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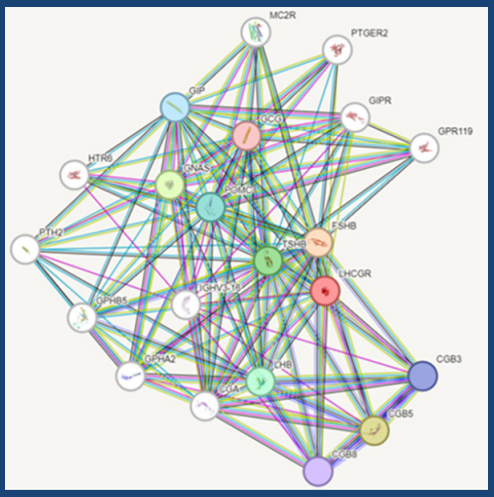

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Cover Picture: Thomas and Veerabathiran. Meta-analysis and power analysis of *LHCGR* gene polymorphism on PCOS

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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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The ''Journal of the Turkish-German Gynecological Association'' (EISSN 1309-0380; Abbreviated as "J Turk Ger Gynecol Assoc") is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and the Turkish-German Gynecological Association. Formerly named "ARTEMIS", the journal is published quarterly (March, June, September, December) in English and publishes original peer-reviewed articles, reviews, and commentaries in the fields of Gynecology, Gynecologic Oncology, Endocrinology & Reproductive Medicine and Obstetrics. Case reports are not accepted for publication. Reviews will be considered for publication only if they are prepared by authors who have at least three published manuscripts in international peer reviewed journals and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer review from qualified experts in the area.

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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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Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

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Contents

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Gynecological Association

Contents

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Editorial

Dear Colleagues,

It is my great pleasure to introduce the last issue of the "Journal of the Turkish-German Gynecological Association (J Turk Ger Gynecol Assoc)" in the publishing year of 2024. This issue is consisted of seven articles, and two reviews that we hope you will read with interest. Also you may have the opportunity to read the quiz. Here we share some of our favorite articles that were published in this issue of the journal.

Although the precise etiology of polycystic ovary syndrome (PCOS) is still unknown, research has indicated that both genetic and environmental factors play a role in its development. Research on PCOS has long focused on genetics because the condition's symptoms often run in families. The luteinizing hormone (LH)/choriogonadotropin receptor is encoded by the LH/choriogonadotropin receptor (*LHCGR*) gene. During the last stages of preovulatory follicles, this gene is primarily active in granulosa cells. You will have the opportunity to read a meta-analysis evaluating the pattern of the association between LHCGR and PCOS.

Pregnancy-related spontaneous hyperglycemia is a common consequence of gestational diabetes mellitus (GDM). Hemoglobin A1C, fasting glucose, a two-hour 75 gr oral glucose tolerance test (OGTT), or a two-step test are some of the several screening methods. You will also have the opportunity to read an article determining whether maternal subcutaneous, visceral, and total adipose tissue measurements, as well as the ratios between them, could be utilized as a substitute for OGTT in the early stages of GDM prediction.

I would also like to invite you to join us for our prestigious 15th Turkish-German Gynecology Congress which will be held in Antalya between April 23-27 of 2025. As of before, our congress will be held to the highest scientific standards with a rich scientific program and pre-congress courses. At this year's congress we will be having lectures with the world's most reputable speakers; Prof. Gunter Noe, Prof. Ceanea Nezhat, Prof. Cristoph Berg, Prof. Karl Oliver Kagan, Prof. Ertan Sarıdoğan and more.

Dear Esteemed Readers, Authors and Reviewers,

Our objective is to reduce turnaround times inside the editorial system, with a focus on providing comprehensive justifications for unfavorable decisions (particularly those made without external review) for assistance with revision and resubmission elsewhere. Beyond this, we are creating chances for the author to reach a wider audience by having their work shared by our social media editors. Please visit our website at www.jtgga.org, and follow us on Twitter at @JtggaOfficial to stay up to date.

I would like to wish you a happy new year in 2025 and we are looking forward to receiving your valuable submissions, thank you in advance for your contributions.

Sincerely,

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

Basal serum luteinizing hormone, total testosterone, and free testosterone levels do not impact IVF outcomes in patients with polycystic ovary syndrome

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*The first two authors had equal contribution

Abstract

Objective: To assess the influence of basal serum levels of luteinizing hormone (LH), total testosterone (TT), and free testosterone (FT) on in vitro fertilization (IVF) success rates in patients with polycystic ovary syndrome (PCOS).

Material and Methods: A retrospective cohort analysis of PCOS patients who underwent freeze-all, gonadotropin releasing hormone (GnRH) antagonist IVF protocols from January 2013 to December 2019. Patients were grouped based on median basal serum levels of LH, TT, and FT to compare their IVF outcomes.

Results: A total of 76 women with PCOS diagnosed as per the 2003 Rotterdam criteria were included. When analyzed by LH levels, groups had similar baseline characteristics except for higher mean ± standard deviation TT (1.4±0.9 vs. 1.9±0.9 nmol/L, p=0.02) and FT (0.6±0.5 vs. 0.9±0.5 nmol/L, p=0.03) in the elevated LH group. However, clinical pregnancy rates (CPR) (34.2% vs. 44.7%, p=0.35) and live birth rates (LBR) (21.0% vs. 31.6%, p=0.29) were not different. The group with lower TT had more previous pregnancies (0.9±1.2 vs. 0.3±0.7, p=0.02) and shorter infertility duration (2.3±2.0 vs. 3.7±2.7 years, p=0.04), but again CPR (46.8% vs. 42.8%, p=0.90) and LBR (37.5% vs. 25.7%, p=0.33) were similar. FT analysis revealed no significant differences in CPR (48.2% vs. 36.7%, p=0.36) and LBR (23.2% vs. 37.9%, p=0.22) despite higher TT (1.1±0.4 vs. 2.2±1.1 nmol/L, p<0.001) and LH (6.1±3.8 vs. 11.2±7.2 IU/L, p<0.001) in the high FT group.

Conclusion: Basal serum levels of LH, TT, and FT did not significantly affect IVF outcomes in patients with PCOS using GnRH antagonist, freeze-all protocols. (J Turk Ger Gynecol Assoc. 2024; 25: 192-9)

Keywords: Polycystic ovary syndrome, in vitro fertilization, luteinizing hormone, total testosterone, free testosterone

Received: 16 July, 2024 **Accepted:** 11 October, 2024

Introduction

The correlation between initial serum levels of luteinizing hormone (LH), total testosterone (TT), and free testosterone (FT) and in vitro fertilization (IVF) outcomes remains a contentious issue (1). Women with polycystic ovary syndrome (PCOS) often exhibit increased amplitude and frequency of LH secretion (2). Given that PCOS is a predominant contributor to infertility through its disruption of ovulation, understanding the impact of these hormone variations is important in clinical practice (3). Many IVF centers delay ovarian stimulation until early follicular phase basal levels of LH, TT, or FT are reduced, based on concerns that elevated levels might negatively influence miscarriage rates, oocyte quality and quantity, and

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overall pregnancy success (4-7). However, some studies suggest that high basal LH levels may not compromise IVF outcomes (8).

The rationale for delaying stimulation based on basal hormone levels is the expectation of better outcomes in subsequent cycles. However, significant hormonal fluctuations mean a single measurement will not accurately represent a patient's hormonal profile (9). In addition, previous research was conducted before the widespread adoption of gonadotropin releasing hormone (GnRH) antagonist protocols for PCOS, which minimize the risk of ovarian hyperstimulation syndrome (OHSS) (10). Recent but limited research has suggested that hormone level variations may be temporary and less critical to pregnancy outcomes than previously thought. The present study assessed how initial serum concentrations of LH, TT, and FT affect IVF success rates in women with PCOS.

Material and Methods

This study was a retrospective cohort analysis performed at a university-affiliated fertility center, using electronic medical records collected from January 1, 2013, to December 31, 2019. The study protocol received approval from the McGill University Health Centre Institutional Review Board Ethics Committee (approval number: REB 2020-5971, date: 31.10.2019), and informed consent was waived owing to the study's retrospective design.

The study included women diagnosed with PCOS who participated in freeze-all GnRH antagonist IVF treatments. Participants were categorized into two groups depending on whether their basal serum levels of LH, TT, and FT, measured between days 2 and 5 of a natural or progestin-induced cycle, fell above or below the median. Each participant's PCOS diagnosis was confirmed via chart review following the Rotterdam criteria (11). Individual subjects were only included once in this study.

Inclusion criteria were patients who had undergone a GnRH antagonist IVF cycle with all embryos cryopreserved, followed by a frozen embryo transfer, and a confirmed PCOS diagnosis. Exclusion criteria included patients with untreated uterine pathologies, such as intra-cavitary polyps, fibroids, or ultrasound-visible hydrosalpinxes, and individuals with severe male infertility necessitating surgical sperm retrieval. Additionally, to exclude non-classical congenital adrenal hyperplasia, only patients with 17-hydroxyprogesterone levels below 2 ng/mL were included in the study, ensuring that all participants were within the normal range for women of reproductive age. Serum dehydroepiandrosterone sulfate levels were also measured to rule out an androgen secreting adrenal tumor. However, these results were not measured close to the IVF cycle and as such could not be compared in this study. They were often performed one or more years before care was initiated.

Demographic and baseline characteristics collected included female age, duration of infertility, gravidity, parity, serum estradiol, basal follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), prolactin, TT, FT, antral follicle count (AFC), male age, and semen analysis. The primary outcomes were clinical pregnancy rates (CPR) and live birth rates (LBR). Secondary outcomes included the total number of oocytes retrieved, the number of mature (MII) oocytes, the number of embryos reaching the two pronuclei (2PN) stage, the total number of blastocysts cryopreserved, and miscarriage rates.

A fixed antagonist protocol was used, initiating gonadotropins on the third day of the menstrual cycle, whether spontaneous or progestin induced. Experienced sonographers conducted baseline ultrasounds during the early follicular phase to check for anatomical abnormalities (such as fibroids, adenomyosis, ovarian cysts, and signs of endometriosis) and determine the AFC. GnRH antagonist treatment, using either Orgalutran (Organon, Canada) or Cetrotide (Merck Serono, Canada), began on the sixth day of gonadotropin therapy. Ovarian stimulation was performed using recombinant FSH alpha (Merck Serono Canada), recombinant FSH beta (Organon, Canada), or menopausal gonadotropins (Ferring, Canada). The ovulation trigger primarily involved Buserelin (Suprefact, Sanofi-Aventis, Canada), with some patients receiving recombinant beta-human chorionic gonadotropin (β-hCG) 250 mcg subcutaneously (Ovidrel, Serono, Canada). Egg collection was executed 36 hours following the ovulation trigger. Fertilization was performed either through conventional IVF or intracytoplasmic sperm injection (ICSI), with fertilization assessments conducted 16-18 hours post-retrieval. ICSI was performed primarily in cases of poor motility (<30%), oligospermia, abnormal strict morphology, and after unsuccessful fertilization in previous IVF attempts without ICSI. Due to the risk of OHSS, all embryos were cryopreserved. Only embryos graded 3BB or higher according to Gardner's criteria (12) were frozen at the blastocyst stage.

Future research should focus on the impact of peri-trigger serum progesterone levels on IVF outcomes in patients with PCOS. Moreover, further studies should investigate the stimulation-induced production of hormones such as LH and testosterone, and their subsequent influence on IVF outcomes. Pregnancy was confirmed by a serum β-hCG level of ≥20 IU/L sixteen days after transfer. Clinical pregnancy was confirmed by the presence of an intrauterine gestational sac with a fetal pole and heartbeat observed through transvaginal ultrasound between 6 and 7 weeks. Miscarriage was defined as the spontaneous loss of a pregnancy before 20 weeks of gestation, including

biochemical pregnancies, which were characterized by a positive pregnancy test but no clinical evidence of a gestational sac on ultrasound. Live birth was defined as the birth of a live infant after 20 weeks of gestation.

Statistical analysis

SPSS, version 23.0 (IBM Inc., Armonk, NY, USA) was used. Participants were categorized into two groups based on their serum LH levels: the lower 50% (1.0-7.0 IU/L) and the higher 50% (7.1-29.0 IU/L). A similar classification was applied to serum TT levels (0.30-1.50 nmol/L vs. 1.6-5.6 nmol/L) and FT levels (0.07-0.50 nmol/L vs. 0.51-2.70 nmol/L). Comparisons between the two groups for each serum marker were performed using independent t-tests for continuous variables and chi-squared tests for categorical variables. A p-value <0.05 was deemed statistically significant.

Results

Over the study period, 76 women meeting the inclusion criteria were identified. Analysis based on basal serum LH levels showed no significant differences in baseline characteristics between groups, except for mean ± standard deviation (SD) TT levels (1.4±0.9 vs. 1.9±0.9 nmol/L, p=0.02) and FT levels (0.6±0.5 vs. 0.9±0.5 nmol/L, p=0.03), which were higher in the elevated LH group (Table 1). The mean ± SD counts for oocytes retrieved (26.8±7.4 vs. 27.3±9.6, p=0.82), mature MII oocytes (19.9±6.8 vs. 20.2±7.8, p=0.88), and cryopreserved high-quality blastocysts (6.9±4.4 vs. 8.2±5.1, p=0.25) showed no significant differences between the groups. Similarly, the fertilization rate of MII oocytes (75.1±16.2% vs. 73.9±18.8%, p=0.78), the percentage of 2PN embryos developing into blastocysts (47.4±23.0% vs. 55.0±27.0%, p=0.18), and the proportion of MII oocytes progressing to blastocysts (34.9±18.0% vs. 39.8±18.8%, p=0.25) were comparable. Following embryo transfer, CPR (34.2% vs. 44.7%, p=0.35) and LBR (21.0% vs. 31.6%, p=0.29) were not significantly different between the groups. Miscarriage rates were found to be 52% in the lower LH group and 39% in the higher LH group, with no statistically significant difference between the groups (p=0.40) (Table 2).

When examining baseline characteristics based on TT levels, the lower TT group had a greater mean ± SD number of prior pregnancies (0.9±1.2 vs. 0.3±0.7, p=0.02), a shorter duration of infertility (2.3±2.0 vs. 3.7±2.7 years, p=0.04), and lower TSH (1.7±0.9 vs. 2.4±1.9 nmol/L, p=0.04) (Table 3). The counts of collected oocytes (27.5±7.5 vs. 28.3±9.7, p=0.73), mature MII oocytes (20.5±6.9 vs. 20.4±8.3, p=0.98), and cryopreserved

Table 1. Baseline characteristics of PCOS patients categorized by luteinizing hormone levels: upper 50% (7.1-29.0 IU/L) vs. lower 50% (1.0-7.0 IU/L)

Variable	Lower 50% LH, (n=38)	Upper 50% LH, (n=38)	p-value
Female age (years)	29.9±3.7	30.9±2.7	0.21
Duration of infertility (years)	3.2±2.6	3.0±2.1	0.82
Parity	0.2±0.5	0.2±0.5	0.82
Gravidity	0.7±0.9	0.6±1.1	0.65
Baseline FSH (IU/L)	5.4±1.5	5.9±1.3	0.15
Baseline LH (IU/L)	4.2±1.4	13.0±5.7	0.001
Baseline estradiol (pmol/L)	193.6±121.3	277.6±364.2	0.20
Prolactin (mcg/L)	10.0±3.5	10.7±5.6	0.50
TSH (mIU/L)	2.2±1.8	3.3±8.2	0.40
Total testosterone (nmol/L)	1.4±0.9	1.9±0.9	0.02
Free testosterone (nmol/L)	0.6±0.5	0.9±0.5	0.03
Antral follicle count	42.3±15.2	50.9±22.6	0.09
Male age (years)	33.7±5.6	34.7±4.9	0.46
Sperm concentration (mil/mL)	38.8±32.3	41.4±36.9	0.75
Ejaculate volume (mL)	2.6±1.2	2.7±1.3	0.61
Semen motility (%)	40.1±26.6	44.9±27.6	0.46
Total motile sperm count (Mil)	53.4±77.7	75.1±97.7	0.31
FSH total dose (IU)	1287.1±518.4	1311.2±458.5	0.84
Peak estradiol during stimulation (nmol/L)	10980.1±4760.9	13982.7±6613.4	0.03
Peak endometrial thickness (mm)	10.1±2.0	10.3±2.1	0.81

Data are presented as mean ± standard deviation, PCOS: Polycystic ovary syndrome, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, TSH: Thyroid stimulating hormone

Table 2. Embryologic and pregnancy outcomes of PCOS patients categorized by luteinizing hormone levels: upper 50% (7.1-29.0 IU/L) vs. lower 50% (1.0-7.0 IU/L)

Variable	Lower 50% LH, (n=38)	Upper 50% LH, (n=38)	p-value
Number of oocytes collected	26.8±7.4	27.3±9.6	0.82
Number of MII oocytes	19.9±6.8	20.2±7.8	0.88
MII fertilized (%)	75.1±16.2	73.9±18.8	0.78
2PN grew to blastocyst (%)	47.4±23.0	55.0%±27.0	0.18
MII grew to blastocyst (%)	34.9±18.0	39.8±18.8	0.25
Number of embryos frozen	6.9±4.4	8.24±5.1	0.25
Clinical pregnancy no. (%)	13 (34.2)	17 (44.7)	0.35
Miscarriage no/total pregnancies (%)	12/23 (52%)	7/18 (39%)	0.40
Live birth no. (%)	8 (21.0)	12 (31.6)	0.29

Data are presented as mean ± standard deviation, PCOS: Polycystic ovary syndrome, LH: Luteinizing hormone, MII oocytes: Mature oocytes, 2PN: 2 pronuclei stage

Table 3. Baseline characteristics of PCOS patients by total testosterone levels: upper 50% (1.6-5.6 nmol/L) vs. lower 50% (0.30-1.50 nmol/L)

Variable	Lower 50% TT, (n=32)	Upper 50% TT, (n=35)	p-value
Female age (years)	30.1±3.2	30.8±3.3	0.37
Duration of infertility (years)	2.3±2.0	3.7±2.7	0.04
Parity	0.3±0.6	0.1±0.4	0.11
Gravidity	0.9±1.2	0.3±0.7	0.02
Baseline FSH (IU/L)	5.7±1.1	5.6±1.6	0.78
Baseline LH (IU/L)	6.9±5.2	10.0±6.8	0.04
Baseline estradiol (pmol/L)	168.6±82.4	302.0±377.7	0.06
Prolactin (mcg/L)	10.0±3.1	10.4±5.5	0.70
TSH (mIU/L)	1.7±0.9	2.4±1.9	0.04
Total testosterone (nmol/L)	0.9±0.4	2.3±0.9	0.001
Antral follicle count	45.6±22.7	48.9±17.7	0.53
Male age (years)	34.5±5.4	33.9±4.6	0.68
Sperm concentration (mil/mL)	35.0±28.3	46.1±44.0	0.24
Ejaculate volume (mLl)	2.9±1.2	2.4±1.2	0.12
Semen motility (%)	39.1±29.3	44.9±30.2	0.41
Total motile sperm count (Mil)	58.7±76.3	77.3±119.9	0.47
FSH total dose (IU)	1199.5±424.4	1402.1±552.2	0.11
Peak estradiol during stimulation (nmol/L)	11561.9±3802.5	13318.9±7716.2	0.24
Peak endometrial thickness (mm)	10.1±2.2	10.3±1.9	0.69

Data are presented as mean ± standard deviation, PCOS: Polycystic ovary syndrome, TT: Total testosterone, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid stimulating hormone

high-quality blastocysts (6.9±4.0 vs. 8.1±5.7, p=0.32) showed no significant differences between the groups. Furthermore, the fertilization rate of MII oocytes (73.3±18.8% vs. 73.5±16.7%, p=0.96), the percentage of 2PN embryos developing into blastocysts (48.3±22.9% vs. 52.5±27.5%, p=0.50), and the proportion of MII oocytes advancing to the blastocyst stage (34.1±17.0% vs. 38.2±19.1%, p=0.36) were similar. CPR (46.8% vs. 42.8%, p=0.90) and LBR (37.5% vs. 25.7%, p=0.33) did not significantly differ among patients who underwent embryo transfer. Miscarriage rates in the lower and upper TT groups were 45% and 50%, respectively (p=0.77) (Table 4).

The impact of FT levels on IVF outcomes in PCOS patients was also evaluated. Significant differences in baseline characteristics were observed for serum TT (1.1±0.4 vs. 2.2±1.1 nmol/L, p<0.001) and serum LH levels (6.1±3.8 vs. 11.2±7.2 IU/L, p<0.001) (Table 5). The counts of retrieved

Table 4. Embryologic and pregnancy outcomes of PCOS patients by total testosterone levels: upper 50% (1.6-5.6 nmol/L) vs. lower 50% (0.30-1.50 nmol/L)

Variable	Lower 50% TT, (n=32)	Upper 50% TT, (n=35)	p-value
Number of oocytes collected	27.5±7.5	28.3±9.7	0.73
Number of MII oocytes	20.5±6.9	20.4 3±8.3	0.98
MII fertilized (%)	73.3±18.8	73.5±16.7	0.96
2PN grew to blastocyst (%)	48.3±22.9	52.5±27.5	0.50
MII grew to blastocyst (%)	34.1±17.0	38.2±19.1	0.36
Number of embryos frozen	6.9±4.0	8.1±5.7	0.32
Clinical pregnancy no. (%)	15 (46.8)	15 (42.8)	0.90
Miscarriage no/total pregnancies (%)	10/22 (45%)	9/18 (50%)	0.77
Live birth no. (%)	12 (37.5)	9 (25.7)	0.33
Data are presented as mean ± standard deviation, PCOS: Polycystic ovary syndrome, TT: Total testosterone, LH: Luteinizing hormone, MII oocytes: Mature oocytes, 2PN: 2 pronuclei stage			

Table 5. Baseline characteristics of PCOS patients by free testosterone levels: upper 50% (0.51 to 2.70 nmol/L) vs. lower 50% (0.07-0.50 nmol/L)

Variable	Lower 50% FT, (n=29)	Upper 50% FT, (n=30)	p-value
Female age (years)	30.4±3.3	30.4±3.5	0.99
Duration of infertility (years)	2.4±1.9	3.6±2.5	0.66
Parity	0.2±0.6	0.1±0.4	0.41
Gravidity	0.6±0.9	0.6±1.1	0.84
Baseline FSH (IU/L)	5.7±1.3	5.8±1.5	0.73
Baseline LH (IU/L)	6.1±3.8	11.2±7.2	<0.001
Baseline estradiol (pmol/L)	176.0±81.8	283.3±389.5	0.19
Prolactin (mcg/L)	10.0±3.2	10.7±5.6	0.56
TSH (mIU/L)	1.8±1.1	2.2±2.0	0.33
Total testosterone (nmol/L)	1.1±0.4	2.2±1.1	<0.001
Antral follicle count	43.6±18.2	54.5±20.9	0.06
Male age (years)	33.4±4.7	34.8±5.8	0.30
Sperm concentration (mil/mL)	38.6±33.7	43.2±40.2	0.64
Ejaculate volume (mL)	2.7±1.4	2.9±1.5	0.50
Semen motility (%)	34.6±26.2	43.7±26.0	0.054
Total motile sperm count (Mil)	51.4±86.2	67.6±107.8	0.36
FSH total dose (IU)	1235.7±491.9	1391.6±520.4	0.26
Peak estradiol during stimulation (nmol/L)	12397.5±4123.0	13680.9±7805.8	0.44
Peak endometrial thickness (mm)	10.3±2.5	10.4±1.6	0.81
Data are presented as mean ± standard deviation, PCOS: Polycystic ovary syndrome, FT: Free testosterone, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid stimulating hormone			

oocytes (26.3±7.4 vs. 29.8±8.7, p=0.10), mature MII oocytes (19.9±6.0 vs. 21.3±9.0, p=0.50), and cryopreserved high-quality blastocysts (7.0±4.3 vs. 8.4±6.0, p=0.30) did not differ between groups. The fertilization rate of MII oocytes (75±17% vs. 73±19%, p=0.42), the percentage of 2PN embryos developing into blastocysts (49±19% vs. 51±24%, p=0.63), and the proportion of MII oocytes advancing to the blastocyst stage (37±17% vs. 36±16%, p=0.77) were comparable. Once more the CPR (48.2% vs. 36.7%, p=0.36) and LBR (23.2% vs. 37.9%, p=0.22) did not significantly differ in patients who underwent embryo transfer. Miscarriage rates were 58% in the lower FT group compared to 68% in the higher FT group (p=0.45) (Table 6).

Table 6. Embryologic and pregnancy outcomes of PCOS patients by free testosterone levels: upper 50% (0.51 to 2.70 nmol/L) vs. lower 50% (0.07-0.50 nmol/L)

Variable	Lower 50% FT, (n=29)	Upper 50% FT, (n=30)	p-value
Number of oocytes collected	26.3±7.4	29.8±8.7	0.10
Number of MII oocytes	19.9±6.0	21.3±9.0	0.50
MII Fertilized (%)	75±17	73±19	0.42
2PN grew to blastocyst (%)	49±19	51±24	0.63
MII grew to blastocyst (%)	37±17	36±16	0.77
Number of embryos frozen	7.00±4.3	8.40±6.0	0.30
Clinical pregnancy no. (%)	14 (48.2)	11 (36.7)	0.36
Miscarriage no/total pregnancies (%)	15/26 (58)	15/22 (68)	0.45
Live birth no. (%)	11 (37.9)	7 (23.3)	0.22
Data are presented as mean ± standard deviation, FT: Free testosterone, LH: Luteinizing hormone, MII oocytes: Mature oocytes, 2PN: 2 pronuclei stage			

Discussion

The aim of this study was to assess if elevated basal serum levels of LH, TT, and FT influenced outcomes in freeze-all, GnRH-antagonist IVF cycles for patients with PCOS. The results revealed no significant differences in CPR, LBR, or IVF stimulation outcomes across varying hormonal levels. Notably, patients in the elevated basal serum LH group had higher TT and FT levels. Analysis of TT groups showed differences in baseline characteristics and clinical parameters such as serum TSH levels, duration of infertility, and prior pregnancies, but these did not impact overall success rates. Similar trends were observed within the FT groups, which were mainly distinguished by their higher basal serum TT and LH levels without impacting key treatment outcomes.

Earlier research primarily focused on PCOS patients undergoing IVF before the common use of freeze-all, GnRH-antagonist protocols. Many clinics have used basal serum LH values on day 3 of the menstrual cycle to decide on cycle cancellations, as reported in previous studies (8,13). However, these studies may not be relevant to current practice with changes in management. Recent research, such as the study by Singh et al. (1), presents different findings, likely due to advances in GnRH-antagonist protocols and frozen-embryo transfer cycles. Unlike Singh et al.'s (1) study, the present study included only frozen embryo transfers, limiting our outcomes to the effects on oocyte and embryo potential. A recent randomized controlled trial by Chen et al. (14) demonstrated that PCOS patients undergoing frozen embryo transfer experienced higher live birth rates and a reduced risk of OHSS compared to those receiving fresh embryo transfers.

The higher peak estradiol levels in the higher LH group likely indicate stronger follicular activity. However, this did not translate into significantly different clinical outcomes, such as the number of oocytes retrieved, fertilization rate, embryo quality, CPR, miscarriage rate, or live birth rate. This observation is consistent with other studies suggesting that while estradiol levels are indicative of follicular activity, they may not directly correlate with pregnancy success in PCOS patients (15,16).

The pulsatile nature of LH means that a single measurement may not accurately represent an individual's hormonal status. In PCOS patients, LH levels are generally higher with an accelerated pulse frequency compared to normal controls. However, at any given time, these patients' serum LH levels could be low, normal, or high, depending on the steroid pulse curve (17). This variability highlights the lack of reliability in using single LH measurements to categorize hormonal status. PCOS patients often exhibit significant variability in hormonal levels due to the pulsatile nature of hormone secretion. This variability suggests that a single measurement may not capture the hormonal environment and may lead to misinterpretation of the hormonal status. This is particularly relevant for LH, where levels can fluctuate widely within a short period (18,19).

Freeze-all cycles create a separation between basal serum levels of LH, TT, and FT and the endometrial environment at the time of embryo transfer. While elevated serum LH is known to alter the ovarian hormonal environment (20), the impact of basal serum levels of LH, TT, and FT on fresh embryo transfer success remains unexplored, and is thus an important area for future research. Nevertheless, our findings suggest that ovarian stimulation outcomes, oocyte quality, and embryo development do not significantly differ across groups categorized by basal serum levels of LH, TT, or FT. This indicates that while hormonal levels may vary, their influence on core aspects of IVF treatment, such as stimulation response, oocyte, and embryo development, remains consistent regardless of basal hormone levels.

Study limitations

This study is subject to limitations inherent in a retrospective cohort design, including the reliance on previously documented data and the inability to control for various confounding factors, which may introduce potential biases and influence the generalizability of our findings. The accuracy and completeness of the information are particularly limited concerning factors such as anti-Mullerian hormone (AMH) levels, which were not performed at our institution until more recently due to high cost, and body mass index (BMI), which was not reliably recorded during the study period. The absence of AMH levels, an important marker for ovarian reserve and PCOS, restricts our ability to fully assess PCOS status. Similarly, the lack of accurate BMI data impedes a comprehensive analysis of the impact of body weight in PCOS on IVF outcomes. Moreover, there was insufficient data on complications, such as obstetrical outcomes, including pre-eclampsia, preterm birth, and placental pathology. Peri-trigger serum progesterone levels, which might influence several examined outcomes, were not routinely measured during the IVF process in our clinic. This could represent a potential confounding factor. Furthermore, the small sample size limits our ability to conclusively identify differences, and outcomes may have varied if fresh embryo transfers had been included. This study did not account for variations in stimulation protocols within the same patients, which could provide more insight into the consistency of hormonal impacts on IVF outcomes. Longitudinal studies are recommended to explore this aspect.

Future research should focus on the impact of peri-trigger serum progesterone levels on IVF outcomes in patients with PCOS. In addition, further studies should investigate the stimulation-induced production of hormones, including LH and testosterone, and their subsequent influence on IVF outcomes.

Conclusion

In PCOS patients undergoing freeze-all, GnRH antagonist IVF and frozen embryo transfer, basal serum levels of LH, TT, and FT did not show significant differences in oocyte/embryo quality, CPR, or LBR. This suggests that cancelling cycles for women with PCOS and high basal LH and testosterone levels, in anticipation of improved outcomes in future cycles, may not be necessary for those undergoing frozen embryo transfers.

Ethics Committee Approval: *The study protocol received approval from the McGill University Health Centre Institutional Review Board Ethics Committee (approval number: REB 2020-5971, date: 31.10.2019).*

Informed Consent: *Informed consent was waived owing to the study's retrospective design.*

Footnotes

Author Contributions: *Surgical and Medical Practices: N.K., A.D., M.H.D.; Concept: N.K., A.D., M.H.D.; Design: N.K., A.D., M.H.D.; Data Collection or Processing: N.K., A.D., K.R.O., V.B., A.P., W.Y.S.; Analysis or Interpretation: N.K., A.D., K.R.O.; Literature Search: N.K., A.D.; Writing: N.K., A.D., K.R.O., V.B., A.P., W.Y.S., M.H.D.*

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Family planning behavior before and during the COVID-19 pandemic

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Abstract

Objective: Contraception use and follow-up visit data from before and in two periods during the coronavirus disease-2019 (COVID-19) pandemic were compared to investigate change in behavior.

Material and Methods: A retrospective study of women aged 18-49 years from New York City during three one-year time periods: pre-COVID-19 pandemic [(COV-PRE); n=4,261], early COVID-19 pandemic when the COVID-19 vaccine was not available [(COV-VACNO); n=3,365], and later COVID-19 pandemic when the COVID-19 vaccine was available [(COV-VACAV); n=4,170].

Results: There were higher odds of implant use [odds ratio (OR): 1.42, 95% confidence interval (CI): 1.05, 1.93, p=0.02] during COV-VACNO. There were lower odds for any contraception (OR: 0.88, 95% CI: 0.79, 0.98, p<0.001) or intrauterine device (IUD) (OR: 0.73, 95% CI: 0.61, 0.86, p<0.001) use during COV-VACAV. No differences occurred for bilateral tubal ligation, pill, patch, injection, medical elective abortion, or surgical elective abortion. There was a greater percentage of follow-up visits for any contraception (p=0.02) and IUD (p=0.02) use during COV-VACNO and COV-VACAV than COV-PRE.

Conclusion: When COVID-19 vaccines were unavailable, there were higher odds for use of implants. Once COVID-19 vaccines were available, there were lower odds for any contraception and IUD use. These findings highlight changes in behavior in terms of contraceptive concerns and preferences during a public health crisis that should be planned for by healthcare providers. (J Turk Ger Gynecol Assoc. 2024; 25: 200-6)

Keywords: Contraception, family planning services, COVID-19

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Introduction

In the United States (US) at the beginning of the coronavirus disease-2019 (COVID-19) pandemic, there were delays or cancellations of sexual and reproductive healthcare visits and decreased access to birth control for 33% of women (1). Women had increased levels of fear, stress, and anxiety regarding pregnancy due to concerns about the potential negative risks of COVID-19 on maternal and fetal health (2). The COVID-19 pandemic affected family planning and contraception access due to lockdown measures, overwhelmed healthcare systems, and restricted access to contraceptive services. This led to delays or cancellations of appointments, reduced availability of certain contraceptive methods, and limited access to essential sexual and reproductive health services (3).

A nationwide US study compared the time periods from before the COVID-19 pandemic and the first year of the COVID-19

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pandemic and found that contraception visits declined for the first year of the COVID-19 pandemic for tubal ligations, long-acting reversible contraception, pills, patches, rings, and injectables (4). A review on the impact of COVID-19 mitigation measures on sexual and reproductive health in low- and middle-income countries found that there was an overall reduction in the uptake and delivery of services, including family planning clinics, health facility deliveries, and post-abortion care services (5). A study from the southwest US found that during the beginning of the COVID-19 pandemic that there was a greater desire to become pregnant during the first few months which then changed to a greater desire to not become pregnant over the next few months (6).

