kg/m^2 Baseline mean (SD) 25(OH)D: Vitamin D3 and EPO arm: 14.0±10.1 ng/mL; placebo arm: 11.4±4.3 ng/mL	Vitamin D3 and EPO arm: 1,000 IU of vitamin D and 1,000 mg of EPO: daily (60) Placebo arm. Total vitamin D received in six weeks: 42,000 IU.	1. GW 2. BMI 3. 25(OH)D
Jamilian et al. (35)	Randomized, placebo-controlled clinical trial Blinding: double blinded No. of treatment arms: four Single centered trial Study duration: six weeks. Country where trial was conducted: Iran Ethical permission: obtained Consent from participants: obtained Information regarding funding: provided Clinical trial registration number: IRCT201605135623N78	Diagnosis: GDM (using ADA criteria) Number of participants randomized: 140 Vitamin D and omega-3 fatty acid arm (n): 35 Vitamin D arm (n): 35 Omeag-3 fatty acid arm (n): 35 Placebo arm (n): 35 Average age of vitamin D and omega-3 fatty acid arm: 31.2±4.3 years Average age of vitamin D arm: 31.5±7.0 years Average age of omega-3 arm: 30.7±3.5 years Average age of placebo arm: 30.7±4.1years Missing outcome data: 6 (all withdrawn from the trial due to personal reasons) Baseline mean (SD) GW: Vitamin D and omega-3 fatty acid arm: 77.3±9.9 kg Vitamin D arm: 78.4±15.2 kg Omeag-3 fatty acid arm: 75.0±5.8 kg Placebo arm: 75.9±7.1 kg Baseline mean (SD) BMI: Vitamin D and omega-3 fatty acid arm: 29.7±3.9 kg/m^2 Vitamin D arm: 29.7±5.1 kg/m^2 Omeag-3 fatty acid arm: 28.8±2.4 kg/m^2 Placebo arm: 29.2±3.4 kg/ m^2 Baseline mean (SD) 25(OH)D: Vitamin D and omega-3 fatty acid arm: 15.5±3.1 ng/mL; vitamin D arm: 15.2±3.8 ng/mL; Omeag-3 fatty acid arm: 16.9±3.5 ng/mL; placebo arm: 16.6±2.6 ng/mL	Vitamin D and omega-3 fatty acid arm: 50,000 IU of vitamin D (two weekly) and 1000 mg omega-3 fatty acid (twice daily) Vitamin D arm: 50000 IU vitamin D (two weekly) Omega-3 fatty acid arm: 1000 mg omega-3 fatty acids Placebo arm. Total vitamin D received in six weeks: 150,000 IU	1. GW 2. BMI 3. 25(OH)D

ADA: American Diabetes Association (52,53); EPO: evening primrose oil, GDM: Gestational diabetes mellitus, BMI: Body mass index, SD: Standard deviation, GW: Gestation weight, 25(OH)D: 25-hydroxyvitamin D

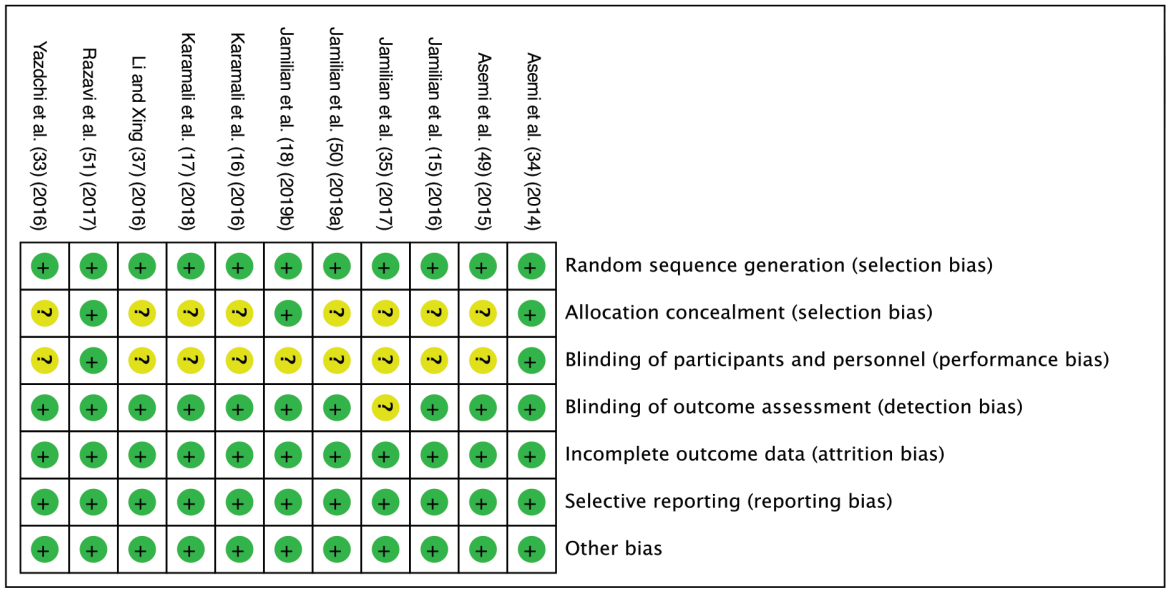
Implications and strengths

The chief inference of this paper is that it informs about the rigor of the current evidence of the maternal benefits of prenatal vitamin D supplementation in GDM patients. From the perspective of maternal health, this study may help health authorities to determine if large scale supplementation for all GDM pregnancies will be an appropriate public health initiative or not, given the current evidence. Moreover, as the reviewed trials were primarily Iran-based, this paper might encourage

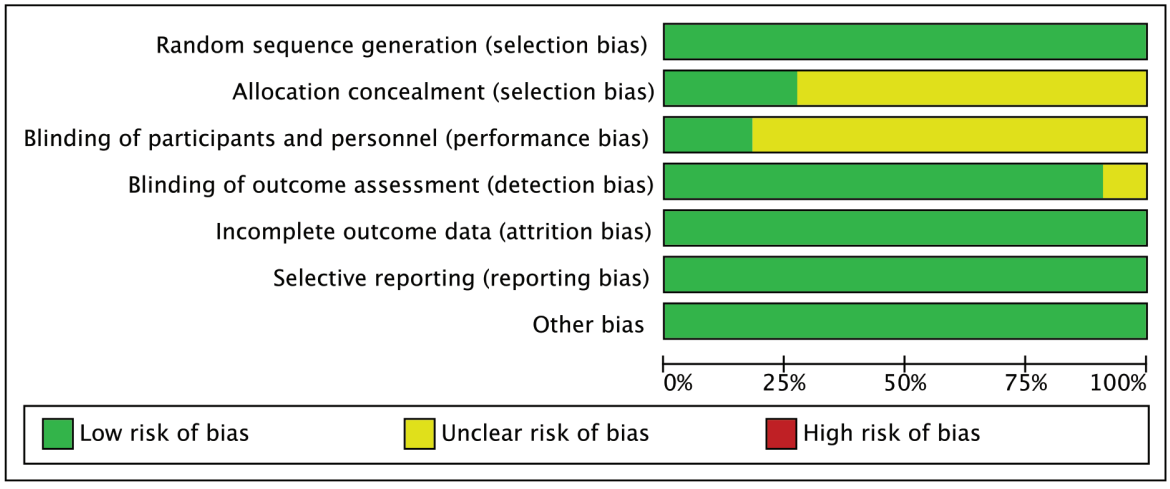
Table 2. Risk of bias assessment

Trial#	Selection bias (Random sequence generation)	Selection bias (Allocation concealment)	Performance bias Outcome: BMI, GW, and 25(OH)2D	Detection bias Outcome: BMI, GW, and 25(OH)2D	Attrition bias	Reporting bias	Other bias
Karamali et al. (16)	**Low**	**Unclear**	**Unclear**	**Low**	**Low**	**Low**	**Low**
	Authors' comment: Allocation concealment: it's not clear if the midwife who measured did the random allocation of participant (in an unblind manner) was related the study personnel or the outcome assessor; Performance bias: Participants were blinded by making the placebos identical to the supplements. However, it's not clear if study personnel were adequately blinded or not.						
Karamali et al.(17)	**Low**	**Unclear**	**Unclear**	**Low**	**Low**	**Low**	**Low**
	Authors' comment: Allocation concealment: Precise mechanism not clear; Performance bias: Participants were blinded by making the placebos identical to the supplements. However, it's not clear if study personnel were adequately blinded or not.						
Asemi et al. (49)	**Low**	**Unclear**	**Unclear**	**Low**	**Low**	**Low**	**Low**
	Authors' comment: Allocation concealment: Precise mechanism not clear; Performance bias: Participants were blinded by making the placebos identical to the supplements. However, it's not clear if study personnel were adequately blinded or not.						
Jamilian et al. (50)	**Low**	**Unclear**	**Unclear**	**Low**	**Low**	**Low**	**Low**
	Authors' comment: Allocation concealment: Precise mechanism not clear; Performance bias: Participants were blinded by making the placebos identical to the supplements. However, it's not clear if study personnel were adequately blinded or not.						
Jamilian et al. (18)	**Low**	**Low**	**Unclear**	**Low**	**Low**	**Low**	**Low**
	Authors' comment: Performance bias: Participants were blinded by making the placebos identical to the supplements. However, it's not clear if study personnel were adequately blinded or not.						
Li and Xing (37)	**Low**	**Unclear**	**Unclear**	**Low**	**Low**	**Low**	**Low**
	Authors' comment: Participants were blinded by using coded labels on the interventions. However, it remains unclear if study personnel were adequately blinded or not.						
Razavi et al. (51)	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**
Yazdchi et al. (33)	**Low**	**Unclear**	**Unclear**	**Low**	**Low**	**Low**	**Low**
	Authors' comment: Allocation concealment: Precise mechanism not clear; Performance bias: Participants were blinded by making the placebos identical to the supplements. However, it's not clear if study personnel were adequately blinded or not.						
Asemi et al. (34)	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**
Jamilian et al. (15)	**Low**	**Unclear**	**Unclear**	**Low**	**Low**	**Low**	**Low**
	Authors' comment: It remains unclear if the midwife responsible for random sequence generation and its allocation concealment was also the study personnel or anyway could have broken the blinding of the study personnel.						
Jamilian et al. (35)	**Low**	**Unclear**	**Unclear**	**Unclear**	**Low**	**Low**	**Low**
	Authors' comment: The precise mechanism used to keep the allocation sequence of the computer-generated random numbers concealed from the participants was not clear. It's not clear how were study personnel and participants blinded in this trial as we couldn't find a clear mention about it. It also remains unclear if the nutritionist and the midwife measuring weight and height of participants were part of the intervention providing team or anyway their blinding might have been broken about the interventions received by the participants.						

#1st author's last name and publication year.
BMI: Body mass index, 25(OH)2D: 1,25-dihydroxyvitamin D, GW: Gestation weight



2a.



2b.

Figure 2. a) Risk of bias graph: review authors' evaluation of respective risk of bias items presented across all studies included in the review. b) Risk of bias summary: review authors' evaluation of respective risk of bias item for each included study

future trialists to conduct identical trials globally to generate generalizable evidence. Concerning the strength, this systematic review is one of the preliminary efforts to investigate the maternal effects of antenatal vitamin D supplementation in GDM patients. A further strength was the unbound nature of our electronic database searche to include any date, language, or geographical boundary, thus adding comprehensiveness to our review. Finally, evidence generated from this systematic review and meta-analysis is likely to be rigorous, as it's grounded on the highest level of epidemiological evidence, randomized controlled trials.

Despite these strengths, this paper has a few weaknesses. As most trials were conducted in Iran, the external validity of this review is likely to be compromised. The heterogeneity observed for the 25(OH)D levels might have increased the risk of bias in our estimates, and this can be because one of the studies was not from Iran. Besides, the maternal health effects of vitamin D supplementation remains inseparable from other supplements that were simultaneously given to the participants in most trials.

Conclusion

Using vitamin D as the chief ingredient of antenatal supplements favors in blood 25(OH)D level rise in GDM patients. However, the effect of these supplements on GW and BMI was not distinguishable from those subjects who did not receive supplementation.