Regarding contraception receipt and use, one study from California, US found that there was a lower percentage for planned use of top-tier contraception, of either sterilization, an intrauterine device (IUD), or an implant at admission for delivering a baby during the first few months of the COVID-19 pandemic compared to pre-COVID-19 pandemic (7). One study from Massachusetts, US found that at the beginning of the COVID-19 pandemic there was an increase compared to the pre-COVID-19 period in terms of receiving postpartum progesterone-only pills, combined oral contraceptives, rings, patches, and injections while receipt of IUDs, implants, and sterilization were similar (8). However, another study from Massachusetts found that at the beginning of the COVID-19 pandemic there was an increase in use of immediate postpartum long-acting reversible contraception, while overall contraception use at 10 weeks postpartum did not change (9).

There are a number of studies that compare family planning approaches between the initial period or first year of the COVID-19 pandemic to the pre-COVID-19 pandemic period (4,7-9). The first year of the COVID-19 pandemic was a time when COVID-19 vaccines were unavailable. It is possible that once COVID-19 vaccines were available, family planning approaches may have changed even though the COVID-19 pandemic was still negatively impacting health. We are unaware of any studies comparing family planning approaches during the years of the COVID-19 pandemic when vaccines were and were not available. Thus, this study was designed to compare family planning approaches and follow-up visits for family planning between three different time periods: one year before the COVID-19 pandemic; the first year of the COVID-19 pandemic when vaccines were unavailable; and the second year of the COVID-19 pandemic when vaccines were available.

Material and Methods

Setting

This was a retrospective study of all women of reproductive age (18-49 years) seen at the obstetrics and gynecology department at a New York City State Hospital. This hospital typically serves patients of lower socioeconomic status. We compared three different time periods: before the COVID-19 pandemic impacted New York City (March 2019-February 2020; COV-PRE); initial phase of the COVID-19 pandemic when COVID-19 vaccine access was not readily available (March 2020-February 2021; COV-VACNO); and a phase of the COVID-19 pandemic when COVID-19 vaccine access was typically available (March 2021-February 2022; COV-VACAV). The study was ethically conducted, received New York City Health + Hospitals Institutional Review Board approval [approval number: BRANY IRB File # 23-12-003-378(HHC), date: 01.10.2023], and was conducted in accordance with the Helsinki Declaration. A waiver for informed consent was obtained due to the retrospective nature of the study.

Variables

Demographic variables consisted of age (years), race/ethnicity (white, black, Hispanic, Asian, other), and preferred language (English vs. non-English). Other data items collected included body mass index [(BMI) in kg/m^2], current cigarette smoking status, and parity. Medical history variables consisted of type 1/type 2 diabetes mellitus (DM), gestational diabetes mellitus (GDM), hypertension, thrombophilia, thyroid issue, uterine anomaly, and gonorrhea/chlamydia, all measured as no versus yes. Use of prenatal care and telehealth visits were recorded.

The primary outcome was overall contraception use including bilateral tubal ligation (BTL), oral contraceptive pill, patch, IUD, injection, and/or implant during the three different time periods. The secondary outcomes were use of each of the above individual contraception approaches, medical elective abortion, or surgical elective abortion. Another secondary outcome compared attending a follow-up visit during the three different time periods of three months for BTL and 12 months for pill, patch, IUD, injection, or implant. Follow-up visits for any contraception use summarized content from of any of the above six contraception types.

Statistical analysis

The continuous variables are presented as mean and standard deviation and these were compared using analysis of variance (ANOVA) tests. Categorical variables are presented as frequency and percentage and these were compared using the Pearson chi square test. Variables that differed significantly between the time periods were included as covariates in the multivariate logistic regression analyses. Missing BMI values were entered using the sample mean value of 28.392. All p-values were two-sided with alpha level for significance at $p<0.05$. IBM SPSS, version 29 was used for all analyses (IBM Corporation, Armonk, NY, USA).

Results

The sample consisted of 4,261 patients in COV-PRE, 3,365 patients in COV-VACNO, and 4,170 patients in COV-VACAV. Table 1 shows the sample characteristics of the three time periods. Many variables significantly differed between the time periods. Mean age (p<0.001), and percentages for non-English language (p<0.001), receipt of prenatal care (p<0.001), and telehealth visit (p<0.001) had the highest values in the COV-PRE period. Race/ethnicity (p<0.001) had the greatest percentage of Hispanics in COV-VACAV. Mean BMI (p<0.001) had the highest values during COV-VACNO and COV-VACAV. DM (p<0.001), GDM (p=0.002), and gonorrhea/chlamydia (p=0.03) had the highest percentages during COV-VACNO. Hypertension (p=0.003) had the highest percentage in the COV-VACAV period.

Table 2 shows the univariate comparisons for the time periods and family planning behavior. Any contraception use differed significantly (p=0.01) with the greatest percentage for COV-VACNO. IUD use significantly differed (p<0.001) with the greatest percentage for COV-PRE. Injection significantly differed (p=0.03) with the greatest percentage for COV-VACAV. Implant significantly differed (p=0.002) with the greatest percentage for COV-VACNO. BTL, pill, patch, medical elective abortion, and surgical elective abortion did not significantly differ between the time periods.

Table 3 shows the multivariate analyses for any contraception and IUD. For any contraception, COV-VACAV significantly differed (p=0.02) with lower odds than COV-PRE. COV-VACNO did not significantly differ from COV-PRE. For IUD, COV-VACAV significantly differed (p<0.001) with lower odds than COV-PRE while COV-VACNO did not differ from COV-PRE. Table 4 shows the multivariate analyses for injection and implant. For injection, there were no significant differences between the time periods. For implant, COV-VACNO exhibited significantly higher odds (p=0.02) than COV-PRE, while COV-VACAV did not differ from COV-PRE.

There were significant findings for the covariates from the multivariate analyses (Tables 3, 4). Increased age was significantly associated with lower odds for any contraception, IUD, injection, and implant. Black and Hispanic race/ethnicity significantly differed from whites with higher odds for any contraception, lower odds for IUD, higher odds for injection, and higher odds for implant. BMI significantly differed with increased values significantly associated with lower odds for

Table 1. Sample characteristics of the three time periods

Variable	COV-PRE, mean (SD) or n (%) (n=4,261)	COV-VACNO, mean (SD) or n (%) (n=3,365)	COV-VACAV, mean (SD) or n (%) (n=4,170)	p-value
Age (years) (mean)	36.0 (7.29)	35.5 (7.44)	35.1 (7.69)	<0.001
Race/ethnicity				
White	1,065 (25.0)	773 (23.0)	864 (20.7)	<0.001
Black	486 (11.4)	428 (12.7)	535 (12.8)	
Hispanic	1,423 (33.4)	1,235 (36.7)	1,801 (43.2)	
Asian	405 (9.5)	311 (9.2)	366 (8.8)	
Other	882 (20.7)	618 (18.4)	604 (14.5)	
Body mass index (kg/m^2) (mean)	28.0 (5.85)	28.6 (5.77)	28.6 (5.92)	<0.001
Language (non-English)	2,499 (58.6)	1,809 (53.8)	2,357 (56.5)	<0.001
Smoking	238 (5.6)	189 (5.6)	210 (5.0)	0.43
Parity (mean)	2.1 (1.49)	2.0 (1.51)	2.0 (1.50)	0.15
Diabetes mellitus	489 (11.5)	503 (14.9)	580 (13.9)	<0.001
Gestational diabetes mellitus	323 (7.6)	302 (9.0)	283 (6.8)	0.002
Hypertension	160 (3.8)	154 (4.6)	221 (5.3)	0.003
Thrombophilia	13 (0.3)	11 (0.3)	14 (0.3)	0.97
Thyroid issue	306 (7.2)	238 (7.1)	253 (6.1)	0.09
Uterine anomaly	11 (0.3)	9 (0.3)	7 (0.3)	1.00
Gonorrhea/chlamydia	107 (2.5)	118 (3.5)	138 (3.3)	0.03
Prenatal care	1,292 (30.3)	910 (27.0)	1,059 (25.4)	<0.001
Telehealth visit	206 (4.8)	50 (1.5)	99 (2.4)	<0.001

COV-PRE: Pre-COVID-19 pandemic, COV-VACNO: Initial phase of COVID-19 pandemic when no COVID-19 vaccines were available, COV-VACAV: During COVID-19 pandemic when COVID-19 vaccines were available, SD: Standard deviation

Table 2. Univariate comparisons for the time periods and family planning behavior

Variable	COV-PRE, n (%) (n=4,261)	COV-VACNO, n (%) (n=3,365)	COV-VACAV, n (%) (n=4,170)	p-value
Any contraception	1,011 (23.7)	875 (26.0)	959 (23.0)	0.01
Bilateral tubal ligation	92 (2.2)	67 (2.0)	66 (1.6)	0.14
Intrauterine device	356 (8.4)	272 (8.1)	256 (6.1)	<0.001
Pill	308 (7.2)	292 (8.7)	340 (8.2)	0.06
Patch	70 (1.6)	49 (1.5)	67 (1.6)	0.80
Injection	241 (5.7)	227 (6.7)	293 (7.0)	0.03
Implant	80 (1.9)	90 (2.9)	79 (1.9)	0.002
Abortion: medical	12 (0.3)	13 (0.4)	20 (0.5)	0.34
Abortion: surgical	89 (2.1)	73 (2.2)	75 (1.8)	0.47

COV-PRE: Pre-COVID-19 pandemic, COV-VACNO: initial phase of COVID-19 pandemic when no COVID-19 vaccines were available, COV-VACAV: during COVID-19 pandemic when COVID-19 vaccines were available

Table 3. Multivariate logistic regression analysis for any contraception and intrauterine device

Variable	Any contraception, OR (95% CI)	p-value	IUD, OR (95% CI)	p-value
Time period				
COV-PRE	1.00		1.00	
COV-VACNO	1.09 (0.98, 1.21)	0.13	0.97 (0.82, 1.14)	0.69
COV-VACAV	0.88 (0.79, 0.98)	0.02	0.73 (0.61, 0.86)	<0.001
Age (years)	0.947 (0.941, 0.953)	<0.001	0.97 (0.96, 0.98)	<0.001
Race/ethnicity				
White	1.00		1.00	
Black	1.23 (1.03, 1.45)	0.02	0.38 (0.28, 0.52)	<0.001
Hispanic	1.76 (1.56, 1.99)	<0.001	0.59 (0.49, 0.71)	<0.001
Asian	1.15 (0.97, 1.37)	0.11	1.13 (0.90, 1.42)	0.29
Other	1.23 (1.06, 1.41)	0.01	0.73 (0.59, 0.89)	0.002
Body mass index (kg/m^2)	0.991 (0.984, 0.999)	0.03	1.01 (1.002, 1.03)	0.03
Language (non-English)	1.10 (1.00, 1.21)	0.06	1.07 (0.92, 1.24)	0.42
Diabetes mellitus	0.73 (0.62, 0.88)	<0.001	0.85 (0.65, 1.12)	0.26
Gestational diabetes mellitus	2.80 (2.28, 3.43)	<0.001	1.64 (1.20, 2.24)	0.002
Hypertension	1.47 (1.19, 1.83)	<0.001	1.70 (1.23, 2.35)	0.001
Gonorrhea/chlamydia	1.35 (1.08, 1.69)	0.01	0.78 (0.49, 1.22)	0.27
Prenatal care	0.80 (0.72, 0.89)	<0.001	0.91 (0.77, 1.07)	0.23
Telehealth visit	1.38 (1.09, 1.75)	0.01	1.21 (0.83, 1.75)	0.32

COV-PRE: Pre-COVID-19 pandemic, COV-VACNO: Initial phase of COVID-19 pandemic when no COVID-19 vaccines were available, COV-VACAV: During COVID-19 pandemic when COVID-19 vaccines were available, IUD: Intrauterine device, OR: Odds ratio, CI: Confidence interval. Nagelkerke R square: Any contraception=0.07, IUD=0.03. Analysis of variance inflation factor values indicated no multicollinearity concerns

any contraception and injection and slightly higher odds for IUD. Non-English language was significantly associated with higher odds for injection and higher odds for implant. DM was significantly associated with lower odds for any contraception. GDM was significantly associated with higher odds for any contraception, IUD, injection, and implant. Hypertension was significantly associated with higher odds for any contraception, IUD, and injection. Gonorrhea/chlamydia was significantly associated with higher odds for any contraception. Prenatal care was significantly associated with lower odds for any contraception. Telehealth visit was significantly associated with higher odds for any contraception and injection.

The Figure 1 shows univariate comparisons for follow-up visits. Any contraception significantly differed (p=0.02) with COV-VACNO and COV-VACAV having greater percentages than COV-PRE. IUD significantly differed (p=0.02) with COV-VACNO and COV-VACAV having greater percentages than COV-PRE. Follow-up for all time periods did not approach 100%. Injection had the highest percentage of follow-up for the time periods, ranging from 53.9-60.4%.

Table 4. Multivariate logistic regression analysis for injection and implant

Variable	Injection, OR (95% CI)	p-value	Implant, OR (95% CI)	p-value
Time period				
COV-PRE	1.00		1.00	
COV-VACNO	1.14 (0.94, 1.38)	0.17	1.42 (1.05, 1.93)	0.02
COV-VACAV	1.09 (0.91, 1.30)	0.36	0.79 (0.57, 1.09)	0.14
Age (years)	0.94 (0.93, 0.95)	<0.001	0.91 (0.89, 0.93)	<0.001
Race/ethnicity				
White	1.00		1.00	
Black	2.56 (1.90, 3.44)	<0.001	3.86 (2.11, 7.08)	<0.001
Hispanic	2.57 (2.03, 3.24)	<0.001	4.96 (3.04, 8.09)	<0.001
Asian	0.89 (0.61, 1.30)	0.56	0.66 (0.26, 1.66)	0.37
Other	1.09 (1.82, 1.47)	0.55	2.30 (1.32, 4.01)	0.003
Body mass index (kg/m^2)	0.98 (0.97, 0.997)	0.02	1.01 (0.99, 1.03)	0.35
Language (non-English)	1.43 (1.20, 1.72)	<0.001	1.89 (1.38, 2.58)	<0.001
Diabetes mellitus	0.80 (0.59, 1.08)	0.14	0.77 (0.46, 1.28)	0.31
Gestational diabetes mellitus	2.27 (1.60, 3.21)	<0.001	3.34 (1.91, 5.83)	<0.001
Hypertension	2.13 (1.54, 2.95)	<0.001	1.45 (0.77, 2.74)	0.26
Gonorrhea/chlamydia	1.33 (0.96, 1.86)	0.09	0.80 (0.44, 1.46)	0.47
Prenatal care	0.84 (0.70, 1.01)	0.06	0.74 (0.54, 1.00)	0.053
Telehealth visit	1.46 (1.002, 2.13)	0.049	1.09 (0.54, 2.17)	0.82

COV-PRE: Pre-COVID-19 pandemic, COV-VACNO: Initial phase of COVID-19 pandemic when no COVID-19 vaccines were available, COV-VACAV: During COVID-19 pandemic when COVID-19 vaccines were available, IUD: Intrauterine device, OR: Odds ratio, CI: Confidence interval. Nagelkerke R square: Injection=0.07, Implant=0.11. Analysis of variance inflation factor values indicated no multicollinearity concerns

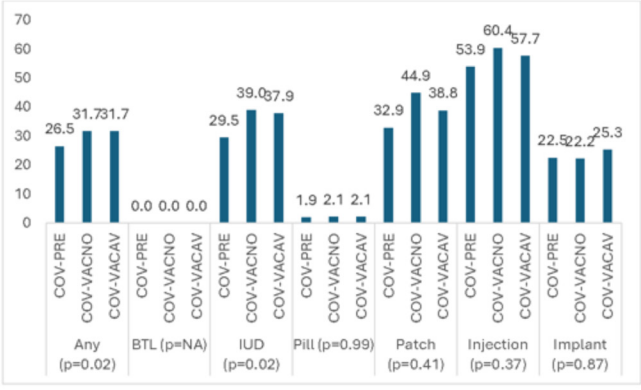

Figure 1. Univariate percentage comparisons for the time periods for contraception follow-up visits
COV-PRE: Pre-COVID-19 pandemic, COV-VACNO: Initial phase of COVID-19 pandemic when no COVID-19 vaccines were available, COV-VACAV: During COVID-19 pandemic when COVID-19 vaccines were available, BTL: Bilateral tubal ligation, IUD: Intrauterine device

Discussion

We found that for the category "any contraception" and IUD that COV-VACAV had significantly lower odds for use than COV-PRE while no differences occurred between COV-VACNO and COV-PRE. We found for implants that COV-VACNO had significantly higher odds than COV-PRE for use while no differences occurred between COV-VACAV and COV-PRE. We did not find any differences among the time periods for BTL, pill, patch, injection, medical elective abortion, and surgical elective abortion. For both "any contraception" and IUD, there were significantly greater percentages for follow-up visits during COV-VACNO and COV-VACAV than COV-PRE.

We found for any contraception use that COV-VACAV had significantly lower odds than COV-PRE while COV-VACNO did not significantly differ from COV-PRE. Previous research into any contraception use found that there were no differences between pre-COVID-19 pandemic and the beginning of the COVID-19 pandemic (9). Our finding for the first year of the COVID-19 pandemic when COVID-19 vaccines were unavailable is similar to this pattern. However, our finding for the second year of the COVID-19 pandemic when COVID-19 vaccines were available differs. We suggest that once COVID-19 vaccines were available, there were lower levels of contraception use since women and their partners felt more comfortable with a pregnancy, which would involve doctor and hospital visits.

We found for IUD use that COV-VACAV had significantly lower odds than COV-PRE while COV-VACNO did not significantly differ from COV-PRE. We found a different pattern for implant use where COV-VACNO significantly differed with higher odds

than COV-PRE while COV-VACAV did not significantly differ from COV-PRE. Previous research for the time period of the first year of the COVID-19 pandemic report variable findings, with some reporting lower percentages of IUD use and implants (7) while others report no differences in use (8,10) when compared to the pre-COVID-19 period. Our findings for IUD use in the first year of the pandemic are similar to those reporting no difference between pre-COVID-19 pandemic and the first year of the COVID-19 pandemic. However, our findings for implants differ from the above studies as we found increased use during the first year of the COVID-19 pandemic. We suggest that during the first year of the COVID-19 pandemic women were uncomfortable becoming pregnant due to concerns about contracting COVID-19 at physician or hospital visits. Women chose implant use because it has a longer active time for contraception impact than pills, patches, and injections which require more regular visits for additional prescriptions. Moreover, more clinicians may have recommended implant use since there would be less requirement for follow-up. Clinicians may also have thought that there may be decreased access to care due to shortages of healthcare providers from possible illness during the COVID-19 pandemic. In addition, once COVID-19 vaccines were available, there was an increased interest in becoming pregnant as the health risks associated with COVID-19 became better understood. This may be a reason for the decreased IUD use during the second year of the COVID-19 pandemic. Furthermore, placing an IUD may be considered invasive and may have been avoided or not preferred once other contraception options were available.

We did not find any differences among the time periods for BTL, pill, patch, injection, medical elective abortion, and surgical elective abortion. Previous research that included many contraception choices found increased use at the beginning of the COVID-19 pandemic as compared to pre-COVID-19 pandemic for pills, patches, and injections while there was no change in use for sterilization (8). Our findings for pills, patches, and injections differ from this pattern. These contraception options require more frequent healthcare visits. Patients at our hospital may have been concerned about contracting COVID-19 by attending such visits and did not choose to increase use of these options during the pandemic.

For both the categories “any contraception” and IUD, there were significantly greater percentages for follow-up visits during COV-VACNO and COV-VACAV than COV-PRE. Previous research reports that 72.9% attended a follow-up visit for an IUD (11). Our follow-up visit findings for all three time periods are much lower, and ranged from 29.5-39.0%. We suggest that there may have been cultural differences since the earlier study included mostly white race/ethnicity while our sample was mostly from those of non-white race/ethnicity. Regarding the higher follow-up rates for IUD in our sample during the COVID-19 pandemic as compared to pre-COVID-19 pandemic, we speculate that patients wanted to ensure that their IUD was working properly since they were very concerned about becoming pregnant during the pandemic. The reason for the high rates of follow-up for injections in all three time periods ranging from 53.9-60.4% is likely due to the need for short-term follow-up every 10-12 weeks and this may have been a concern that patients were aware of and did not ignore.

We found that those of black and Hispanic race/ethnicities had significantly higher odds for use of any contraception, injection, and implant than whites. However, those of black and Hispanic race/ethnicities had lower odds of IUD use than whites. Previous research reports that blacks had lower odds for use of any contraception when compared to whites (12). Our study differs from this pattern. A possible reason is that the previous study (12) included all types of contraception ranging from the least effective (e.g., condom), through moderately effective (e.g., injection), to highly effective (e.g., sterilization, IUD) while our study only included moderately and highly effective contraception use. Our study has positive findings in that there were no health disparities for the use of moderately or highly effective contraception among blacks and Hispanics. Instead, blacks and Hispanics choose better contraception use than whites.

DM had significantly decreased odds for any contraception use while no significant association with use of IUD, injection, or implant. GDM had significantly greater odds for use of any contraception, IUD, injection, and implant. Previous research reports no difference in any contraception use (both sterilization and reversible methods) between women with DM and gestational diabetes, while reversible contraception use was higher among those with gestational diabetes as compared to those with DM (13). Our findings differ for any contraception use but are similar for reversible contraception use. We suggest that the complications experienced during pregnancy among those with gestational diabetes are associated with patients being more cautious about becoming pregnant.

Telehealth use was low during all time periods and ranged from 1.5-4.8% and was lower during the COVID-19 pandemic as compared to pre-COVID-19 pandemic. Telehealth visits had a significant association with increased odds for use of any contraception and injection while there was no association with use of IUD or implant. Previous research reports a significant increase in obstetric and gynecologic telehealth visits during the first year of the COVID-19 pandemic as compared to pre-COVID-19 pandemic with negligible use pre-COVID-19 pandemic, ranging from 6.1-11.8%, depending upon the hospital location for the obstetrics and gynecology setting during the first year of the COVID-19 pandemic (14).

Our telehealth use findings differ from this pattern. We suggest that at our hospital healthcare workers during the COVID-19 pandemic focused on acute inpatient care and not on outpatient care, such as telehealth visits. For those telehealth visits that were for contraception, we suggest there were increased odds for injection since this approach required more follow-up visits and patients preferred telehealth to minimize possible contraction of COVID-19 because of in-person visits.

Study limitations

A study strength is the investigation of the time period when the COVID-19 vaccine was available. This study has several limitations due to the retrospective study design. We were unable to determine reasons why people chose a particular family planning method during a particular time period. We were also unable to ask people why they did not attend a follow-up appointment. Future research should study reasons for lower follow-up rates and identify interventions for improved follow-up rates.

Conclusion

We found that during the first year of the COVID-19 pandemic when COVID-19 vaccines were unavailable and the risks of contracting COVID-19 were not clear, there were significantly higher odds for use of implants. However, there was a different pattern during the second year of the pandemic when COVID-19 vaccines were available, with lower odds of any contraception use and IUD use. These findings highlight changes in behavior and preferences in terms of contraceptive concerns in an urban setting during a public health crisis that should be planned for by healthcare providers.

Ethics Committee Approval: *The study was ethically conducted, received New York City Health + Hospitals Institutional Review Board approval [approval number: BRANY IRB File # 23-12-003-378(HHC), date: 01.10.2023].*

Informed Consent: *A waiver for informed consent was obtained due to the retrospective nature of the study.*

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Evaluating the impact of *LHCGR* gene polymorphism on polycystic ovary syndrome: a comprehensive meta-analysis and power assessment

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Abstract

Objective: Polycystic ovary syndrome (PCOS) is prevalent among reproductive-aged women and is categorized by hormonal imbalances, irregular menstrual cycles, and challenges with fertility. PCOS affects approximately 3.6% of women globally, with prevalence varying by region. The luteinizing hormone/choriogonadotropin receptor *(LHCGR)* gene, which encodes the LHCGR, has been implicated in PCOS pathophysiology. This study investigated the association between the *LHCGR* gene polymorphism rs2293275 and PCOS through a meta-analysis.

Material and Methods: An extensive literature review was carried out using Embase, PubMed, and Google Scholar databases to identify research studies exploring the association between *LHCGR* gene variants and PCOS. The review was conducted based on the PRISMA checklist. Eligible case-control studies from 2016 to 2024 were chosen based on predefined criteria. Quantitative data analysis was performed using MetaGenyo software, employing a significance threshold of $p<0.05$. Odds ratios (OR) and confidence intervals (CI) were calculated to evaluate the relationships. G*Power 3.1 software was employed for statistical power analysis to assess the study's strength. The meta-analysis explored the link between *LHCGR* gene variant rs2293275 and PCOS across diverse ethnic groups and genetic models.

Results: Analyzing data from 10 studies involving 1,431 PCOS cases and 1,317 controls, the findings revealed no significant associations in most genetic models: allele (OR: 0.89, 95% CI: 0.54-1.49), dominant (OR: 0.74, 95% CI: 0.47-1.18), recessive (OR: 0.80, 95% CI: 0.41-1.57), and over-dominant (OR: 1.13, 95% CI: 0.69-1.85). Subgroup analyses by ethnicity (Arabs, Asians, Caucasians) consistently showed no significant correlations, except a protective effect in Caucasians (OR: 0.57, 95% CI: 0.34-0.95) in the AA vs. aa comparison. Sensitivity analyses confirmed robustness, and there was no indication of publication bias. Power analysis validated adequate sample sizes, and protein-protein interaction networks underscored biological relevance.

Conclusion: The meta-analysis concluded that no significant connection was observed between the *LHCGR* gene variant rs2293275 and the risk of PCOS among different populations. This suggests a complexity in PCOS etiology and indicating that *LHCGR* may not be a significant genetic marker for PCOS. Future research should explore other genetic and environmental factors contributing to PCOS, emphasizing the importance of genetic and ethnic variability in such studies. (J Turk Ger Gynecol Assoc. 2024; 25: 207-18)

Keywords: Polycystic ovary syndrome, *LHCGR* gene, genetic polymorphism, reproductive health and susceptibility

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Introduction

Polycystic ovary syndrome (PCOS) is commonly acknowledged as a predominant endocrine disorder and was initially described by Stein and Leventhal in 1935 (1). Mostly women of reproductive age (18-44 years) are affected by this very prevalent endocrine (2), metabolic, and reproduction condition. PCOS is mainly associated with hormonal disturbances that alter the menstrual cycle, thereby causing irregular monthly cycles and eventually affecting the fertility profile of women (3).

According to the World Health Organization estimates, 116 million women, approximately 3.6% of women globally, are in the reproductive age range and have PCOS. The global incidence of PCOS ranges from 2.2% to 26% (1). In India, the prevalence of PCOS varies between 3.7% and 22.5%, according to population studies and diagnostic standards (4). Based on the Rotterdam criteria, PCOS is identified as a diverse syndrome characterized by the occurrence of at least two of these criteria: persistent lack of ovulation or irregular ovulation; clinical or biochemical signs of excess androgens; and polycystic ovarian morphology which may be observed using ultrasound (5). This morhology is illustrated in Figure 1. Hyperandrogenism may halt folliculogenesis, leading to multifollicular morphology, which disrupts the menstrual cycle and causes anovulatory infertility (6).

The imbalance in female sex hormones causes the development of a cyst in the ovarian antral follicle. The cyst, an egg-containing sac filled with fluid, is ordinarily released during fertilization. The transformation of the egg into a functional cyst usually prevents the ovulation process. As a result of ovulation inhibition, the development of several cysts takes place, leading to amenorrhea (7). PCOS is often characterized by increased levels of testosterone and luteinizing hormone (LH) in the blood. In addition, individuals with PCOS commonly experience metabolic disruptions, including as insulin resistance, high levels of insulin in the blood, and irregularities in energy expenditure. A recent study indicated a 2 to 3 times higher likelihood of developing cardiovascular diseases and type 2 diabetes in PCOS-affected individuals in the later stages of their lives (8). The etiology and progression of type 2 diabetes mellitus are complex and include several environmental variables, including but not limited to physical inactivity, excessive dietary glucose intake, poor eating habits, smoking, alcohol use, obesity, and hereditary factors (9). A strong association between PCOS and obesity is known in the affected population of reproductive-aged females (10).

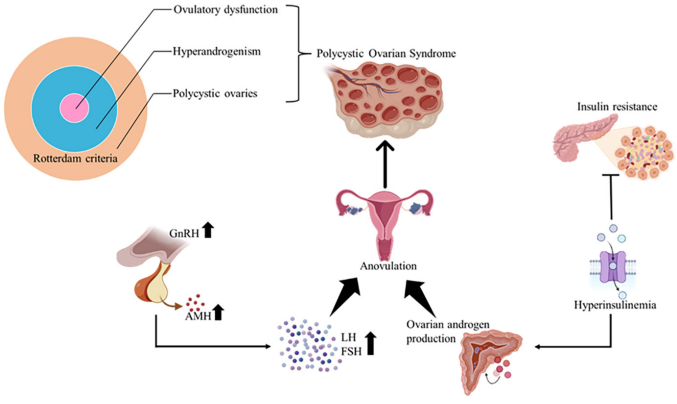

Figure 1. Pathophysiology of PCOS and the Rotterdam criteria
PCOS: Polycystic ovary syndrome, GnRH: Gonadotropin-releasing hormone, AMH: Anti-Mullerian hormone, LH: Luteinizing hormone, FSH: Follicle stimulating hormone

The underlying mechanisms of PCOS involve ovarian dysfunction influenced by external factors, such as the hypothalamic-pituitary-ovarian axis and hyperinsulinemia. Elevated levels of gonadotropin-releasing hormone lead to increased secretion of LH, affecting androgen production and ovulation. Insufficient feedback communication between the ovaries and the hypothalamic-pituitary unit exacerbates the suppression of gonadotropin secretion. Insulin resistance, induced by compensatory hyperinsulinemia, is identified as a major contributor to the development of symptoms of PCOS. Hyperinsulinemia promotes androgen production and reduces levels of sex hormone-binding globulin, resulting in hyperandrogenism and its associated clinical symptoms (11). This leads to an increased risk for the development of several gynecological cancers, including an elevated incidence rate of endometrial cancer (12). Among the gynecological cancers, cervical neoplasia is the second most common malignancy threatening women and is also associated with fertility issues among reproductive-aged women (13).

The luteinizing hormone/choriogonadotropin receptor *(LHCGR)* gene codes for the LH/choriogonadotropin receptor and is situated on chromosome 2p16.3, spanning over 70 kbp with 11 exons. This gene is mainly active in granulosa cells during the final phases of preovulatory follicles. Its primary role is to generate receptors for human chorionic gonadotropin (hCG) and LH (14). LH and hCG receptor functions allow the body to respond to these hormones functionally and structurally appropriately. In males, hCG supports Leydig cell growth in the testes, while LH prompts these cells to produce androgens. Androgens, like testosterone, are crucial for regulating male sexual development and reproductive processes (15).

The LHCGR protein functions as a receptor for glycoprotein hormones and is found in granulosa cells in the late stages of preovulatory follicles. LH stimulates the ovarian theca cells to produce testosterone, which is converted into estrogen. The expression of *LHCGR* during granulosa cell maturation allows the pre-ovulatory follicle to respond to the mid-cycle LH surge, leading to ovulation and the release of the mature egg cell (16).

Irregular *LHCGR* expression correlates with elevated LH levels, enlarged ovaries, infrequent menstrual cycles, and resistance to LH and/or hCG, all of which contribute to infertility. During pregnancy, women produce hCG, which promotes the continuation of the pregnancy (17).

The expression of *LHCGR* mainly occurs in the granulosa cells and theca of the ovary, and studies have shown increased expression in individuals with PCOS. When LH binds to its receptor LHCGR, a structural change activates the receptor, leading to signaling cascades involving cAMP and specific kinases. These signals regulate the expression of genes essential for steroid production. Furthermore, LH-induced activation of LHCGR plays a crucial role in follicle development and ovulation. Therefore, any genetic variation affecting the structure or function of the protein product of *LHCGR* could directly impact ovarian function and related conditions, such as PCOS (18).

Various studies have demonstrated that the *LHCGR* single nucleotide polymorphism (SNP) rs2293275 (p. Ser312Asn polymorphism) is significantly associated with women with PCOS. The fertility profile was also examined, revealing a notable correlation with infertile women with PCOS. In the Bulgarian population, a significant association was explicitly observed with infertile women (19). The Egyptian population also showed a significant association between PCOS and several anthropometric and biochemical characteristics, including an elevated free androgen index and hirsutism index (20).

The correlation of the rs2293275 variation with many cases of PCOS and the evident connection between *LHCGR* and reproductive signaling pathways indicates that it might constitute a fundamental and common mechanism contributing to the development of PCOS. Determining the practical implications of the *LHCGR* rs2293275 variation may clarify a typical reproductive phenotype that connects the etiology of various reproductive issues. Consequently, an extensive meta-analysis was conducted to determine the relationship pattern between *LHCGR* and PCOS.

Material and Methods

The study adhered to the PRISMA checklist, a recognized guideline for conducting meta-analyses and systematic reviews. Furthermore, the study's prospective review protocol (ID: 559449) was registered with the International Prospective Register of Systematic Reviews (PROSPERO), confirming the study's validity.

Literature analysis

The literature search for this study was conducted from January 1, 2014, to December 31, 2024, using the Google Scholar, Embase, and PubMed databases. The search focused on identifying relevant studies on the *LHCGR* gene, PCOS, polymorphisms, SNPs, rs2293275, and genetic variations. MeSH terms and commonly used keywords were combined with Boolean logic operators (AND, OR) to refine the search results. For instance, in PubMed, the search string included terms such as "*LHCGR* gene," "PCOS," "rs2293275," and "genetic variations." Filters were applied to include only studies published in English within the specified timeframe. The extensive search and exploration of the research articles were independently conducted by [Author 1 (Thomas)], and Author 2 (Veerabathiran) who independently screened the included articles, and any disagreements were resolved through discussion and consensus between the authors. The inclusion criteria focused on studies published in English that investigated the association between *LHCGR* gene polymorphisms, particularly rs2293275, and PCOS. Studies that did not meet these criteria or were published outside the designated search period were excluded. The complete search strategy for PubMed, including the detailed search string and filters used, is provided in the supplementary materials.

Criteria for inclusion and exclusion

A thorough assessment of the inclusion criteria ensured that the selected papers met the criteria for this meta-analysis. Specifically, studies employing case-control or cohort study design to investigate the association among the *LHCGR* genetic variants and PCOS and offering allelic and genotypic frequency data for determining 95% confidence intervals (CI) and odds ratios (OR), with corresponding p values were among the critical prerequisites for study selection. Female candidates aged 18-40 years were included in the studies, with a focus on their fertility profiles and hormonal ranges for data analysis. Infertile females and those with a family history of PCOS were also considered. The research considered hormonal profiles, with elevated hormone levels and irregular menstrual cycles indicative of the condition. The studies that reported adjusted estimates from the multivariable analysis were also included in this study to ensure the robustness of the findings.

Excluded studies included inadequate data or did not meet the following criteria. Using predetermined inclusion and exclusion criteria, we assessed the publications. For an article to be accepted, it had to fulfil two requirements: the study required using a case-control research design and the presentation of genotype frequencies for both cases and controls. Exclusion criteria included: studies using animal models or cell lines; case reports; no control group; or there was inadequate data.

Extraction of data

Based on predetermined criteria, the data were chosen from relevant publications, and the required data was then

meticulously acquired as described. A thorough analysis of the gathered articles was conducted to extract information regarding the genotypic and allelic frequencies among individuals in the case and control groups. The research was deemed ineligible if it failed to submit complete genotypic information, including allelic frequencies, or could not gather the information required from the patient and control groups. Every study used several data, including the Hardy-Weinberg equilibrium (HWE) value, language, initial author name, publication year, ethnicity, sample size, and PubMed ID.

Methodological quality evaluation using Hardy-Weinberg equilibrium and Newcastle Ottawa Scale scoring

The criteria employed to evaluate the quality of the chosen analysis were the Newcastle Ottawa Scale (NOS) and the HWE. A control genotype assignment was necessary to meet HWE (>0.05). The NOS rating, which has a maximum possible score of nine, considers three factors: relevance, equivalency, and selection. Studies with a score of six or above were included in this meta-analysis.

Statistical analysis

MetaGenyo software was used to set a statistical significance criterion of $p<0.05$ for all genomic changes during the data analysis. Specific protocols and resources are essential for conducting thorough meta-analyses on genetic interactions, evaluating genetic variations for potential therapeutic applications, ensuring rigorous significance testing in large-scale genetic studies, and maximizing statistical power. Previous research has used the Q statistic test, based on chi-square analysis, to interpret the heterogeneity assumption, as indicated by the I^2 metric. If the I^2 value was below 50, a fixed-effect model was applied to determine the OR and the 95% CI. Conversely, a random-effect model was employed if the I^2 value exceeded 50. The HWE technique used chi-square testing. We performed a subgroup analysis on the entire population to explore our analysis further. The sensitivity plot was also examined to determine the effects of leaving out individual studies, especially those where the controls deviated from the HWE. Egger's regression technique was applied to identify any possible publication bias.

Power analysis

Power analysis was performed on the metadata, using a 0.05 α error and a 95% CI. For the designated genes, the power of the sample size in each study-which included both case and control groups-was assessed separately. G*Power 3.1 was the program used to calculate power.

Protein-protein interactions

For the discovered SNPs linked with PCOS, the STRING (v11.0) online search tool database can predict functional proteins and protein-protein interactions (PPIs) with a minimum score of ≥0.4. STRING applies a minimum score criterion of ≥0.4 to signify the confidence level of projected interactions and aggregates large-scale experimental data, text mining, and computational predictions to gather information on protein interactions. STRING facilitates the visualization, interpretation, and analysis of intricate biological relationships by building networks of interacting proteins and offering functional annotations for proteins. Based on the predicted PPIs, it supports the identification of essential proteins, pathways, or molecular mechanisms associated with, in this case, PCOS through integrating various data sources and providing visualization tools, ultimately facilitating a deeper understanding of the condition's underlying biology.

Results

Search results

The literature search identified ten studies that examined the *LHCGR* gene, including information from 1,431 PCOS patients and 1,317 control participants. Once the articles were gathered, they were scrutinized to select the ones pertinent to this research and with important information. Figure 2 displays the research approach for *LHCGR*. Ten research studies examined the relationship between the collected data and PCOS severity, with ten of them focusing on the SNP rs2293275 polymorphism.

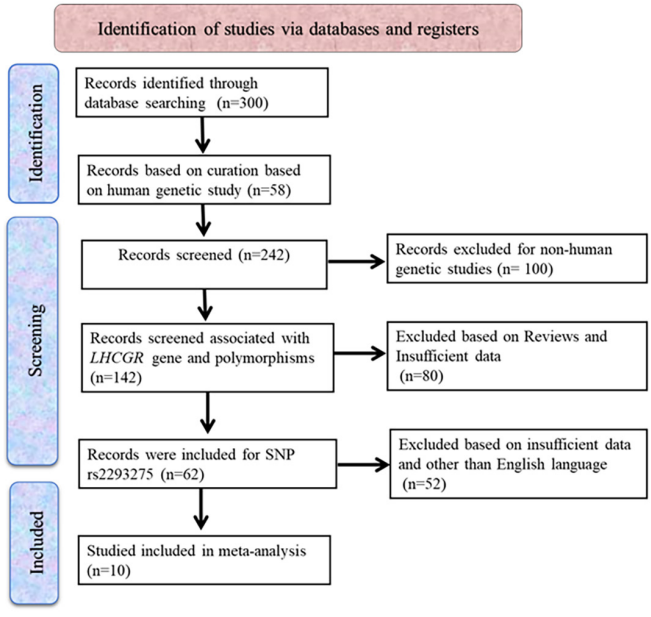

Figure 2. A flow diagram shows the overview of the study selection
LHCGR: Luteinizing hormone/choriogonadotropin receptor, SNP: Single nucleotide polymorphism

Table 1 presents the details of all the studies we looked at, including the traits of the controls and patients, to explore the connection between PCOS condition susceptibility and *LHCGR* polymorphisms. Participants in the 10 study projects were from various ethnic backgrounds (21-30).

Assessment of methodological quality

The methodological quality of the findings included in this meta-analysis was evaluated using the NOS and HWE criteria, including papers with a NOS score of six or higher guaranteed the selection of high-caliber research using dependable methods, lowering the possibility of bias. The NOS ratings primarily evaluate the research quality by reflecting varying degrees of methodological correctness among the included studies. Two studies out of the ten that were reviewed had an NOS score of six, indicating a moderate quality rating, and four studies received an NOS score of seven, indicating a somewhat superior methodological quality. Four research publications obtained an 8 on the NOS scale, signifying outstanding methodological excellence. These scores indicate that most of the studies meet the requirements in a good to exceptional manner for comparison, research group selection, and outcome evaluation. It is feasible to thoroughly examine the body of evidence and pinpoint the advantages and disadvantages of the research to this distribution. To preserve the accuracy of the genetic data and prevent biases, we only included and displayed in Table 1 studies whose control genotype distributions fulfilled HWE ($p>0.05$). These strict standards will improve the validity and robustness of the meta-analysis findings.

Quantitative data analysis

Ten studies were assessed to investigate the link between the rs2293275 polymorphism in the *LHCGR* gene and the propensity for PCOS. The results revealed no significant connection between PCOS risk and *LHCGR* polymorphisms. Based on the I^2 value, the models were obtained from random effect values. These models are the following: the allele model, G vs. A ($I^2=93\%$), with an OR of 0.89, 95% CI: 0.54-1.49, and $p=0.81$ and is depicted in Figure 3; the dominant model, GG + GA vs. AA ($I^2=55\%$), with an OR of 0.74, 95% CI: 0.47-1.18, and $p=0.03$ which is depicted in Figure 4; the recessive model, GG vs. GA + AA ($I^2=93\%$), with an OR of 0.80, 95% CI: 0.41-1.57, and $p=0.81$, shown in Figure 5; and the over-dominant model, GA vs. GG + AA ($I^2=86\%$), with an OR of 1.13, 95% CI: 0.69-1.85, and $p>0.05$, depicted in Figure 6.

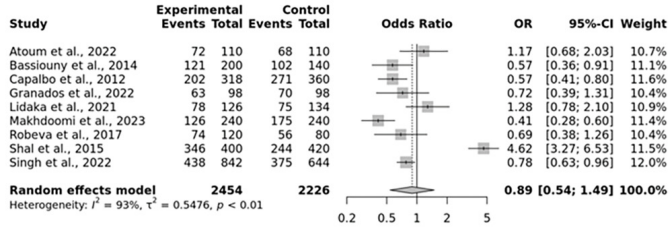

Figure 3. The forest plot showing an association between *LHCGR* gene polymorphism and PCOS in the allele model
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

Table 1. The characteristics of selected case-control studies of *LHCGR* (rs2293275) gene polymorphism and PCOS and HWE score

Author &year	Genotypic frequency						Allele frequency				Sample size		Ethnicity	NOS scoring	HWE
	Case			Control			Case		Control						
	GG	GA	AA	GG	GA	AA	G	A	G	A	Case	Control			
Atoum et al. (21), 2022	26	20	9	23	22	10	72	38	68	42	55	55	Arab	7	0.2575
Bassiouny et al. (22), 2014	47	27	26	42	18	10	121	79	102	38	100	70	Arab	8	0.0034
Capalbo et al. (23), 2012	63	76	20	103	65	12	202	116	271	89	159	180	Caucasian	8	0.6891
Alarcón-Granados et al. (24), 2022	20	23	6	23	24	2	63	35	70	28	49	49	Caucasian	7	0.1615
Lidaka et al. (25), 2021	26	26	11	22	31	14	78	48	75	59	63	67	Caucasian	7	0.6161
Makhdoomi et al. (26), 2023	10	106	4	58	59	3	126	114	175	65	120	120	Asian	8	0.0080
Robeva et al. (27), 2017	27	20	13	21	14	5	74	46	56	24	60	40	Caucasian	6	0.2918
El-Shal et al. (28), 2015	146	54	0	75	94	41	346	54	244	176	200	210	Arab	7	0.2424
Singh et al. (29), 2022	138	162	121	120	135	67	438	404	375	269	421	322	Asian	8	0.0131
Thathapudi et al. (30), 2015	59	124	21	22	155	27	242	166	199	209	204	204	Asian	6	0

LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, HWE: Hardy-Weinberg equilibrium, NOS: Newcastle Ottawa Scale

Subgroup analysis

The results of the meta-analysis provide a complex relationship between genetic variations and the desired outcome for various genetic models across several ethnic populations. The results for the allele comparison model (A vs. a) in the ten selected studies showed no substantial correlation, with an OR of 0.9476 (95% CI: 0.6025-1.4904, p=0.8158). Furthermore, non-significant relationships were found in subgroup analyses by ethnicity, with ORs for Arabs (1.4681, 95% CI: 0.3839-5.6145, p=0.575), Asians (0.7967-95% CI: 0.4170-1.5222, p=0.49), and Caucasians (0.7639, 95% CI: 0.5236-1.1146, p=0.16) all showing non-significant connections. Likewise, no significant correlations were seen overall (OR: 0.9249, 95% CI: 0.4804-1.7804, p=0.815) or among subgroups in the recessive model (AA vs. Aa + aa).

The analysis for Caucasians neared significance (OR: 0.6362, 95% CI: 0.3914-1.0340, p=0.0680); however, no substantial relationships were detected in subgroups in the dominant model (AA + Aa vs. aa). The total OR in the model was 0.8077 (95% CI: 0.5239-1.2454, p=0.33). An overall OR of 1.0306 (95% CI: 0.6502-1.6336, p=0.9) for the overdominant model (Aa vs. AA + aa) indicated no significant connection.

Except for the comparison of AA vs. aa in Caucasians, which revealed a substantial protective effect (OR: 0.5656, 95% CI: 0.3376-0.9474, p=0.0304), pairwise comparisons (AA vs. aa, AA vs. Aa, and Aa vs. aa) likewise generally showed no significant relationships within subgroups or overall. In many situations, heterogeneity was negligible across studies (I^2=0%), and no discernible publication bias was found (p>0.05 for the Egger's test). Therefore, the meta-analysis across different genetic models and ethnic subgroups did not reveal a substantial link between the genetic variants and the result, except for an essential protective finding in the pairwise comparison of AA vs. aa in the Caucasian subgroup. These results underscore the importance of considering genetic and ethnic heterogeneity in genetic association studies. Table 2 depicts the data included in the subgroup analysis.

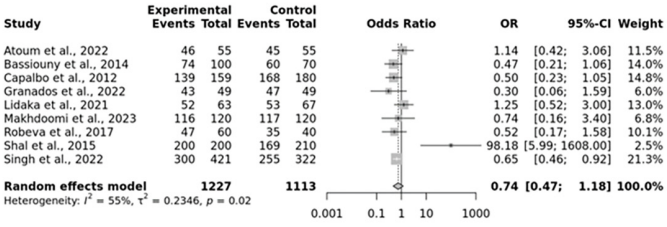

Figure 4. The forest plot showing an association between *LHCGR* gene polymorphism and PCOS in the dominant model
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

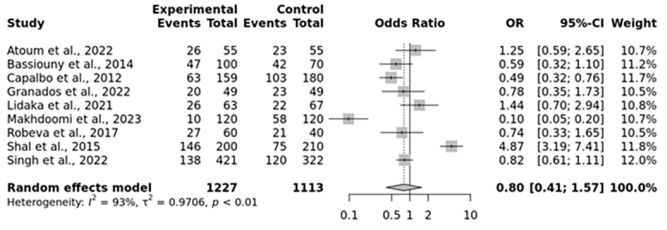

Figure 5. The forest plot showing an association between *LHCGR* gene polymorphism and PCOS in the recessive model
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

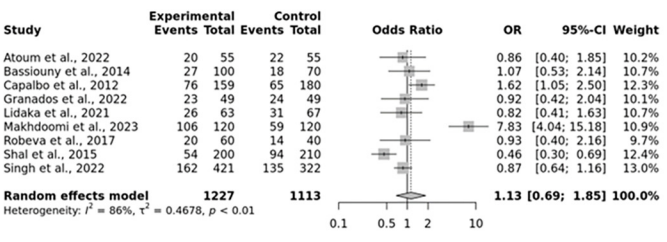

Figure 6. The forest plot showing an association between *LHCGR* gene polymorphism and PCOS in the over-dominant model
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

An examination of sensitivity analysis and publication bias

A sensitivity analysis examined the inconsistent findings from several investigations, particularly concerning departures from HWE. Research with intervention changes or non-compliance with HWE criteria was omitted from the analysis. As Figure 7 shows, removing these studies had no discernible impact on the final p-value. In addition, a funnel plot was used to detect publication bias and validate the results, and showed no evident bias, as illustrated in Figure 8.

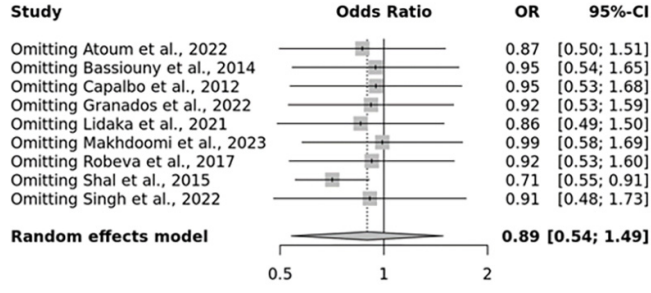

Figure 7. Sensitivity analysis was performed for *LHCGR* rs2293275 gene polymorphism among PCOS cases and controls
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

Power analysis and PPI interaction evaluation

A power analysis was conducted to assess the significance level of each research study related to the chosen SNPs. After conducting an inquiry, we discovered that the sample sizes in the examined literature, with an α error probability of 0.05, satisfied the necessary significance level. The outcomes of a two-tailed hypothesis test are displayed in the power analysis graph. The graph indicates that the hypothesis test is conducted at a significance level (α) of approximately 0.05, corresponding to a 95% confidence level, with a critical z-value of 1.95996. Thus, the graph underscores the need for careful study design to balance the risks of type 1 and type 2 errors, ensuring that the study has sufficient power to detect true associations while minimizing the risk of false positives. This sort of analysis (Figure 9) evaluates the probability of finding an effect of a certain magnitude under specific variables, such as sample size, effect size, and significance level. Table 3 provides specifics of the power analysis.

Twenty-one nodes and 131 edges in the PPI network were built using the STRING database. Its low PPI enrichment p-value was less than 1.0e-16, and its clustering coefficient was 0.814. Its average node degree was 12.5. This suggests that the proteins interact more than one could anticipate from a randomly chosen protein group of comparable size and distribution from the genome. Figure 10 shows the network of other genes and proteins associated with the *LHCGR* gene. Such enrichment suggests a degree of biological interconnectedness among the proteins. These findings also suggest a tightly integrated

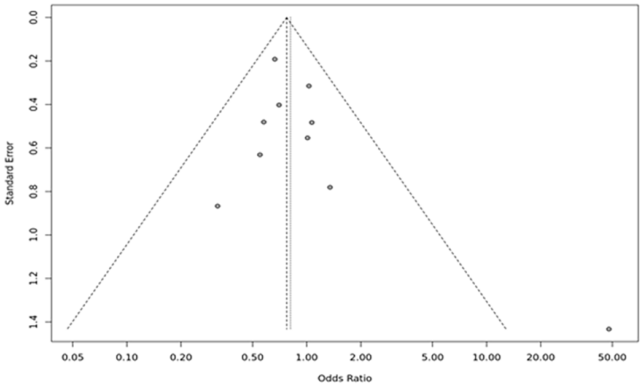

Figure 8. Publication bias in association between *LHCGR* gene polymorphism and PCOS in all models
LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome

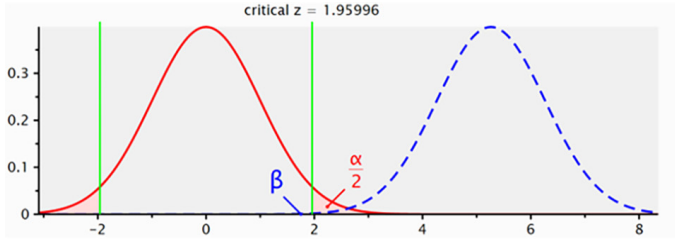

Figure 9. The graphical representation of a power analysis plot depicts how statistical power is affected by either the sample size or effect size in a two-tailed hypothesis test for the *LHCGR* rs2293275 gene polymorphisms
LHCGR: Luteinizing hormone/choriogonadotropin receptor, ß: Beta

Table 2. Subgroup meta-analysis of the association between *LHCGR* (rs2293275) gene polymorphism with PCOS susceptibility

Model	Ethnicity	Number of studies	Test of association			Test of heterogeneity			Publication bias
			OR	95% CI	p value	Model	p	I^2 value	p value (Egger's test)
Allele contrast (A vs. a)	Overall	10	0.9476	(0.6025; 1.4904)	0.815807	Random	0	0.9313	0.9062
	Arab	3	1.4681	(0.3839; 5.6145)	0.574787	Random	0	0.9627	0.3915
	Asia	3	0.7967	(0.4170; 1.5222)	0.491407	Random	0	0.9376	0.8021
	Caucasian	4	0.7639	(0.5236; 1.1146)	0.162334	Random	0.0709	0.5734	0.5142
Recessive model (AA vs. Aa + aa)	Overall	10	0.9249	(0.4804; 1.7804)	0.815211	Random	0	0.9301	0.6288
	Arab	3	1.5565	(0.3921; 6.1785)	0.529334	Random	0	0.9397	0.3813
	Asia	3	0.6606	(0.1370; 3.1859)	0.60547	Random	0	0.9657	0.8215
	Caucasian	4	0.7597	(0.4640; 1.2438)	0.274576	Random	0.0883	0.541	0.2612
Dominant model (AA + Aa vs. aa)	Overall	10	0.8077	(0.5239; 1.2454)	0.333777	Random	0.0099	0.5851	0.3368
	Arab	3	2.1265	(0.3296; 13.7177)	0.42765	Random	0.0012	0.8511	0.1027
	Asia	3	0.7726	(0.5768; 1.0349)	0.08365	Fixed	0.1331	0.5041	0.7065
	Caucasian	4	0.6362	(0.3914; 1.0340)	0.067985	Fixed	0.3103	0.1625	0.6232

Table 2. Continued

Model	Ethnicity	Number of studies	Test of association			Test of heterogeneity			Publication bias
			OR	95% CI	p value	Model	p	I^2 value	p value (Egger's test)
Overdominant (Aa vs. AA + aa)	Overall	10	1.0306	(0.6502; 1.6336)	0.898061	Random	0	0.8646	0.4151
	Arab	3	0.701	(0.3967; 1.2386)	0.221271	Random	0.0766	0.6107	0.2146
	Asia	3	1.4433	(0.4159; 5.0084)	0.563231	Random	0	0.9588	0.5195
	Caucasian	4	1.2005	(0.8798; 1.6380)	0.249145	Fixed	0.2846	0.2093	0.0776
pairw1 (AA vs. aa)	Overall	10	0.8412	(0.4311; 1.6416)	0.612205	Random	0	0.7975	0.5944
	Arab	3	2.6908	(0.3116; 23.2387)	0.368201	Random	0.0003	0.8787	0.0931
	Asia	3	0.7636	(0.1768; 3.2984)	0.717866	Random	0	0.9026	0.9837
	Caucasian	4	0.5656	(0.3376; 0.9474)	0.03036	Fixed	0.1272	0.4737	0.917
pairw2 (AA vs. Aa)	Overall	10	0.9623	(0.5251; 1.7635)	0.901082	Random	0	0.9063	0.5542
	Arab	3	1.5197	(0.5619; 4.1098)	0.409698	Random	0.0009	0.8566	0.2739
	Asia	3	0.6918	(0.1387; 3.4495)	0.653102	Random	0	0.9651	0.7459
	Caucasian	4	0.7368	(0.5300; 1.0243)	0.069237	Fixed	0.1496	0.4364	0.1519
pairw3 (Aa vs. aa)	Overall	10	0.7736	(0.6023; 0.9936)	0.044424	Fixed	0.1774	0.2904	0.2043
	Arab	3	1.7294	(0.3339; 8.9584)	0.513906	Random	0.0138	0.7667	0.0671
	Asia	3	0.7666	(0.5599; 1.0496)	0.097305	Fixed	0.3774	0	0.3608
	Caucasian	4	0.7085	(0.4220; 1.1895)	0.192387	Fixed	0.6308	0	0.287

LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

Table 3. Power analysis for *LHCGR* (rs2293275) gene polymorphism and PCOS

Gene	SNP	No. of studies	Cases	Controls	A- err prob	Power (1β err prob)
LHCGR	rs2293275	10	1431	1317	0.05	0.99955

LHCGR: Luteinizing hormone/choriogonadotropin receptor, PCOS: Polycystic ovary syndrome, SNP: Single nucleotide polymorphism, β: Beta

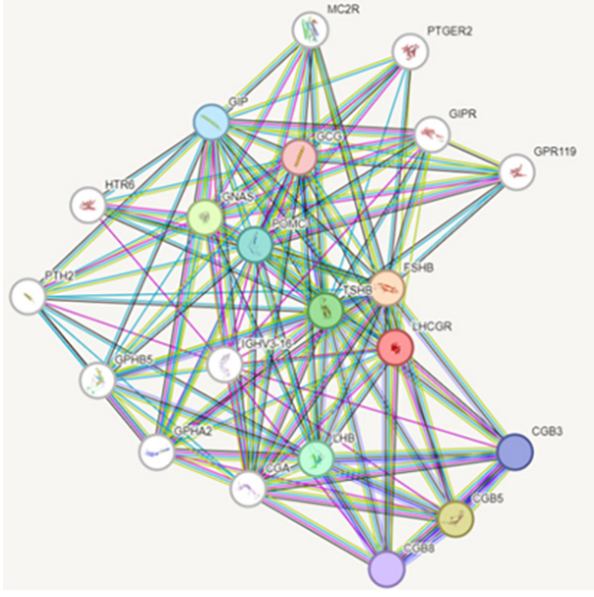

Figure 10. The protein-protein interaction network of differentially expressed genes of the selected gene associated with PCOS
PCOS: Polycystic ovary syndrome

network of proteins, indicating that the proteins in this network interact more extensively and are more interconnected than would be anticipated by chance. The PPI network reveals a substantial level of biological interrelationships among the associated proteins, underscoring the importance and complexity of their interactions and potential functional relevance in the context of the *LHCGR* gene.

Discussion

The *LHCGR* gene produces a G protein-coupled receptor that binds to both LH and hCG. In the ovary, the expression of *LHCGR* is central to the interaction of pre-ovulatory follicles and the mid-cycle LH surge, facilitating ovulation during the differentiation of granulosa cells. While women with inactivating *LHCGR* mutations may experience elevated LH levels and enlarged ovaries with irregular menstrual cycles, they do not typically exhibit significant reproductive issues, as seen in males with similar mutations who present with early puberty (31). Studies have associated *LHCGR* variations, particularly the rs2293275 SNP in exon 10, causing an S312N

amino acid change with PCOS. Although this SNP does not seem to affect glycosylation, its functional implications in PCOS remain unclear. Interestingly, unlike in males, where activating LHCGR mutations trigger early puberty and increased testosterone production, these mutations are not linked to ovarian hyperandrogenemia in women. This challenges the assumption that "hyper-responsive" *LHCGR* isoforms are associated with the dysfunction of theca cells (27).

There is strong evidence suggesting that PCOS has a genetic basis despite its diverse manifestations. Recent genome-wide association studies (GWAS) have identified multiple genetic regions associated with PCOS, strengthening the hypothesis of a likely oligogenic or polygenic model, although evidence suggests autosomal dominant inheritance. Environmental factors, incomplete penetrance, and epigenetic modifications require intricate efforts to understand the inheritance patterns fully. Despite advances in genetic research, the identification of definitive PCOS susceptibility genes remains limited. Though much research has been done on candidate gene connections, only a few have shown consistently reproduced statistically significant relationships (32).

The study conducted by Branavan et al. (33) showed no substantial link between the *LHCGR* gene and PCOS patients. This study was conducted in Sri Lankan women of 16-19 years of age, and genotype associations were analyzed using amplification-refractory mutation system - polymerase chain reaction. Although this study did not show any association between genetic polymorphisms and the *LHCGR* gene, in contrast, various other studies have shown a substantial correlation between the *LHCGR* genetic variant and PCOS, some reporting that the *LHCGR* gene was strongly associated with PCOS, thereby playing an essential part in the pathophysiology of PCOS (33). Capalbo et al. (23) reported a substantial correlation between Sardinian PCOS patients and the *LHCGR* gene. This study identified the *LHCGR* gene as a functional solid candidate for susceptibility to PCOS, showing the highest relative risk among specific genotypes. The findings suggest that assessing the *LHCGR* genotype, particularly the 312N allele, in PCOS patients and their family members could be valuable (23).

Based on the studies conducted, an association between the *LHCGR* gene and PCOS was demonstrated through GWAS analysis (34,35). The initial report found that granulosa and theca cells from PCOS patients expressed higher levels of *LHCGR* compared to normal control cells. In a subsequent study, using data from the Gene Expression Omnibus (GEO) database to confirm their findings, the authors identified an increased expression of *LHCGR* in cumulus cells from women with PCOS, regardless of obesity status. Women diagnosed with PCOS, especially those who were not overweight, demonstrated heightened secretion of LH from the pituitary gland, enhanced bioactivity of LH, and increased androgen production in response to LH stimulation. The researchers proposed that increased receptors due to LHCGR overexpression led to heightened androgen production from the theca cells, thereby increasing ovarian sensitivity to LH. Moreover, in non-obese women with PCOS, there was evidence of reduced methylation and increased expression of *LHCGR* in adipose tissue, suggesting a consistent gene regulatory profile across different tissues. To validate their finding of *LHCGR* overexpression as a unique feature of PCOS, the researchers analyzed GEO datasets that allowed stratification, based on traits such as obesity and insulin sensitivity. They found no differences in *LHCGR* expression between lean and obese subjects in three adipose GEO datasets stratified by obesity or in three datasets stratified by insulin sensitivity. These findings suggest that the observed variations in *LHCGR* expression are specific to PCOS and not merely a result of metabolic differences within the cohort.

LHCGR genetic polymorphisms and PCOS did not correlate in the current investigation. The I^2 value surpassed 50%, indicating no substantial linkages at the allele level, recessive relationships, over-dominant associations, or dominant associations between the *LHCGR* genetic variant and PCOS. Therefore, these results suggest that the *LHCGR* gene polymorphism and PCOS symptoms are unrelated. A NOS rating was used to select studies with high-quality methodologies and guarantee high-quality research to minimize the possibility of bias. The study concluded that the *LHCGR* gene polymorphism was congruent with the HWE value principle. Both Egger's test and a funnel plot were used to evaluate publication bias, but neither revealed any bias. The PPI network indicates that the proteins show more interactions than one would anticipate from a haphazardly chosen set of proteins with comparable sizes and genomic distributions. The degree of biological interconnectedness between the proteins and the network of other genes and proteins that share the *LHCGR* gene is shown by this enrichment.

This meta-analysis examined various genetic models and ethnic subgroups, revealing no significant association between genetic variants and outcomes, except for a notable finding in comparing AA vs. aa within the Caucasian subgroup. These findings underscore the importance of accounting for genetic and ethnic diversity in genetic association studies. PCOS susceptibility may be influenced by the *LHCGR* polymorphism across different populations, where its impact could vary due to diverse genetic backgrounds, environmental exposures, and lifestyles. These factors, serving as confounding variables across broader populations, may weaken the observed associations. However, the link becomes more apparent and

detectable in more homogeneous subgroups with reduced variability in these factors. In addition, interactions with other genetic or hormonal factors prevalent in these subgroups could enhance the biological significance of the *LHCGR* polymorphism. The subgroup analysis highlighted a significant relationship, emphasizing the need for further investigation into how specific factors, such as genetic diversity, environmental influences, hormone levels, and lifestyle choices amplify the impact of the *LHCGR* polymorphism on PCOS susceptibility. The statistical data strongly supports these conclusions, with rigorous data extraction and analysis methods ensuring reliable study outcomes.

This study advances our understanding of the genetic underpinnings of PCOS, potentially contributing to improved diagnosis and treatment strategies in the future. Despite inconsistent results addressing the minor relationship between *LHCGR* gene polymorphisms and PCOS risk due to the inadequate and short sample size, our investigation emphasized the need to investigate the process of *LHCGR* genetic variation. Recognizing genetic indicators could aid in early detection, personalized treatment, and risk assessment. Ultimately, we believe our research contributes to elucidating the connection between the risk of PCOS and *LHCGR* gene polymorphisms, highlighting the growing importance of addressing these challenges.

Study limitations

This study is subject to several limitations. Variations in different populations may impact outcomes differently. First, various populations may have distinct genetic origins, and although ethnicity-based subgroup analysis was attempted, it could not include all ethnic groups. This restriction emphasized how important it is to carry out more inclusive research encompassing a more comprehensive range of genetic varieties. Furthermore, publication bias is a risk, which might distort the overall results since research with unfavorable outcomes can be under-represented. Therefore, the subgroup analysis based on ethnicity could not encompass all ethnic groups. Another significant limitation was the relatively small sample sizes in some of the included studies, which may reduce the statistical power and limit the generalizability of the findings. Small sample sizes can also contribute to more significant variability and uncertainty in the results. Furthermore, the reported relationships between *LHCGR* gene variants and PCOS may have been impacted by confounding factors, which may not have been adequately controlled across investigations. The study's emphasis on specific demographics further restricts the generalizability of findings. Subsequent investigations must bridge these gaps by executing extensive, multi-ethnic investigations encompassing heterogeneous populations and accommodating plausible confounding factors. Understanding the temporal link between *LHCGR* gene variants and the onset of PCOS might also benefit from longitudinal research. Furthermore, investigating gene-environment interactions and broadening the study scope to encompass a greater variety of genetic variants may contribute to a more thorough knowledge of the genetic foundation of PCOS. Recognizing these constraints underscores the necessity for more extensive research with larger sample sizes to enhance our understanding of the role of *LHCGR* gene variations in PCOS.

The implications of this study on the correlation between *LHCGR* gene polymorphisms and PCOS have significant ramifications, particularly in genetics and personalized medicine. Understanding how *LHCGR* variations contribute to susceptibility to PCOS may lead to the development of more precise diagnostic techniques, enabling early detection of high-risk individuals. Furthermore, a more detailed study could pave the way for tailored treatments that address the genetic aspects of PCOS, potentially improving therapeutic outcomes. By identifying these correlations, healthcare professionals can adopt a more personalized approach to managing PCOS, tailoring prevention and treatment strategies to each patient's unique genetic profile. Future studies need to be conducted to explore the genetic underpinnings of PCOS, fostering advances in precision medicine and improving patient outcomes.

Conclusion

This study used a thorough meta-analysis spanning many ethnic backgrounds to investigate the relationship between a single *LHCGR* gene polymorphism, rs2293275, and PCOS. Except for a small finding in the Caucasian subgroup, our study revealed no substantial correlation between this polymorphism and PCOS across various genetic models and ethnic groupings, despite several reports pointing to a connection. The robustness of the conclusions was underscored by adherence to strict methodological criteria. The results demonstrated the complexity and variety of PCOS, indicating that more research is required to identify additional genetic and environmental variables contributing to the syndrome, even though the *LHCGR* gene may not be a significant marker for PCOS risk. This work adds to the continuing efforts to understand and manage PCOS better and highlighted the need to take genetic and ethnic heterogeneity into account in genetic association studies.