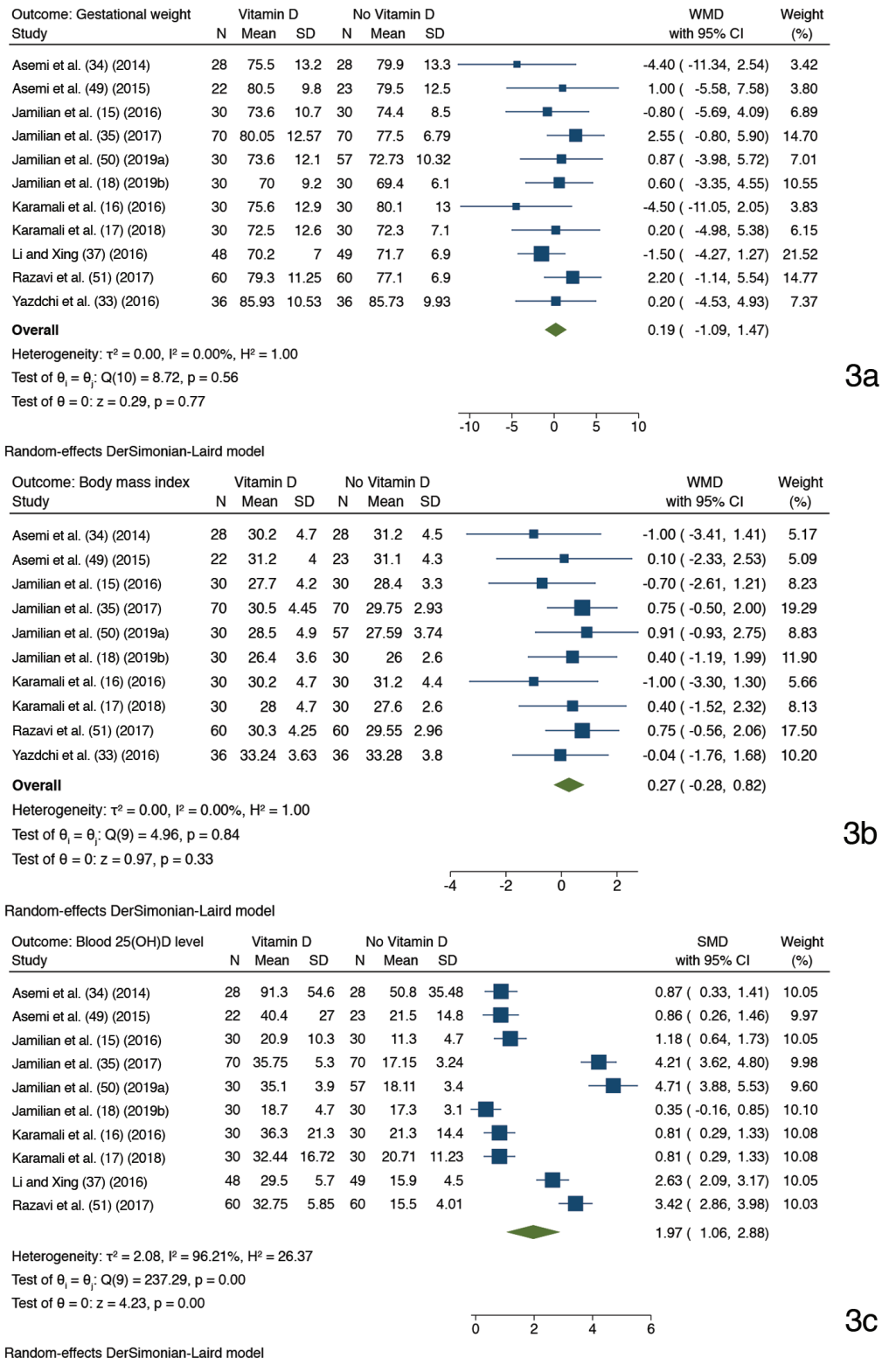


Figure 3. Forest plots depicting meta-analysis findings (random-effect model). Outcome: gestational weight a), body mass index b), and 25(OH)D level in blood c). A comparison between antenatal vitamin D supplementation (as the only or co-supplement with other supplements) and non-vitamin D based supplementation; Two trials had with identical trial author name and year have been suffixed with alphabet "a" (50) and "b" (18) after the study name and year

SD: Standard deviation, CI: Confidence interval, SMD: Standardized mean difference, 25(OH)D: 25-hydroxyvitamin D

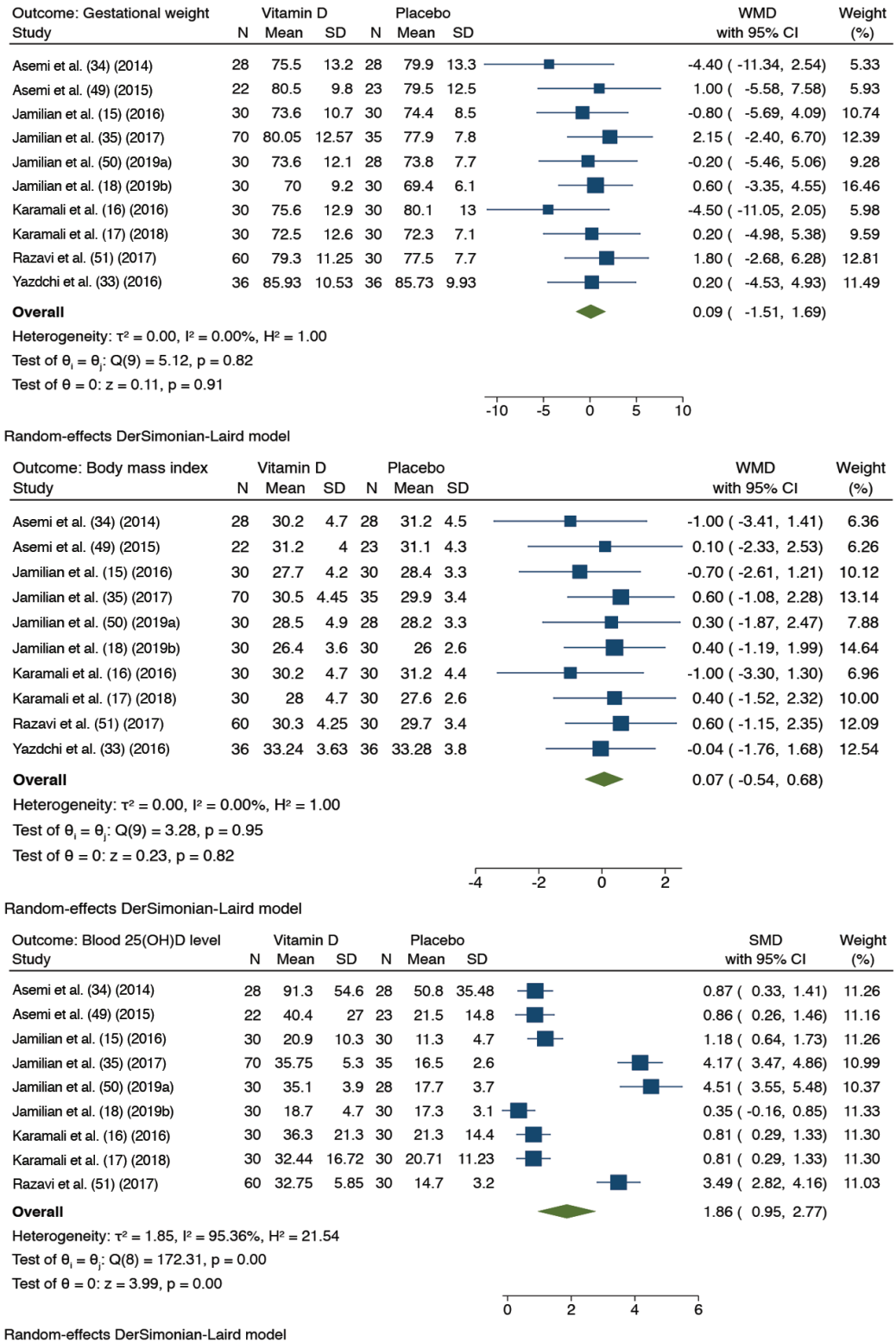


Figure 4. Forest plots depicting meta-analysis findings (random-effect model). Outcome: gestational weight a), body mass index b), and 25(OH)D level in blood c). A comparison between antenatal vitamin D supplementation (as a sole or co-supplement with other supplements) and placebo; Two trials had with identical trial author name and year have been suffixed with alphabet "a" (50) and "b" (18) after the study name and year

SD: Standard deviation, CI: Confidence interval, SMD: Standardized mean difference, 25(OH)D: 25-hydroxyvitamin D

Peer-review: *Externally peer-reviewed.*

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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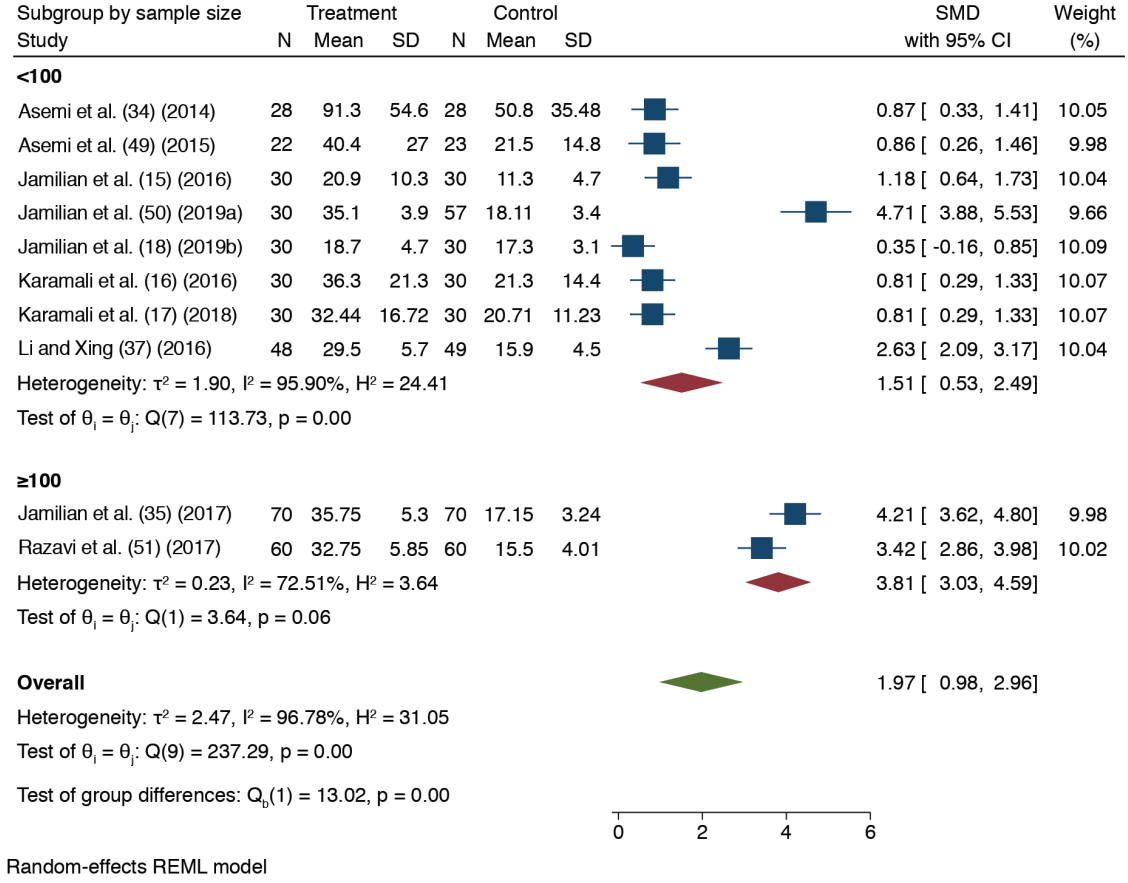
Supplement Table 1. PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7-8
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8-9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	10
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	10
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	10
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	10-11
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	12
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	11-12
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11-12

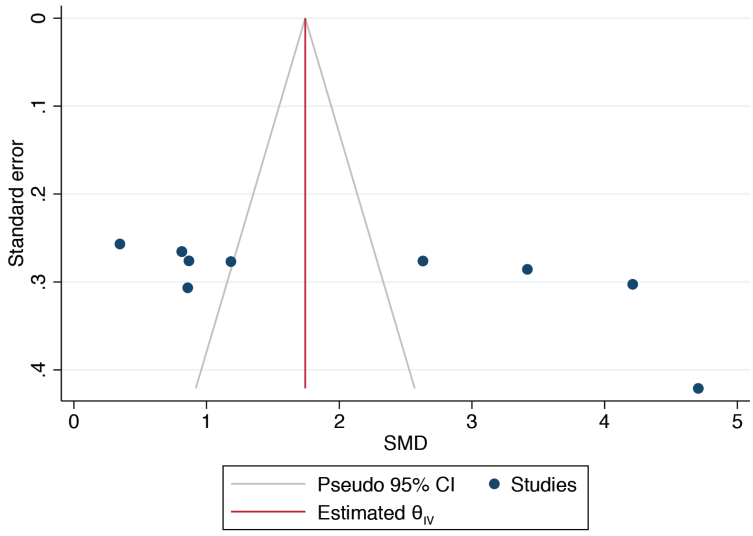
Supplement Table 1. Continued

Section/topic	#	Checklist item	Reported on page #
Results			
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	11-12
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	14
From: Moher D, Liberati A, Tetzlaff J, Altman DG; The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009; 6: e1000097.			