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Testing the role of unstimulated in vitro maturation for potential development of immature oocytes in women with oocyte maturation abnormalities

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Abstract

Objective: The aim of this study was to investigate the developmental potential of immature oocytes and evaluate whether unstimulated in vitro maturation (IVM) could serve as a treatment option for women with oocyte maturation abnormalities (OMAs).

Material and Methods: This cohort study was conducted between September 2019 and December 2022, and included women who underwent unstimulated, non-human chorionic gonadotropin (hCG) priming IVM. Oocytes were incubated with IVM medium for 26-48 hours and evaluated to compare their maturation profiles with the immature oocytes retrieved from the same patients in their previous in vitro fertilization cycles.

Results: Among the twelve women in the study, eleven (91.6%) underwent whole exome sequencing analysis. Of these, 18 variants were identified in 10 individuals, excluding case 1, who had no previous mutation analysis. Of the mutations identified, 9 (50%) were located in *FSHR*, 5 (27.8%) in *TUBB8*, 1 (5.6%) in *ZP1*, 1 (5.6%) in *SLFN14*, 1 (5.6%) in *AR*, and 1 (5.6%) in *STEAP3*. Apart from one woman with resistant ovary syndrome (ROS), none treated with unstimulated IVM had oocyte maturation. Remarkably, the only patient to achieve oocyte maturation in an unstimulated IVM cycle was case 11, who had ROS and a single *FSHR* variant.

Conclusion: Unstimulated, non-hCG primed IVM does not appear to be effective in the treatment of OMAs, perhaps with the exception of women with ROS. However, this study led our team to develop novel treatment options based on physiological mechanisms for some subtypes and supraphysiological approach for other subtypes of OMAs. (J Turk Ger Gynecol Assoc. 2024; 25: 219-23)

Keywords: Oocyte maturation arrest, oocyte maturation abnormalities, unstimulated in vitro maturation, mutation

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Introduction

Recurrent immature oocyte retrieval in at least two consecutive in vitro fertilization (IVF) cycles is defined as oocyte maturation abnormalities (OMAs) (1). OMAs were initially described by Rudak et al. (2) as an oocyte factor in four cases. Subsequently, Levran et al. (3) reported different types of OMAs in eight women with unexplained infertility. Hourvitz et al. (4) defined OMA as “bad egg syndrome” and reported the first pregnancies from women with genuine empty follicle syndrome (G-EFS). Beall et al. (5) and Hatirnaz et al. (6) further defined the subtypes of OMA by excluding G-EFS, resistant ovary syndrome

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(ROS), and premature ovarian failure (POF), and included only the cases with intrinsic, that is mutation-related, factors. Galvão et al. (7) reported the first pregnancies from ROS cases through in vitro maturation (IVM) in their report.

Recent publications have provided support for the notion that OMAs extend beyond OMA. The spectrum of OMAs has been expanded to include oocyte degeneration, oocyte dysmorphism, EFS, ROS, certain forms of premature ovarian insufficiency or POF, zygotic cleavage failure, and early embryonic arrest (1,8,9).

The objective of this study was to investigate the developmental potential of oocytes obtained through unstimulated, unprimed IVM from women with OMA.

Material and Methods

This retrospective cohort study was conducted between September 2019 and December 2022, and it included women who underwent unstimulated, non-human chorionic gonadotropin (hCG) priming IVM. The study received approval from the Ethical Committee of Medicana Samsun International Hospital (approval number: 7159, date: 27.12.2021). Written informed consent was obtained from all women with OMAs for all procedures. These procedures were recorded in their respective files.

The selected patients for this study had a history of recurrent OMAs in at least two IVF cycles. Patients were selected to cover the spectrum of OMAs as far as possible. However, patients with G-EFS were not included in the evaluation during the study period, despite the fact that IVM is considered the gold standard treatment for G-EFS.

The primary objective of this study was to demonstrate the developmental potential of oocytes in unstimulated IVM procedures and to evaluate the distribution of mature and immature oocytes after the IVM process.

Unstimulated IVM refers to the IVM of oocytes retrieved from natural cycles, with or without the use of an hCG trigger, as defined by Dahan et al. (10). In this study, we used unstimulated IVM (without an hCG trigger) to assess the developmental potential of immature oocytes obtained from women with OMAs. The laboratory procedures employed for OMAs in this study followed the standard protocols used in previous IVM studies (11).

Statistical analysis

The data in the study were analyzed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp, Armonk, NY, USA). In the tables, the quantitative data are presented as mean ± standard deviation and median (minimum-maximum) values, and the categorical data as number (n) and percentage (%). Mann-Whitney U test was used to compare the independent groups, and Pearson's chi-square test and Fisher's exact test to compare the categorical variables. Data were determined at the 95% confidence level, and a $p<0.05$ was accepted as statistically significant.

Results

During the study period, a total of 12 women were enrolled for unstimulated IVM after previous unsuccessful IVF cycles. Table 1 presents the demographic, laboratory, and clinical data of the patients.

Patients with OMAs, ranging from necroptosis to *TUBB8* mutation, were enrolled in the study. Among these twelve, eleven (91.6%) underwent whole exome sequencing (WES) analysis. Of these eleven, 18 variants were identified in ten women. The exception was, case 1 who had no previous mutation analysis. Interestingly, case 6, who had a history of necroptosis, did not show any detected mutations (8.3%) in the WES analysis.

Of the mutations identified, 9 (50%) were in *FSHR*, 5 (27.8%) in *TUBB8*, 1 (5.6%) in *ZP1*, and 1 (5.6%) each in *SLFN14*, *AR* and *STEAP3*. Case 4, who exhibited variable oocyte dynamics and an unclassified form of OMAs, had two *FSHR* mutations. Similarly, case 7, experiencing metaphase I-metaphase II (MI-MII) arrest, also presented two *FSHR* mutations. In cases 5 and 8, two *FSHR* mutations and one *TUBB8* mutation were detected, respectively. Case 2, who experienced MI arrest in previous IVF attempts, showed both an *AR* and a *TUBB8* mutation.

Table 1. Demographic, laboratory, and clinical data of patients[a]

	Patients (n=12)
Female age, years	32.25±4.11
Male age, years	34.50±5.46
Time of marriage, years	6.87±4.34
Infertility duration, years	6.41±4.40
BMI, kg/m^2	38.70±7.47
Basal serum FSH, IU/L	16.86±5.68
Basal serum LH, IU/L	12.47±6.12
Basal serum estradiol, pg/mL	59.49±50.55
Basal serum progesterone, ng/mL	0.59±0.36
Basal serum TSH, mU/mL	1.43±0.43
Basal serum AMH, ng/mL	2.54±1.77
Basal serum prolactin, ng/mL	20.54±13.43
AFC	12.58±7.6

[a]Data are given as mean±standard deviation. BMI: Body mass index, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone, AMH: Anti-Müllerian hormone, AFC: Antral follicle count

Table 2. Distribution of mutations in twelve women with OMAS

Patients	Diagnosis (OMAS)	Mutation 1	Gene 1	Nucleotide 1	Mutation 2	Gene 2	Nucleotide 2	Mutation 3	Gene 3	Nucleotide 3
Case 1	MI arrest	Not analyzed	-	-	-	-	-	-	-	-
Case 2	MI arrest	TUBB8	*Exon 4*	C.400C>T	AR	*Exon 4*	C.1913A>G	-	-	-
Case 3	GV arrest/zonafree	ZP1	*Exon12*	C1775-3C>A	-	-	-	-	-	-
Case 4	GV-MII arrest	FSHR	*Exon 10*	C.919G>A	FSHR	*Exon 10*	C.2039G>A	-	-	-
Case 5	GV-MI arrest	TUBB8	*Exon 4*	C.928T>C	FSHR	*Exon 10*	P.5680N	FSHR	*Exon 10*	P.A307T
Case 6	Gv arrest/necroptosis	Normal genome	-	-	-	-	-	-	-	-
Case 7	MI-MII arrest	FSHR	*Exon 10*	C.919G>A	FSHR	*Exon 10*	C.2039G>A	-	-	-
Case 8	Mixed arrest	TUBB8	*Exon 4*	C.721C>t	FSHR	*Exon 10*	P.5680N	FSHR	*Exon 10*	P.A307T
Case 9	GV arrest/zonafree	TUBB8	*Exon 4*	C.959G>A	-	-	-	-	-	-
Case 10	POF	SLFN14	*Exon 3*	C.1513A>T	STEAP3	*Exon 4*	C.907G>A	-	-	-
Case 11	ROS	FSHR	*Exon 10*	C.1412T>G	-	-	-	-	-	-
Case 12	Mixed arrest	TUBB8	*Exon 4*	C.535G>A	-	-	-	-	-	-

GV: Germinal vesicle, MI: Mitosis I, MII: Mitosis II, POF: Premature ovarian failure, ROS: Resistant ovary syndrome, TUBB8: Tubulin beta 8B, ZP1: Zona pellucida glycoprotein 1, FSHR: Follicle-stimulating hormone receptor, SLFN14: Schlafen family member 14, AR: Androgen receptor, STEAP3: Six-transmembrane epithelial antigen of prostate 3-metalloreductase

Remarkably, the only patient to achieve oocyte maturation in an unstimulated IVM cycle was case 11, who had ROS and a single *FSHR* mutation. On the other hand, case 10, the patient with POF and very small preantral follicles resistant to previous IVF attempts did not yield any oocytes in the unstimulated IVM cycle and harbored *SLFN14* and *STEAP3* variants.

Lastly, case 3 and case 9, diagnosed with germinal vesicle (GV) arrest and a zonafree phenotype had only one *ZP1* and *TUBB8* mutation, respesctively, while case 12 exhibited only a *TUBB8* mutation in the WES analysis. Table 2 provides a summary of the distribution of these mutations.

Discussion

This study revealed that immature oocytes obtained from women with OMAs exhibit limited developmental potential. In addition, it was observed that the distribution of immature oocytes in IVF cycles showed more progress compared to unstimulated, non-hCG primed IVM. These findings suggest that ovarian stimulation, whether mild or standard, might have a positive impact on the developmental potential of oocytes in women with OMAs.

The initial report by Rudak et al. (2) in 1990 was significant as it expanded the understanding of OMAs beyond the conventional classifications of GV, MI, and MII arrest studied and classified by Beall et al. (5) and Hatirnaz et al. (1). Since then, recent studies have explored the genetic variants that contribute to a wider spectrum of OMAs (5,8,12).

Levran et al. (3) conducted a study on OMAs in ICSI cycles involving eight women with history of unexplained infertility. The study identified one case of GV arrest, four cases of MI arrest, and three cases of MII arrest, which were categorized under the term “oocyte factor”. Furthermore, the study also noted atypical findings, such as one MI arrest case with four GV and 17 MI oocytes, and one MII arrest case with 13 MII and 2 MI oocytes, which did not fit into the conventional classification of oocyte maturation profiles.

The golden era of IVM was between 2000 and 2013 until when the ASRM practice committee released an opinion paper that IVM was an experimental procedure (ASRM 2013 Practice Committee). Within that time frame, many studies were conducted on the use of IVM in other indications and many treatment modalities were used rather than unstimulated IVM (13).

The first papers related to the use of IVM in women with OMAs were published in 2010 (4,5). Hourvitz et al. (4) reported on seven women who had experienced three failed IVF attempts due to “oocyte-related factors” before undergoing scheduled IVM cycles. In these cases, all women received FSH-hCG primed IVM cycles prior to oocyte retrieval, and the Canadian IVM protocol was followed for clinical and laboratory procedures (14).

Notably, this report was the first to include EFS as part of the spectrum of OMAs.

Beall et al. (5) introduced the first classification system for OMA, which was derived from analysis of previous case reports and animal studies. This classification system categorized OMAs into four distinct subtypes: type 1, characterized by GV arrest; type 2, denoting MI arrest; type 3, representing MII arrest; and type 4, encompassing mixed arrest (5). The present study primarily focused on intrinsic factors associated with OMAs and did not include other etiological factors related to OMAs. Subsequently, Hatirnaz et al. (6) recognized the limitations of the Beall et al. (5) classification system and so developed the Hatirnaz et al. (6) and Dahan et al. (10) definition system. This novel classification excluded other factors related to OMAs in their subsequent studies and reported outcomes of FSH-hCG primed IVM cycles. However, their studies did not report any clinical pregnancies, and the oocyte maturation profiles did not significantly differ from the previous IVF cycles of the same women (6).

Galvão et al. (7) conducted a study including 28 patients who underwent 49 IVM cycles to evaluate the impact of IVM in patients with ROS and women with deficient oocyte maturation. Among them, nine patients had ROS and underwent 24 IVM cycles. From these cycles, 23 cleavage-stage embryos were obtained, and eight patients achieved pregnancy, resulting in five healthy live births. The remaining 19 patients had OMAs and underwent 25 IVM cycles. In 11 of these cycles, oocyte retrieval was not successful. In 10 cycles, mature oocytes were retrieved, but fertilization failed after ICSI. Fertilization occurred in only four of the OMAs cycles, resulting in a single good quality embryo transfer, which ultimately led to a negative beta-hCG test. No live births were reported among the OMAs cases. Based on their findings, IVM may be a valuable option for women with ROS. However, they recommended caution and further improvements in the procedure before considering IVM as a suitable option for women with OMAs.

Prior to 2016, research on genetic mutations associated with OMAs primarily relied on animal studies. However, significant advances have been made since then in understanding the mechanisms and phenotypical characteristics of human genetic mutations linked to OMAs. This progress is evident in the growing number of publications focusing on human genetic variants associated with OMAs (9,15,16).

The results of mutation analysis have provided insights into the genetic basis of various forms of OMAs. Interestingly, certain severe forms of OMAs, such as GV arrest and oocyte degeneration, as well as necroptosis, have been found to have no detectable mutations. Conversely, some cases of POF/primary ovarian insufficiency have been associated with significant mutations, while others exhibit no detectable mutations at all. These findings have led to a re-evaluation of the spectrum of OMAs, and all forms of OMAs, including oocyte degeneration, EFS, ROS, and both classified and unclassified OMA, have been combined and classified as OMAs (8). Furthermore, Sang et al. (9) expanded the spectrum of OMAs in their mutation study by including zygotic cleavage failure and early embryonic arrest as additional components of OMAs.

Gulekli et al. (17) published a case report focusing on two women who had experienced MI arrest in their previous IVF cycles. In their clinical practice, they used unstimulated IVM as an alternative approach to address OMA but failed to achieve oocyte maturation. As a result, they concluded that the application of unstimulated IVM did not lead to significant outcomes for patients with OMAs (17). It is important to note that the cases included in their study specifically involved MI arrest, which is recognized as a particularly challenging subtype among OMAs cases.

This study aimed to evaluate the potential competence of oocytes from women with OMAs and to assess the role of ovarian stimulation in their development. We found that unstimulated IVM was only effective for patients with ROS. During the study, we made some valuable observations, including the observation of zona pellucida covering the oocytes of women with zona-free oocytes due to *TUBB8* mutation. We also found that in women with *TUBB8* mutation, we were unable to progress immature oocytes to MI and MII stages. Furthermore, in one woman with POF, we were not able to retrieve any oocytes both in unstimulated and letrozole primed IVM cycles. Although the results of this study do not provide any value for clinical use in OMAs, this study inspired the us to develop novel treatment options to overcome OMAs.

Study Limitations

The retrospective design and the small sample size are significant limitations of this study. The study team halted the study after realizing that unstimulated IVM was not effective in many cases, but this is the first study to examine the use of unstimulated IVM in women with different subtypes of OMAs. This experience has provided important insights into the limitations of unstimulated IVM for women with OMAs. Furthermore, this led the team to develop both physiological and supraphysiological IVM treatment modalities for women suffering from OMAs.

Conclusion

Unstimulated IVM does not appear to be an effective therapeutic option for women with OMAs. However, it is important to note that the study has limitations but it is stil the

first study to examine the use of unstimulated IVM in women with OMAs. Moreover, the study has provided the stimulus for us to develop of promising new treatment options.

Ethics Committee Approval: *The study received approval from the Ethical Committee of Medicana Samsun International Hospital (approval number: 7159, date: 27.12.2021).*

Informed Consent: *Written informed consent was obtained from all women with OMAs for all procedures.*

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

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Effectiveness of first trimester maternal fat tissue measurement in prediction of gestational diabetes: a prospective cohort study

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Abstract

Objective: The aim was to find a cost-effective, more practical method to be used in the early gestational weeks as an alternative to the oral glucose tolerance test (OGTT) for predicting gestational diabetes mellitus (GDM). The method selected was adipose tissue measurements made in the first trimester.

Material and Methods: The study was designed as a prospective, cohort study. Ultrasound images were used to calculate abdominal visceral (VAT) and subcutaneous adipose tissue (SAT) thicknesses of the first trimester pregnant women. Two groups were formed: those who were diagnosed with GDM and those who were not, based on the results of the OGTT performed in the same patients at 24th-28th weeks of gestation. Ultrasonographic records were examined and compared between these two groups using received operator characteristic curves and logistic regression analyses.

Results: A total of 292 pregnant women were included, of whom 21.2% were diagnosed with GDM. In the group diagnosed with GDM, SAT, VAT and total adipose tissue (TAT) values were significantly higher than the women who did not have GDM. Threshold values for SAT, VAT and TAT were 18 mm, 55 mm and 55 mm.

Conclusion: First trimester SAT, VAT and TAT measurements of pregnant women with GDM were significantly higher than those without GDM diagnosis. Although our results showed that adipose measurements cannot be an alternative to OGTT; they may be a powerful aid in identify at-risk pregnant women, suggesting to perform an early OGTT in the first trimester. (J Turk Ger Gynecol Assoc. 2024; 25: 224-30)

Keywords: Gestational diabetes, subcutaneous adipose tissue, visceral adipose tissue, first trimester screening

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Introduction

Gestational diabetes mellitus (GDM) is a serious public health problem that can cause adverse perinatal complications (1,2). Therefore, standardized screening, diagnosis and treatment for GDM are also important. Classically, screening has been performed between the 24th and 28th weeks in pregnant women who showed no evidence of glucose intolerance in the early pregnancy period. However, there is no consensus on the optimal approach among national and international organizations, and the choice often depends on local choices.

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Maternal obesity has been reported to negatively affect the early and late prognosis of mother and fetus (3). The World Health Organization (WHO) classification defines a body mass index (BMI) above 30 as obesity (4). However, maternal obesity does not affect every mother and fetus to the same extent. Furthermore, it is thought that the distribution of fat around the body may be more important than the total fat mass in terms of risk factors associated with obesity. Abdominal fat storage is more strongly linked to metabolic diseases (5,6).

Studies have found that central fat storage was more closely correlated with perinatal diseases, such as preeclampsia, GDM, and preterm birth, compared to peripheral lipidosis (7-10). In non-pregnant women, increased abdominal adipose tissue was associated with an increased risk of diabetes, atherosclerosis, dyslipidemia, and metabolic syndrome (11,12).

Abdominal adipose tissue has two compartments, namely visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). Some previous studies have shown that visceral and subcutaneous fat tissue measurements can provide early predictions regarding glucose intolerance, metabolic syndrome and insulin resistance (13-15). However, it is not yet clear which fat compartment increase is associated with the development of GDM in pregnant women.

The aim of this study was to examine the effectiveness of first trimester maternal SAT, VAT and total adipose tissue (TAT) measurements and the ratios of these measurements, in predicting GDM in the early period, and whether they can be used as an alternative to oral glucose tolerance test (OGTT).

Material and Methods

This was designed as a prospective cohort study. Ethics committee approval was obtained from the Ethics Committee of University of Health Sciences Turkey, Prof. Dr. Cemil Taşçıoğlu City Hospital (approval number: 14, date: 31.01.2022). Based on the prevalence of GDM in society, the sample size required to investigate the role of adipose tissue thickness in predicting GDM was calculated to be 225 pregnant women (two sided α=0.05, power=95%). Women who attended the University of Health Sciences Turkey, Prof. Dr. Cemil Taşçıoğlu City Hospital Perinatology Outpatient Clinic between 07.02.2022 and 07.08.2022 for 11^{th}-14^{th} weeks screening, were 18 years of age and older, had a single pregnancy, did not have any known systemic or chronic disease, were otherwise healthy and did not have a pre-existing diagnosis of diabetes mellitus (DM) or a history of drug use related to it, and who did not have any scarring in the area to be measured, were included in the study. Pregnant women were excluded from the study in the presence of any structural anomaly of the fetus or in the absence of fetal heartbeat. Written informed consent was obtained from all participants.

Demographic information of all participants was recorded, including age, gravida, parity, and weight and height before conception. Maternal SAT thickness and VAT thickness were measured ultrasonographically, and these measurements, the sum of these measurements and their ratio to each other were noted. All fetal and maternal measurements were made by the same perinatologist (H.A.Ş.) with the same ultrasonography device (Mindray Resona 7, 1.2-6 MHz convex abdominal probe). Maternal VAT and SAT measurements were made as described by Armellini et al. (16) and shown in Figure 1 and noted.

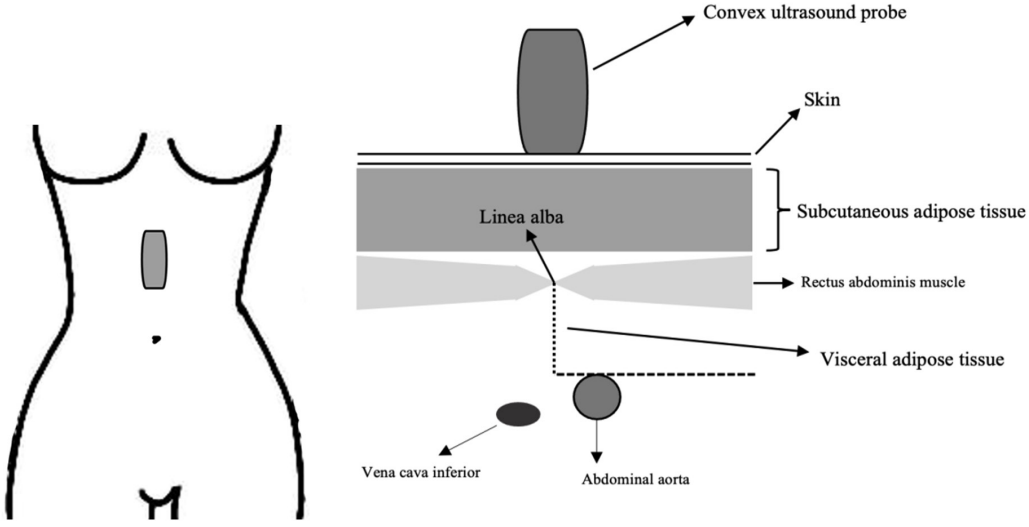

Figure 1. Ultrasonographic measurement of maternal subcutaneous and visceral fat tissue thickness

The measurement was made with a convex ultrasonography probe placed on the xipho-umbilical axis at the end of expiration while the mother was lying in a rested, supine position. The distance from the probe to the rectus abdominis muscle was measured as SAT, and the vertical distance from the linea alba to the abdominal aorta was measured as VAT. The maximum measurable values were obtained after repeated measurements. All pregnant women included in the study underwent 75-gram OGTT as a direct diagnostic test between 24th and 28th weeks for GDM screening. The test was performed after at least eight hours of fasting. The criteria suggested by the International Association of the Diabetes and Pregnancy Study Groups, which are fasting plasma glucose <92 mg/dL, <180 mg/dL at 1 hour, and <153 mg/dL at the second hour, were used as diagnostic criteria (17). GDM was diagnosed if at least one of these values was at the threshold value or above. Pregnant women who were not diagnosed with GDM constituted the control group.

Statistical analysis

Statistical analyzes were performed with Number Cruncher Statistical System (NCSS) 2007 Statistical Software (NCSS, Utah, USA). In addition to descriptive statistical methods including (mean ± standard deviation and median with interquartile range), the distribution of variables was examined with the Shapiro-Wilk normality test. Data were compared using the independent t-test for the comparison of normally distributed variables between paired groups, and the Mann-Whitney U test was used for comparison of non-normally distributed variables between the paired groups. The chi-square test was used to compare qualitative data. Since the group of patients with a history of GDM was a small group, Fisher's exact test was used for this group to compare the qualitative data. Univariate and multivariate logistic regression analysis was performed to separate the influential factors in the patient group with GDM. The areas under the received operator characteristic (ROC) curve were calculated for differential diagnosis of GDM, and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), logistic regression (+) values, and cut-off values of the variables were determined. The results were evaluated at the significance level of $p<0.05$.

Results

Maternal SAT and VAT were measured in the first trimester in a total of 369 pregnant women. However, 77 were excluded from the study for reasons including withdrawal from the study, refusing the OGTT, not tolerating the OGTT, moving out of the city, diagnosis of fetal anomaly in the late period, abortion, and loss of communication with the patient. Thus the study cohort numbered 292 pregnant women. Of these, 62 (21.2%) were diagnosed with GDM, and 230 did not have a diagnosis of GDM. The comparison of the demographic characteristics of these two groups is shown in Table 1. There was no significant

Table 1. Demographic characteristics of the groups

		GDM (-) (n=230)	GDM (+) (n=62)	p
Age (years)	Mean ± SD	30.64±4.94	31.49±5.24	0.233
Gravida	Mean ± SD	2.3±1.24	2.32±1.44	0.825
	Median (IQR)	2 (1-3)	2 (1-3)	
Parity	Mean ± SD	0.96±0.88	1±0.99	0.937
	Median (IQR)	1 (0-2)	1 (0-2)	
Abortus	Mean ± SD	0.33±0.68	0.29±0.64	0.864
	Median (IQR)	0 (0-0)	0 (0-0)	
BMI (kg/m²)	Mean ± SD	25.43±4.85	27.84±3.91	<0.001*
BMI (kg/m²)	<30	196 (85.23%)	42 (67.74%)	0.002
	>30	34 (14.78%)	20 (32.26%)	
Family history of DM	(-)	166 (72.17%)	40 (64.52%)	0.24
	(+)	64 (27.83%)	22 (35.48%)	
GDM in previous pregnancy	(-)	228 (99.13%)	60 (96.77%)	0.199
	(+)	2 (0.87%)	2 (3.23%)	
SAT† (mm)	Mean ± SD	18.7±9.55	22.51±7.66	0.004*
VAT‡ (mm)	Mean ± SD	35.37±14.89	43.72±17.38	<0.001*
VAT/SAT	Mean ± SD	2.19±1.03	2.15±1.13	0.798
TAT§ (mm)	Mean ± SD	54.06±18.76	66.23±19.45	<0.001*

*Statistically significant, SAT†: Subcutaneous adipose tissue, VAT‡: Visceral adipose tissue, TAT§: Total adipose tissue, GDM: Gestational diabetes mellitus, SD: Standard deviation, IQR: Interquartile range, BMI: Body mass index, DM: Diabetes mellitus

difference between the two groups in terms of age, gravida, parity, abortion, family history of DM, history of GDM in previous pregnancy, and VAT/SAT ratios. However, the BMI of the pregnant women in the GDM (+) group was significantly higher than the pregnant women in the GDM (-) group (p<0.001). Mean SAT, VAT and TAT measurements in the GDM (+) group were significantly larger than the means in the GDM (-) group (p=0.004, p<0.001 and p<0.001, respectively).

Univariate logistic regression analysis was performed for the factors affecting the diagnosis of GDM (Table 2). The following factors were identified as significant: BMI >30 BMI odds ratio (OR): 0.36 (0.19-0.69) (p=0.002); SAT measurement (defined threshold value was 18 mm) OR: 1.04 (1.01-1.08) (p=0.006); VAT measurement (defined threshold value was 55 mm) OR: 1.03 (1.02-1.05) (p<0.001); and TAT measurement (defined threshold value was also 55 mm) OR: 1.03 (1.02-1.05) (p<0.001). Multivariate logistic regression analysis was then performed to assess the strength of the relationship between outcome and predictor variables as well as the importance of each of the predictors to the relationship, for the factors affecting the diagnosis of GDM. Having a BMI >30 lost its significance but all the other factors identified by univariate regression analysis retained significance (Table 2).

Among the factors in the prediction of GDM positivity, the area under the ROC curve for BMI was 0.673 (0.616-0.726), the area under the curve for SAT was 0.723 (0.686-0.767), for VAT it was 0.738 (0.680-0.763) and for TAT it was 0.781 (0.684-0.797). In general, for the area under the ROC curve, 0.7 to 0.8 is considered acceptable and the higher this value, the more valuable the result is considered. The areas under the ROC curve of SAT, VAT and TAT variables all exceeded the 0.7 limit (Figure 2, Table 3).

Based on the ROC curves optimal threshold values were calculated to predict GDM. The optimal threshold value for SAT was >18 mm with a sensitivity of 67.74%, specificity of 60.87%, PPV of 41.8%, NPV of 87.5%, and a likelihood ratio (LR) of 1.73. Similarly, for VAT the threshold was 55 mm, with a sensitivity of 52.26%, specificity of 75.43%, PPV of 54.6%, NPV of 83.2%, and LR of 3.37 for VAT >55 mm. Thus, a pregnant woman with a VAT measurement >55 mm was found to be 3.37 times more likely to have GDM than a pregnant woman with a VAT measurement of <55 mm.

In addition, the same calculations were made for the sum of SAT and VAT. The optimal threshold value calculated for TAT thickness was also 55 mm, similar to that for VAT alone. Thus for a TAT thickness >55 mm, sensitivity was 77.42%, specificity was 56.52%, PPV was 42.4%, NPV was 90.3%, and the LR was 1.78 (Table 4).

Discussion

Our intention was to examine measurement of SAT and VAT as a cheap, practical and effortless method that would be useful for screening for GDM in pregnant women attending for first trimester screening. Our findings showed that the risk of

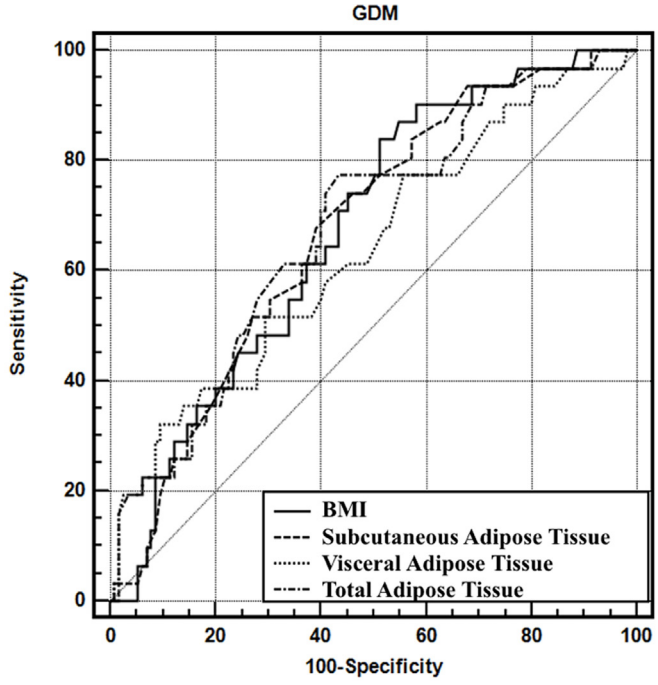

Figure 2. ROC curves for body mass index, subcutaneous adipose tissue, visceral adipose tissue, and total adipose tissue variables
ROC: Receiver operating characteristic, GDM: Gestational diabetes mellitus, BMI: Body mass index

Table 2. Logistic regression analysis for factors affecting gestational diabetes mellitus positivity

	Univariate		Multivariate	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
>30 BMI (kg/m^2)	0.36 (0.19-0.69)	0.002*	0.64 (0.31-1.34)	0.237
SAT (>18 mm)	1.04 (1.01-1.08)	0.006*	1.03 (1.00-1.06)	0.036*
VAT (>55 mm)	1.03 (1.02-1.05)	<0.001*	1.03 (1.01-1.02)	0.005*
TAT (>55 mm)	1.03 (1.02-1.05)	<0.001*	1.04 (1.00-1.06)	0.006*

*Statistically significant, OR: Odd ratio, BMI: Body mass index, SAT: Subcutaneous adipose tissue, VAT: Visceral adipose tissue, TAT: Total adipose tissue, CI: Confidence interval

developing GDM was higher in pregnant women with a SAT thickness of over 18 mm in the first trimester compared to pregnant women with a thickness below 18 mm. It was also found that a cut-off value of 55 mm for VAT and TAT tissue measurements may be a valuable indicator that will warn of an increased risk of GDM. Although the areas under the curve for SAT, VAT and TAT measurements were above the 0.700 limit, these thresholds had relatively good NPVs but poor PPVs so these measurements will not replace OGTT screening. However, these results would be available earlier in pregnancy than the OGTT screening is usually performed, so it would be feasible to identify women at greater risk of GDM and perhaps bring forward the OGTT test in patients with SAT, VAT and TAT values above the thresholds identified.

Furthermore, our study suggests that VAT, SAT and TAT are related to GDM and these measurements, which are available from routine ultrasonographic pregnancy monitoring, may be a more valuable tool than BMI in predicting GDM. All three values remained significant in multivariate regression analysis. In contrast, BMI, specifically being obese with a BMI >30 kg/m^2, lost significance in multivariate regression analysis. TAT seems to be more valuable than the other two abdominal compartments in terms of predicting GDM by looking at the areas under the curve calculated after the ROC curves. In a large-scale study conducted by Bourdages et al. (18) with 1048 pregnant women, it was reported that SAT, VAT and total fat tissue thickness measurements in the first trimester could be used to predict GDM, and a significant relationship was found, especially with those with GDM who needed insulin.

Although there are various studies on the use of abdominal adipose tissue to predict GDM, there are differences and contradictions between studies regarding which compartment is useful. Gur et al. (13) measured visceral and subcutaneous fat tissues of 94 pregnant women and then the women were examined in two groups; those who were diagnosed with GDM and those who were not. While VAT thickness was found to be more valuable than BMI in predicting GDM, no significant difference was found in the two groups in terms of SAT thickness, which is inconsistent with our findings. Similar findings were reported by D'Ambrosi et al. (19), who looked at SAT and VAT thicknesses of 295 pregnant women. These authors also found VAT thickness to be significantly increased in those diagnosed with GDM, while SAT thickness did not differ significantly between the two groups. In contrast, the studies of Yang et al. (20) and Kansu-Celik et al. (21) examined the relationship between SAT and GDM, but did not examine the visceral component and its relationship with GDM. In these two studies, SAT thickness was found to be significantly higher in pregnant women diagnosed with GDM, and it was reported that measurement of SAT could be used to predict GDM.

Some studies examining maternal adipose tissue to predict GDM have also proposed threshold values and suggested that pregnant women with measurements above the determined thresholds should be followed more closely. Thaware et al. (22) investigated 80 pregnant women and a threshold value of 42.7 mm was reported for VAT and it was suggested that this value may be used as a tool to predict GDM with high sensitivity and specificity. Similarly, threshold cut-off values were reported in the studies of Kansu-Celik et al. (21) (SAT thickness 16.75 mm), Yang et al. (20) (SAT thickness 24 mm), and Bourdages et al. (18) (TAT 61 mm).

The meta-analysis of Rahnemaei et al. (23) included 56,438 pregnant women from 29 studies and evaluated the relationship of various body compartments of the mother with GDM. VAT and SAT thickness emerged as parameters with utility in identifying women at risk of GDM and it was suggested that these measurements could help in managing GDM with low cost.

Our study, including 292 pregnant women is one of the largest patient populations published to date. In terms of measurement standardization, all measurements were made using the measurement method described by Armellini et al. (16), by the same perinatologist (H.A.Ş.) and using the same ultrasonography device.

Table 3. ROC curves and areas under the curve

	AUC	S.E.	95% CI
BMI (kg/m^2)	0.673	0.041	0.616-0.726
Subcutaneous adipose tissue (18 mm)	0.723	0.041	0.686-0.767
Visceral adipose tissue (55 mm)	0.738	0.042	0.680-0.763
Total adipose tissue (55 mm)	0.781	0.041	0.684-0.797

ROC: Receiver operating characteristic, AUC: Area under the curve, CI: Confidence interval, BMI: Body mass index

Table 4. Optimal threshold values and analysis results at 95% confidence interval to predict gestational diabetes mellitus in the first trimester

	Cut-off value (mm)	Sensitivity (%)	Specificity (%)	PPV* (%)	NPV† (%)	LR‡ (+)
SAT	>18	67.74 (54-79)	60.87 (54-67)	41.8 (39-43)	87.5 (81-92)	1.73
VAT	>55	52.26 (47-59)	75.43 (65-83)	54.6 (48-63)	83.2 (83-88)	3.37
TAT	>55	77.42 (65-87)	56.52 (50-63)	42.4 (37-54)	90.3 (84-94)	1.78

*PPV: Positive predictive value, †NPV: Negative predictive value, ‡LR: Likelihood ratio

Study limitations

This study has several limitations. It was single center and the patient population consisted of pregnant women living in the same region, with similar characteristics in terms of race and ethnicity, thus limiting the generalizability of the findings. The other major limitation was that pregnant women were not classified according to their insulin needs after the diagnosis of GDM. The final limitation was that the measurements made were only made at a single time point.

Conclusion

Maternal central obesity appears to be associated with an increased risk of GDM, as evidenced by several studies, including the findings of the present study. As we lacked a sufficiently diverse patient group to generalize to all populations, these results suggest that measurements of SAT, VAT and TAT have low sensitivity and specificity in predicting GDM and are not an alternative to OGTT. However, for early identification of pregnant women at increased risk for GDM, and these measurements may be useful in determining who should have an early OGTT in the first trimester.

Ethics Committee Approval: *This was designed as a prospective cohort study. Ethics committee approval was obtained from the Ethics Committee of University of Health Sciences Turkey, Prof. Dr. Cemil Taşçıoğlu City Hospital (approval number: 14, date: 31.01.2022).*

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

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Does COVID-19 reduce anti-Mullerian hormone levels in women of reproductive age in late periods of infection?

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Abstract

Objective: The question of whether severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection influences ovarian function and oocyte quality has arisen as angiotensin converting enzyme-2 receptors, which facilitates viral infection, are found on reproductive system tissues, including the vagina, placenta, uterus, and ovaries. The primary objective of this prospective study was to evaluate the impact of SARS-CoV-2, on ovarian function, with a focus on anti-Mullerian hormone (AMH) and acute phase reactant levels in patients well after recovery from coronavirus disease-2019 (COVID-19).

Material and Methods: This prospective cohort study was conducted in the department of obstetrics and gynecology at a single center between October 2020 and June 2021. In order to investigate the impact of COVID-19 on ovarian reserve, 34 non-pregnant women of reproductive age (24-38 years) with COVID-19 polymerase chain reaction positivity were included.

Results: The difference between AMH levels measured 6 months after COVID-19 infection and baseline AMH levels was -0.31±0.80 ng/dL on average and -0.25 (-2.1-1.3) ng/dL on median. Significant correlations were observed between the change in AMH levels and white blood cell levels (r=-0.434, p=0.010), lymphocyte levels (r=-0.361, p=0.036), C-reactive protein levels (r=0.542, p=0.001), ferritin levels (r=0.570, p=0.001) and procalcitonin levels (r=0.598, p=0.001).

Conclusion: We believe this is the first study to examine whether there is a correlation between the late results of COVID-19 and ovarian function. In this cohort, AMH values decreased 6-months after recovery from COVID-19 and a correlation was found between measures of disease severity and the magnitude of decrease in AMH. However, the study was underpowered and future larger studies are required to validate these findings. (J Turk Ger Gynecol Assoc. 2024; 25: 231-7)

Keywords: AMH, COVID-19, ovarian function, ovarian reserve, SARS-COV-2

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Introduction

The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), first detected in Wuhan, China in December 2019, caused a global coronavirus disease-2019 (COVID-19) pandemic. As of June 2022, 532,887,351 cases and 6,307,021 deaths have been detected worldwide (1).

SARS-CoV-2 exploits membrane bound angiotensin converting enzyme-2 (ACE2) in order to infect host cells. The fusion of the virus and the host cell occurs with the transmembrane serine protease 2 receptor (2). By targeting the vascular endothelium, SARS-CoV-2, known to cause severe harm to the respiratory system, can also lead to thrombosis, pulmonary embolism and high blood pressure. Immune system dysfunction contributes to the current condition by enhancing microvascular permeability and vascular inflammation (3).

Additionally, there is evidence that the heart, bowels, testicles, and ovaries may be target tissues for SARS-CoV-2 (4) ACE2 receptors on the ovaries have functions including gonadotropin response, modulation of steroidogenesis, follicle growth and angiogenesis (5,6). However, the current data on whether SARS-CoV-2 infection has any impact on the female reproductive system is quite limited.

Anti-Mullerian hormone (AMH) is an important biomarker of ovarian follicle reserve and quality (7). During the early 1990s, it was discovered that blood AMH concentration might serve as a measure of ovarian reserve by providing an indirect estimate of the total number of potential follicles (8).

The primary objective of this prospective study was to evaluate the impact of SARS-CoV-2 on ovarian function by measuring AMH and acute phase reactant levels in patients after complete recovery from COVID-19. The study also aimed to investigate the effect of severity of COVID-19 on ovulation and follicular function. We believe that this is the first study to investigate the relationship between severity of COVID-19 and ovarian function.

Material and Methods

After receiving University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital Local Ethics Committee approval (approval number: 2020-22-07, date: 02.11.2020), a prospective cohort study was conducted by the department of obstetrics and gynecology of a single center between October 2020 and June 2021. Written informed consent was obtained from all participants before starting the study.

In order to investigate the impact of COVID-19 on ovarian reserve, non-pregnant women of reproductive age (24-38 years) with COVID-19 polymerase chain reaction positivity and with regular (24 to 38 day) menstrual cycles were included in the study. Exclusion criteria were: pregnancy or lactation; pre-existing conditions that may affect ovarian function (ovarian surgery, pelvic region radiotherapy, systemic chemotherapy); endocrine disease (thyroid dysfunction, hyperprolactinemia or Cushing syndrome); diagnosis of premature ovarian failure; suspicion of adnexal malignancy; presence of ovarian endometrioma; history of infertility or pregnancy via assisted reproductive technique; and women with severe COVID-19 requiring intensive care.

On the day of COVID-19 diagnosis, blood samples were taken from each participant (day 0). The following parameters were measured: plasma AMH (ng/dL); hemoglobin (Hb, g/dL); hematocrit (Htc, %); white blood cell count [(WBC), 10^3/uL]; lymphocyte proportion of WBC (%); neutrophil proportion of WBC (%); eosinophil proportion of WBC (%); C-reactive protein [(CRP), mg/L]; ferritin (ng/mL); procalcitonin (ng/mL); aspartate aminotransferase [(AST), U/L]; alanine aminotransferase [(ALT), U/L]; gamma glutamyl transferase [(GGT), IU/L]; lactate dehydrogenase [(LDH), U/L]; urea (mg/dL); and creatinine (mg/dL).

Patients were reviewed six months following infection, and blood tests were recollected to determine AMH levels. Plasma was centrifuged at 2500 rpm for 10 minutes and then stored at -80 °C. Serum AMH concentrations were measured by an enzyme immunoassay kit (EIA AMH/MIS; Immunotech, Chantilly, VA, USA) with a detection limit of 0.006 ng/mL. All hormone assays were processed by the same reference laboratory. Patients who experienced any adverse condition that could adversely affect AMH levels and/or ovarian reserve during this 6-month period were excluded. The effect of COVID-19 on ovarian reserve was evaluated by comparing day 0 and 6-month AMH levels.

Sample size and power analysis

The 2021 study by Kolanska et al. (9) provides comprehensive descriptive statistics for AMH levels. Specifically, the study reported initial median AMH levels of 2.87 ng/dL [interquartile range (IQR): 1.69-3.99] and subsequent levels of 1.51 (IQR: 0.82-2.38). In light of these values, a power analysis was conducted to assess the statistical significance of the difference between the initial and subsequent AMH measurements. This analysis was carried out under conditions of 80% power and 5% types 1 error rate.

Given the uncertainties regarding whether the data set follows a normal distribution, and considering that the study of Kolanska et al. (9) used non-parametric descriptive statistics, such as IQR, a non-parametric approach was adopted for the analysis. This methodology abstains from making any assumptions about the data distribution and offers a more robust alternative when parametric assumptions are not met.

In our initial sample size calculations, we anticipated achieving a power of 0.80 with 21 participants. However, our post-

hoc power calculations revealed a power of 0.618 with 34 participants. This discrepancy appears to be related to the observation of a smaller effect size than the one estimated based on previous studies, as well as an unforeseen variance. These findings emphasize that the expectations set for sample size and effect size in a study may not always match the actual outcomes. Nonetheless, considering the constraints imposed by the pandemic, our results can still be deemed noteworthy.

The analytic process was executed using the Python programming language and the SciPy statistical library, establishing a robust foundation for making more precise estimations of sample size in future research.

Statistical analysis

In order to perform statistical analysis, the Number Cruncher Statistical System 2007 (Kaysville, Utah, USA) program was used. Descriptive statistical methods (mean, standard deviation, median, range, frequency, and percentage) were used whilst evaluating the study data. The Shapiro-Wilk test and graphical analysis were used to assess normality of data distribution of the data sets. The Mann-Whitney U test was used to compare two data sets when at least one was non-parametric. The associations between the quantitative variables were examined using Spearman correlation analysis. The threshold for statistical significance was set at 0.05.

Results

In total, 34 women with a mean age of 26.79±4.87 years. The mean body mass index (BMI) was 23.35±2.98 kg/m^2. The parity was 0 in 22 (64.7%), 1 in 3 (8.8%), 2 in 7 (20.6%), and 3 in 2 (50.9%).

The distribution of Hb, Htc, WBC, lymphocyte, neutrophil, eosinophil, CRP, ferritin, procalcitonin, AST, ALT, GGT, LDH, urea, creatinine values of the patients participating in the study is shown in Table 1.

Table 2 shows the changes in AMH levels at baseline and at 6-months following SARS-CoV-2 infection. The mean decrease of 0.31±0.80 units in the month 6 measurements was significant compared to the day 0 AMH levels (p=0.025) (Table 2, Figure 1).

No significant correlation was found between the first and second AMH concentrations of the patients and WBC, lymphocyte, neutrophil, eosinophil, CRP, ferritin and procalcitonin measurements (p>0.05). There was no relationship between neutrophil and eosinophil levels of women participating in the study and the changes in the AMH values (p>0.05) (Table 3).

A weak negative correlation (r=-0.361; p=0.036) was identified between the baseline lymphocyte measurements and the value of the difference between the baseline and 6-month

Table 1. Summary of baseline hematological parameters and biochemical markers in COVID-19 patients

	Mean ± SD	Median (min.-max.)
Hematological parameters		
Hb (g/dL)	12.86±0.96	13 (10.9-15)
Htc (%)	38.61±2.95	38.9 (31-44)
WBC (10^3/uL)	11.12±4.05	9.8 (5.9-21.4)
Lymphocyte (10^3/uL)	2.18±1.00	2.1 (0.1-4.5)
Neutrophil (10^3/uL)	6.11±2.68	5.4 (3.3-16.3)
Eosinophil (10^3/uL)	0.21±0.17	0.2 (0-0.6)
Inflammation and infection markers		
CRP (mg/L)	11.09±11.87	4.1 (0.5-43)
Ferritin (ng/mL)	61.94±43.66	59.8 (6.6-157)
Procalcitonin (ng/mL)	0.06±0.09	0 (0-0.5)
Liver function tests		
AST (U/L)	18.24±9.94	16 (10-68)
ALT (U/L)	19.18±16.72	14 (8-91)
GGT (U/L)	19.38±6.42	18 (11.4-41)
LDH (U/L)	200.21±45.57	200 (139-338)
Renal function parameters		
Urea (mg/dL)	24.03±7.31	24.3 (10-43)
Creatinine (mg/dL)	0.63±0.09	0.6 (0.4-0.9)

Hb: Hemoglobin, Htc: Hematocrit, WBC: White blood cell, CRP: C-reactive protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase, LDH: Lactate dehydrogenase, SD: Standard deviation, min.: Minimum, max.: Maximum

AMH concentrations. Similarly, a moderate negative correlation (r=-0.434; p=0.010; p<0) was found between WBC and the variation of difference between baseline and 6-month AMH measurements (Figure 2). This finding is perhaps not unexpected, as a major proportion of the WBC will consist of lymphocytes.

In terms of acute phase reactants, moderate positive correlations were found for CRP (r=0.542; p=0.001; Figure 3), ferritin (r=0.570; p=0.001; Figure 4) and procalcitonin (r=0.598; p=0.001; Figure 5) and the differences in baseline and 6-month AMH values.

Table 2. Evaluation of variation between AMH measurements

		Day 0	Month 6	Difference	Test statistic/p-value
AMH	Mean ± SD	3.58±1.51	3.27±1.53	-0.31±0.80	Z: -2.248
	Median (min.-max.)	3.01 (1.94-8.41)	3.01 (1.4-9.74)	-0.25 (-2.1-1.3)	[a]**0.025***

[a]Wilcoxon signed-ranks test, *p<0.05, min.-max.: Minimum-maximum, SD: Standard deviation, AMH: Anti-Mullerian hormone

Table 3. Correlation between change in AMH at baseline and at 6-months and parameters of inflammation

		1. Measurement AMH	2. Measurement AMH	Change in AMH
WBC (10^3/uL)	r	0.170	-0.005	-0.434
	p	0.336	0.978	**0.010**
Lymphocyte (%)	r	-0.152	0.034	-0.361
	p	0.392	0.849	**0.036**
Neutrophil (%)	r	-0.136	-0.007	-0.116
	p	0.443	0.968	0.515
Eosinophil (%)	r	0.047	0.158	-0.111
	p	0.793	0.371	0.531
CRP (mg/L)	r	0.254	0.080	0.542
	p	0.147	0.652	**0.001**
Ferritin (ng/mL)	r	0.312	0.015	0.570
	p	0.072	0.934	**0.001**
Procalcitonin (ng/mL)	r	0.151	-0.154	0.598
	p	0.393	0.385	**0.001**

r: Spearman correlation coefficient, WBC: White blood cell, CRP: C-reactive protein, AMH: Anti-Mullerian hormone

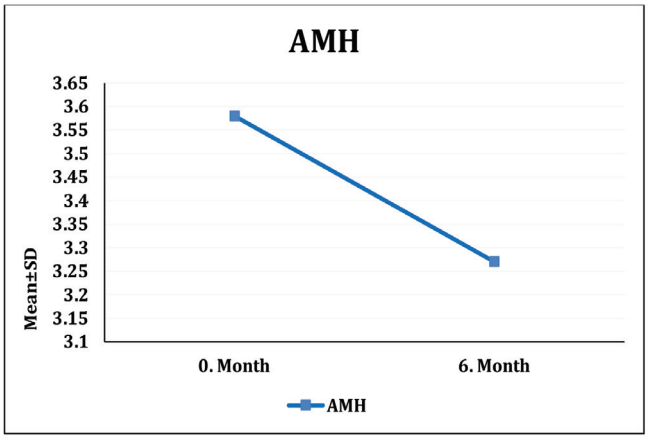

Figure 1. Evaluation of variation between AMH measurements
AMH: Anti-Mullerian hormone

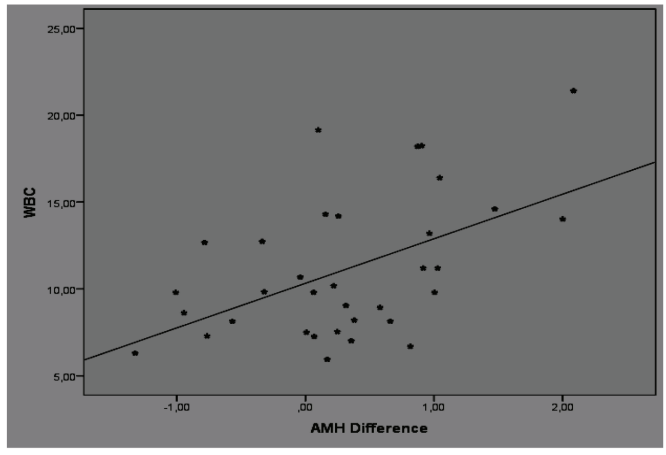

Figure 2. Distribution of the relationship between WBC values and the changes in 1st and 2nd AMH measureme
WBC: White blood cell count, AMH: Anti-Mullerian hormone

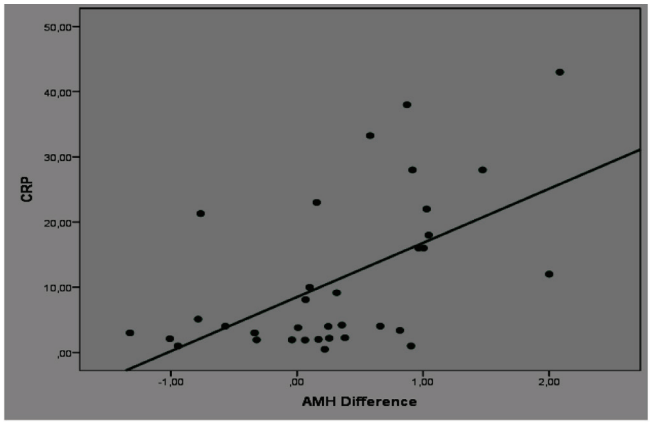

Figure 3. Distribution of the relationship between CRP concentrations and the differences in 1st and 2nd AMH measurements
CRP: C-reactive protein, AMH: Anti-Mullerian hormone

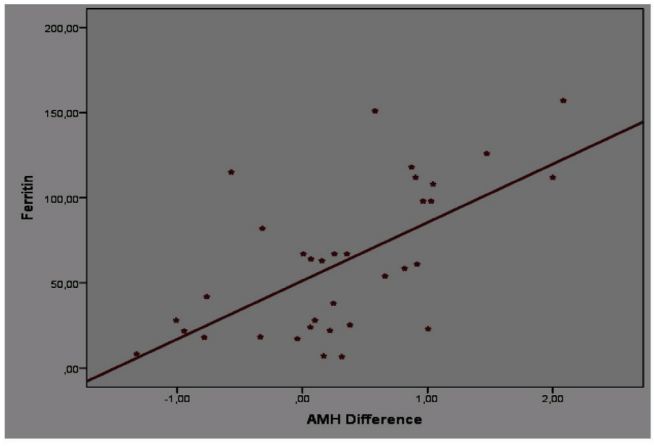

Figure 4. Distribution of the relationship between ferritin levels of participants and the variations in 1st and 2nd AMH measurements
AMH: Anti-Mullerian hormone

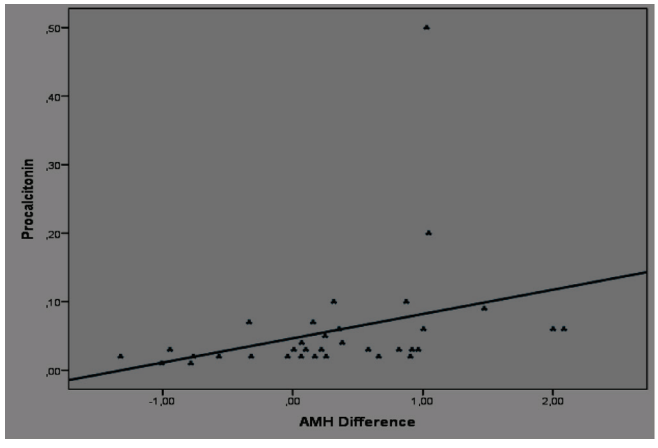

Figure 5. Distribution of the correlation between procalcitonin levels of the patients and the changes in AMH concentrations measured at day 0 and month 6
AMH: Anti-Mullerian hormone

Discussion

COVID-19 continues to affect tens of thousands of individuals worldwide, causing severe morbidity and mortality in certain cases. However, limited data exist regarding the early and long-term impacts of COVID-19 on ovarian function.

AMH is the most sensitive indicator of ovarian reserve, recognized as an early marker during its assessment (10,11).

In our study group, while some women saw their AMH levels rise and others fall, we observed a clear trend towards decreased AMH levels 6-months after COVID-19 infection compared to initial measurements. This suggests a significant effect of COVID-19 on ovarian reserve over time. A correlation was identified between decreased AMH values and increased acute phase reactants, suggesting an effect of severity of COVID-19 on ovarian function and/or reserve. This suggests a relationship between the severity of disease and thus impact of systemic inflammation on ovarian reserve in the chronic process, as well as in the acute infection period. We speculate that this may be due to severe oophoritis or multisystem inflammatory syndrome in some cases of COVID-19.

In a study of 78 female patients, COVID-19 positive patients had significantly lower serum AMH levels (0.19 vs. 1.12 ng/mL, $p=0.003$) and higher serum testosterone (0.38 vs. 0.22 ng/mL, $p<0.001$), FSH (FSH ≥10 mIU/mL: 53.8% vs. 34.7%, $p=0.041$) and prolactin levels (25.43 vs. 12.12 ng/mL, $p<0.001$). Changes in menstrual characteristics, such as menstrual irregularities and increased premenstrual symptoms, were also observed in this study (12). Furthermore, an animal study reported that SARS-CoV-2 infection of the ovarian granulosa cells via ACE2 receptors may lead to loss of ovarian reserve and adverse outcomes (13).

In studies, SARS-CoV-2 RNA was not detected in the follicular fluids of COVID-19 positive cases, while SARS-CoV-2 immunoglobulin G (IgG) was positive (14) IgG positivity can be interpreted as evidence of inflammation, which may cause tissue damage and hence a decrease in ovarian reserve. A study conducted by Herrero et al. (15) documented lower interleukin-1 and vascular endothelial growth factor (VEGF) levels in follicular fluid (14). In previous studies higher IL-1 and VEGF levels were associated with higher IVF success rates (16,17). Thus, oocyte quality may have deteriorated due to COVID-19. However, in a study by Li et al. (18), the concentrations of sex hormones [follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, progesterone, testosterone and AMH] in women of childbearing age with COVID-19 were compared to those of age-matched controls. These authors found no difference between the groups, although some of the women with COVID-19 exhibited a menstrual volume decrease or cycle prolongation (17).

Similarly, in another study by Madendag et al. (19) on 132 patients with COVID-19 examining the blood levels of AMH, FSH, LH, and estradiol following disease, at 3 months after recovery from COVID-19 no negative impact on the ovarian reserve was reported. Yet, irregular menstruation and decrease in bleeding volume were identified on review of patients' menstrual cycles. The cause of these changes was attributed to the immune response and inflammation (19).

Same study also assessed serum and follicular fluid samples for anti-COVID IgG as well as estrogen, progesterone and heparan sulfate proteoglycan 2 concentration in three study groups, women recovering from confirmed COVID-19, vaccinated women and uninfected, non-vaccinated controls suggested that SARS-CoV-2 had no influence on ovarian functions on hospitalized patients with moderate symptoms of the disease (18).

One study represented that a mild COVID-19 infection did not significantly alter ovarian reserve in women undergoing assisted reproductive technology (ART) treatment, as measured by AMH levels. AMH levels were comparable among COVID-19 positive and negative groups, before and during ART treatment. This suggests that mild COVID-19 infection may not have a detrimental effect on ovarian reserve (20).

The common feature of these studies, and ours, is the investigation of ovarian reserve and quality 3-9 months following infection. However, if it is acknowledged that inflammation is a mechanism of injury, longer-term studies are needed and may yield different results. Many studies have shown that oxidative stress may increase due to inflammation and adversely affect oocyte quality, female fertility and the number of healthy embryos (21). SARS-CoV-2 also triggers a systemic inflammatory response, which may cause oxidative stress. Even though the harmful effects of COVID-19 on the human body have not been fully elucidated, there is a clear need for further research with more cases and longer follow-up is to evaluate the influence of COVID-19 on both the menstrual cycle and ovarian reserve.

Study limitations

Our study found a post-hoc power value of 0.62, which is lower than the often-recommended value of 0.8. According to fundamentals of statistical analysis (22), while higher power is preferred, lower power can still be meaningful, especially in early-stage or constrained research. Our study's design, a before-and-after approach conducted during the challenging times of the COVID-19 pandemic, faced unique hurdles, such as difficulties in recruiting participants and collecting data. These challenges mean we should interpret our results carefully, recognizing they still contribute valuable insights despite the lower power value. This situation highlights the need to be flexible and realistic about what can be achieved under less-than-ideal research conditions. Another limitation of our study is the short follow-up period. In assessing the long-term effects of COVID-19 on health outcomes, a longer follow-up period would have been ideal to understand the persistence or evolution of impact over time fully. The rapid emergence of the pandemic and the urgent need for timely data contributed to the decision to use a shorter observation window. Future research should aim for longer follow-up periods to capture the full range of effects of COVID-19 and allow a more detailed assessment of its long-term health consequences.

Conclusion

Reflecting on the outcomes of our investigation and considering the broader spectrum of research, our study delves into the consequences of COVID-19 on ovarian reserve, acknowledging that while some reports indicate minimal impact, particularly in cases of mild infection, our observations point towards a discernible decrease in AMH levels post-infection. This trend is particularly pronounced in more severe instances of the disease, suggesting that the extent of systemic inflammation could play a significant role in this outcome. Such variability underscores the intricate ways in which COVID-19 can affect reproductive health, highlighting the critical need for more comprehensive, long-term studies to unravel the complex interplay between infection severity, inflammation, oxidative stress, and reproductive function.

Ethics Committee Approval: *The study protocol was reviewed and approved by University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee (approval number: 2020-22-07, date: 02.11.2020).*

Informed Consent: *Written informed consent was obtained from all participants before starting the study.*

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

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

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Factors affecting obstetric outcomes in patients who underwent cold-knife and loop electrosurgical excision procedure conization due to cervical intraepithelial neoplasia 2 or cervical intraepithelial neoplasia 3

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Abstract

Objective: To determine factors affecting obstetric outcomes in pregnancies after conization by loop electrosurgical excision procedure (LEEP) or cold-knife conization (CKC) due to cervical intraepithelial neoplasia.

Material and Methods: The maternal and clinical characteristics and obstetric outcomes of CKC, LEEP and control groups were evaluated and compared. Risk factors for adverse pregnancy outcomes were evaluated using multiple logistic regression analyses.

Results: The incidence of preterm delivery, preterm premature rupture of membranes (PPROM), low APGAR scores, fetal mortality, and late-period spontaneous abortus was highest in patients who underwent CKC ($p<0.05$). Cone depth of CKC was greater than LEEP ($p=0.025$). Cervical length (CL) at pregnancy was CKC < LEEP < controls ($p=0.003$). Shorter CL at pregnancy and time from conization to pregnancy (t-CP) was correlated with a high incidence of preterm delivery and PPROM ($p<0.05$). To predict preterm delivery, t-CP <14 months had 63.16% sensitivity and 77.42% specificity [area under the curve (AUC): 0.714, 95% confidence interval (CI): (0.603-0.809); $p=0.005$], and CL at pregnancy <31 mm had 65% sensitivity and 71.78% specificity [AUC: 0.731, 95% CI: (0.675-0.782); $p<0.001$]. To predict PPROM, t-CP <15 months had 85.71% sensitivity and 65.22% specificity [AUC: 0.730, 95% CI: (0.603-0.809); $p=0.024$], and CL <32 mm had 72.73% sensitivity and 61.89% spcificity [AUC: 0.685, 95% CI: (0.675-0.782); $p=0.007$].

Conclusion: Compared with CKC, LEEP has shorter cone depth and fewer adverse pregnancy outcomes. The t-CP <14 months was a risk for preterm delivery and <15 months was a risk for PPROM. CL at pregnancy <31 mm was a risk for preterm delivery and <32 mm was a risk for PPROM. (J Turk Ger Gynecol Assoc. 2024; 25: 238-46)

Keywords: Cervical intraepithelial neoplasia, conization, cold-knife conization, loop electrosurgical excision procedure, obstetric outcome

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Introduction

Cervical cancer screening and follow-up treatment have been implemented in routine healthcare. As a result, most cases are detected and treated in the pre-malignant phase, known as cervical intraepithelial neoplasia (CIN). Thus, the incidence of cervical cancer has been significantly decreased from 14.8 per 100,000 in 1975 to 6.6 per 100,000 in 2013 (1,2). The majority of CIN 2 (peaking at the age of 25 to 29 years) and CIN 3 (peaking at the age of 25 to 40 years) occur in childbearing age (3). Cold-knife conization (CKC) and loop electrosurgical excision procedure (LEEP) conization are both excisional procedures and are the most widely accepted and used for the treatment of CIN 2 and CIN 3. However, both CIN and conization alter the morphology of the cervix, which holds the fetus in the uterine cavity. Thus, adverse pregnancy outcomes in patients with CIN 2 and CIN 3 who underwent excisional procedures have been reported in previous studies, including late pregnancy loss due to cervical insufficiency, preterm birth, preterm premature rupture of membranes (PPROM), premature rupture of membranes (PROM), increased fetal mortality and second-trimester abortion (4-6). However, some studies attributed these adverse pregnancy outcomes to inherited risks because these patients also have low socioeconomic status and income, advanced maternal age, and high smoking rates (7). In addition, one study affirmed that the risk of preterm delivery in these patients was not due to conization but because of CIN (8). In addition, there is a conflict regarding pregnancy outcomes between studies in respect to the effect of the type of cervical excision procedures (CKC or LEEP) performed, the depth and volume of excised tissue, remaining cervical length, and the time elapsed from the procedure on adverse pregnancy outcomes (4-6,9,10). Based on these findings, it is clear that there is a necessity to bring a clarity to these issues. Further studies will allow the development of strategies for optimizing subsequent pregnancy results after conization.

The aim of this study was to evaluate factors affecting pregnancy outcomes in patients with CIN 2 or CIN 3 who underwent LEEP or CKC.

Material and Methods

This study involved a single centre and retrospectively evaluated the data of singleton pregnancies that reached 16 gestational weeks after conization due to CIN 2 or CIN 3, between January 2010 and July 2020.

The study was approved by the University of Health Sciences Turkey, Etlik Zübeyde Hanım Woman's Health Training and Research Hospital Ethical Committee Local Ethics Committee (approval number: 08/23, date: 23.06.2021).

The inclusion criteria were: patients with singleton fetuses; pathologic diagnoses as CIN 2 or CIN 3; subsequent pregnancy after CKC or LEEP; and reaching at least 16 gestational weeks. The exclusion criteria were: patients who aborted before 16 gestational weeks because measuring the cervical length before this week is problematic and also the relation of spontaneous abortion due to cervical insufficiency is weak (11); patients with known major risk factors for preterm delivery including history of preterm delivery and having multifetal pregnancies; history of repeated conization or ablative treatments; and those with missing data. We documented the maternal age, body mass index (BMI), medico-surgical and obstetric history, smoking habits, gravidity, parity, pathologic diagnoses, times and types of conization, depth and volume of conization specimens, length of cervix measured between the 16^{th} and 24^{th} gestational weeks, weeks of spontaneous abortion and delivery, time interval between conization and pregnancy and fetal outcomes. The cases in the control group were selected among those had no symptoms, such as bleeding or uterine contractions, and were age-matched and had cervical length measured during routine detailed fetal anatomic evaluation.

Deliveries occurring between the 24^{th} and 37^{th} gestational weeks were defined as preterm deliveries. PPROM was defined as the loss of the integrity of membranes before labor began in pregnancies before 37 gestational weeks, PROM was defined as the loss of the integrity of membranes before labor began in pregnancies after 37 gestational weeks (12). Late spontaneous abortion was defined as abortion occurring between 16^{th} and $23^{0/6}$ gestational weeks. Cervical length measurements were obtained using transvaginal ultrasonography after voiding between the 16^{th} and 24^{th} gestational weeks.