Supplement Figure 1. Forest plot showing meta-analysis comparing between antenatal vitamin D supplementation (as a sole or co-supplement with other supplements) and non-vitamin D supplementation results on 25(OH)D level in blood using random-effect model. Subgroup by sample size (<100 and ≥100 category); Two trials have identical trial author name and year that have been suffixed with alphabet "a" (61) and "b" (27) after the study year

SD: Standard deviation, CI: Confidence interval, SMD: Standardized mean difference, 25(OH)D: 25-hydroxyvitamin D



Supplement Figure 2. Funnel plot assessing publication bias between vitamin D supplemented and not supplemented GDM mothers for 25(OH)D levels in the blood

GDM: Gestational diabetes mellitus, SMD: Standardized mean difference, CI: Confidence interval, 25(OH)D: 25-hydroxyvitamin D

Supplement Table 2. Univariate meta-regression analysis

Category		**Univariate model**		
		Estimate	**p-value**	**95% CI**
Participant attrition	No	1	-	-
	Yes	1.05	0.308	-0.97, 3.07
Sample size	<100	1	-	-
	≥100	2.31	0.028*	0.25, 4.37

*p<0.05, CI: Confidence interval

The experience of in vitro fertilization data collection in Turkey

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Abstract

Collecting and reporting data is a crucial aspect of in vitro fertilization (IVF) practice. During the following two decades after the first report of the European IVF-monitoring Consortium (EIM) on IVF data, the number of contributing countries increased gradually reaching nearly forty. For the first seven years of publication, between 2001 and 2007, Turkey did not provide IVF data to the European registry. Turkey first took part in the European registry in 2008 and thus also in the World registry. The addition of Turkish data to EIM was an important milestone, since Turkey appeared as the country with the sixth highest number of cycles, performing nearly eight percent of all European assisted reproductive technology (ART) cycles. Turkey continued contributing to the European registry for the following four years consecutively but after 2012 the input of Turkish IVF data stopped. Strikingly, between 2008-2012 Turkey became one of the main contributors to the registry with an ability to give a full report. So far, we do not have a complete European set of data and the number of cycles reported by European Society for Human Reproduction and Embryology (ESHRE) EIM can easily be said to be an underestimation of the actual number of cycles. IVF data from Turkey - a country having the 17th highest population in the World and appearing among the first six countries in Europe in terms of the number of ART cycles per year- will definitely contribute very much to ESHRE EIM database. It is now time to turn the tide and restart submitting Turkish data to European registry, but this time regularly and in a systematic method. Such an achievement will greatly contribute to the aim of EIM of achieving a complete data set. (J Turk Ger Gynecol Assoc 2021; 22: 235-41)

Keywords: IVF, assisted reproduction, data collection

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Introduction

After the report of the first successful in vitro fertilization (IVF) treatment more than forty years ago (1), practitioners in the field focused on optimization of the laboratory set up and improving treatment protocols as the primary goals. This has resulted in a gradual evolution of the technique during the following four decades (2). As the technique started to be used more extensively in all geographical regions of the world, concerns about creating a database arose. Australia was the first country to establish a data registry in 1992. The initial regional data came from Australia-New Zealand (3), Latin America (4) and the USA and Canada (5). The first global data were presented at congresses in the early 1990s (6,7) and published as an article in 1997 (8). Recently China presented assisted reproductive technology (ART) data for the first time, showing that nearly one-third of all global cycles were performed in mainland China (9). Europe, a region performing roughly another one-third of all global ART treatments and with the largest number of ART cycles compared with the other regions of the World (10), started to contribute to the world registry a couple of years later (11). This delay of the European registry data compared to other regions is probably due to the difficulty of creating a consortium and collaborative work. Europe consists of many countries practising with heterogenous dynamics in the continent and these countries have diverse cultural, political, economic and legal systems, often lacking national data registers dealing with reproduction.

When we look back at the history of data collection process in Europe, this started with contact between the European Society

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for Human Reproduction and Embryology (ESHRE) and either national registers or key persons of all European countries in 1999. Initially eighteen countries responded and the first report was produced in 2001 pertaining to cycles performed in 1997 (11). In this first report, France appeared as the leading country regarding the total number of ART cycles, followed by the United Kingdom and Germany was third, with all three countries reporting >50% of all cycles. During the following two decades, the number of contributing countries increased gradually, reaching nearly forty with slight fluctuations in the number of countries reporting on a yearly basis (12,13).

So far seventeen countries have been contributing to the registry regularly from the very beginning, with some countries joining after several years and continuing regularly and some others providing data irregularly for a few years either consecutively or separately (Figure 1, 2).

The first successful IVF treatment in Turkey was accomplished a decade after the birth of the world's first IVF baby (14). During the following years, the number of IVF clinics, as well as the number of IVF cycles in the country increased steadily and rapidly. For the first seven years of the European IVF-monitoring Consortium (EIM) registry pertaining to the period 1997-2003, Turkey did not provide IVF data to the European registry. In 2005 Mete Işıkoğlu from Turkey contacted the chairman of the consortium, Karl Nygren, personally enquiring as to the reasons of failure to submit data and what the current situation was. Prof. Nygren kindly gave a prompt response with a suggestion of collaboration and sent his suggestions. After mutually checking all the probabilities for a feasible solution via e-mail, as a next step, Işıkoğlu brought the issue for discussion in the excecutive committee-meeting of Society of IVF Centers, Turkey *(SICT-Formerly Society of Private IVF Centers)* for which he was a delegate and is currently the president. After negotiations, upon the decision of SICT he was charged to lead the process and participated in the EIM meeting held in Lausanne in 2007 as the Turkish representative. Soon after this meeting, SCIT invited all IVF centers in the country via e-mail and regular mail to submit their data voluntarily. In the end, four out of 78 IVF centers, each from four major cities (İstanbul, Ankara, İzmir, Antalya) provided their data. In 2008 for the first time, Turkey took part in the European registry, reporting data pertaining to 2004 (15) and, with its inclusion in the collective European data, in the World registry (16).

Starting the submission of Turkish data to EIM was an important milestone since Turkey carried out the sixth highest number of cycles, performing nearly eight percent of all European ART cycles (Table 1). Turkey continued contributing to the European registry for the following four years consecutively, through

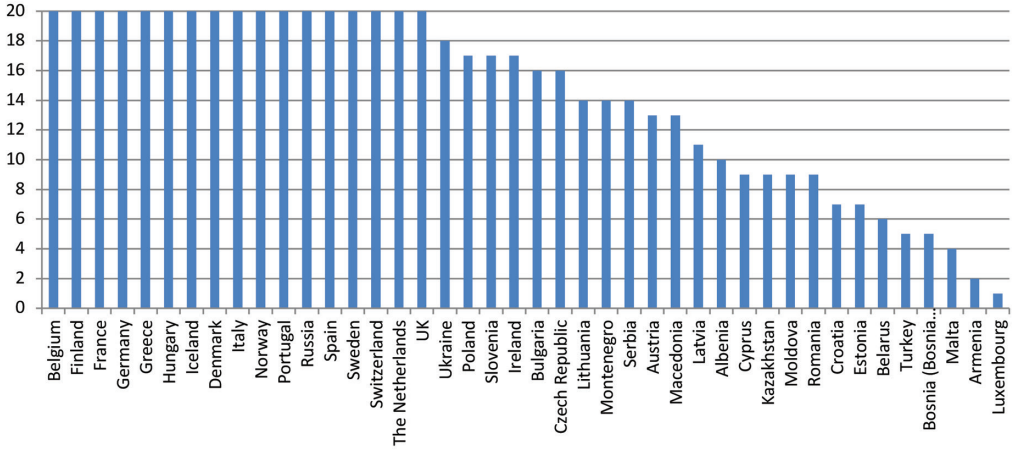

Figure 1. Number of years regarding the contribution of each country in decreasing order between 1997-2016

Table 1. Percentage of cycles from Turkey in total over five years

Year	#IVF Clinics in Turkey		Total cycles in Europe	Cycles reported from Turkey	Rank in row from the top	% of Turkish cycles in total
	Total	Reporting				
2004	78	4	367066	3575	17*	0.97*
2005	93	61	418111	28417	6	6.80
2006	77	77	458759	37468	6	8.17
2007	92	92	493184	35386	6	7.18
2008	107	107	531260	43928	5	8.27

*Note the limited number of clinics reporting.
IVF: In vitro fertilization

the efforts of Timur Gurgan from the Society of Reproductive Medicine, Turkey. However, after 2012 the input of Turkish IVF data stopped again (17). Data on birth outcome and frozen embryo replacement cycles were not available. Strikingly, for the database pertaining to treatments between 2004-2008 Turkey became one of the main contributors to the registry with an ability to give a full report. When we take into consideration that nearly 5-7% of the cycles in Europe are egg donation treatments and that third party reproductive treatments are not allowed in Turkey, actual contribution of Turkish data to non-donor cycle pool of the European registry is probably higher.

The current situation

At the moment there are five regional registries in the World, namely ESHRE EIM, Society of Assisted Reproductive Technology, Australia and New Zealand Assisted Reproduction Database [formerly National Perinatal Epidemiology and Statistics Unit, since 2004 known as the Australia and New Zealand Assisted Reproduction Database (ANZARD)], Latin America and The African Network and Registry for Assisted Reproductive Technology. The Middle East Registry used to provide data but does not work regularly at the moment. The International Committee for Monitoring Assisted Reproductive Technology is the organization collecting worldwide data since 1989 (18) and reporting up-to-date data almost regularly every other year. To date, we do not have a complete European set of data and the number of cycles reported by ESHRE EIM is very probably an underestimation of the actual number of cycles. When the number of countries that have contributed so far (n=42) is multiplied by the number of years data published (n=20), it makes 840 country-years but the actual reported country-years so far (sum of the number of contributing countries of all years) is 603 which means that the available database so far represents nearly 71% of the performed cycles by the reporting clinics during the whole period. The fact that not all the clinics are reporting (roughly 82%) from every country is an additional weakness of the registry.

Only seventeen countries have contributed to the registry regularly every single year from the beginning, while some others also provided data regularly albeit having joined the consortium several years later. Considering the last five years, six countries dominate by the number of cycles (France, Germany, Italy, UK, Russia, and Spain) constituting nearly two thirds of the grand total (13,19-23).

Currently the only available data source in Turkey is the official records administered and kept by the relevant department of Government of Health (24) which annually collects IVF data pertaining to the previous year from all private and government based clinics. These data used to be collected as paper-work up to 2011 and online thereafter. The obvious advantage of this system is that the data is gathered regularly from all clinics since it is a compulsory reporting system. On the other hand, there are some potential shortcomings of this existing system which weaken the usefulness and reliability of the data quality. Firstly, since the data represents the previous year, all pregnancy variables and outcome cannot be obtained accurately (obstetric and neonatal outcome is not obtained from a national based birth registry, but is provided by the individual IVF clinics instead). Secondly, the data collecting authority is also the law maker and the inspector of the health care system. Furthermore data is not submitted in an anonymous or voluntary manner. Hence, the clinic directors may feel hesitancy to report some inadvertent events which may result in statistical bias, a fact more or less a universally valid probability for all national registries (25). Finally, this official registry is neither published anywhere nor is available as an open access to professionals or lay people. As the documentation of adverse events is a crucial part of an IVF registry, it is worth remembering the utmost importance of fundamentals i.e. surveillance and vigilance while collecting data which is valid in any field of medicine. Thus, even though data submission in a voluntary manner may theoretically overcome such a handicap in some countries, compulsory submission may work better in other societies.

Up to now, there has been no collaboration between the government authority and any of the national societies regarding data collection. Since there is no collaboration between the national authority and ESHRE either, the possibility of a regular data flow from the current Turkish database to ESHRE EIM registry seems quite low. From the very beginning of the negotiations regarding the issue of creating a national IVF data registry, special sessions have been held in almost all extended national IVF congresses. Strenuous efforts of the delegates of the national societies have not been able to achieve the initiation of a collaborative work between the national IVF societies and the national health authorities. Even so, trying to convince the national authorities may be an option to resume the submission of national IVF data to international database.

Future perspectives

In general, collecting data is important in many ways: instead of guessing what is going on, robust data allows the storage and analysis of important information about the existing situation and helps to plan for a potential future. An IVF database not only reveals the clinical pregnancy variables but also the side effects and the follow up of children's health. Long term data also reveals the progress of IVF outcome variables, provides an available source of research and helps to inform patients who may have questions about the IVF process. Although the history of IVF dates back more than forty years ago, ESHRE EIM has

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Albenia	-	-	-	-	-	-	-	123	146	141	161
Armenia	-	-	-	-	-	-	-	-	-	-	-
Austria	-	-	-	-	-	-	4887	4504	-	5177	5528
Belarus	-	-	-	-	-	-	-	-	-	-	-
Belgium	7552	10529	10511	11823	12205	12877	15594	19759	22012	22730	24459
Bosnia*	-	-	-	-	-	-	-	-	-	-	162
Bulgaria	-	-	-	-	396	877	880	1003	886	1387	1369
Croatia	-	-	-	-	-	2621	2707	-	2807	-	-
Cyprus	-	-	-	-	-	1032	-	-	-	1432	1590
Czech Republic	7940	7943	8718	2605	-	-	-	-	5168	13707	15060
Denmark	7855	8530	8793	9682	10305	11321	10893	11518	11931	12618	14067
Estonia	-	-	-	-	-	-	-	-	-	-	-
Finland	7909	7877	7320	7489	7980	8352	7533	9204	8202	9116	8935
France	45697	46720	51868	56754	54462	59296	60681	69746	71278	65749	67572
Germany	27927	46132	60723	63005	71752	84819	102426	56813	53378	54695	62322
Greece	7277	7388	6776	5888	4063	5589	9790	9810	10110	3971	2503
Hungary	1747	2099	2024	2157	6277	6814	2850	2878	3563	3307	3128
Iceland	384	422	415	364	360	352	387	316	583	530	665
Ireland	-	-	1338	1570	1724	1912	2058	2580	2860	3232	3565
Italy	9570	13680	15316	19835	18602	18948	25877	26099	34541	40748	43708
Kazakhstan	-	-	-	-	-	-	-	-	-	-	-
Latvia	-	-	-	-	116	-	147	184	-	280	352
Lithuania	-	-	-	-	-	-	82	83	68	413	425
Luxembourg	-	-	-	-	-	-					
Macedonia**	-	-	-	-	-	241	383	522	638	911	1008
Malta	-	-	-	-	-	-	-	-	-	-	-
Moldova	-	-	-	-	-	-	-	-	-	-	-
Norway	3562	3643	4029	4340	4396	4180	5314	6078	6672	7134	7871
Poland	-	-	-	3728	4262	4303	4163	5059	5962	6223	7515
Portugal	1183	1217	1760	2079	2208	2955	3108	2904	3806	3871	5236
Romania	-	-	-	-	-	-	-	-	-	-	-
Russia	3123	4692	4789	6363	7665	8667	10819	14872	17553	21274	26983
Montenegro	-	-	-	-	-	-	380	187	164	245	278
Serbia	-	-	-	-	-	-			250	526	1126
Slovenia	-	-	-	2374	2237	2576	2643	2725	2907	2807	3428
Spain	12603	9962	11616	14519	13355	15030	17011	40956	41689	49943	54620
Sweden	8424	8381	8660	9205	10082	11081	11736	12871	13647	14931	15061
Switzerland	3346	4002	4166	4644	4929	5395	5628	5718	6126	7109	7815
The Netherlands	13700	13965	14378	15062	15335	16273	17649	15366	17462	17770	19699
Turkey	-	-	-	-	-	-	-	3575	28417	37468	35386
UK	34398	35261	30215	34634	35492	37083	37348	39981	41768	43953	46688
Ukraine	-	-	914	1147	1487	1694	2132	1632	3517	5361	4899

Figure 2. Total number of ART cycles in European countries between 1997-2016.

(Bosnia Herzegovina after 2013), **(North Macedonia in 2020)

	2008	2009	2010	2011	2012	2013	2014	2015	2016
Albenia	164	-	-	-	289	139	153	178	175
Armenia	-	-	-	-	-	-	-	1465	346
Austria	6540	6277	6402	6676	6822	7173	7326	8778	9721
Belarus	-	-	-	2216	2098	2451	2739	2969	2997
Belgium	28751	27674	28521	29130	28578	28854	28845	30300	30929
Bosnia*	180	-	-	-	-	-	598	280	135
Bulgaria	3297	1797	5030	2101	7162	5380	6314	9849	11009
Croatia	-	4296	-	-	3413	4818	2115	-	-
Cyprus		1421	-	2046	-	1850	1739	1737	1727
Czech Republic	18607	19431	20020	20319	22716	25318	28759	30107	32543
Denmark	13476	14992	15954	14560	15142	15143	16167	17454	15917
Estonia	2259	-	-	2474	2715	2887	2884	2955	2952
Finland	8997	8637	9312	9019	8824	8587	8642	9343	9191
France	68446	74475	79427	85253	85594	84214	90434	93918	104773
Germany	69902	67349	62571	67354	71251	76422	81177	96512	99226
Greece	2476	2310	3693	5185	8207	18278	24120	27149	27976
Hungary	3197	7068	5562	4681	4874	6152	5626	6262	5608
Iceland	700	806	824	741	733	789	706	739	644
Ireland	3489	4065	4078	3042	2843	1566	1513	-	706
Italy	47829	52032	58860	63777	64197	64446	68896	73405	77559
Kazakhstan	1465	1474	2276	3209	3143	4612	3937	5020	4460
Latvia	340	762	-	-	-	674	1390	2143	1528
Lithuania	463	131	131	115	173	380	381	655	758
Luxembourg									980
Macedonia**	1536	2065	1497	-	-	1699	1987	2136	2934
Malta	-	-	-	-	-	100	176	311	359
Moldova	613	625	624	632	1187	966	843	993	934
Norway	8535	8544	9007	8927	8982	8169	10925	10324	10280
Poland	10490	12068	13325	15507	16849	20968	23594	26491	31349
Portugal	5569	6077	7179	7107	7444	7362	7786	8660	9365
Romania	1143	1052	1151	1553	1956	2444	3357	3935	5009
Russia	31217	42110	34026	57094	62620	67861	94985	110723	121235
Montenegro	370	482	452	445	540	475	442	506	566
Serbia	1574	1232	1484	1560	2064	2720	278	488	286
Slovenia	3705	3680	4419	4069	4597	4755	4684	4649	4725
Spain	38245	54266	58735	68756	69699	78152	109275	119875	140909
Sweden	16107	16714	17628	18562	18280	18266	18213	18603	18989
Switzerland	8477	9099	9540	9456	9546	9554	9922	10038	10960
The Netherlands	21164	22061	23627	24182	25173	24951	25141	26136	27901
Turkey	43928	-	-	-	-	-	-	-	-
UK	50555	54314	57856	60377	60151	61728	63504	65461	68308
Ukraine	7454	8077	7085	9851	12282	15968	16983	19264	20411

been collecting European IVF data for the last twenty years and so far has revealed data pertaining to these last 20 years.

There are more than fifty countries on the European continent, some of which are small states without IVF clinics and some only partially located in Europe (26). During the twenty-year history of the EIM IVF registry, 40 of these countries submitted national data. Since the data reporting system shows diversity in all these countries, a uniform quality assurance protocol is still lacking. There are two main concerns regarding the achievement of an ideal registry: first, reaching the ultimate aim of gathering *complete IVF data from all European countries* in a *regular pattern*. Second, the reliability of the registry should be as high as possible. Although it is not easy to reach the ideal point in practice regarding these two issues, one should keep in mind the saying that I first heard from Prof. Dr. Carl Nygren which I like and use frequently *"little data is better than no data"*. In order to be able to improve something one should certainly have a draft at hand.

EIM recently analysed the achievements and potential deficiencies in the twenty year registration process comparatively with registries from two other regions. They aimed to identify similarities and discrepancies between these registries in order to further improve data recording and interpretation. When the ESHRE/EIM registry is compared to the register of the Centres for Disease Control and Prevention and ANZARD, it was found that adverse events, such as maternal death, ovarian hyperstimulation syndrome and infections, were recorded sporadically and only by EIM and ANZARD. Although improvements are recorded in the three regional registers over time, inconsistencies and inaccuracies still remain and need to be identified. This reality necessitates the use of some caution when analyzing the data. EIM also defines an ultimate target of a continuous recording system, rather than the existing cross-sectional one, to achieve greater accuracy, independent of time span and borders (25).

Conclusion

The IVF data from Turkey - a country having the seventeenth highest population in the World and appearing among the first six countries in Europe in terms of the number of ART cycles per year- will definitely contribute greatly to the ESHRE EIM database. Then, it is time to turn the tide and restart submitting our data to the European registry, but this time regularly and in a systematic manner. In order to achieve this aim, a two-step approach would be simple and effective in solving the problem: the first step is the collaboration of the national IVF societies for a joint effort and construction of a national working group on data collection. There are four existing national societies in the field, one of which is the Society of Clinical Embryologists while the other three are general IVF societies, namely Society of Reproductive Health and Infertility *(Üreme Sağlığı ve İnfertilite Derneği)*, Society of Reproductive Medicine *(Üreme Tıbbı ve Cerrahisi Derneği)* and Society of IVF Centers *(Tüp Bebek Merkezleri Derneği)*. One representative from each society is sufficient to accomplish this task. The next step should be simply inviting all clinics to submit data in a voluntary reporting system. Collecting the data in accordance with the datasets used by EIM will overcome the shortcoming of inability to provide data, such as for delivery outcome and frozen embryo replacement cycles. Such an achievement will greatly contribute to the aim of EIM of achieving a complete European data set.

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A short review of current implementations of sentinel lymph node mapping in gynecologic cancers

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Abstract

Lymph node metastasis both increases disease stage and alters adjuvant treatment plans in gynecologic cancers. Since a minority of the patients have nodal metastasis, many patients unnecessarily undergo complete lymphadenectomy and are exposed to the subsequent morbidities. Sentinel lymph node (SLN) mapping is an alternative for evaluation of lymph nodes with lesser side effects. Although it is yet an experimental approach in ovarian cancer, it has been incorporated into guidelines for endometrial, cervical and vulvar cancers. We aimed to summarize the current situation of SLN mapping in gynecologic cancers. (J Turk Ger Gynecol Assoc 2021; 22: 242-8)

Keywords: Sentinel lymph node, endometrial cancer, cervical cancer, vulvar cancer, ovarian cancer

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Introduction

Lymphadenectomy is a part of surgical staging in gynecologic cancers since it is important to determine the nodal status for guiding adjuvant treatment. However, only a minority of the cases have nodal metastasis and many patients face morbidities associated with complete lymphadenectomy unnecessarily. Sentinel lymph node (SLN) mapping has been proposed as a less invasive technique used for assessment of lymph nodes in gynecologic cancers. The lymph node that has direct connection to the tumor site, and most likely to receive any metastasis first is called the SLN. Therefore, with SLN mapping while nodal metastasis is identified, morbidities of complete lymphadenectomy can be avoided in node negative patients.

SLN mapping has gained importance in staging of gynecologic cancers in the last decade and it has been incorporated into National Comprehensive Cancer Network (NCCN) Guidelines for endometrial, cervical and vulvar carcinomas (1-3). Currently, in ovarian carcinoma SLN mapping remains an experimental approach.

Technetium-99m (99mTc), indocyanine green (ICG) and blue dyes can be used alone or combined for identifying SLNs. All suspicious lymph nodes must be removed besides SLNs and side-specific lymphadenectomy should be performed in case of mapping failure.

SLN mapping allows detection of uncommon drainage sites, such as internal iliac lymph nodes, that may not otherwise have been resected. Another advantage of SLN mapping is detection of more nodal metastasis by pathologic ultrastaging which cannot be identified by routine hematoxylin and eosin (H&E) staining. To do so, SLNs are cut at 50-200 µm intervals and two paraffin embedded slides are prepared from each section. One slide is stained with H&E and the other with immunohistochemistry stains (AE1 and AE3 anticytokeratin antibodies) if no metastasis is identified by H&E examination. Tumor deposits >2 mm are defined as macrometastasis. Micrometastasis is defined as metastatic deposits ranging from 0.2 mm to no more than 2 mm and isolated tumor cells (ITCs) are defined as single tumor cells or clusters <0.2 mm.

Although it has been shown that the SLN algorithm is a highly sensitive method and has high detection rate and negative predictive value (NPV), survival data comparing only SLN removal and complete lymphadenectomy is insufficient (4-6).

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This review aims to summarize current SLN mapping implementations in gynecologic cancers.

Endometrial cancer

Endometrial cancer is surgically staged, which includes hysterectomy with or without oophorectomy and lymphadenectomy. Nodal status not only changes the stage of the disease but also guides the adjuvant treatment plans. However, most endometrial cancer patients are diagnosed in the early-stage when the disease is limited to the uterus and many patients undergoing complete lymphadenectomy face intraoperative and postoperative complications (eg. neurovascular injuries, lymphedema and lymphocele formation) unnecessarily (7,8). Moreover, two randomized studies have shown that lymphadenectomy does not provide survival advantage in early-stage patients (9,10). Therefore, the approach to lymph node evaluation is an issue of debate.

Many centers perform selective lymphadenectomy as the uterus is sent for intraoperative frozen section evaluation and no further lymphadenectomy is performed when the analysis reveals grade 1 or 2 tumors invading less than 50% of the myometrium and smaller than 2 cm (11). These criteria are also known as the Mayo criteria and the risk of nodal metastasis in this low-risk population is less than 5% (11,12). However, only 20% of high-risk patients have nodal metastasis and many patients still undergo unnecessary lymphadenectomies (13). Moreover, accuracy of frozen section is higher in more experienced centers and not all centers may have a frozen section unit to guide the surgery (14,15).

The SLN mapping algorithm is a less invasive technique for evaluation of nodal status. Both in prospective and retrospective studies, sensitivity and NPV of the SLN mapping algorithm were reported between 84-97% and 97-99%, respectively (4,16-19). It has been shown that the SLN algorithm does not compromise overall detection of stage IIIC disease, both in low-risk and high-risk endometrial cancer patients (20,21). In a meta-analysis including 55 studies, bilateral and overall detection rate of SLN mapping were 50% and 81%, respectively (4). With these promising data, the SLN mapping algorithm is also incorporated into the NCCN guidelines, despite a lack of randomized studies comparing survival outcomes of SLN mapping and complete lymphadenectomy (1).

99mTc, blue dyes (1% methylene blue, 1% isosulfan blue or 2.5% patent blue sodium) or ICG can be used for lymphatic mapping. ICG requires a near-infrared camera for visualization, but it is shown to be superior to blue dye alone and equal to a combination of 99mTc and blue dye in terms of SLN detection (22). Whichever dye is used, 1 mL deep (1 cm) and 1 mL superficial (3-4 mm) cervical injections are made at 3 o'clock and 9 o'clock positions before hysterectomy. Although fundal or hysteroscopic injections lead to higher mapping rates in the para-aortic area, they do not provide higher detection rates in the pelvic area compared to cervical injection (23). Optimal detection of SLNs occurs 15-60 minutes after the injection. Besides all identified SLNs and any enlarged or suspicious lymph nodes should be removed. Side-specific lymphadenectomy is required if a hemipelvis does not map. Para-aortic lymphadenectomy is not an essential step in the SLN algorithm and can be performed at the surgeon's discretion (Figure 1).