CKC was performed in the operating room and all patients were treated by experienced gynecologic oncologists who have performed at least 60 conization per year. Under spinal anesthesia, a surgical margin of 2 mm was created using a scalpel, and interrupted vertical sutures with Dexon-1 were used for hemostasis. All LEEPs were performed by experienced gynecologic oncologists using the same technique; first, Lugol iodine was applied and then a 2% lidocaine-containing solution was also applied. Cone size was based on loop dimension: small, ≤10×10 mm; middle-sized, 15×12 mm, and the current was set to cut and coagulate.

The volume of the elliptical cone = (D.d.ϖ / 4) X h / 3 h: height of the cone; D: major axis of the ellipse; d: minor axis of the ellipse (ϖ=2.622).

The primary outcomes of the study were rates of preterm birth (between 24-36 gestational weeks) and PPROM, and the secondary outcomes were spontaneous abortion (between 16-24 gestational weeks) and fetal mortality.

Statistical analysis

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test the normality of data distribution. Appropriate tests were selected according to the results. Continuous variables that satisfied the assumption of normal distribution were compared using Student's t-test and the others by using the Mann-Whitney U test among categories of groups such as LEEP + CKC and controls. Homogeneities of variances were tested using the Levene's test. For comparisons of more than two independent groups, ANOVA or the Kruskal-Wallis tests were used. Mean ± standard deviation and median (range) are given as descriptive statistics for these variables. The differences in proportions between groups were compared using the chi-square or Fisher's exact tests, where appropriate, and the results were summarized using column percentages with frequency distributions. To define independent risk factors of outcome variables, such as LEEP and CKC, we ran multiple logistic regression (LR) analyses and odds ratios with associated confidence intervals (CI) were calculated. Correlations between variables were examined against the multicollinearity problem and a candidate model was defined accordingly. Variance inflation factor and tolerance values and model fit statistics were acceptable and multiple LR was used with the backward LR method. P values of less than 0.05 were considered statistically significant. The IBM SPSS Statistics for Windows, version 26.0. (2) package was used for all statistical analyses (IBM Inc., Armonk, NY, USA).

Results

The data of 1,069 pregnant women who underwent conization due to CIN 2 and CIN 3 were evaluated. Among them, 598 were CKC and 471 were LEEP. Seventy-two patients who underwent CKC and 45 patients who underwent LEEP became pregnant. Twenty-one women who underwent CKC and 15 who underwent LEEP were excluded due to histories of preterm delivery, early pregnancy losses, and losses to follow-up. As a result, 51 pregnancies with a history of CKC and 30 with a history of LEEP were included in the study (Figure 1).

The basic maternal characteristics, including maternal age at pregnancy, BMI, gravidity, parity, method of conception, and rates of smoking of all groups showed no differences ($p>0.05$). The incidence of complications such as diabetes, hypertension, preeclampsia, oligohydramnios, polyhydramnios, and placenta previa of all groups was also similar ($p>0.05$). In addition, gestational weeks at the time of cervical length measurements of all groups were similar ($p>0.05$) and thus the baseline characteristics of patients in each group were comparable.

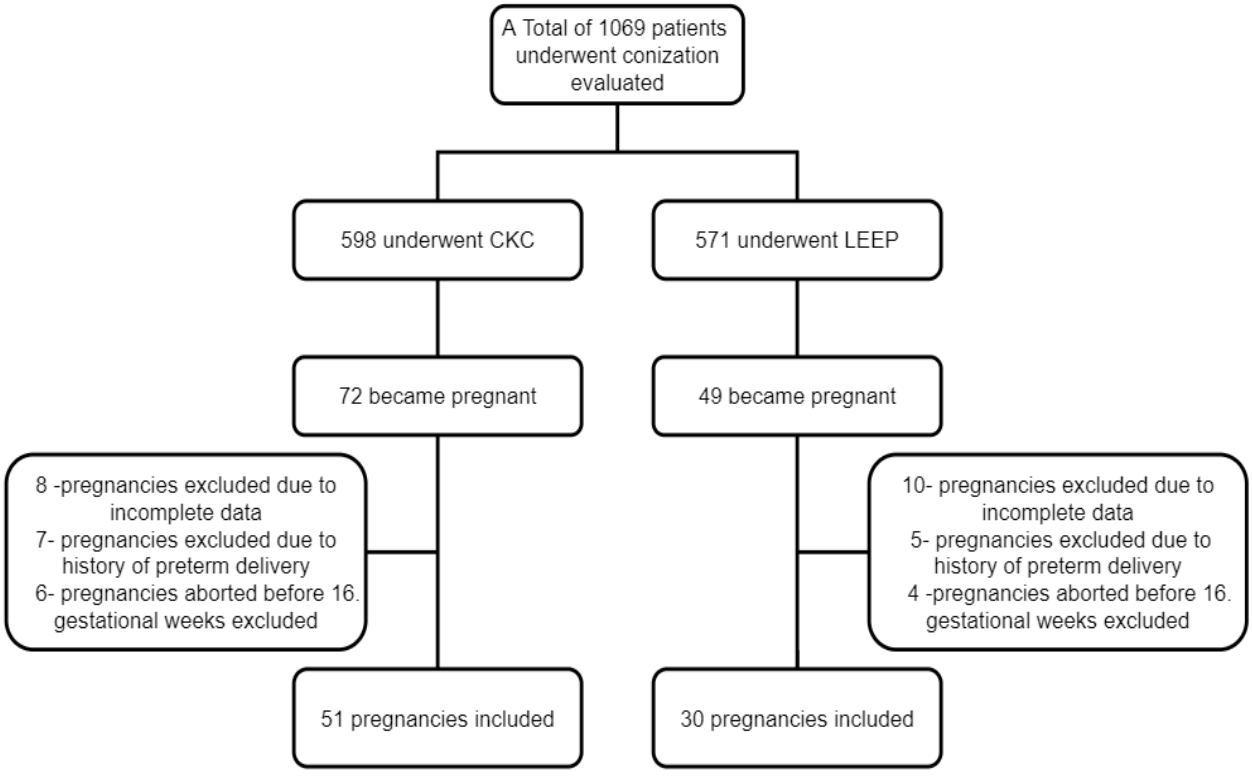

Figure 1. Description of the study cohort
CKC: Cold knife conization, LEEP: Loop electrosurgical excision procedure

To minimize the effect of factors on obstetric outcomes, maternal age when conization was performed, time from conization to last menstrual period, and rates of CIN 2 and CIN 3 were compared between the CKC and LEEP groups, and no significant difference was found between them (p>0.05) (Tables 1, 2). Thus, the CKC and LEEP groups were comparable. Although the mean cone volume by CKC was greater (5.59±5.28 cm^3) than in LEEP (2.96±3.14 cm^3), the difference was not statistically significant. The depth of tissue was greater in the CKC group than in the LEEP group (p=0.025). The calculated length of cervix was CKC = LEEP < controls (p=0.003) (Table 1). Although conization was not seen as a factor affecting the total duration of pregnancy (p=0.294) (Table 1), the number of preterm deliveries was higher in the CKC and LEEP groups than in the control group (p=0.014). When we analysed the reason of preterm delivery, five (38%) patients in CKC group and one (16%) in LEEP group were due to PPROM (Table 2). Pregnancies with a history of CKC were more likely to be complicated by PPROM and low 1st and 5th minute APGAR scores than pragnancies with a history of LEEP and the controls (p=0.007, p=0.015 and p=0.001, respectively) (Tables 1, 2). The incidence of low 1st and 5th min APGAR scores was more common in preterm and PPROM cases, which was the main reason for the difference between the CKC and LEEP groups and the control group. The rate of overall mortality, which included late spontaneous abortion and fetal mortality, in the CKC group was also higher than in the LEEP and control groups (p=0.004) (Table 2).

We evaluated the effect parameters, such as cone volume and depth, time elapsed from conization to pregnancy, cervical length, smoking, and type of CIN (CIN 2 CIN 3) on adverse pregnancy outcomes including preterm delivery, PPROM, PROM, and fetal mortality. The time from conization to pregnancy (t-CP) in patients with PPROM and preterm delivery were significantly shorter than in those who delivered at term and without PPROM (p=0.005 and p=0.046, respectively). A shortened cervix was associated with preterm delivery, PPROM, and fetal mortality (p<0.001, p=0.037, and p=0.005). As the volume of excised tissue increased, the rate of fetal mortality also increased (p=0.019) (Table 3). Using the receiver operating characteristics (ROC) curve, a cervical length under 31 mm and t-CP under 14 months was observed to be the most relevant value for the prediction of preterm delivery, with 63.16% sensitivity and 77.42% specificity [AUC: 0.714, 95% CI: (0.603-0.809); p=0.005], and <31 mm had 65% sensitivity and 71.78% specificity [AUC: 0.731, 95% CI: (0.675-0.782); p<0.001], respectively (Figure 2). For the prediction of PPROM, t-CP of <15 months had 85.71% sensitivity and 65.22% specificity [AUC: 0.730, 95% CI: (0.603-0.809); p=0.024], and cervical length of <32 mm had 72.73% sensitivity and 61.89% specificity [AUC: 0.685, 95% CI: (0.675-0.782); p=0.007], (Figure 3).

Table 1. Comparison of the groups regarding fetal and maternal characteristics

	CKC		LEEP		Control		P
	Mean ± SD	Median (range)	Mean ± SD	Median (range)	Mean ± SD	Median (range)	
Maternal age at conisation (years)	31.61±3.97	32 (18)	31.53±4.21	32 (16)	NA	NA	0.875*
Maternal age at delivery (years)	34.12±3.54	34 (19)	34.57±3.11	34.5 (12)	33.86±3.97	34 (20)	0.620+
BMI (kg/m^2)	27.82±3.72	27 (17)	28.07±3.05	28 (12)	27.24±4.59	26.79 (26.7)	0.236
Gravidity	3.49±1.63	3 (9)	3.2±1.37	3 (5)	3.69±2.15	3 (19)	0.614
Parity	1.78±1.15	2 (5)	1.47±1.11	2 (4)	1.82±1.21	2 (5)	0.405
Volume of cone (cm^3)	5.59±5.28	4.39 (18.71)	2.96±3.14	2.43 (11.64)	NA	NA	0.061*
Depth of cone (cm)	1.11±0.39	1 (1.7)	0.96±0.35	0.8 (1.2)	NA	NA	0.025*
Time from conisation to delivery (month)	30.12±18.00	24 (72)	36.33±31.32	2 (8)	NA	NA	0.960*
Time from conisation to LMP (month)	22.47±14.88	18 (57)	28.27±28.34	17.5 (91)	NA	NA	0.984*
Cervical length (mm)	32.12±5.56	32 (28)[a]	32.97±3.92	32 (14)[a]	34.91±6.37	36 (30)[b]	0.003
Pregnancy weeks at cervical length measurement	18.43±2.69	17 (8)	17.87±2.45	17 (8)	17.8±2.14	17 (10)	0.582
Duration of pregnancy (days)	254.43±41.23	266 (241)	262.13±29.3	270.5 (151)	260.99±26.85	266.0 (175)	0.294
APGAR 1'	8.5±1.56[a]	9 (9)	8.83±0.54[b]	9 (2)	8.88±0.53[b]	9 (3)	0.015+
APGAR 5'	9.46±1.64[a]	10 (10)	9.93±0.26[b]	10 (1)	9.89±0.52[b]	10 (3)	0.001+

P<0.05 means there is significantly statistical difference between groups. *P-values from Mann-Whitney U test, +p-values from ANOVA and all others from Kruskal-Wallis test[a,b]. Medians or means with the same indices are the same, with different indices are statistically different from each other. CKC: Cold knife conization, LEEP: Loop electrosurgical excision procedure, SD: Standard deviation, BMI: Body mass index, LMP: Last menstrual period

Table 2. Comparison of the groups according to maternal characteristics and obstetric outcomes

		CKC		LEEP		Control		p
		n	%	n	%	n	%	
CIN	CIN 2	21	41.18	19	63.33	-	-	0.068
	CIN 3	30	58.82	11	36.67	-	-	
Method conception	Spontaneous	48	94.12	28	93.33	191	95.50	0.574*
	IUI	1	1.96	0	0.00	4	2.00	
	IVF	2	3.92	2	6.67	5	2.50	
Smoking	No	29	56.86	23	76.67	134	67.00	0.173
	Yes	22	43.14	7	23.33	66	33.00	
Preterm delivery	No	38	74.51	24	80.00	179	89.50	**0.014**
	Yes	13	25.49	6	20.00	21	10.50	
Mode of delivery	VD	24	47.06	12	40.00	110	55.00	0.096*
	C/S	24	47.06	17	56.67	88	44.00	
	Abortus	3	5.88	1	3.33	2	1.00	
PPROM	No	45	88.23	28	93.33	196	98.00	**0.007***
	Yes	6	11.76	2	6.66	4	2.00	
PROM	No	48	94.12	27	90.00	194	97.00	0.126*
	Yes	3	5.88	3	10.00	6	3.00	
HT	No	48	94.10	26	86.70	185	92.50	0.450*
	Yes	3	5.90	4	13.30	15	7.50	
Placenta previa	No	49	96.08	30	100.00	195	97.50	0.683*
	Yes	2	3.92	0	0.00	5	2.50	
Preeclampsia	No	50	98.04	26	86.67	189	94.50	0.123*
	Yes	1	1.96	4	13.33	11	5.50	
GDM	No	47	92.16	28	93.33	182	91.00	1,000*
	Yes	4	7.84	2	6.67	18	9.00	
Oligohydramnios	No	47	92.20	29	96.67	196	98.00	0.075*
	Yes	4	7.80	1	3.33	4	2.00	
Polyhydramnios	No	50	98.04	27	90.00	194	97.00	0.108*
	Yes	1	1.96	3	10.00	6	3.00	
IUGR	No	47	92.16	27	90.00	177	88.50	0.746
	Yes	4	7.84	3	10.00	23	11.50	
Gender	Female	23	45.10	15	50.00	99	49.50	0.845
	Male	28	54.90	15	50.00	101	50.50	
NICU admission	No	42	82.35	26	86.66	184	92.90	0.067*
	Yes	9	17.64	4	13.33	14	7.10	
RBC Tx	No	49	96.10	30	100.00	192	96.00	0.761*
	Yes	2	3.90	0	0.00	8	4.00	
Foetal mortality	No	46	90.2	28	93.30	198	99.00	**0.004**
	Yes	5	9.80	2	6.70	2	1.00	
	Abortus	3	5.88	1	3.33	2	0.00	

P<0.05 means there is significantly statistical difference between groups. CKC: Cold knife conization, LEEP: Loop electrosurgical excision procedure, CIN: Cervical intraepithelial neoplasia, IUI: Intrauterine insemination, IVF: In-vitro fertilization, VD: Vaginal delivery, C/S: Cesarean section, PPROM: Preterm premature rupture of membranes, PROM: Premature rupture of membranes, HT: Hypertension, GDM: Gestational diabetes mellitus, IUGR: Intrauterine growth restriction, NICU: Neonatal intensive care unit, RBC: Red blood cell, Tx: Transfusion

Table 3. Effect of some parameters on pregnancy outcomes

			Volume of cone (cm^3)	Depth of cone (mm)	Time from conization to pregnancy (months)	Cervical length (mm)	Smoking (no)	Smoking (yes)	CIN 2	CIN 3
Preterm delivery	No	Mean	4.14	1.06	27.76	34.92	160 (86.02)	81 (85.26)	32 (80.00)	30 (73.17)
		SD	4.22	0.40	22.43	5.75				
		Median	2.58	0.80	23.00	35.00				
		Range	18.76	1.70	91.00	32.00				
	Yes	Mean	6.15	1.02	14.37	29.80	26 (13.98)	14 (14.74)	8 (20.00)	11 (26.83)
		SD	6.08	0.30	9.67	6.43				
		Median	2.73	0.80	10.00	29.50				
		Range	18.40	0.90	31.00	29.00				
p			0.210	0.995	**0.005**	**<0.001**	0.863		0.601	
PPROM	No	Mean	4.53	1.06	25.58	34.44	174 (96.13)	91 (95.79)	34 (89.47)	35 (92.11)
		SD	4.74	0.39	21.50	6.10				
		Median	2.64	0.80	18.00	35.00				
		Range	18.76	1.70	91.00	32.00				
	Yes	Mean	5.80	0.96	12.71	30.27	7 (3.87)	4 (4.21)	4 (10.53)	3 (7.89)
		SD	5.72	0.26	9.83	5.78				
		Median	2.73	0.80	9.00	31.00				
		Range	14.91	0.70	28.00	20.00				
p			0.403	0.685	**0.046**	**0.037**	1,000*		1,000*	
PROM	No	Mean	4.82	1.06	24.43	34.28	179 (96.24)	90 (94.74)	36 (90.00)	39 (95.12)
		SD	4.83	0.39	21.71	6.15				
		Median	2.76	0.80	17.00	35.00				
		Range	18.71	1.70	91.00	32.00				
	Yes	Mean	2.05	0.98	27.00	32.33	7 (3.76)	5 (5.26)	4 (10.00)	2 (4.88)
		SD	2.96	0.27	3.58	4.85				
		Median	0.85	0.90	27.00	32.00				
		Range	7.77	0.70	10.00	14.00				
p			0.069	0.929	0.100	0.202	0.547*		0.432*	
Foetal mortality	No	Mean	4.19	1.03	25.26	34.40	180 (96.72)	92 (96.84)	36 (90.00)	38 (92.68)
		SD	4.39	0.37	21.22	6.00				
		Median	2.52	0.80	18.00	35.00				
		Range	18.76	1.70	91.00	30.00				
	Yes	Mean	9.10	1.27	17.86	27.89	6 (3.23)	3 (3.16)	4 (10.00)	3 (7.32)
		SD	6.46	0.39	17.16	6.25				
		Median	8.48	1.50	9.00	30.00				
		Range	16.17	0.90	48.00	21.00				
p			**0.019**	0.069	0.198	**0.005**	1.000*		0.712*	

*Fisher's exact p-value and all others from Mann-Whitney U test, $p<0.05$ means there is significantly statistical difference between groups. SD: Standard deviation, CIN: Cervical intraepithelial neoplasia, PPROM: Preterm premature rupture of membranes, PROM: Premature rupture of membranes

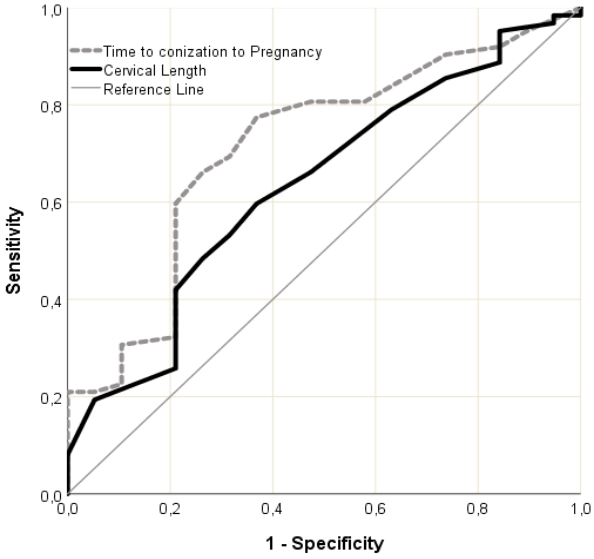

Figure 2. ROC analysis of cervical length and time from conization to pregnancy and preterm delivery
ROC: Receiver operating characteristics

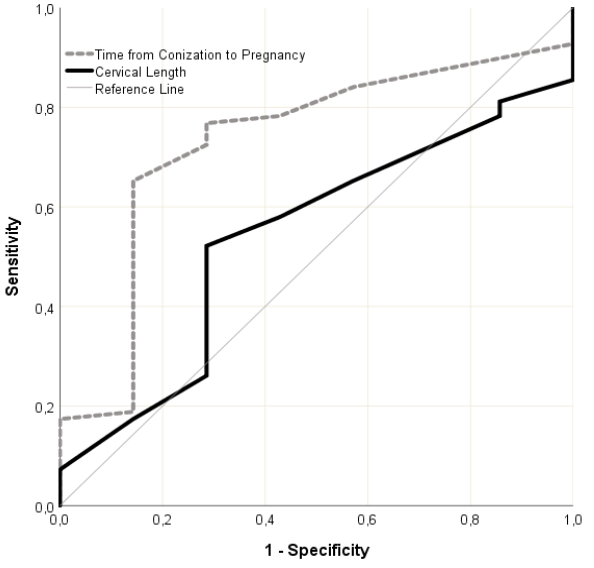

Figure 3. ROC analysis of cervical length and time from conization to pregnancy and PPROM
ROC: Receiver operating characteristics, PPROM: Preterm premature rupture of membranes

Table 4 shows the results of multivariate LR that included the risk of admission to the neonatal intensive care unit, PPROM, delivery mode, preterm delivery, cervical length, and low APGAR 1st and 5th-minute scores. According to the final model, PPROM and cervical length were significant (p=0.024 and p=0.048, respectively); patients with PPROM were 4.3 times more likely to be in conization group. For each one millimetre shortening of the cervix, the likelihood of PPROM was increased 1.01 times.

Discussion

The aim of this study was to focus on obstetric outcomes and factors affecting subsequent pregnancies after conization due to CIN. The one difficulty in evaluating factors affecting obstetric outcomes is that there are numerous potential factors. The well-known risk factors of adverse obstetric outcomes are increased maternal age, smoking, multifetal gestation, and obstetric complications including polyhydramnios, hypertension, and preeclampsia, which were similar between all groups in our study. Furthermore, we did not include patients with a history of preterm delivery and multifetal gestation. Moreover, obstetric complications including gestational diabetes mellitus, hypertension, preeclampsia, polyhydramnios, oligohydramnios, and placenta previa were similar in all groups of this study. The majority of published studies compared conization groups and control groups, meaning that the control and conization groups were different in respect to the history of preterm delivery. Thus, the outcomes of these studies are debatable. In this respect, the present study is valuable.

The outcomes of this study can be summarized as cone volume removed during CKC or LEEP was similar; however, cone depth in CKC was longer. CKC was related to a higher incidence of preterm delivery, PPROM, low 1st and 5th minute APGAR scores, fetal mortality, and late spontaneous abortion. When we evaluated factors that affected preterm delivery and PPROM, shorter cervical length and less time elapsed from conization to pregnancy were correlated, rather than cone volume and depth. Cone volume was correlated with overall fetal mortality including late spontaneous abortion and fetal mortality.

As a structure that holds the fetus in the uterine cavity and protects the fetus, both anatomically and by secreting cervical mucus, which contains several antimicrobial agents and forms

Table 4. Multiple logistic regression analysis results to identify risk factors for being conization

Variables	B	Standard error	p	Exp(B) O.R.	O.R. lower limit	O.R. upper limit
PPROM	1,472	0.652	0.024	4,357	1,214	15,643
Cervical length	-0.046	0.024	0.048	0.988	1,000	1,101
PPROM: Preterm premature rupture of membranes						

It is known that some bacteria, such as *Bacteroides fragilis* and group B *Streptococcus,* can cause PPROM or preterm delivery by secreting phospholipase or proteolytic enzymes (17). Conization alters the cervical tissue anatomically, physiologically, and histologically. As a result of conization, the internal orifice of the cervical canal can be damaged and the cervical gland, which secretes mucus with a protective effect against ascending infectious agents, can be destroyed (11). LEEP and CKC are both effective, safe methods in the treatment of CIN and have similar rates of recurrence (18). LEEP controls the maximum size of the cone; however, cone biopsy by CKC can either be too large or too deep (6). In the present study, although the mean cone volume by CKC and LEEP were similar (p=0.061), the cone depth in CKC was longer than in LEEP (p=0.025). Considering the damage to the cervical canal and the secretory function of cervical glands, cone biopsy depth is more important than cone volume. The other evidence that supports this opinion is that although cervical cerclage supports the cervix mechanically, it is not effective in pregnancies with a history of conization (14,19). Recently, Liverani et al. (13) reported that cone depth was correlated with preterm delivery in pregnancies after conization due to CIN, but not cone volume. Liu et al. (6) conducted a prospective randomized controlled study comparing 124 pregnancies with a history of LEEP and 120 pregnancies with a history of CKC and they found that compared with LEEP, cone biopsy depth by CKC was deeper and in parallel with the incidence of preterm delivery, and PPROM was more common with CKC compared with LEEP. However, they did not report the cone volumes (6). Although studies found a similar incidence of preterm delivery and PPROM between CKC and LEEP, a link was reported between cone depth and preterm delivery (9,10). This disparity might result from different cone sample sizes, depths, and diameters, and times elapsed from conization to pregnancy.

It has been shown that cervical tissue is highly regenerable. As expected, deeper and wider wounds to the cervix require more time. Accordingly, a study that investigated the minimum time that should elapse from conization to pregnancy found the time for CKC was nine months and LEEP was six months, which is compatible with the volume and depth of excised tissue (11). Similarly, a study found that immediate pregnancy after LEEP increased the risk of preterm delivery (20). This is borne out by the results of the present study as the t-CP was significantly shorter in those with preterm delivery and PPROM compared with those without. ROC analysis showed that the t-CP under 14 months was a risk for preterm delivery and under 15 months was a risk for PPROM. These times were longer than those reported in a previous study (11). Although pregnancy outcomes improved over time, this should be balanced by the fact that the patients who undergo conization due to CIN are older than the general pregnant population and advanced age in women is related to low fertility rates and poorer pregnancy outcomes. Thus, recommendations for the optimal time that should elapse from conization to pregnancy must consider the patient's age, cone depth, and the desired number of children. Further studies are needed in this regard.

The relationship between cervical length and preterm delivery has been well established in obstetric care. However, there is no consensus on the exact length, ranging from 15 mm to 30 mm. Some authors accept 25 mm for those with a history of preterm delivery and 20 mm for those without a history of preterm delivery (21,22). In the present study, for patients who underwent conization, using ROC analysis, cervical length <31 mm was a risk for preterm delivery and <32 mm was a risk for PPROM. These differences between conization and non-conization cases may result from altering the physiologic and histologic nature of cervical tissue by conization.

Study Limitations

The limitation of this study is that although the patients had good documentation, there is a possibility of missing patients, which creates selection bias due to the nature of the retrospective analysis.

Conclusion

CKC results in deeper cone depth and shorter cervical length. The incidence of PPROM, preterm delivery, low APGAR scores, and fetal mortality were higher in patients with a history of CKC. The t-CP and cervical length at pregnancy are determinant factors for preterm delivery and PPROM. Cervical length at pregnancy <31 mm was a risk for preterm delivery and <32 mm was a risk for PPROM. It is important to consider this when advising patients about the optimal time to become pregnant because the t-CP under 14 months was a risk for preterm delivery and 15 months was a risk for PPROM. Strategies that regulate the vaginal microbiota and prevent infectious morbidity is also a reasonable management approach because one of the most prevalent complications of pregnancies with conization is PPROM. However, future randomized controlled studies are needed before these suggestions can be fully accepted.

Ethics Committee Approval: *The study was approved by the University of Health Sciences Turkey, Etlik Zübeyde Hanım Woman's Health Training and Research Hospital Ethical Committee Local Ethics Committee (approval number: 08/23, date: 23.06.2021).*

Informed Consent: *Retrospective study.*

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The role of leptin in the male reproductive system

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Abstract

Leptin is a hormone produced from adipose tissue, targeting the hypothalamus and regulating energy expenditure, adipose tissue mass, and reproductive function. Leptin concentration reflects body weight and the amount of energy stored, as well as the level of reproductive hormones and male fertility. In this review, the aim was to focus on leptin signaling mechanisms and the significant influence of leptin on the male reproductive system and to summarize the current knowledge of clinical and experimental studies. The PubMed database was searched for studies on leptin and the male reproductive system to summarize the mechanism of leptin in the male reproductive system. Studies have shown that obesity-related, high leptin levels or leptin resistance negatively affects male reproductive functions. Leptin directly affects the testis by binding to the hypothalamic-pituitary-gonadal axis and the receptors of testicular cells, and thus the location of leptin receptors plays a key role in the regulation of the male reproductive system with the negative feedback mechanism between adipose tissue and hypothalamus. Based on the current evidence, leptin may totally inhibit male reproduction, and investigation of this role of leptin has established a potential interaction between obesity and male infertility. The mechanism of leptin in the male reproductive system should be further investigated and possible treatments for subfertility should be evaluated, supported by better understanding of leptin and associated signaling mechanisms.
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Keywords: Leptin, male reproductive system, obesity

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Introduction

Leptin is a hormone largely produced by adipocytes (1). Leptin receptors are widely spread in many tissues, cells, and endocrine glands and perform vital functions by binding to leptin and activating several pathways including Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3), extracellular signal-regulated kinases 1/2 (ERK 1/2), and phosphoinositide 3-kinases (PI3K)/AKT signal pathways (2,3). Leptin receptors are particularly concentrated in the hypothalamus, and by binding to them, leptin stimulates neuronal pathways that control body weight and energy expenditure and stimulate the pituitary gland to release gonadotropin hormones. Gonadotropin hormones play a crucial role in regulating the timing of puberty and reproductive functions, which means that leptin plays a role in regulating fertility and body weight simultaneously and across common pathways (4,5). Leptin levels correlate positively with fat mass. Excess body weight and obesity lead to increased secretion of leptin, and this usually causes resistance to leptin (6,7). Moreover, low body weight leads to a lack of leptin, and therefore it is normal for reproductive function disorders in obese or thin men to be caused by excess, deficiency, or resistance to leptin (8). Leptin resistance is not only related to obesity, but may also result from a genetic defect in leptin receptors, and variants may occur in the leptin gene (*ob*) that lead to the failure to produce leptin (9,10). In addition, leptin plays a role independent of the hypothalamus in regulating testicular functions and steroidogenesis through its association with its receptors throughout all testicular and sperm cells (11,12). Leptin is also involved in the negative effects of some diseases on reproductive functions (13,14). In this review, we aim to summarize the role of the physiological leptin in reproductive function, the relationship between leptin level and fertility, and the risk of subfertility.

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Leptin

Leptin is a protein hormone produced from white adipose tissue by the *Ob* gene. Like many other hormones, leptin is secreted in a pulsatile fashion at higher levels in the evening and early morning hours (15). It is released into the bloodstream and binds to its receptors in the hypothalamus, creating a feeling of satiety, and therefore it was previously called the "satiety hormone" (1,16,17). Leptin maintains its functions by binding to specific leptin receptors (Ob-Rs) expressed in peripheral tissues, as well as in the brain. There are several isoforms of Ob-Rs. The Ob-Ra isoform (short leptin receptor isoform) plays an important role in the transport of leptin across the blood-brain barrier (BBB) (18). The Ob-Rb isoform (long leptin receptor isoform) is strongly expressed and mediates signal transduction in the hypothalamus, a region important for the regulation of neuroendocrine and energy homeostasis (19,20). Leptin is also secreted in small quantities from gastric mucosa, brown adipose tissue, bone marrow, striated muscle cells, mammary gland, ovaries, brain tissue, lymph tissue, placenta, and spermatozoa (21-23). Leptin regulates its vital functions by binding to ob-Rs, which are found on neuronal and non-neuronal cells. The most important roles of leptin are to regulate fat storage, energy consumption, neuroendocrine function, immunity, reproduction, bone metabolism, angiogenesis, inflammation, growth hormone secretion, and improvement in insulin sensitivity (24-26).

Leptin receptors

Leptin receptors are divided into six types, one of them is long (ob-Rb), four short (ob-Ra, ob-Rc, ob-Rd, ob-Rf), and one soluble (ob-Re), according to the length of their domains inside the cell (27). The long, active isoform of Ob-Rb is expressed primarily in the hypothalamus, and plays an important role in the regulation of endocrine organs and energy homeostasis. Also, it has been reported that leptin receptors located in the uterine artery during the ovarian cycle and pregnancy regulate angiogenesis in uterine artery endothelial cells (28). Ob-Rb is found in all immune cells related to adaptive and innate immunity (29-31). Another study demonstrated that leptin/ob-Rb signaling plays an important role in the pathogenesis of obesity-associated neutrophilic airway inflammation in women by promoting M1 macrophage polarization (32). Lack of full-length Ob-Rb receptor in obese rats and db/db mice induces the development of early obesity phenotype. In db/db mice, the presence of a short Ob-Ra isoform with limited activity causes morbid obesity, diabetes, and developmental disorders in adolescence. Furthermore, the db/db mouse phenotype lacks leptin receptors but exhibits a significantly higher blood leptin concentration (33). The cytoplasmic domains of the long receptor contain segments capable of activating the JAK-STAT3 pathway and are found largely in the hypothalamus, and in small amounts in the lungs, pancreas, muscles, ovaries, testes, blood, kidney, heart, BBB, and sperm (2,4). The cytoplasmic domains of the short receptor lack the segments that activate the JAK/STAT3 pathway, but it can activate leptin signals via the adenosine monophosphate kinase (AMPK) pathways, and it is found in the liver, pancreas, gonad, and BBB (4,34). The soluble receptor lacks both cytoplasmic and membrane segments and plays a role as a leptin-binding protein in blood circulation and regulates its bioavailability and is also found in the seminal plasma (35,36). The majority of Ob-R isoform receptors are intracellular, with only 5-25% found on the cell surface. After ligand binding, the receptors are internalized into endosomes via clathrin-coated vesicles. The receptor is broken down or recycled to the cell membrane. A decrease in Ob-Rb expression is much greater than changes in Ob-Ra expression, and the short isoform Ob-Ra is recycled much more rapidly to the cell membrane (37-39).