Compared to complete lymphadenectomy, although fewer nodes are removed, 4.5-8% additional lymphatic metastasis is detected with the SLN algorithm as a result of ultrastaging (18,24). One step nucleic acid amplification assay (OSNA) is a new method for intraoperative SLN assessment which detects cytokeratin 19 messenger RNA in metastatic lymph nodes. Compared to classic ultrastaging, more nodal metastasis is detected by OSNA, but no nodal tissue is left for postoperative assessment. Despite this, the prognostic significance of these metastases detected by OSNA is not known (25,26).

Currently, micrometastasis is regarded as node positive and managed accordingly, but prognostic significance of ITCs is unknown and adjuvant treatment of patients with ITCs is given according to primary tumor characteristics (27). Yet, more studies are needed to clarify the clinical relevance and treatment of patients with ITCs.

In some retrospective series, it has been shown that removal of SLNs alone does not have a negative effect on

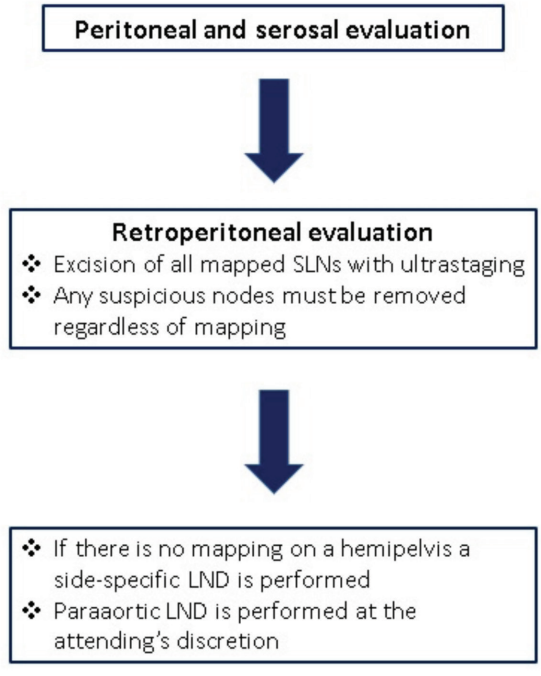

Figure 1. SLN mapping algorithm in endometrial cancer

SLN: Sentinel lymph node, LND: Lymph node dissection

oncological outcomes, both in low- and high-risk pathologies as 3-year overall survival and disease-free survival (DFS) were comparable between the SLN algorithm group and lymphadenectomy groups (21,28-31).

Cervical cancer

Lymphatic metastasis has a negative effect on prognosis and alters treatment plans in cervical cancer. Sensitivity of preoperative imaging studies for nodal metastasis is reported below 75% and surgical evaluation is generally needed (32). The risk of nodal involvement is <1% in stage IA1 cervical cancer and there is no need for lymphadenectomy in this stage when there is no lymphovascular space invasion. However, when the disease has been staged as IA2 and beyond, at least SLN removal is needed.

SLN biopsy in cervical cancer appears promising. Cervical injections are done with ICG, blue dye or 99mTC at two or four points (2). All enlarged and suspicious nodes must be removed in addition to the SLNs. Side-specific lymphadenectomy is mandatory if SLN is not detected. One hundred and thirty-nine women with stage IA1 to IB2 cervical cancer were recruited to the SENTICOL study, at least one SLN was detected in 98% of the patients, and sensitivity and NPV were 92% and 98.2%, respectively (33). In another study, SLN detection rate, sensitivity and NPV were 88.6%, 77.4% and 94.3%, respectively (34). Although the SLN technique can be used in tumors up to 4 cm, the best detection rates, sensitivity and NPV are achieved in tumors smaller than 2 cm (5,34). Moreover, studies have demonstrated that SLN biopsy is completely reliable if bilateral SLNs are detected (33,35).

Similar to endometrial cancer, ultrastaging is performed if no metastasis is identified in SLN with routine H&E staining. A recent study showed that patients with micrometastasis and ITCs had similar DFS compared to node negative patients (92.7% vs 93.6%) and DFS did not improve with adjuvant treatment in these patients (36). Yet more studies are needed to clarify clinical significance and treatment of low-volume metastasis. The ongoing prospective randomized SENTICOL III and SENTIX studies aim to find out whether DFS, recurrence rate and quality of life differs between patients with cervical cancer undergoing only SLN biopsy and complete bilateral pelvic lymphadenectomy after SLN biopsy.

Vulvar cancer

Lymphatic metastasis is the most important prognostic feature in vulvar cancer and the 5-year disease specific survival for node positive and negative patients were reported between 70-93% and 25-41%, respectively (37). The primary tumor should be resected with at least 1 cm clear margins and either unilateral or bilateral inguinofemoral lymph nodes or in selected patients SLNs should be removed. There is no need for inguinofemoral lymphadenectomy for stage IA patients since risk for nodal involvement is <1% (38). But patients with stage IB or higher stages have ≥8% risk of nodal metastasis and lymphadenectomy is mandatory (39). Unilateral lymphadenectomy can be performed in case of clinically negative lymph nodes when the tumor is <4 cm and ≥2 cm lateral to vulvar midline (3).

20-70% of the patients experience surgical morbidities, such as wound breakdown and lymphedema, due to inguinofemoral lymphadenectomy (40). SLN biopsy is an option for inguinofemoral lymphadenectomy in selected early-stage patients whose tumors are unifocal and smaller than 4 cm and in whom imaging and/or clinical examination reveals negative lymph nodes (41,42). For tumors >4 cm, the SLN technique is both associated with reduced sensitivity and higher groin recurrences (43). It is also recommended to perform complete inguinofemoral lymphadenectomy after SLN biopsy in at least 10 cases before performing SLN biopsy alone. The best SLN detection rates are observed when 99mTC and blue dye are used together (42,44).

99mTC is injected 2-4 hours prior to the operation and a gamma probe is used to detect the SLN. The most commonly used blue dye is isosulfan blue 1%. 4 cc blue dye is injected intradermally into the normal tissue around the tumor at 2, 5, 7 and 10 o'clock positions. It is recommended to perform SLN biopsy prior to excision of vulvar tumor so the lymphatic network will not be disturbed. Side-specific inguinofemoral lymphadenectomy is recommended when SLN is not detected.

In the GOG-173 study, SLN biopsy was prospectively compared to inguinofemoral lymphadenectomy in 452 patients with squamous cell carcinoma with tumors ranging between 2-6 cm in diameter and at least 1 mm depth of stromal invasion (42). At least one SLN was identified in 92% of the cases, and sensitivity and NPV were 91.7% and 96%, respectively. In another prospective study, the GROINSS-V I study, investigating clinical safety and utility of SLN biopsy in early-stage vulvar cancer, the false negative rate of SLN biopsy was found to be 3% (44). Four hundred and three women with stage I-II squamous cell tumors <4 cm were enrolled in this study, and no further lymphadenectomy was performed if SLNs were negative. In a study evaluating oncologic outcomes of the GROINSS-V I study participants, groin recurrence rates for SLN negative and positive patients at 5 years were 2.5% and 8%, respectively (45).

The ongoing GROINSS-V II study is a prospective study which aims to find out whether complete inguinofemoral lymphadenectomy can be replaced by adjuvant radiotherapy (RT) in patients with metastatic SLNs undergoing only

SLN biopsy. Preliminary results of this study showed that inguinofemoral lymphadenectomy can be safely replaced by RT in patients with low-volume metastasis, but not for patients with macrometastasis (46). Therefore, when SLN metastases are >2 mm, it is recommended to perform complete ipsilateral lymphadenectomy. In such cases, contralateral lymph nodes should also be resected or treated with external beam radiation therapy. Frozen section of SLNs may be used to for deciding to perform complete lymphadenectomy.

The aim of GROINSS-V III will be to examine the effectiveness and safety of chemoradiation in patients with macrometastatic SLNs.

Ovarian cancer

In apparently early stage ovarian cancer, bilateral pelvic and para-aortic lymphadenectomy is indicated to detect occult lymphatic metastasis, since 1/3 of these patients have nodal metastasis and these metastases can be seen in bilateral pelvic or para-aortic nodes (47,48). It has been shown that early-stage ovarian cancer patients who had undergone systematic lymphadenectomy had better survival outcomes compared to no lymphadenectomy (49).

SLN biopsy is investigational in ovarian cancer. 99mTC, blue dye or ICG can be used as tracers alone or in combination (50). Whichever dye is used, the best detection rates were reported with injections at the utero-ovarian and infundibulopelvic (IP) ligaments, or only at the IP ligament if hysterectomy had been performed previously, just underneath the peritoneum. Mesovarium, ovarian hilum and ovarian cortex are alternative injection sites, but lower detection rates were reported, and the latter technique may result in tumor rupture.

In a systematic review including 145 patients, overall SLN detection rate was found to be 90.3% (range; 40-100%) (50). While ICG alone resulted in 93.3% of detection rate, with ICG + 99mTC combination, it was 100%. Mean detection rate in the pelvic and para-aortic region were 44% (range; 25-87.5%) and 82% (range; 70-91%), respectively. In a recent prospective cohort study including 20 patients, first unilateral salpingo-oophorectomy was performed and SLN mapping was performed with 99mTC plus 0.5 mL of ICG afterwards, if the frozen section revealed malignancy (51). Hence, injections were done into both utero-ovarian and IP ligament stumps or only into the IP ligament stump according to previous hysterectomy status. SLNs were identified in all patients in the para-aortic region and in 93% of the patients in the pelvic regions. The ongoing prospective SELLY trial is a phase II trial, which aims to evaluate accuracy of SLN biopsy in diagnosing nodal metastasis in early-stage ovarian cancer patients using ICG. The preliminary results of this study that included 31 patients revealed overall detection rate of 67.7% and detection rate was significantly higher in patients undergoing immediate staging surgery compared to delayed surgery. All four patients with lymphatic metastasis had metastatic SLNs, yielding 100% sensitivity and 100% NPV (52).

Conclusion

Table 1 summarizes the NCCN and European Society of Gynaecological Oncology recommendations for SLN mapping in gynecologic cancers.

Table 1. NCCN and ESGO recommendations for SLN mapping in gynecologic cancers

	NCCN	ESGO
Endometrial cancer	- SLN mapping can be considered for apparently uterine-confined disease when there is no metastasis demonstrated by imaging studies or at exploration - SLN mapping may also be used in high-risk histologies	- SLN mapping is still experimental for apparently uterine-confined disease and systematic lymphadenectomy is recommended
Cervical cancer	- SLN mapping can be considered in stage IA1 with LVSI, IA2, IB1 and select IB2 cases - SLN mapping can be used in tumors up to 4 cm, but best detection rates are achieved in tumors <2 cm	- SLN biopsy is an acceptable method of LN staging for stage IA1 patients with LVSI and IA2 - SLN biopsy in addition to BPLND is strongly recommended for stage IB1-IIA1 patients. - Intraoperative assessment of SLNs is also recommended for stage IB1-IIA1 patients
Vulvar cancer	- SLN biopsy is an alternative standard of care approach to inguinofemoral lymphadenectomy in select cases - SLN biopsy can be used in patients with negative clinical examination or imaging and unifocal tumors <4 cm	- (At least) unilateral SLN biopsy for tumors >1 cm from the midline and bilateral SLN biopsy for tumors within 1 cm of the midline should be performed for unifocal T1, <4 cm tumors in case of no suspicious nodes preoperatively, and IFL should be performed if SLN is not detected or positive
Ovarian cancer	- Experimental	- Experimental

NCCN: National Comprehensive Cancer Network, ESGO: European Society of Gynaecological Oncology, SLN: Sentinel lymph node, LN: Lymph node, BPLND: Bilateral pelvic lymphadenectomy, IFL: Inguinofemoral lymphadenectomy, LVSI: Lymphovascular space invasion

Endometrial cancer

SLN mapping is an alternative method for lymph node assessment in staging of apparently early-stage low-risk endometrial cancer patients when no metastasis is detected by imaging modalities or intraoperative exploration. Recent studies showed that SLN mapping can also be considered in high-risk histologies (type 2 endometrial cancer). To date, patients with macrometastasis and micrometastasis are treated in the same way, but adjuvant treatment of patients with ITCs is given according to primary tumor characteristics. Current data showed that removal of SLNs alone does not have a negative effect on oncological outcomes compared to complete lymphadenectomy, but more data are needed.

Cervical cancer

Both prospective and retrospective series have demonstrated that SLN mapping have high NPV in cervical cancer. However, SLN biopsy is completely reliable if bilateral SLNs are detected. SLN mapping may be used in tumors up to 4 cm, but best detection rates are observed in tumors <2 cm.

Vulvar cancer

SLN biopsy is an option for inguinofemoral lymph node dissection in selected early-stage patients with unifocal tumors <4 cm and in whom imaging and/or clinical examination reveals negative lymph nodes. Both prospective and retrospective series have demonstrated that SLN mapping has high NPV in vulvar cancer. For tumors >4 cm, SLN technique is both associated with reduced sensitivity and higher groin recurrences.

Ovarian cancer

SLN biopsy is an investigational approach in ovarian cancer. The best detection rates were observed with injections at the utero-ovarian and IP ligaments, or only at the IP ligament if hysterectomy had been performed before, just underneath the peritoneum. Pilot studies with limited number of patients showed moderate detection rate of SLNs in the pelvic region and high detection rate in the para-aortic region.

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What is your diagnosis?

A 33-year-old woman, P2L2, presented with complaints of intractable dry cough for three months with nausea, vomiting, loss of appetite, and occasional episodes of fever. She had no menstrual complaints. Her past and family histories were unremarkable. On clinical examination, her performance status score was "2" according to the Eastern Cooperative Oncology Group (ECOG). Vital signs were normal, except for low-grade fever and World Health Organization grade 3 anemia (hemoglobin: 7.6% gm). Abdominal examination revealed a firm to hard, abdominopelvic mass of 22-24 week's size occupying hypogastrium, right and left iliac fossa with restricted mobility. Cervix and vagina were healthy on pelvic examination. Vaginal examination revealed a similar mass, uterus was not felt separately, pouch of Douglas and bilateral fornices were full. These findings were reconfirmed on rectal examination. Rectal mucosa, recto-vaginal septum, and parametrium were found to be healthy on P/V/R examination.

Complete fever profile including blood culture was normal. She tested negative for Coronavirus disease-2019 (COVID-19). Chest X-ray and contrast-enhanced computed tomography (CECT) of the chest were ordered to look for a cause of refractory cough, but the reports were absolutely normal. Any possibility of tuberculosis was also ruled out.

Ultrasonography (USG) of the abdomen and pelvis was suggestive of a large solid-cystic mass occupying the whole pelvic cavity. CECT showed an 18.5x16.5x15 cm lobulated heterogeneous mass lesion with multiple peripheral enhancing excrescences and nodules with a large, central, non-enhancing area suggestive of neoplastic etiology, uterus, and ovaries were not visualized separately (Figure 1). No enlarged lymph nodes were reported. Serum tumor markers including CA125 (408.2 U/mL) and CA19-9 (312.3 U/mL) were markedly raised while lactate dehydogenase, carcinoembryonic antigen, alpha-fetoprotein, and human chorionic gonadotropin were within normal limits. Pap smear was negative for malignant cells. USG of breast, and upper and lower gastrointestinal endoscopies were insignificant.

Her cough could not be relieved with broad-spectrum antibiotics, cough suppressants, and other conservative measures advised by a chest physician. She started having fever more frequently and her total leucocyte count was rising. Eventually, a plan of staging laparotomy was made because of high suspicion of malignancy and deterioration in the general condition of patient.

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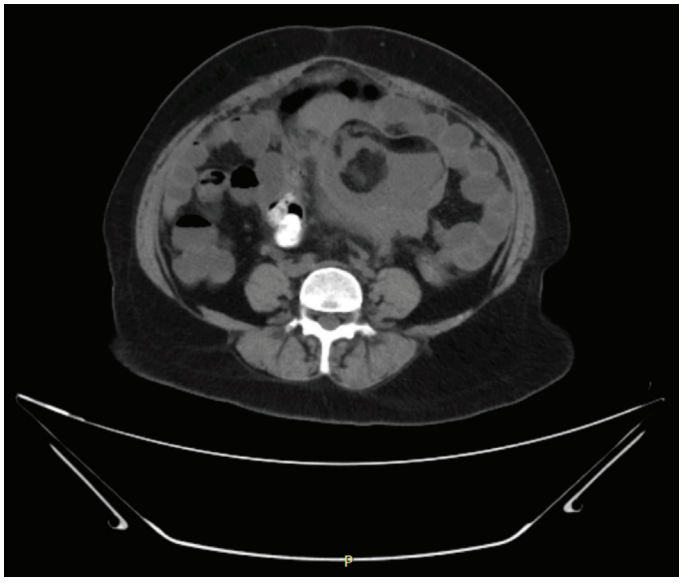

Figure 1. CECT abdomen & pelvis; axial section suggestive of neoplastic ovarian mass

CECT: Contrast-enhanced computed tomography

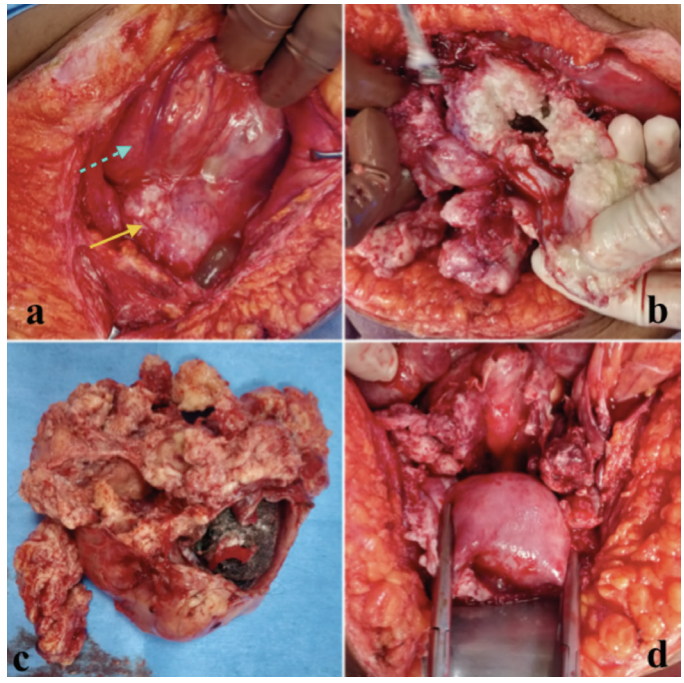

Figure 2. Intraoperative images; a,b) a huge, poorly defined, necrotic, friable tumor was found occupying the whole abdominopelvic cavity, mass (yellow arrow) adhered to bowel loops and omentum (shown by dotted arrow); c) Image shows tumor specimen with an irregular surface, solid-cystic areas containing sebaceous fluid and hair tuft; d) After removal of tumor, uterus was seen deep in the pelvis

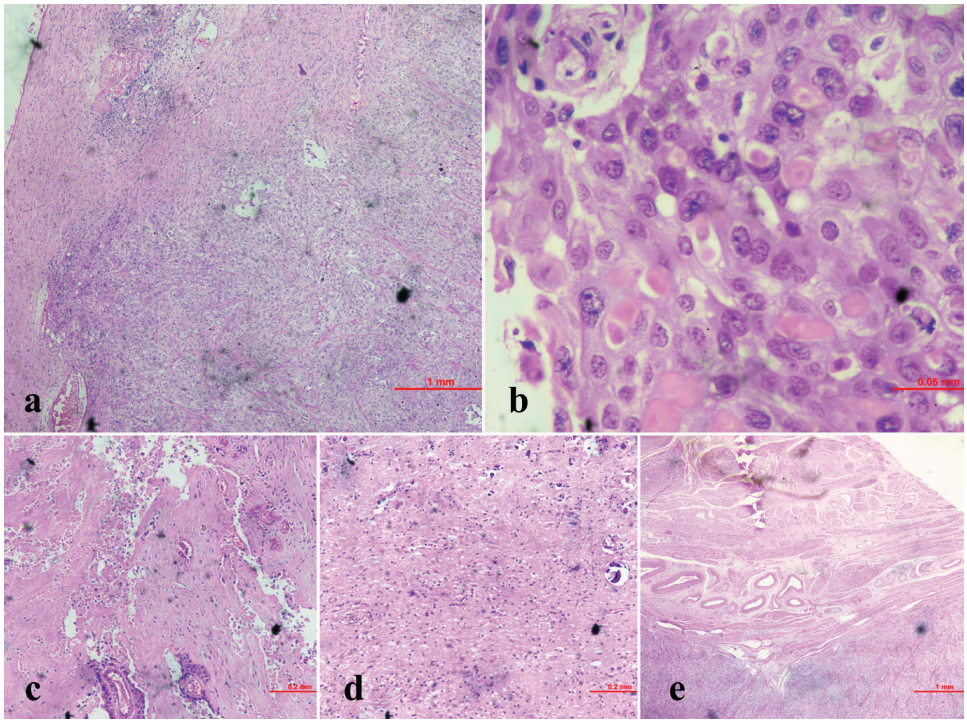

Figure 3. Histopathological images; a) 40x haemotoxylin & eosin (H&E) stain, showing squamous cell differentiation; b) 40x H&E stain, showing squamous cells with individual cell keratinization; c) H&E stain, showing necrotic areas; d) H&E stain, showing glial tissue (neuroectoderm) with calcification; e) H&E stain, showing myometrial involvement

Answer

After obtaining informed written consent, she was taken up for surgery under combined spinal-epidural anesthesia. She was considered to be at very high risk of respiratory failure with general anesthesia due to persistent cough. The abdomen was entered with a midline vertical infra-umbilical incision. Intraoperatively, a huge, poorly defined, necrotic, friable tumor was found occupying the whole abdominopelvic cavity (Figure 2a,b). Fat planes were lost with anterior abdominal wall, omentum, and surrounding bowel loops. Adhesions were released and approximately 1000 mL sebaceous content was drained from the tumor. Macroscopically, the tumor measured 20x18x15 cm with an irregular surface, solid-cystic areas containing sebaceous fluid, and hair tuft (Figure 2c). There was no well-defined plane and the tumor mass was resected in pieces. The tumor was sent for frozen section to look for malignancy but yielded inconclusive results. After piecemeal removal of tumor mass, uterus could be seen deep down in the pelvis (Figure 2d). Bilateral tubes and ovaries were replaced by the tumor. Total abdominal hysterectomy with bilateral adnexectomy was performed. The omentum and the bowel loops were conglomerated with each other and the entire peritoneum was inflamed and friable. Therefore, pelvic and para-aortic lymph node dissection could not be carried out. However, lymph nodes were not enlarged on examination. Partial omentectomy was performed.

Interestingly, the patient was completely relieved of her cough immediately after surgery and there were no fever episodes in the postoperative period. Her postoperative ECOG score was 1. Histopathology suggested stage IIIC squamous cell carcinoma (SCC) arising from mature cystic teratoma (MCT) involving bilateral ovaries and posterior wall of the uterus up to an outer third of myometrium and atypical cells in omental tissue (Figure 3). Cytology for malignant cells was negative. The peritoneal and cystic fluids were sterile and negative for mycobacterium tuberculosis. Tumor markers were repeated on postoperative day 2 and showed a significant fall in CA125 (87.9 U/mL) and CA19-9 (12.46 U/mL) values. The case was discussed in the institutional tumor board and a decision was taken to give adjuvant chemotherapy with paclitaxel and carboplatin. She was discharged on the 5th postoperative day in stable condition.

Discussion

MCT is the most common ovarian germ cell tumor. It can occur in 10-20% of women in their lifetime (1). The biological behavior of most MCTs is benign and the clinical course remains uneventful. However, malignant transformation of ovarian MCT is extremely rare (0.17-2%) (2). Most MCTs are diagnosed during reproductive age, while malignant transformation usually occurs in postmenopausal women. Patients with malignant transformation usually present with abdominal pain and mass (2). Whereas, in our case, MCT malignant transformation occurred in a young, reproductive age woman who presented with a complaint of intractable cough.

MCT can have a malignant transformation to various types such as SCC, adenocarcinoma, sarcoma, small cell carcinoma, or malignant melanoma (3). SCC is the most common among all histological types, accounts for >80% of all the malignant transformations (2).

The detection of SCC transformation in MCT is often incidental during examination, imaging or postoperative pathological examination as the presenting complaints are non-specific (4). Patients usually present with abdominal pain, palpable mass, and bloating (2,5). Sometimes, a patient may present with an acute abdomen due to torsion or rupture of tumor (6,7). Our case is unique as the patient's chief complaint was intractable cough. In the literature review, we could not find any patient with malignant transformation with such presenting complaints. The exact reason behind her cough is unclear. Still, it might be because of diaphragmatic irritation due to tumor cells or the other possibility is prior rupture of MCT, but less likely as there was no feature of peritonitis, such as high-grade fever or acute abdominal pain.

Like any other ovarian cancer, SCC transformation in MCT should be treated with staging surgery including total hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and lymph node dissection. Li et al. (2) described that hysterectomy and omentectomy may improve survival while lymph node dissection doesn't affect long-term survival. Nevertheless, they recommend lymphadenectomy as complete cytoreduction should improve the outcome. Fertility sparing surgery may be offered in women <45 years of age with stage IA/IC malignancy. In our case, hysterectomy and partial omentectomy were performed due to high suspicion of advanced malignancy.

The role of adjuvant chemotherapy and the ideal regimen is not well recognized in SCC transformation of MCT. In a systemic review by Li et al. (2), overall survival was not improved with adjuvant chemotherapy. On the contrary, Hackethal et al. (8) reported that adjuvant chemotherapy (paclitaxel and carboplatin) may have a better prognosis in the advanced stage.