Leptin signaling pathways

Leptin causes JAK/STAT3 signal activation by binding to long receptors (ob-Rb) with intracellular signaling capabilities. JAK2 phosphorylates Tyr985, Tyr1138, and Tyr1077 tyrosine localize in the intracellular domain. Two units of STAT3 bind to phosphorylated tyrosine residues and are phosphorylated to form the STAT3 dimer. The dimer migrates to the nucleus and binds to target genes. If this signal occurs in the hypothalamus, the dimer activates cocaine- and amphetamine-regulated transcript (CART) and pro-opiomelanocortin (POMC) neurons and inhibits agouti-related peptide (AGRP) and neuropeptide Y (NPY) neurons. Moreover, the dimer causes transcription of suppressors of cytokine signaling 3 (SOCS3) which prevents excessive activation of leptin by inhibiting JAK2, so this protein is part of the negative feedback mechanism (35,40).

When leptin binds to the receptor, a second signal pathway, ERK1/2 [also known as Ras/Raf/mitogen-activated protein kinase (MAPK)], is also activated. Tyrosine-protein phosfatase (SHP2) and growth factor receptor-bound protein 2 bind to Tyr985 residue phosphorylated by JAK2. Then the enzyme, ERK, initiates a protein chain. Afterward, the activated mammalian target of rapamycin complex 1 (mTORC1) inhibits the AMPK signal. ERK also activates MAPK (3,40). AMPK enzyme functions as an energy sensor. When the energy level inside the cell decreases and the ATP ratio increases, AMPK is activated by phosphorylation of the α subunit, allowing amino acids and glucose to enter the cell for energy synthesis. Thus, leptin must inhibit this enzyme to secure energy consumption (41,42). AMPK is also found in the midpiece and flagellum of the sperm and plays a role in motility modulation. Therefore, in the absence of leptin

expression or if mTORC1 is deleted, AMPK is not inhibited, resulting in decreased sperm motility (43).

The third signaling pathway that may be activated by leptin is the PI3K/AKT signaling pathway. Insulin receptor substrate (IRS) is phosphorylated and activates PI3K which stimulates the protein kinase (AKT). AKT activates mTORC1 and inhibits FoxO1, which inhibits POMC neurons and activates AGRY neurons (42,44).

In a study, it was shown that the proliferation and neuronal differentiation of neural stem cells were supported by the cooperative effect of MAPK/ERK1/2, JAK2/STAT3 and PI3K/AKT signaling pathways induced by leptin (45). In addition, these signaling pathways induced by leptin play an important role in many cyclic activities, such as development, differentiation, renewal and repair. Dysregulation of these leptin-induced signaling pathways leads to pathological processes (46). In one study, dysregulation of leptin signaling in Alzheimer's disease was reported as evidence of neuronal leptin resistance (47).

Leptin - hypothalamus - adipocyte axis

When we eat food, the energy obtained may be greater than the energy consumed. To maintain the balance of this energy, fatty acids and glucose in the blood are stored as triglycerides in adipocyte droplets within the white adipose tissue. After about two hours, fat mass increases and leptin is released. Leptin and insulin in the blood both bind to cognate receptors on the hypothalamus, inhibiting anabolic reactions by inhibiting neuropeptides, such as NPY and AGRP and initiating catabolic reactions by stimulating neuropeptides likely POMC and CART. POMC is cleaved by proteolytic enzymes into adrenocorticotropic, β-lipotropic, and α-melanocyte stimulating hormone (α-MSH). These hormones and CART reduce the appetite and increase energy expenditure. After energy consumption and increased lipolysis, fat mass decreases and leptin release stops. In this way, leptin plays a role in maintaining energy balance and regulating body mass (Figure 1) (4,48,49). As a result, blood leptin levels are positively correlated with bodily fat mass (2,6). When a mutation occurs in the *Ob* gene, the energy balance may be disturbed leading to increased food intake and potentially resulting in severe obesity (Figure 1) (16).

Leptin resistance

Leptin resistance is a major biological factor in cases of obesity. Leptin resistance, in which the body becomes insensitive to leptin, will prevent the feeling of satiety and lead to increased food intake. SOCS3 and protein tyrosine phosphatase 1B (PTPB1), which are part of the negative feedback mechanism after leptin is expressed, inhibit JAK2 phosphorylation, preventing leptin overactivation. When leptin expression increases significantly in obese men, SOCS3 and PTPB1 concentrations increase significantly, permanently inhibiting leptin expression (50,51).

All excess fatty acids combine with glycerol and are stored in adipocyte tissue in the form of triglyceride. When some people eat too much, for unknown reasons these fatty acids turn into diacylglycerol, ceramide, or acetyl-CoA and are stored in different locations, such as the liver, kidney, or hypothalamic neurons leading to lipotoxicity. In the hypothalamic neurons, these molecules cause stress of the endoplasmic reticulum. PTPB1, which is located on the surface of the endoplasmic reticulum, and SOCS3 expressions increase, permanently inhibiting leptin expression. Furthermore, endoplasmic reticulum stress in POMC neurons leads to incorrect or absent folding of MSH, so appetite is not reduced and energy is not consumed (52,53). Moreover, in obese persons, matrix metalloproteinase 2 is activated in the hypothalamus. This enzyme cleaves leptin receptors and leads to inhibition of leptin expression (54).

Another reason for leptin resistance may be the incapacity of leptin to cross the BBB. If leptin cannot pass through the BBB, it will not reach the hypothalamus and exert its effects. A high triglyceride level inhibits this crossing of the BBB (35,55). Triglycerides may cross the BBB and regulate central leptin receptor resistance (55). The relationship between leptin and triglycerides is not fully known, but in obese rats, fasting reduced triglyceride levels and increased leptin transport across the BBB and satiety increased triglyceride levels and reduced leptin transport across the BBB, so it is thought that the leptin transporter may have a regulative site controlled by the triglyceride (55,56). A study showed that leptin resistance protected mice from hyperoxia-induced acute lung injury (57).

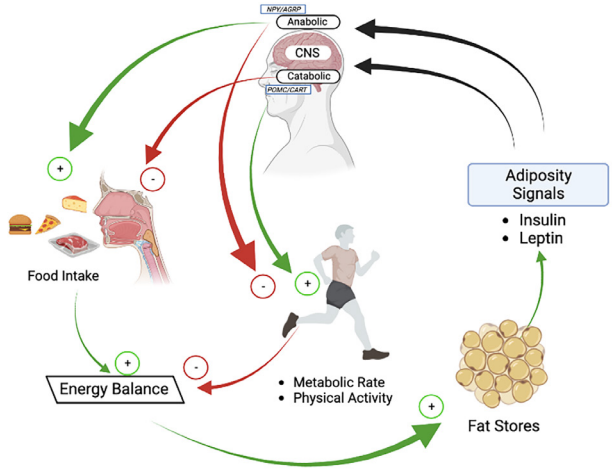

Figure 1. Leptin plays a role in maintaining energy balance and regulating body mass
AGRP: Agouti-related peptide, NPY: Neuropeptide Y

In addition, in the presence of low gene expression or gene mutation in leptin receptors, leptin resistance occurs (9). Blood-testicular barrier (BTB) does not play a role in leptin resistance, indicating that Sertoli, Leydig, and germ cells are exposed to high concentrations of leptin (35).

Interaction of leptin and the male HPG axis

Leptin regulates neural pathways that have multidirectional effects, linking energy storage with other physiological activities. It plays a main and important role in regulating reproductive function and securing the vital energy needed for it (3,36). The leptin released from adipose tissue travels through the blood and reaches the hypothalamus by transport across the BBB. It stimulates POMC, CART, and kisspeptin neurons by binding to the ob-Rb in the hypothalamic paraventricular and arcuate nuclei and inhibits NPY and AGRP, which suppress gonadotropin production. POMC, CART, and kisspeptin stimulate gonadotropin-releasing hormone (GnRH) that transfers to the anterior lobe of the pituitary gland and triggers the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH bind to their receptors in the testis inducing steroidogenesis and spermatogenesis (Figure 2) (12,58).

Leptin affects the testis through the HPG axis and by binding directly to its receptors in the testis and sperm (59). Kisspeptin functions as a stimulator of steroidogenesis. It has been shown that an interaction between leptin and sex hormones can trigger KISS-1/GPR54 signaling to GnRH neurons, suggesting novel mechanisms regulating the onset of puberty (60). Leptin levels peak before puberty, and with the increase in kisspeptin levels, leptin has been reported to be critical for the onset of puberty in males. Studies conclusively showed that kisspeptin neurons are not direct targets of leptin at the onset of puberty. Leptin signaling in kisspeptin neurons occurs only after the completion of sexual maturation and may experience a critical window of sensitivity to the influence of metabolic factors that may alter the onset of fertility (61). Furthermore, altering neonatal leptin fluctuation may alter the timing of pubertal onset and have long-term effects on reproductive and hypothalamic expression of metabolic neuropeptides (62). It also provides the energy and the availability of fats needed for puberty, where some studies have shown that the lack of leptin in boys leads to a delay in puberty (63,64).

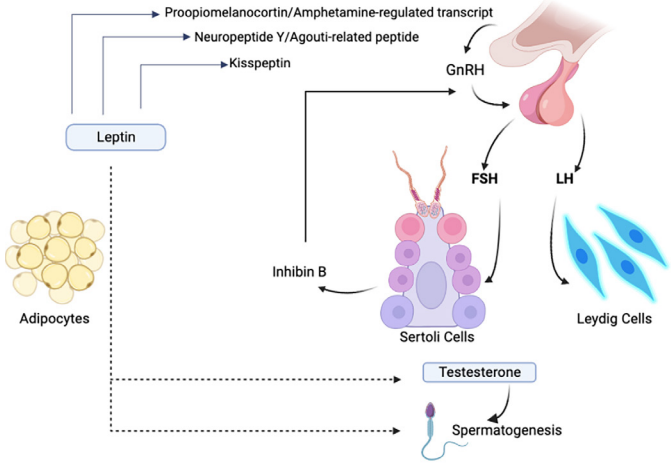

Figure 2. Mechanism of leptin actions on the hypothalamic-pituitary-gonadal axis
GnRH: Gonadotropin-releasing hormone, FSH: follicle stimulating hormone, LH: Luteinizing hormone

Leptin, sertoli, and germ cells

Sertoli cells are supporting cells found in the epithelium of the seminiferous tubules that have an important role in regulating spermatogenesis. The Sertoli cell contains glucose transporters (GLUTs) and ob-Rb. Glucose enters the Sertoli cell and converts to pyruvate via phosphofructokinase. Pyruvate is converted to alanine through alanine aminotransferase, to lactate through lactate dehydrogenase, and, in mitochondria, to acetyl-CoA via pyruvate dehydrogenase. Through monocarboxylate transporters, lactate passes into the adluminal space and enters the germ cells. Lactate is an important energy source for the germ cell and functions as an anti-apoptotic factor through an unknown mechanism. Acetyl-CoA is then converted into acetate that also enters germ cells, but its role is still unknown (Figure 3). However, acetate is considered the most important

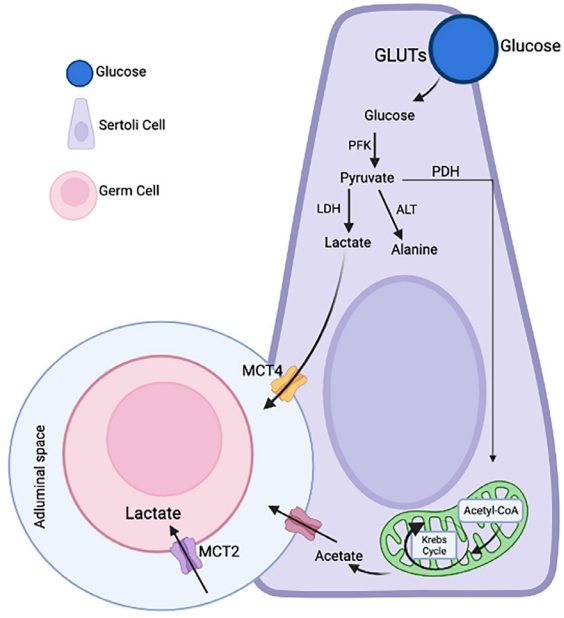

Figure 3. Metabolic cooperation between Sertoli cells (SCs) and developing germ cells
GLUTs: Glucose transporters, PFK: Phosphofructokinase, PDH: Pyruvate dehydrogenase, LDH: Lactate dehydrogenase, ALT: Alanine aminotransferase, MCT: Monocarboxylate transporters

carbon source for the synthesis of lipids and cholesterol that are necessary for germ cell division and spermatogenesis. When the leptin level increases in the Sertoli cell, lipolysis increases, and acetate is consumed to synthesize lipids again. It was also observed that when the leptin level increased, lactate was not produced. Therefore, germ cells are damaged and their concentration decreases (65-67). Thus, leptin triggers the production of factors necessary for spermatogenesis both through the HPG axis and by binding to its receptors in Sertoli cells (68). Moreover, considering the glycolytic flow suitability of Seroli cells, it has been reported that leptin affects mitochondrial physiology in human Sertoli cells and that leptin plays a role in glycolysis (68). Leptin also directly affects germ cells by binding to its receptors in these cells, as it phosphorylates STAT3, which supports stem cell renewal, proliferation, and differentiation (69).

In a study, Sertoli cells and peritubular myoid cells together form the testis microenvironment (TME). It has been shown that the differentiation of Leydig stem cells is severely impaired as a result of the loss of TME (70). This study was supported by other studies suggesting that cells within the TME are involved in the release of paracrine factors, which are very important for stimulating the differentiation of Leydig cells (71). In a study published in 2022, the important role of leptin, which is secreted by the TME and serves as a paracrine factor, on human Leydig cell differentiation and function was detected (72). In the same study, it was shown that low-level leptin treatment in cells taken from male testis biopsies with azoospermia can also increase testosterone levels and Leydig cell differentiation (72).

Leptin and Leydig cells

Leydig cells are interstitial cells located between the seminiferous tubules in the testes which are responsible for spermatogenesis, the biosynthesis and secretion of androgens, and maintaining secondary sexual characteristics in males. Leydig cells express ob-Rb. Leptin triggers the production of testosterone both through the HPG axis and by binding to its receptors in Leydig cells (25,73). In a study, leptin was identified as an important paracrine factor released by cells within the TME, modulating Leydig cell differentiation and testosterone release from mature Leydig cells (74). When LH binds to its receptors on the Leydig cell, cAMP levels increase which causes dissociation of the catalytic unit by binding to PKA. This unit enters the nucleus and phosphorylates the GATA4 transcription factor, allowing the expression of the steroidogenic acute regulatory protein (StAR) that transfers cholesterol from the outer membrane of the mitochondria to the inner membrane to produce testosterone (74,75). Normal levels of leptin are involved in stimulating StAR transcription factors via the PI3K/AKT and ERK1/2 pathways (76).

In order not to produce excessive amounts of steroids, cAMP is converted into AMP by phosphodiesterase. AMP activates AMPK that stops steroidogenesis by inhibiting transcription factors stimulating steroidogenesis and activating transcription factors inhibiting steroidogenesis (77). Since leptin inhibits AMPK when leptin expression is absent, AMPK is not inhibited, and sustained AMPK activity inhibits StAR expression, leading to a decrease in testosterone production (3,42,48,76). A study showed that high leptin levels lead to decreased expression of cAMP-dependent steroidogenic genes (*STAR* and *CYP11a1*) in MA-10 Leydig cells (78). Furthermore, another study showed that leptin inhibits the division of prepubertal Leydig cells through a cyclin D-independent mechanism and that cyclin D1 may play a role in leptin-induced differentiation of Leydig cells (79).

The role of leptin on male reproductive function

Since leptin hormone acts by crossing the BTB, it is present in the testicular fluid and seminal plasma and has receptors in spermatozoa, sperm, germ cell, somatic cell, epididymis, Leydig cell, Sertoli cell and epithelial cells of seminal vesicles and prostate (11,25,80,81). Leptin induces FSH and LH release via the HPG axis. Therefore, leptin plays a role in the production of testosterone in Leydig cells and androgen binding protein, testicular fluid, inhibin, activin, and factors necessary for spermatogenesis in Sertoli cells (Figure 2) (12,82). In an in vitro study, it was shown that leptin application reduced oxidative stress and apoptosis of sperm and positively affected mitochondrial function and energy source (83). Therefore, when leptin is absent or present at very low concentration due to being underweight, the level of steroid hormones decreases, germ cell apoptosis and the expression of pro-apoptotic genes in the testes increase (84) and vacuolization occurs in Sertoli cells (8,85,86). In the absence of leptin in *ob/ob* mice, fertility was restored with leptin therapy (87). Furthermore, when leptin concentration is elevated, the rate of apoptosis of all testis cells and the number of abnormal spermatazoa increases, sperm motility, concentration, and progressive motility decrease, and the BTB is disrupted, especially in the VIII of seminiferous epithelium stages, which is restructured for the pre-leptotene spermatocytes to pass through to enter the stage of meiosis (84,87,88). Another study indicates significant morphological, hormonal and enzymatic changes in leptin-deficient mouse testes. Alterations in the enzymatic steroidogenic pathway and enzymes involved in spermatic activity support insights into the fertility failures of these animals (85). In addition, a study has shown that leptin deficiency in mice was associated with impaired spermatogenesis, increased germ cell apoptosis, and upregulated expression of pro-apoptotic genes within the testes (86). It has been reported that dysfunction of

spermatogenesis in infertile men associated with varicocele was associated with an increase in leptin concentration and leptin receptor expression, and leptin had local effects on the function of testicles and spermatogenesis (89). Furthermore, a study reported that leptin and leptin receptor expression in the testicles of fertile and infertile patients is due to a systemic effect related to the central neuroendocrine system, androgen levels, or spermatogenic presence, rather than a direct effect on testicular tissue (16).

The interaction of leptin and obesity on the male reproductive system

Leptin has many effects on the reproductive system, and studies have shown that it provides a link between infertility and obesity. The obese male body is resistant to leptin. When leptin is not expressed, AMPK increases, leading to StAR production decreases, and thereby testosterone production decreases. Obesity also reduces the expression of steroidogenic factor-1 which is necessary to produce StAR and P450 side-chain cleavage enzyme which is involved in the synthesis of testosterone (3,76,90,91).

When leptin concentration increases, it decreases the activities of antioxidant enzymes in the cytosol and mitochondria via the PI3K/AKT signaling pathway and increases respiratory chain enzymes in the mitochondria. When the level of antioxidant is decreased in the cytosol, oxidative stress occurs and activates the pro-apoptotic molecules BAX and BAK that enter the mitochondria by changing the permeability of the outer mitochondrial membrane. The increased ROS in the mitochondria crosses into the cytosol and damages DNA. In addition, apoptosis-inducing factor and serine protease high-temperature requirement A2 (HtrA2) from mitochondria pass to the cytosol and cause cell apoptosis by breaking DNA. HtrA2 also separates the cytoskeleton and other cell substrates (Figure 4) (2). Protamine replaces histone during spermiogenesis. This is an important process for protecting the DNA because protamine is capable of packing longer sections of DNA than histone. In an unknown way high leptin levels in sperm reduces the replacement of histone by protamine, so that a smaller number of unpackaged DNA fragments are packaged. Consequently DNA is easily vulnerable to ROS damage (Figure 4) (2,59,92). Thus, ROS decreases the concentration of sperm and increases the percentage of abnormal sperm (25,93). In addition, ROS causes apoptosis of Leydig cells, Sertoli cells, and especially germ cells by damaging DNA, and in so doing also reduces sperm concentration and increases the percentage of abnormal sperm. Moreover, ROS disrupts the tight junction-related proteins (occludin, claudins, and ZO-1), disrupting the BTB. This also causes germ cell damage and negative changes in sperm parameters (88,94).

Since too much fat accumulates around the testis, the scrotal temperature (hyperthermia) increases, causing ROS to increase (95). In obesity, when adipocytes enlarge, the blood supply to them decreases, causing hypoxia and an increase in the accumulation of macrophages in the adipose tissue, which leads to adipocyte inflammation. Under normal conditions, a small amount of interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) is produced from adipose tissue, and during inflammation the levels of these proteins increase significantly, also causing ROS (96-98). The increased ROS oxidizes unsaturated fatty acids in the plasma membrane of the sperm, which leads to formation of malondialdehyde that causes DNA fragmentation and so the concentration of healthy sperm in obese men decreases. Moreover, ROS changes the phospholipid membrane of mitochondria and inhibits oxidative phosphorylation. Hence ATP and then the activity of mitochondria decreases, resulting in decreased sperm motility and progressive motility (99-101). ROS has been shown in a number of studies to disrupt the tight junction-related proteins of the BTB causing damage to germ cells and increasing the rate of apoptosis in Sertoli and Leydig cells (102-104).

In the adipocyte cell, testosterone is produced and converted to estradiol by an aromatase. Estradiol inhibits the HPG axis through a negative feedback mechanism and stimulates the proliferation of adipocyte cells. In obese men, increased adipose tissue produces high levels of estradiol which in

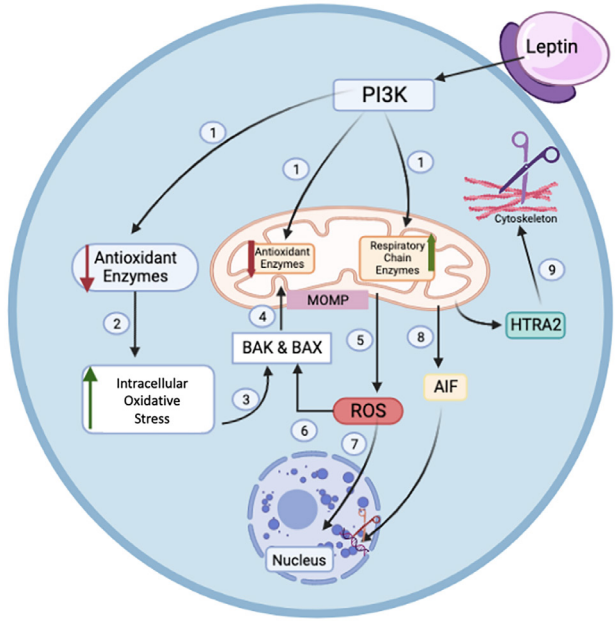

Figure 4. Excess leptin leads to increased ROS via the PI3K\AKT signaling pathway

ROS: Reactive oxygen species, PI3K: Phosphoinositide 3-kinases, HTRA2: High-temperature requirement-A2, AIF: Apoptosis-inducing factor

turn inhibits the HPG axis completely. Therefore testosterone production in the testicle is greatly reduced. Moreover, because testosterone reduces triglyceride accumulation and increases lipolysis in visceral adipose tissue by inhibiting lipoprotein lipase activity, a lack of testosterone leads to increased accumulation of these tissues which causes more estradiol production (105-107). Furthermore, obese men have low levels of inhibin B, sex hormone-binding globulin, FSH, LH, and androgen receptors (59,91,104,108).

There is a positive correlation between leptin and adipose tissue mass in normal men. In a state of positive energy balance, the body increases the size and number of adipocytes to store excess energy. Thus, the more adipose tissue in the body, the more leptin is released, and the man has no impaired reproductive function associated with leptin (6,109). In individuals with homozygous *Ob* gene mutation (*Ob*/*Ob*), no leptin is produced. Therefore, the satiety signal does not interact with the hypothalamus and so the person continues to eat food and gains weight, and this person has reproductive failure associated with leptin deficiency (110). When the person continues to eat constantly, too much fat is stored, hence leptin is released at a high level. In this case, the body becomes unresponsive to leptin to protect itself from high leptin concentration, so the satiety signal is again not detected and the person continues to eat and gain excess weight, and this person has impaired reproductive function associated with leptin deficiency (7,87,111).

Clinical and experimental studies

Studies demonstrated that seminal plasma leptin and its receptors in the testis were elevated in a varicocele patient and this elevation was inversely correlated with sperm density, sperm motility, the weight of testis, the diameter of seminiferous tubules and the thickness of the seminiferous epithelium, and positively correlated with ROS levels and the rate of sperm apoptosis, and it was concluded that leptin was the cause of sperm apoptosis by raising ROS levels (13,24,89).

In patients with leukocytospermia, studies have shown that seminal plasma leptin was elevated and that this elevation was inversely associated with sperm motility, and positively associated with ROS levels, TNF-α levels, and rate of sperm apoptosis. Thus, leptin was the cause of sperm apoptosis by raising ROS and TNF-α levels. Leukocytes migrate to the inflammation area in the genital system in leukocytospermia and phagocytose the damaged cells. Leptin receptors are found in macrophages and monocytes. When inflammation occurs, leptin binds to these receptors, causing macrophages and monocytes to proliferate, produce and release IL-1 and TNF-α and initiate apoptosis. TNF-α activates the caspase system by binding to its receptors in damaged cells. When the macrophage phagocytoses apoptotic bodies, which formed as a result of apoptosis, ROS is released. ROS causes apoptosis of sperm. In this way, leptin increases the release of TNF-α and IL-1. These also increase leptin mRNA expression in adipose tissue for an unknown reason, which means that there is a positive relationship between them. Also, leptin receptors are found in neutrophils and when leptin binds to them, it causes ROS production. As a result, leptin contributes to immune responses affecting fertility (13,112-114).

The leptin level was low in male Akita type 1 diabetic mice and leptin monotherapy was proven to rescue spermatogenesis in these mice. Akita mice have a mutation in the *insulin 2* gene that results in hyperglycemia and eventually type 1 diabetes. In Akita homozygous mice, body mass index, testicular and seminal vesicle weights, LH, testosterone, leptin, and insulin levels are low and spermatogenesis is absent (14,115). There is a relationship between insulin and leptin, as they converge at the PI3K signaling pathway in hypothalamic neurons. When they bind to their receptors, they initiate this signal and activate AKT, which stimulates mTORC1, which contributes to leptin secretion and inhibits FoxO1, which works against STAT3. In this way, insulin increases leptin secretion and expression (14,29,116). In adipocyte cells, insulin activates the vesicle containing GLUT4 through the same signaling pathway, causing GLUT4 to open and glucose to enter the cell. Moreover, insulin stimulates the formation of fatty acids by increasing the activity of fatty acid synthase and acetyl-CoA carboxylase through the same signaling pathway (117,118). Every three fatty acid molecules combine with glycerol, which is synthesized from glucose, to form triglycerides, which are stored in lipid droplets. Thus, insulin stimulates leptin release by increasing lipid synthesis (119). In type 1 diabetes, the decrease in insulin causes the fatty acid storage to decrease, so less leptin is released from the adipose tissue and infertility occurs (120,121). Low insulin causes infertility through both leptin deficiency and hyperglycemia, as hyperglycemia causes excess ROS production by various mechanisms (122,123). Leptin monotherapy, in the absence of exogenous insulin, in homozygous Akita mice significantly improved reproductive system functions and rescued Spermatogenesis. Consequently, infertility in patients with type 1 diabetes is not due to insulin deficiency but to leptin deficiency (14). In summary, studies on leptin metabolism and molecular signaling mechanism are shown in Table 1.

Conclusion

Leptin plays a unique and critical role in regulating energy expenditure, adipose tissue mass, and reproductive functions in males. It stimulates the hypothalamus to activate neural pathways that reduce appetite and increase energy

Table 1. The effects of leptin on male reproductive system. Studies in which the intracellular, intercellular, metabolic, and systemic effects of leptin are summarized

Effects of leptin and molecular mechanism	
The leptin hormone produced from adipose tissue binds to ob-Rb and causes JAK/STAT3 signal activation. This signal activates POMC and CART neurons in the hypothalamus and inhibits AGRP and NPY neurons.	- Landry et al. (35) - Francisco et al. (40)
Leptin binds to the receptor, ERK1/2-mediated mTORC1 is activated, while AMPK, which functions as an energy sensor, is inhibited. Thus, leptin ensures energy consumption.	- Kwon et al. (41) - Wauman et al. (42)
In the absence of leptin expression or when mTORC1 is deleted, AMPK located in the permine midpiece and flagellum is not inhibited, resulting in decreased sperm motility.	- Martin-Hidalgo et al. (43)
Leptin-mediated IRS is phosphorylated and PI3K is activated. AKT activates mTORC1 and inhibits FoxO1 (FoxO1 inhibits POMC neurons and activates AGRY neurons).	- Wauman et al. (42) - Zhou and Rui (44)
Kisspeptin acts as a stimulator of steroidogenesis. The prepubertal level of leptin reaches its peak and leads to a significant increase in the secretion of Kisspeptin, a stimulator of steroidogenesis. Leptin plays an important role in the onset of puberty in male.	- Elias (64) - Zhang and Gong (63)
Leptin induces the release of FSH and LH through the HPG axis. It plays a role in the production of testosterone in Leydig cells and androgen-binding protein in Sertoli cells, testicular fluid, inhibin, activin and factors necessary for spermatogenesis.	- Ramos and Zamoner (12) - Cheng and Mruk (80) - Zhang and Gong (63)
LH hormone raises cAMP levels in Leydig cell, which in turn binds to PKA. It phosphorylates the transcription factor GATA4, enabling the expression of StAR and producing testosterone.	- Abdou et al. (74) - Martin and Touaibia (75)
Normal leptin levels are involved in the induction of StAR transcription factors via the PI3K/AKT and ERK1/2 pathways.	- Roumaud and Martin (76)
Leptin triggers the production of factors necessary for spermatogenesis by binding to its receptors in Sertoli cells. Since it phosphorylates STAT3, which supports stem cell renewal, proliferation and differentiation, it directly affects germ cells by binding to its receptors in these cells.	- El-Hefnawy et al. (69)
Leptin is secreted by the TMJ and acts as a paracrine factor. It is involved in human LSC function and differentiation.	- Arora et al. (72)
ROS disrupts the tight junction related proteins of the BTB causing damage to germ cells and increases the rate of apoptosis in Sertoli and Leydig cells.	- Zhao et al. (103) - Fan et al. (104)

consumption and stimulates the secretion of gonadotropins that affect the Leydig and Sertoli cells, leading to steroidogenesis and supporting spermatogenesis. Therefore, leptin links body weight and fertility. Although leptin levels increase in weight gain, body weight loss is greatly reduced. Leptin receptors are found in all testicular cells and sperm, as leptin regulates reproductive functions independently of the hypothalamus through direct binding to its receptors. It supports testosterone production in Leydig cells and sperm motility by regulating AMPK levels and also supports germ cell regeneration, proliferation, and differentiation.

The role of leptin remains unclear in germ, sperm and Sertoli cells. In obese men, an increase in fat tissue acts to increase the level of leptin, followed by the occurrence of leptin resistance. When leptin expression decreases, it does not support the HPG axis and thus disrupts reproductive functions. Moreover, a high concentration of leptin leads to a decrease in testosterone secretion in Leydig cells, damage to germ cells, and increased levels of ROS that reduce the concentration, motility, and progressive motility of sperm and increase the percentage of abnormal sperm and apoptosis of Leydig, Sertoli, and germ cells by damaging DNA. High leptin concentration also disrupts the BTB. Given that the BTB does not play a role in leptin resistance, it is usual for testes and sperm cells to be exposed to high leptin levels. Leptin insufficiency due to being underweight or a mutation in the *Ob* gene also leads to a significant reduction in steroidogenesis and infertility.

In general, low leptin impairs reproductive functions by not supporting the HPG axis to secrete gonadotropins, and high leptin impairs reproductive functions by directly affecting the functions of testicular and sperm cells. More studies are still needed to clarify how leptin works and how its levels affect the male reproductive system, as the results of these studies may have a significant impact on treating impaired fertility.

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The biological and psychological impact of the Coronavirus disease-19 pandemic on the characteristics of the menstrual cycle

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Abstract

The Coronavirus disease-19 (COVID-19) pandemic was declared in March 2020 by the World Health Organization. The severe acute respiratory syndrome-coronavirus-2 virus enters host cells through angiotensin-converting enzyme 2 receptors and transmembrane serine protease type II that are expressed in pulmonary alveoli, as well as in hepatocytes, endothelium, ovaries, uterus, vagina, thyroid, and other tissues. In addition to viral injury, the COVID-19 pandemic, through protective measures such as social isolation and lockdown, has promoted a scenario of psychosocial stress, especially in women. In this context of isolation, anxiety, fear, and mental distress, there is dysregulation of the hypothalamic-pituitary-adrenal axis and subsequent gonadal side effects. Furthermore, studies report an association between COVID-19 and temporary menstrual cycle alterations such, as increased cycle duration, decreased cycle duration, increased menstrual flow, dysmenorrhea, and amenorrhea. Regarding COVID-19 vaccination, menstrual irregularities have been observed in about half of the women, predominantly with a decrease in cycle duration and increased menstrual flow, but without fertility sequelae. The aim of this study was to review the most up-to-date information on the relationship between the COVID-19 pandemic and menstrual irregularities. (J Turk Ger Gynecol Assoc. 2024; 25: 259-65)

Keywords: COVID-19, menstrual cycle, menstrual irregularity, SARS-CoV-2, women's health

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Introduction

The novel severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) was first reported from China on December 2019, and in March 2020, the Coronavirus disease-2019 (COVID-19) pandemic was declared by the World Health Organization (WHO) (1,2). As the transmission of this disease occurs through droplets, preventive measures such as social distancing and lockdown were recommended during this pandemic scenario (2,3).

COVID-19 is preceded by the entry of SARS-CoV-2 into host cells, mainly through angiotensin-converting enzyme 2 (ACE2) receptors, but also through transmembrane serine protease type 2 (TMPRSS2) that are abundantly expressed in pulmonary alveoli, as well as in other tissues such as hepatocytes and endothelial cells (2,4,5). Regarding women's health specifically, there have been reports of viral entry into organs of the female reproductive system, including the uterus, vagina, and ovaries, as well as viral involvement of organs, such as the thyroid, that participate in the homeostasis of the female hormonal axis (4,5).

In addition to the direct damage caused by viral infection, the COVID-19 pandemic has indirectly affected mental health because of disease containment policies. Overall, the population has been subjected to anti-social restrictions, such as quarantine, physical and social isolation, together with financial complications that also arose (3). The female population is described as a vulnerable group in this context

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because, as a result of quarantine measures, they were potentially exposed to financial dependence, increased responsibility for children, or domestic violence, which caused significant psychological and emotional distress (6-8).