Prognostic factors are not yet defined for this entity. Staging of the tumor appears to have an impact on the outcome. The reported 5-year survival rate in stage I, II, III, IV is 85.8%, 39.1%, 26.2%, 0% respectively (2). Therefore, diagnosis in early stages is crucial for long-term survival. Early diagnosis is a challenge as there is no specific imaging or laboratory test that can detect malignancy in MCT. Hence, MCT in women >30 years old with solid areas or firm, friable, myxomatous, or variegated areas or

unusual adherence to surrounding structures should be treated with suspicion.

To the best of our knowledge, this is the first case report to describe intractable cough as a presenting complaint in SCC transformation from ovarian MCT. This disease always poses a diagnostic challenge due to its rarity and aggressive clinical course. Diagnostic criteria and treatment protocols are yet to be determined. Available data is limited. Hence, the reporting of each case is important to elucidate its biology and clinical course. Preoperative suspicion of malignant transformation in MCT may result in an optimum outcome. Health care providers should be aware of this disease entity and intractable cough as a presenting complaint.

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Para-aortic lymphadenectomy: step by step surgical education video

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Abstract

Para-aortic lymph nodes are exclusively important for the staging of gynecologic malignancies. This surgical education video describes the step-by-step technique for para-aortic lymphadenectomy with anatomic landmarks in a cadaver.

Keywords: Anatomy, surgery, education, lymphadenectomy, cancer

Received: 03 May, 2019 **Accepted:** 29 July, 2019

Introduction

Para-aortic lymph nodes are exclusively important for the staging of gynecologic malignancies. Uterine fundal, ovarian, and tubal lymphatics and pelvic lymphatic drainage eventually drain into the para-aortic lymph nodes (1). Lumbar lymph nodes around the aorta and inferior vena cava are classified as para-aortic lymph nodes. Para-aortic lymph nodes could also be divided into four zones clinically; high para-aortic and low para-aortic (also called the lateral aortic nodes), precaval-interaortocaval-preaortic and lateral caval (Figure 1) (2).

Boundaries of para-aortic lymphadenectomy (Figure 2) (3):

Right: Right psoas major muscle, ascending colon, right ureter.

Left: Mesentery of descending colon and sigmoid colon, inferior mesenteric vein (IMV), left ureter.

Caudal: Mid common iliac level, below the aortic bifurcation.

Cranial: Left renal vein.

Posterior: Anterior longitudinal ligament.

Anatomic landmarks and step-by-step technique for para-aortic lymphadenectomy

- Small intestines are packed craniolaterally and mesentery of sigmoid colon is retracted caudolaterally.
- Posterior parietal peritoneum is cut from the level of ileocolic junction to the level of ligament of Treitz.
- Paracaval space is developed; right ovarian vessels and right ureter are identified.
- Horizontal part of duodenum is mobilized and retracted superiorly. Left renal vein is identified.
- The areolar tissue between the left common iliac artery and mesentery of sigmoid colon is opened, in the process the left ureter and inferior mesenteric artery are identified.
- The para-aortic lymph node dissection begins over the right common iliac artery from the mid-level, caudad to cephalad direction. Clinical tip: Here, the superior hypogastric plexus which is anterior to the aortic bifurcation and left common iliac vein at the superior part of the precaval space is dissected and preserved if possible.
- Precaval and preaortic lymph nodes are dissected to the level of left renal vein. Lateral caval lymph nodes will be dissected within the precaval lymph nodes. Clinical tip: While dissecting the precaval lymph nodes, the tributaries of inferior vena cava towards the lymphatic tissue called "fellow's vein" should be carefully dissected and ligated to prevent a hemorrhage or injury.
- Lateral aortic lymph nodes are dissected from the infra-mesenteric region, below the level of inferior mesenteric artery.

This video presentation will demonstrate the basic surgical steps of para-aortic lymphadenectomy for fellow gynaecological oncologists and gynaecologists, with a detailed view of anatomical landmarks to improve the surgical education.

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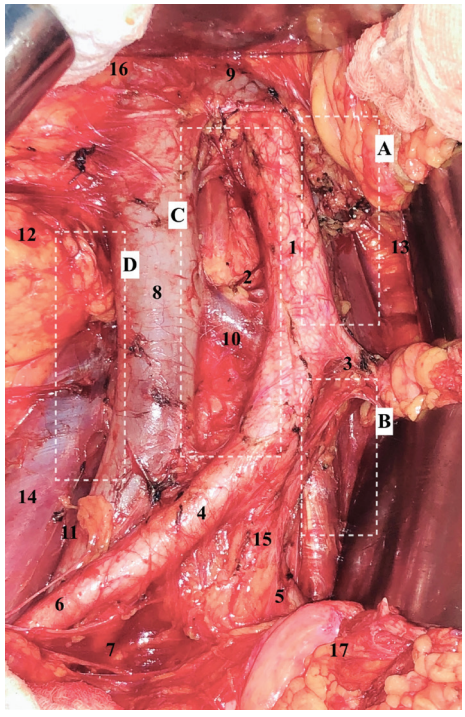

Figure 1. Para-aortic lymph node zones; A. Supramesenteric lateral aortic nodes, B. Inframesenteric lateral aortic nodes, C. Inter-aortocaval nodes, D. Lateral caval nodes

1. Aorta, 2. Lumbar artery, 3. Inferior mesenteric artery, 4. Right common iliac artery, 5. Left common iliac artery, 6. Right external iliac artery, 7. Right internal iliac artery, 8. Inferior vena cava, 9. Left renal vein, 10. Lumbar vein, 11. Right common iliac vein, 12,13. Retracted peri renal adipose tissue, 14. Psoas major muscle, 15. Radiating fibers of superior hypogastric plexus, 16. Retracted duodenum, 17. Retracted sigmoid colon

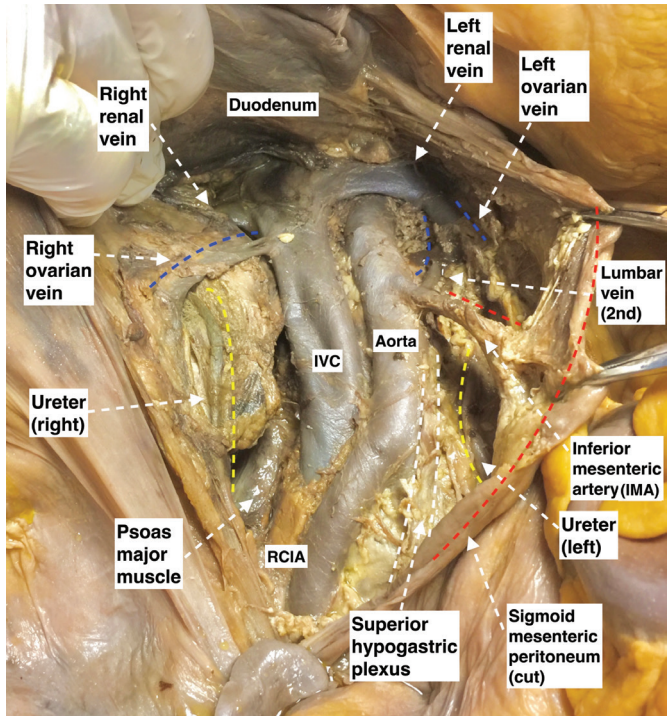

Figure 2. Relevant anatomic landmarks of para-aortic lymphadenectomy on cadaver.

IVC: Inferior vena cava, IMA: Inferior mesenteric artery, RCIA: Right common iliac artery

- Lateral aortic lymph nodes, cranial to the level of inferior mesenteric artery, are identified by sharp and blunt dissection from the mesentery of the descending and sigmoid colon and underlying left Gerota's fascia.
- Lateral aortic lymph nodes are dissected from the supra-mesenteric region, between the inferior mesenteric artery and left renal vein.

Acknowledgement: *This cadaveric dissection was performed at Hacettepe University Faculty of Medicine, Department of Anatomy with the fine contribution of University of Health Sciences Turkey, Zekai Tahir Burak Woman's Health Training and Research Hospital.*

Video 1.

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Vaginal-assisted laparoscopic nerve sparing radical trachelectomy

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Abstract

Fertility-sparing surgery has gained popularity in the last three decades for the management of cervical cancer in women under 40 years of age. Radical trachelectomy is a fertility-sparing surgical technique for women who wish to retain their fertility. Vaginal-assisted laparoscopic radical trachelectomy is feasible in selected patients with early cervical cancer. The aim of this video is to present a nerve-sparing vaginal-assisted laparoscopic radical trachelectomy demonstrating pelvic anatomical structures.

Keywords: Cervical cancer, fertility-sparing surgery, hypogastric nerve, laparoscopic radical trachelectomy, nerve-sparing surgery

Received: 30 June, 2019 **Accepted:** 2 December, 2019

Introduction

Nearly 25% of all cervical cancers are diagnosed in women under 40 years of age (1). Radical trachelectomy by vaginal approach was the first technique to be successfully used (2). Several different procedures have since been described as fertility-sparing surgery techniques (3).

A 38 years-old nulliparous woman with high grade, keratinized, squamous cell carcinoma of the cervix was referred to our clinic. She had undergone a loop electrosurgical excision procedure at another center. The pathological review and clinical examination revealed a FIGO stage IB1 cervical carcinoma without lymphovascular space invasion and less than 2 cm in the greatest diameter. Abdomino-pelvic magnetic resonance imaging showed no residual disease after loop electrosurgical excision procedure. She underwent vaginal-assisted laparoscopic surgery. One 25 mg/vial kit of indocyanine green was reconstituted in 10 mL of aqueous solvent, and then a 2 mL aliquot was further diluted in 2 mL of aqueous solvent. Four mL of indocyanine green was injected into the uterine cervix at the 3 and 9 o'clock positions, submucosally and deep of the cervix to locate the sentinel lymph nodes. In the first step, the round ligaments were coagulated, cut and dissected from the anterior and posterior leaves of the broad ligament. The ureters were identified alongside the posterior leaf of the broad ligament. The uterine arteries were then ligated bilaterally at their origin from the hypogastric artery. Both paravesical and pararectal spaces were opened to gain access to the parametria. The anterior part of the vesicouterine ligament was dissected, and the ureteral tunnel was developed. Colpotomy was performed. All procedures were performed with meticulous dissection of pelvic anatomical structures including hypogastric nerves (Video 1) (Figure 1, 2). Resection of the cervix was performed during the vaginal part of the procedure. Frozen-section examination was performed to ensure negative surgical margins. Finally, the vaginal mucosa was sutured to

This surgical film is also accepted to be presented at 47th American Association of Gynecologic Laparoscopists Global Congress on Minimally Invasive Gynecologic Surgery that will be held during 11-15 November, 2018 at Las Vegas, Nevada, United States of America.

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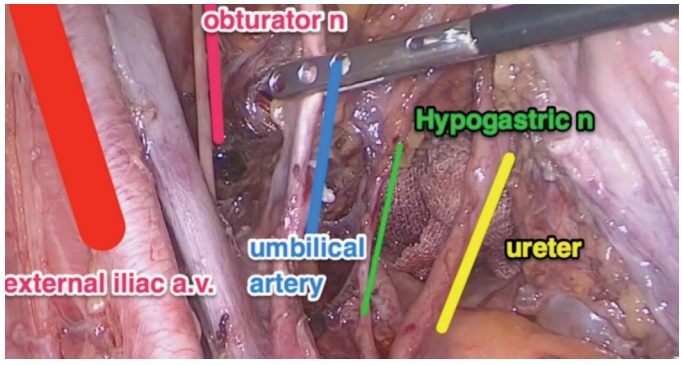

Figure 1. Left pelvic retroperitoneal space demonstrating left external iliac artery and vein, obturator nerve, obliterated umbilical artery, hypogastric nerve, and ureter

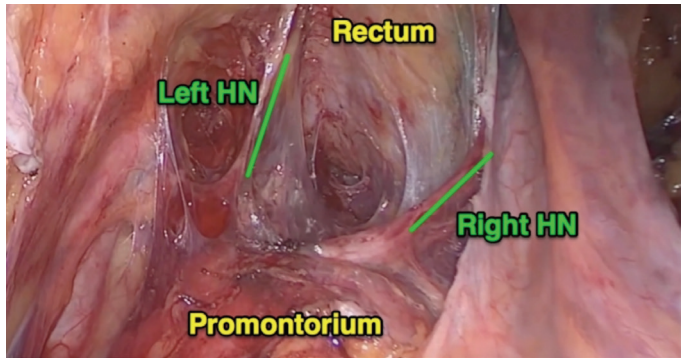

Figure 2. Retrorectal space demonstrating right and left hypogastric nerve

the stroma of the cervix. She was discharged on postoperative day four without any adverse event in the postoperative period. She had no residual tumor on final pathology. There was no metastasis on examination of the lymph nodes.

Vaginal-assisted laparoscopic nerve-sparing radical trachelectomy as a fertility-sparing procedure appears to be a safe and adequate surgical technique in selected young women with early stage cervical cancer.

Video 1.

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Treatment and management of interstitial pregnancy with laparoscopic cornual resection

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Abstract

To show how interstitial pregnancy can be safely managed with a laparoscopic resection.

Keywords: Laparoscopy, ectopic pregnancy, interstitial pregnancy, minimal invaziv surgery

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Introduction

Our aim was to demonstrate how interstitial pregnancy can be treated with laparoscopy. Interstitial pregnancy is one of the more uncommon forms of ectopic pregnancy. It contributes only 2-4% of all ectopic pregnancies (1). Mortality rate is 6-7 times higher than that in classical ectopic pregnancy (2).

The patient was admitted to our clinic with vaginal bleeding. The woman had a 7-week pregnancy in the interstitial region of the uterus. This interstitial pregnancy was safely managed laparoscopically.

We demonstrate a totally laparoscopic approach to an advanced interstitial pregnancy with several key strategies to minimize blood loss:

1. Coagulation of interstitial pregnancy and uterine junction,
2. Use of grasper device to enucleate the gestational sac,
3. Suture the uterine layers with vicryl sutures,
4. Removing the specimen from the optical trocar with a glove bag.

Interstitial pregnancies may be managed with laparotomy. We have demonstrated a safe laparoscopic surgery technique for interstitial pregnancy with minimal instrument use and low cost. The ultrasound images of the patient are shown in Figure 1, 2.

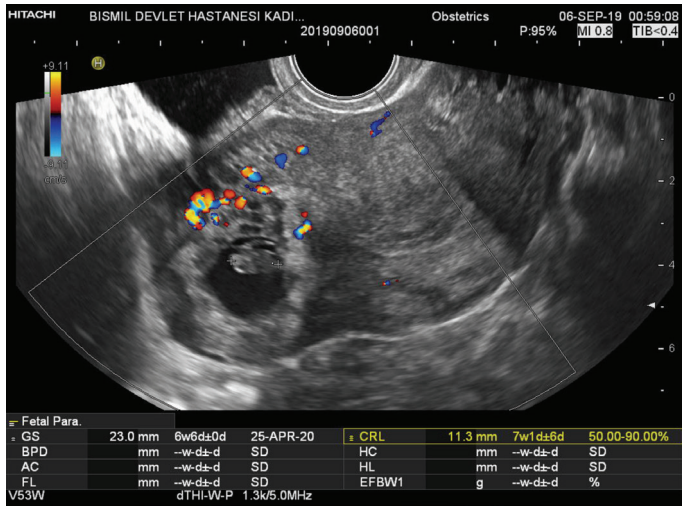

Figure 1. Transvaginal ultrasound image showing a gestational sac in the outer left margin of the uterus

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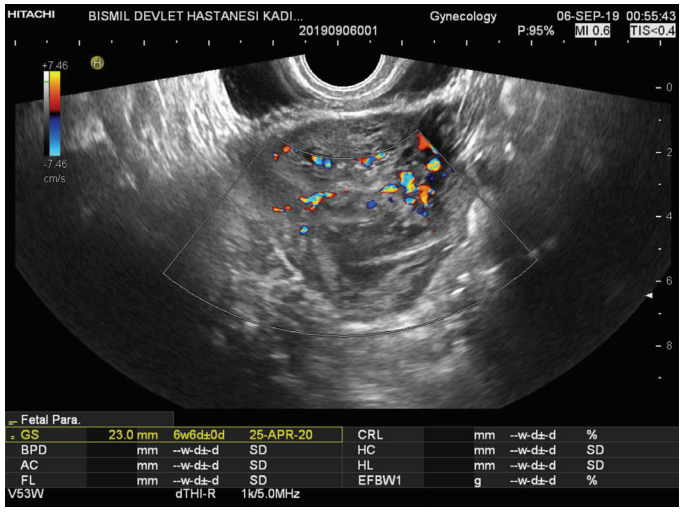

Figure 2. Transvaginal ultrasound revealed vascularity surrounding the gestation

Video 1.

https://www.doi.org/10.4274/jtgga.galenos.2020.2020.0013.video1

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Unicentric Castleman disease; the laparoscopic en bloc resection of a hypervascular giant lymph node in the aortacaval zone

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Abstract

Unicentric Castleman disease (UCD) is a rare disease of the lymph nodes with unknown etiology, most commonly presenting as localized asymptomatic adenopathy incidentally discovered on radiographic imaging. The retroperitoneum is a rare site for UCD, where it can mimic malignant tumors. Complete surgical resection with disease-free margins is considered both diagnostic and curative. However, this may be challenging due to the high vascularity and close proximity of UCD to major vessels. A 42-year-old patient with a 46x44x26 mm mass in the aortocaval area at the level of the renal pelvis underwent surgery with the suspicion of metastatic lymphadenopathy. Laparoscopic excision of the mass was carried out and the histopathological examination revealed the presence of UCD. This video article aimed to demonstrate the surgical steps and techniques used to minimize hemorrhage during dissection of UCD. Laparoscopy is safe and effective in the diagnosis and treatment of UCD, provided the operating surgeons have a thorough knowledge of abdominal anatomy and are aware of the functions and limitations of surgical devices used during laparoscopy.

Keywords: Aortacaval zone, giant lymph node, laparoscopy, unicenteric castleman disease

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Introduction

This video article aimed to demonstrate the laparoscopic complete resection of a giant lymph node located in the aortocaval region, postoperatively diagnosed as Unicentric Castleman disease (UCD). We describe the surgical technique used to minimize blood loss and collateral tissue damage during resection of this rarely encountered and highly vascular mass.

Case Report

A 42-year-old G3P3 patient was admitted to the hospital with postprandial abdominal discomfort and bloating. Abdominal ultrasonography, magnetic resonance imaging, positron emission tomography scan and computed tomography imaging revealed a 50x40 mm mass in the aortocaval area resembling a pathologic lymph node (Figure 1). No other foci of malignancy were detected in other organ systems. Excisional biopsy was

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recommended for diagnosis by the interventional radiologist, due to risk of severe hemorrhage, tumor spread, and loss of cell architecture with fine needle aspiration.

Surgical method

Following exposure of the aortacaval area, the overlying peritoneum was dissected starting from the bifurcation point of the aorta until the level of the renal vessels. Dissection continued until the lateral border of the inferior vena cava (IVC) on the right, and the lateral border of the aorta on the left.

An ultrasonic scalpel was used to dissect the caudal end of the mass. As the vascularity of the mass increased, a Maryland jaw vessel sealer and bipolar forceps were used interchangeably for better hemostasis. The mass was found to be situated between the IVC on the right, aorta on the left, and the lumbar vein at the caudal end. Cranially, it was bordered by the right renal artery on the posterior, and the left renal vein on the anterior aspect. Care was taken to avoid injury to the left renal vein. Since veins lack pulsatility and have thinner vessel walls than arteries, it is harder to obtain a tactile sense of them with laparoscopic devices, making them more prone to injury than arteries. Hence, the accidental incorporation of an artery into the blades of a vessel sealer is more likely to be noticed by the surgeon than the incorporation of a vein. The feeding artery originating directly from the aorta was cut after the placement of hemostatic clips. Another hemostatic clip was placed over the inferior suprarenal artery (Figure 2, 3). The mass was excised completely and externalized using an endobag.

The patient was discharged 48 hours postoperatively without complications. Histopathological examination revealed the diagnosis of Unicentric Castleman disease.

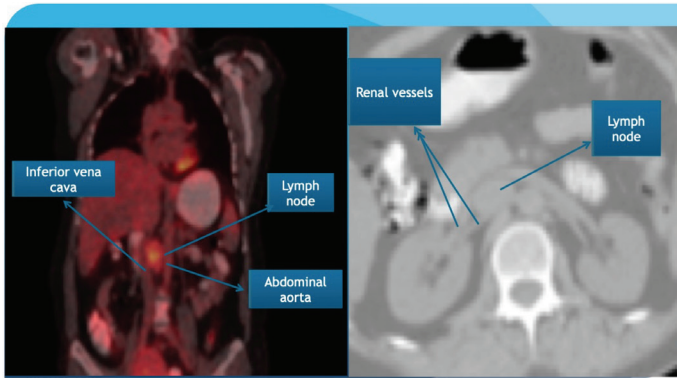

Figure 1. PET-CT images of the giant lymph node

PET: Positron emission tomography, CT: Computed tomography

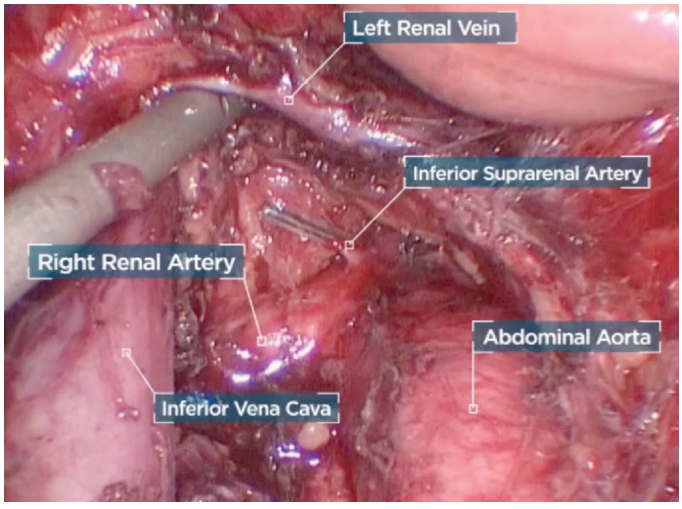

Figure 2. Ligation of the inferior suprarenal artery

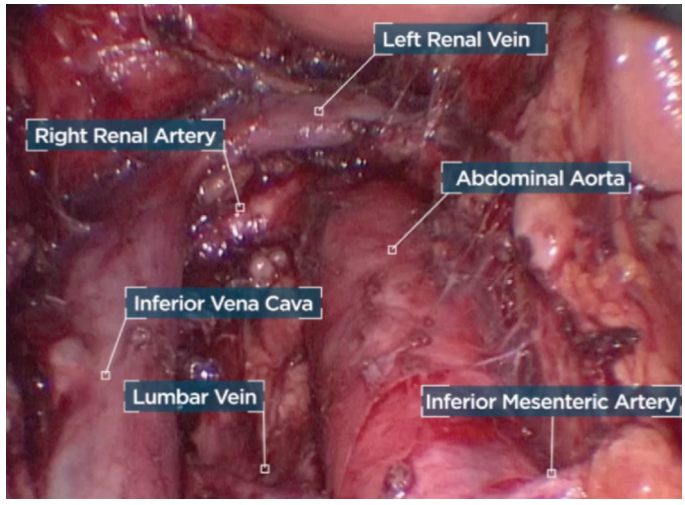

Figure 3. Vascular borders of the resected giant lymph node

Discussion

UCD is an atypical disease of the lymph nodes with unknown etiology (1,2). Although rare, it should be considered in the differential diagnosis of an incidentally detected, solid, intra-abdominal mass resembling an enlargened lymph node with prominent blood flow on Doppler ultrasonography (3). Total resection is curative but challenging due to the high vascularity and tendency of UCD to be located in close proximity to major vessels (4). Although preoperative diagnosis is not possible, having a high index of suspicion will prompt surgeons into taking meticulous care during surgery and help prevent unnecessary hemorrhage. Laparoscopy is safe and effective in the diagnosis and treatment of UCD, provided the operating surgeons have a thorough knowledge of abdominal anatomy and the functions and limitations of laparoscopic devices.

Video 1. Laparoscopic en bloc resection of a hypervascular giant lymph node

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

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CONGRESS CALENDER

INTERNATIONAL MEETINGS

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

October 03-05, 2021	**ESGE 30th Annual Congress, Rome, Italy, onsite + virtual**
October 16-20, 2021	**American Society for Reproductive Medicine (ASRM) 77th Annual Meeting, Baltimore, Maryland, United States**
October 23-25, 2021	**22nd European Congress on Gynaecological Oncology, Prague, Czech Republic + Online**
November 14-18, 2021	**50th American Association of Gynecologic Laparoscopists (AAGL) Global Congress on Minimally Invasive Gynecologic Surgery (MIGS), Austin, Texas, United States**

CONGRESS CALENDER

NATIONAL MEETINGS

(for detailed International Meeting please go website:
http://www.kongre2020.com)

September 10-12, 2021	**9. Acıbadem Kadın Doğum Günleri, İstanbul**
September 23-26, 2021	**3. Obstetrik ve Jinekoloji Tartışmalı Konular Kongresi, KKTC**
October 01-03, 2021	**39. Zeynep Kamil Jineko-Patoloji Kongresi, Online**
October 13-16, 2021	**4. Uluslararası Kozmetoloji ve Kozmetik Jinekoloji Kongresi, İstanbul**
October 28-31, 2021	**Türkiye Maternal Fetal Tıp ve Perinatoloji Derneği Ultrasonografi Kongresi, İstanbul**
October 28-31, 2021	**8. Üreme Tıbbı ve Cerrahisi Derneği Kongresi, Antalya**
November 10-14, 2021	**9. Üreme Sağlığı ve İnfertilite Kongresi – TSRM, Antalya**
December 01-05, 2021	**18. Ulusal Jinekoloji ve Obstetrik Kongresi, Antalya**
December 02-05, 2021	**İstanbul Üniversitesi 10. Kadın Doğum Günleri, İstanbul**
December 03-05, 2021	**Çukurova Kadın Doğum Günleri 2021, Adana**
December 09-12, 2021	**4. Karadeniz Jinekoloji ve Obstetrik Kongresi, Online**