The biological relationship between menstrual irregularities and psychosocial distress is mainly based on cortisol levels, a hormone that increases during psychological stress, which disrupts the hypothalamic-pituitary-adrenal axis and, consequently alters the hormonal regulation of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the menstrual cycle (9-11). Moreover, it has been reported that, in the presence of severe illness or infection, an energy diversion mechanism can occur in the female reproductive system which impacts the immune system or could give rise to compensatory reactions, resulting in menstrual cycle alterations in these patients (9,12,13). In the case of COVID-19, there are reports of ovarian suppression with altered levels of FSH and LH, directly affecting hormonal feedback regulation of menstruation (11,14).

The aim of the present study was to provide an up-to-date review on the biological or psychological impact of the COVID-19 pandemic on women's health, specifically in terms of menstrual cycle alterations.

Physiology of the menstrual cycle

The menstrual cycle is a natural process of the female reproductive system that occurs between menarche and menopause, and it extends from the first day of cyclic uterine bleeding, through endometrial shedding, until the next menstruation. An average menstrual cycle lasts for 28 days but can vary from 21 to 37 days (15,16). Briefly, the regulation of this cycle occurs through the stimulation of gonadotropin releasing hormone (GnRH), a hypothalamic hormone which stimulates the release of pituitary gonadotropin hormones, FSH and LH, which in turn stimulate production and ovarian hormones, estrogen, and progesterone (16,17).

The menstrual cycle is divided into three phases: the follicular phase, ovulation, and the luteal phase. The follicular phase begins with menstruation and lasts until ovulation. It is characterized by the maturation of the ovarian follicle under the influence of FSH and, at the end of this phase, by an estrogen peak that precedes ovulation (15,16). During ovulation, estrogen, in turn, exerts negative feedback on FSH while exerting positive feedback on LH (18,19). Ovulation occurs approximately on the 14th day of the cycle and is characterized by the expulsion of the oocyte from the dominant follicle, which happens after the LH surge. From there, the luteal phase begins, lasting until menstruation, and it is the period during which the remnants of the dominant follicle transform into the corpus luteum, leading to elevated progesterone levels. If there is no ongoing pregnancy, at the end of the luteal phase, endometrial shedding occurs, and a new cycle begins (16,20). Simultaneously with the ovarian cycle, there is also a uterine cycle within the menstrual cycle, as the endometrium prepares for implantation and the continuation of pregnancy. During the follicular phase, the proliferative phase of the endometrium occurs, characterized by an increase in stromal thickness and glandular growth. The secretory phase of the endometrium begins with ovulation and involves endometrial thickening and vascular proliferation (20).

Menstrual cycle alterations are named according to which characteristic of the cycle is affected. In terms of cycle duration, if it is less than 21 days, it is called polymenorrhea, and if it is longer than 37 days, it is called oligomenorrhea (16). Regarding menstrual flow, if it exceeds 150 mL, it is called hypermenorrhea. Uterine bleeding that occurs outside the menstrual cycle is referred to as metrorrhagia. In addition, the absence of the menstrual cycle is called amenorrhea, and the presence of pain during the menstrual cycle is called dysmenorrhea, both of which can be classified as primary or secondary conditions (20,21).

Other hormones can indirectly influence the hypothalamic-pituitary-ovarian axis. For example, in hypothyroidism, there is an increase in the hypothalamic hormone thyrotropin releasing hormone, which elevates levels of pituitary hormones thyroid stimulating hormone and prolactin. Prolactin, in turn, exerts negative feedback on gonadotropin hormones, which can result in menstrual irregularities, such as amenorrhea (18,22).

The relationship between SARS-CoV-2 and the hypothalamic-pituitary-ovarian axis

ACE2 receptors, besides being present in the uterus, vagina, and ovaries, directly participate in the follicular phase of the ovarian cycle, follicular development, ovulation, and luteal angiogenesis. They also participate in the uterine cycle during the secretory phase.

These receptors are used by the SARS-CoV-2 virus to enter cells, and this process itself produces tissue damage that can lead to functional impairment (4,23). Furthermore, ACE2 receptors are also present in the thyroid, which could facilitate the entry of this virus into the organ and cause hypothyroidism, among other conditions. Altered prolactin secretion due to hypothyroidism can subsequently affect ovarian hormone production (4,24).

Hypothalamic-pituitary-adrenal axis

In a context of stress, whether physical or psychological, the hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the pituitary to synthesize and secrete adrenocorticotropin hormone (ACTH), which acts on

the adrenal cortex to increase cortisol levels. The increase in cortisol exerts negative neuroendocrine feedback (9,25). Hypercortisolism affects the reproductive system both through direct action on the ovaries, by reducing gonadotropins, and through reduced LH levels resulting from hypothalamic and pituitary feedback, providing the environment for menstrual irregularities or even amenorrhea to occur (26,27).

There is also literature describing the influence of gonadotropins on the psychological and emotional state of women. Estrogen appears to regulate dopamine levels and has antipsychotic action while progesterone has been reported to have anxiolytic effects (28). Therefore, ovarian suppression due to increased cortisol levels could further promote the psychological stress brought on by the elevation of this hormone, as gonadotropins would not be exerting these actions (29).

Due to hormonal feedback in the hypothalamus and pituitary, there are other axes and hormones that can trigger ACTH stimulation and consequent cortisol elevation, similar to how hypercortisolism can disrupt the levels of these hormones (30-32). It is known that basal cortisol release is controlled by the circadian cycle. Therefore, alterations in the sleep-wake cycle, such as sleep deprivation, prolong cortisol release throughout the day. In a mentally healthy individual, the hypothalamic-pituitary-adrenal axis is inhibited by GnRH during sleep. However, in an individual experiencing psychological distress, there is a predominance of CRH action, promoting wakefulness and compromising pulsatile GnRH secretion, affecting the entire hormonal cascade (29). In addition, both cortisol elevation and sleep deprivation can alter the synthesis of the hormones leptin, which inhibits appetite, and ghrelin, which stimulates appetite, both of which have effects on the hypothalamus (32). Leptin and ghrelin secretion is regulated by the circadian cycle. Therefore, an alteration in the hypothalamic-pituitary-adrenal axis due to elevation of cortisol not only disrupts the circadian cycle but also disrupts the balance between appetite stimulation and inhibition, as cortisol itself also stimulates appetite (31,32). Another component modulated by cortisol is the immune system. The interaction between cortisol and pro-inflammatory cytokines initially plays a role in the mechanism of homeostasis in response to stress, whether it be of immunological, psychological, or emotional origin. However, exposure to excessive or chronic stress, with constantly elevated cortisol levels, disrupts the synthesis of these cytokines, resulting in immunosuppression (34,35). In COVID-19, the use of exogenous corticosteroids in patient management in an attempt to suppress hyper-immune dysregulation was notable (36,37).

Thus, in addition to the viral injury itself and the pandemic scenario, which led to biological and mental stress, the treatment of the disease that may have resulted in elevation of serum cortisol levels, which can result in menstrual irregularities should also be considered (9,38).

Psychological and emotional changes in COVID-19

The experience of the COVID-19 pandemic caused a series of problems that are not limited to SARS-CoV-2 infection. Significant damage and sequelae to mental health have been observed, even in those who did not suffer from viral infection (39,40). The measures proposed by the WHO (41), such as social isolation and lockdown, implemented to curb viral transmission, have resulted in various behavioral, emotional, and cognitive changes, whether directly or indirectly (42,43).

Among the psychosocial consequences, an increase in stress was observed in individuals who were subjected to physical and social distancing. This stress was directly related to isolation, but also to secondary conditions, such as sleep deprivation, sedentary lifestyle, and changes in eating habits (39,44,45). It is important to note that a vicious cycle of stress may be created, in which social isolation promotes stress, which in turn causes sleep disturbances that further increase stress and disrupt appetite. This disruption of eating habits also contributes to hormonal dysfunction in the neuroendocrine axes, leading to increased stimulation of cortisol and thus a rise in its secondary effects, such as ovarian suppression (17,45,46).

Fear and anxiety related to the risk of contracting COVID-19 or, if already infected, developing a severe condition or even dying, as well as fear of seeing friends and relatives under the same risk, are reported as significant causes of psychosocial disorders during the pandemic (47). In the literature, in addition to fear and anxiety, an exacerbation of emotions such as anger, sadness, boredom, and loneliness is described, all of which contribute to mental distress (48). Another source of psychological and emotional distress experienced during the pandemic was the grief over the loss of loved ones, which has caused intense psychological stress for many people (49).

Unfortunately, due to pre-existing gender inequality, women have been more affected by the economic crisis caused by the pandemic, experiencing termination of employment and consequent loss of income, which has led many women to become financially dependent on their partners (6,50). As a result, there has been an increase in cases of domestic violence during the pandemic, including psychological, physical, and sexual violence, subjecting these women to extreme psychosocial and biological stress (6,51). Furthermore, due to the patriarchal nature of some societies, women may have been more exposed to increased domestic demands during the isolation period, as they were assigned responsibilities such as housekeeping and childcare (47,52).

All of these psychological, behavioral, and emotional experiences have served as triggers for the emergence of psychiatric disorders in some individuals. During or after the pandemic, there have been reports of mental health condition diagnoses, with depression and anxiety being the most predominant (53).

Changes in the menstrual cycle in COVID-19

There are reports of positive SARS-CoV-2 real time-polymerase chain reaction results in vaginal samples (54,55), although the possibility of COVID-19 transmission through sexual contact by women is believed to be very low (56). The virus has also been detected in ovarian tissue, which reinforces its potential interference in the female reproductive system (57). However, SARS-CoV-2 has not been detected in endometrial tissue (58,59), although it is known that the uterus expresses ACE2 and TMPRSS2 receptors, which would allow viral entry (4,5,58). In their study, Li et al. (60) reported that women in the menacme who were hospitalized for COVID-19 experienced menstrual irregularities, such as increased cycle duration, variation in cycle length, and decreased menstrual flow, with normalization of the cycle three months after disease. Alessa et al. (61) evaluated 663 menacme women, of whom 206 tested positive for COVID-19, and among these 206, there was a predominance of complaints of dysmenorrhea (73.8%), reduced menstrual flow (51.5%), and polymenorrhea (40.8%). In the study by Lasta et al. (20), out of the 112 women in the sample who previously had regular cycles, 12 experienced a decrease in cycle duration due to COVID-19, and there were also reports of increased menstrual flow. Meanwhile, in a study conducted by Khan et al. (62), in a sample of 127 menacme women who tested positive for SARS-CoV-2, dysmenorrhea (45%) and oligomenorrhea (35%) were predominant. Demir et al. (63) sampled of 263 menacme women, after excluding women with a history of menstrual irregularity or current use of contraceptives, and found a decrease in cycle duration and menstrual flow volume during COVID-19. Notably, this study showed that patients with menstrual changes also mentioned psychological stress as a complaint.

Takmaz et al. (11) conducted a study on menstrual cycle changes in female healthcare professionals who worked in the COVID-19 pandemic setting. Out of a sample of 952 women, 273 experienced menstrual irregularities during the COVID-19 pandemic, with a notable decrease in cycle duration and an increase in menstrual flow. What is noteworthy in this study is that among these 273 women, those with a diagnosis of depression, anxiety, or other mental disorders were prevalent.

Other authors who associate menstrual cycle changes with mental distress in COVID-19 are Ozimek et al. (64), who describe exclusively increased menstrual flow in patients who complained of psychosocial stress, as well as increased cycle duration and dysmenorrhea in women in the sample, regardless of mental distress. In general, these studies reported an increase in behavioral and emotional symptoms, known as premenstrual tension, preceding menstrual cycles during COVID-19.

In a study by Ding et al. (65), which included 78 women with COVID-19, with 17 of them having severe disease, dysmenorrhea, amenorrhea, and hypermenorrhea were reported as changes. In another study by Phelan et al. (66), which had a sample of 1,031 women, 9% reported experiencing their first episode of amenorrhea during COVID-19. Another important finding reported by Nguyen et al. (67) was that in their sample of 18,706 women, regardless of the type of alteration, any COVID-19-related menstrual irregularity ceased shortly after the resolution of the condition in all women, except for one. It is worth noting that several studies describe an association between SARS-CoV-2 infection and coagulation dysregulation, from laboratory abnormalities to hemorrhagic or thromboembolic events (2,68,69).

Vaccination for COVID-19 and menstrual irregularity

There is a description in the literature regarding the association between COVID-19 vaccination and menstrual cycle alterations (9,70). It is essential to remember that in several studies conducted, no negative impact of COVID-19 vaccination on female fertility was detected (5). Laganà et al. (71) reported that around 60% of women, regardless of the vaccine administered, experienced menstrual irregularities, mainly after the second dose, with a predominance of reduced cycle duration and increased menstrual flow. Other studies that found very similar results were those by Lee et al. (72) and Muhaidat et al. (73). In the study by Nazir et al. (74), 39,759 (52.05%) women experienced menstrual irregularities after COVID-19 vaccination, with a high prevalence of polymenorrhea, hypermenorrhea, and metrorrhagia. This latter study highlights the presence of women who had a prior SARS-CoV-2 infection or were under intense psychological stress before vaccination among the most symptomatic patients in terms of menstrual cycle alterations.

Regarding these menstrual cycle alterations after vaccination, it is observed that they cease within a period of up to two subsequent menstrual cycles (5).

Conclusion

It is evident, therefore, that a relationship can be established between the COVID-19 pandemic and apparently temporary menstrual cycle alterations. Both the biological component of SARS-CoV-2 infection or its vaccination and their interaction with organs of the female reproductive system, as well as the

psychosocial component of the social experiences resulting from the pandemic scenario, have descriptions in the literature that support the impact of COVID-19 on menstrual irregularity. However, it is still not possible to establish a predominance of menstrual irregularities present in COVID-19, as a variety of alterations have been reported in the studies published to date. Regarding COVID-19 vaccination, menstrual irregularity was observed in approximately half of the female population, with a predominance of polymenorrhea and/or hypermenorrhea, but without any impact on fertility.

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What is your diagnosis?

A 42-year-old woman presented to the outpatient department with a skin-colored, non-tender, firm, immobile subcutaneous lump on the left corner of the Pfannenstiel scar. The patient noticed the mass eight years back. Initially it was pea sized but gradually increased to approximately 15x15 cm. It was associated with dull aching pain that started two days before menses and lasted five days after completion of menses. This period was also associated with cyclical dyspareunia and swelling around the lump.

Her obstetric history was notable for a full term, normal vaginal delivery 15 years earlier followed by medical termination of pregnancy 12 years earlier because of a malformed fetus. She underwent full term lower segment cesarean section (LSCS) for antepartum hemorrhage 10 years back. Her previous menstrual cycles were regular with average flow. The patient used homeopathic medication for 6-8 months, but did not experience any relief. She had multiple consultations and hospital visits for the same complaint for the last six years. Fine needle aspiration cytology done six years earlier at another center was suggestive of inflammatory cells, while another performed four years before presentation to our department reported degenerated cells.

On abdominal examination, an immobile, non-tender, hard mass of about 15x12 cm was felt above the pubic symphysis with no local rise of temperature. The mass was adherent to the anterior abdominal wall (Figure 1). On per vaginal and per rectal examination, the cervix was firm, regular and pulled anteriorly, the uterus was posterior and adherent to the mass, although bilateral fornices were free.

Given the clinical presentation, the differential diagnosis may include hematoma, stitch granuloma, lymphadenopathy, dermatofibroma, keloid mass, neuroma, abscess, desmoid tumor, or scar endometrioma and imaging will provide additional insight for diagnosing the lesion.

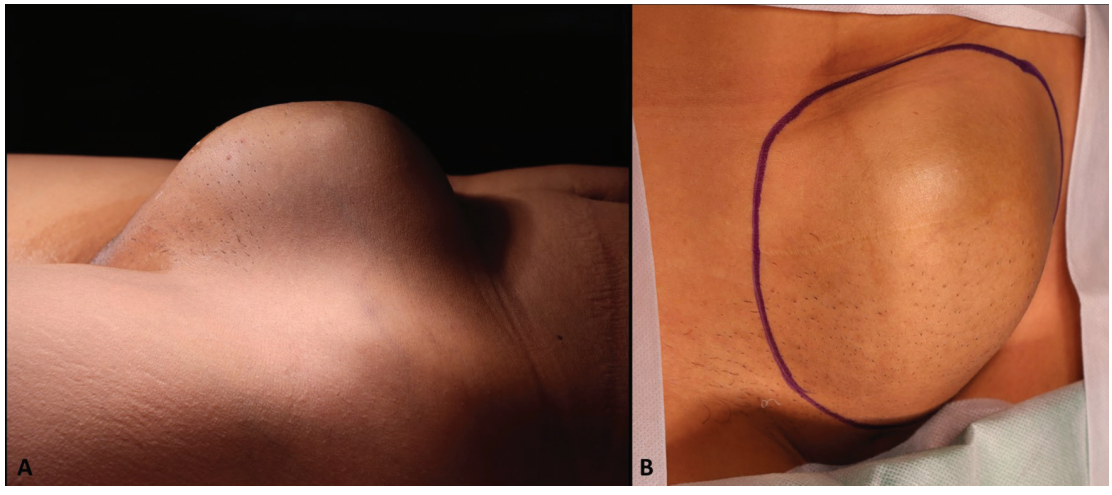

Figure 1. Large, immobile, non-tender mass with restricted mobility at the Pfannenstiel scar site [(A) lateral view, (B) anterior view]

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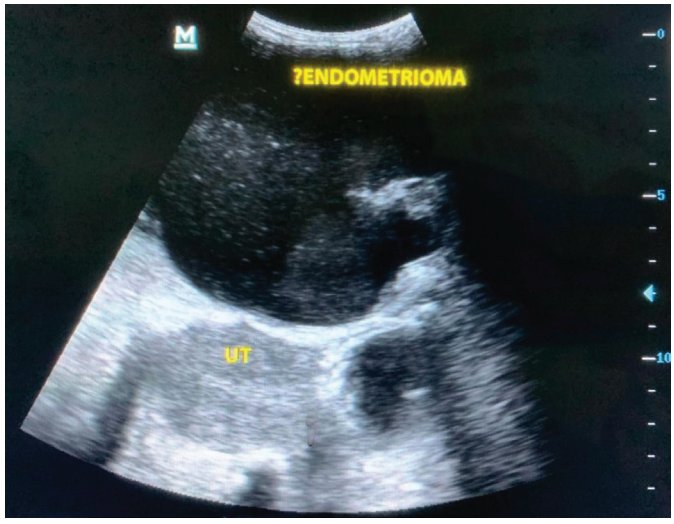

Figure 2. Ultrasonography showing hyperechoic large cystic mass with echogenic contents within with no vascularity

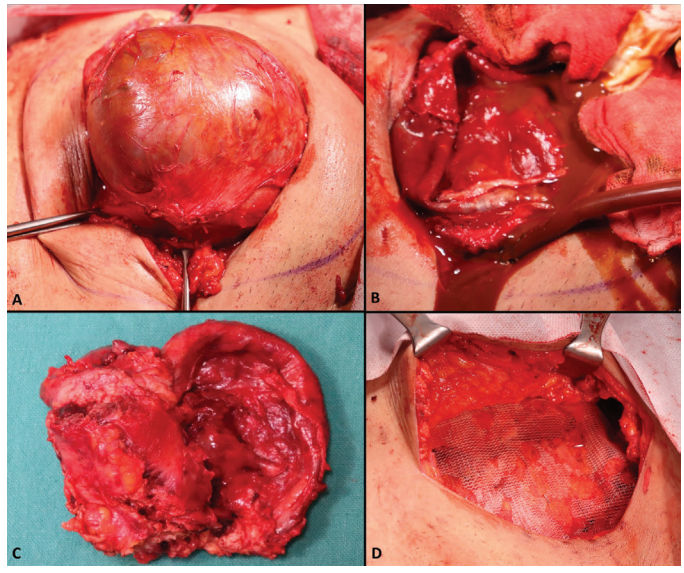

Figure 3. (A) 15x12cm scar site endometrioma adhered to rectus sheath. (B) Drainage of chocolate colored fluid from the endometriotic cyst. (C) Cut section of the specimen. (D) Onlay prolene mesh placed after primary closure of anterior rectus sheath

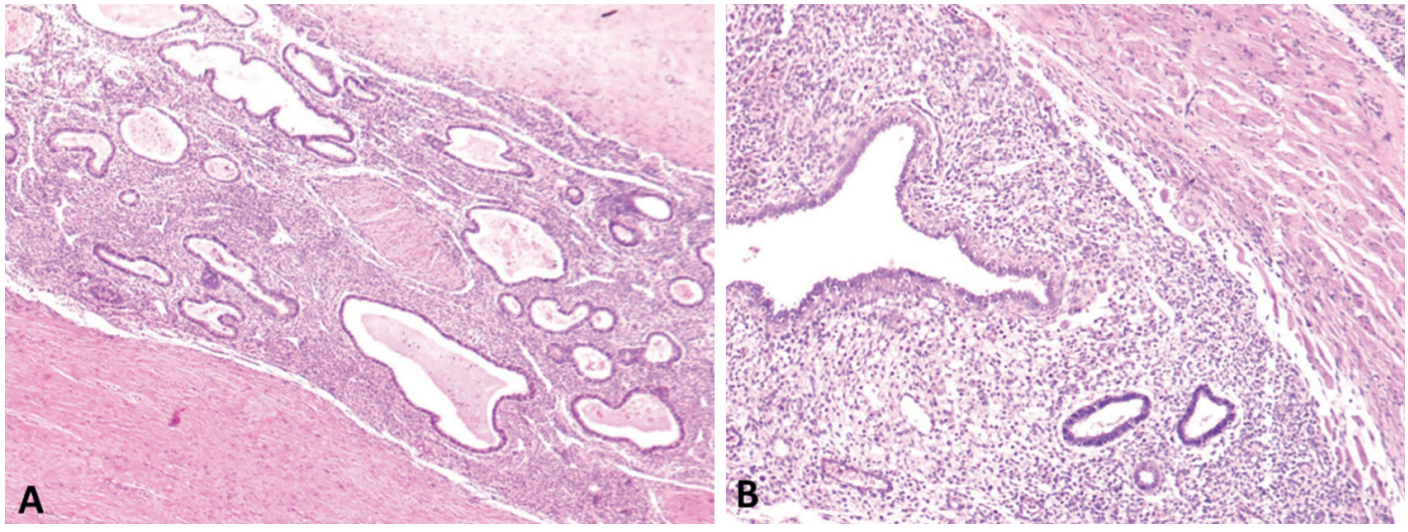

Figure 4. (A) Endometriotic glands and stroma in the subcutaneous tissue consistent with scar endometriosis. (B) Benign endometrial glands and stroma surrounded by scar tissue

Answer

Ultrasonography found a hyperechoic, large, cystic mass with echogenic contents with no vascularity and was suggestive of hemorrhagic cyst (Figure 2). Semisolid components and acoustic shadows were present. The uterus was adenomyotic, bulky and thick. Magnetic resonance imaging (MRI) revealed anterior abdominal wall or scar endometriosis of size 19.3x10.1x9.4 cm.

Based on the characteristic history, examination and radiological findings, the diagnosis of scar endometrioma was made. The patient underwent wide local endometriotic cyst excision followed by onlay prolene mesh repair (Figure 3). The lump was excised in total and final histopathology confirmed scar endometriosis (Figure 4). The patient tolerated the procedure well with an uneventful postoperative course. Currently, the patient is disease free, three years following surgery.

Endometriosis is one of the common gynecological conditions affecting reproductive age women where the non-neoplastic, functional endometrial layer is found outside the uterine cavity. It afflicts at least 11% women in the reproductive age group (1). Endometriosis generally involves pelvic sites, like ovaries, fallopian tubes, pouch of Douglas, uterine ligaments, rectovaginal septum or the pelvic peritoneum (2). Extra pelvic endometriosis is rare and found in unusual sites, such as the bladder, central nervous system, gastrointestinal tract, thorax or cutaneous tissues, including LSCS scar or episiotomy scar, especially after obstetric or gynecological surgical interventions (3).

Scar endometriosis is an extraordinary type of extrapelvic endometriosis with a prevalence reported between 0.03-2% (4). Probable differential diagnoses, including hematoma, stitch granuloma, lymphadenopathy, dermatofibroma, keloid mass, neuroma, abscess or desmoid tumor may cause delayed diagnosis. Depending on the surgical history, cutaneous endometriosis is further divided into primary and secondary cutaneous endometriosis. Primary cutaneous endometriosis occurs when endometriosis develops without any prior surgical intervention whereas secondary cutaneous endometriosis, also known as scar endometriosis, is associated with prior pelvic or abdominal surgery (5). Primary cutaneous endometriosis is less common than secondary cutaneous endometriosis and is thus less likely.

In terms of the pathogenesis of primary and secondary cutaneous endometriosis, the latter is easier to conceptualize. The prevailing hypothesis for secondary cutaneous endometriosis is direct implantation of stromal endometrial cells during surgery, within and adjacent to the incision site, which proliferate under hormonal stimulus; the “cellular transport theory”. However, for primary cutaneous endometriosis, some have proposed that seeding occurs hematogenously or via lymphatics. A third theory, the “coelomic metaplasia” theory proposes that cutaneous endometriosis is the result of metaplasia of pluripotent mesenchymal cells into endometrial tissue (6). The endometrial implant typically appears as a deep-lying or subcutaneous nodule infiltrating the fascia and the muscle, as seen in the present case. The implant was confirmed to be a scar endometrioma rather than an ovarian endometrioma adherent to the anterior abdominal wall, as both ovaries appeared normal and were distinctly separate from the mass. The classical triad is helpful in the diagnosis of subcutaneous endometriosis, which includes menstrual pain, presence of an abdominal wall mass, and history of surgery. However, this triad is only present in 60% of affected women. The frequency of scar endometriosis has increased due to the increased incidence of cesarean sections and laparoscopies performed in recent years. Certain studies have suggested a potentially increased risk of endometriosis associated with a Pfannenstiel incision compared to a midline vertical incision. However, the available evidence is insufficient to draw definite conclusions (7). Scar endometriosis may be noticed after procedures such as amniocentesis or laparoscopy (8). The endometrial implant is commonly observed as a deep-seated or subcutaneous nodule that infiltrates both the fascia and the muscle and during menstruation, there is bleeding into the tissue, leading to cyclic local pain, tenderness, and discoloration. If the nodules are superficial, noticeable signs include cyclic discoloration, bleeding, and ulceration (9). Careful and thorough history taking, physical examination and appropriate imaging modalities like ultrasonography, computed tomography or MRI are key for diagnosis. Ideally, all patients warrant gynaecological workup to rule out concomitant pelvic endometriosis (8). Histopathological examination suggestive of hemosiderin pigment, endometrial glands and stroma in the excised tissue is the diagnostic proof. Local wide excision, with at least 1 cm of margin, is the treatment of choice (9). Large lesions might require placement of synthetic mesh (10). Various protective surgical measures, such as thorough flushing of the wound cavity, eliminating dead space, employing an intro-flexed suture for the uterine incision, and closing both the visceral and parietal peritoneum, have been recommended as strategies to reduce the incidence of cesarean scar endometriosis (8). Postoperative strategies, including the use of combined oral contraceptives or hormonal suppression with gonadotropin-releasing hormone analogs or dienogest, can help mitigate the risk of recurrence and prevent new growth. While these agents are primarily used in the management of pelvic endometriosis, their use in cases of scar endometriosis may also be beneficial, particularly in patients with extensive disease or those who are not candidates for further surgery. However, the supporting evidence for these measures remains limited (11).

A cesarean scar is the most common site for extra pelvic endometriosis. Therefore, it is important to focus on prompt and accurate diagnosis, effective treatment, and preventive measures for scar endometriosis.

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Specialist and general emergency room: from "A to Z" case series of possible misdiagnosis due to the influence of gender

To the Editor,

Gender medicine is an important achievement of the last years. Nevertheless, in the emergency room (ER), women are often referred to gynaecologists even with problems related to other specialities or organ systems, because of their gender. We wanted to focus on the importance of not underestimating the difficulties encountered in general and specialist ERs, taking into account that no physician can be experienced in all fields and cannot know all the typical or atypical presentations of all pathologies.

A routine request for gynaecological counselling is: "I have in the ER a woman with almost certain diagnosis of appendicitis/pancreatitis/or … but I'd rather you evaluate the uterus and the annexes for differential diagnosis". Patient gender and a crowded general ER often lead to a reference to gynaecologist directly, without further examinations. Moreover no one can be experienced in all fields; therefore, especially with the onset of atypical symptoms, an emergency diagnosis can become a real challenge, both for gynaecologists and for colleagues in other disciplines (1,2). This not rare (1,2), as highlighted in our summary report, and may be the result of direct and indirect experience acquired over the years in different hospitals and settings.

1. Pathologies of other branches, referred directly to the obstetrics and gynaecological emergency room

All the cases summarized below were referred directly for gynaecological evaluation, either because of the pregnant state or simply because the patient was female.

Patients from"a to g" were referred just for pregnancy state.

a) Twenty-six-week pregnant woman complaining of confusion.

Diagnosis: Central nervous system stroke, detected by tomography, performed only after gynaecologist's insistence. The radiologist was frightened of potential risks to the foetus.

b) Twelve-weeks pregnant woman with toothache.

Diagnosis: Dental sepsis, treated with a maxillofacial surgery.

c) Thirty-seven-week pregnant woman with toothache, wearing veil and presenting with language barrier.

Diagnosis: Dental abscess treated with urgent tooth extraction and drainage of submandibular abscess.

d) Twelve-week pregnant woman complaining of sensory impairment.

Diagnosis: Cerebellar haemorrhage, diagnosed and treated only thanks to the presence of an experienced anaesthesiologist and gynaecologist in the gynecological ER.

e) Thirty-week pregnant woman with paraparesis of lower extremities, and had landed recently from Africa.

Diagnosis: Vertebral fracture related to bone tuberculosis, diagnosed by a standard X-ray.

f) Ten-week pregnant woman brought to ER by ambulance following car accident.

Diagnosis: Polytrauma. Nevertheless, the first evaluation was assessment of the pregnancy.

g) Eight-week pregnant woman involved in a road traffic accident.

Diagnosis: Bleeding secondary to pelvic fracture. The orthopedist postponed emergency surgery after pregnancy assessment.

Patients from "h to n" were referred to the gynaecologist only because they were female gender or due to recent obstetric or gynaecological diagnoses.

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h) Thirty-five-year-old woman with acute rectal and perineal pain and paraesthesia of the lower limbs.

Referred to gynaecologist due to a recent C-section.

Diagnosis: Dissection of the internal iliac artery diagnosed with a computed tomography scan.

i) Forty-two-year-old woman with haemorrhagic shock, following an accident.

Referred first to the gynaecologist to suture a perineal lesion with slight bleeding.

Diagnosis: Pelvic fracture treated with embolization.

l) Forty-five-year-old women, anaemic and sleepy.

Referred to the gynaecologist for moderate vaginal bleeding but with severe anaemia (hemoglobin 6.6 g/dL).

Diagnosis: Advanced stage of haemolytic uremic syndrome, diagnosed by an older and experienced gynaecologist.

m) Sixty-eight-year-old woman complaining of worsening leg pain.

Referred to the gynaecologist for personal history of gynaecological cancer and chemotherapy.

Diagnosis: Leg ischaemia that required urgent positioning of stent.

n) Eighteen-year-old woman with neurological impairment; relatives who brought her reported severe asthenia, menstrual irregularities and metrorrhagia.

Referred to the gynaecologist for reported menstrual irregularities with metrorrhagia, not present at the time of access.

Diagnosis: Fulminant acute lymphatic leukaemia.

2. Gynaecological-obstetrical cases, misdiagnosed by other specialists

o) Twelve-week pregnant woman with intrauterine gestation with haemorrhagic shock.

Suspect: The surgeon detected abundant free fluid in the abdomen, which was referred to gastrointestinal disease, therefore a laparoscopy was performed.

Diagnosis: Heterotopic pregnancy, carried out by the gynaecologist who was called for consultation in the operating room.

p) Twenty-seven-year-old women with gastrointestinal symptoms associated with lipothymia. She also presented with amenorrhea.

Suspect: She was evaluated for gastrointestinal disease.

Diagnosis: Extrauterine pregnancy with atypical presentation; the gynaecological examination was requested after some delay, following exclusion of other pathologies.

q) Twenty-six-year-old woman with hypovolemic shock with a menstrual delay.

Suspect: Other causes of shock.

Diagnosis: Rupture of ectopic pregnancy; the gynaecological examination was delayed, again because of prior exclusion of other pathologies.

r) Thirty-two-weeks pregnant woman with abdominal pain after Easter lunch.

Suspect: The surgeon thought of indigestion and did not focus on blood pressure of 160/100 mmHg, thinking that the increase in blood pressure was caused by pain.

Diagnosis: HELLP syndrome detected at obstetric assessment that was only requested just before discharge.

s) Thirty-five-week pregnant woman complaining of malaise. Blood pressure was 140/90 mmHg.

Suspect: Other system disease.

Diagnosis: Preeclampsia diagnosed by gynaecological evaluation that was only requested after changes in blood test results.

t) Puerpera 7 days after delivery with visual changes.

Suspect: The ophthalmologist discharged her without any particular indication.

Diagnosis: Post-partum preeclampsia complicated by a posterior reversible encephalopathy syndrome, diagnosed after a second accessment, a few hours later for worsening headache. At that time, the blood pressure detected was 150/100 mmHg and a gynaecological evaluation was requested.

u) Thirty-seven-year-old woman with abdominal pain and lipothymia. She reported amenorrhea for 6 months.

Suspect: Gastrointestinal disease.

Diagnosis: Abdominal pregnancy at 17 weeks, diagnosed by the gynaecologist, called for hemoperitoneum.

v) Puerpera 4 days after delivery with seizure.

Suspect: Epileptic attack not responsive to antiepileptic administration.

Diagnosis: Eclampsia in puerperium.

z) Thirty-weeks pregnant woman with poor hemoperitoneum after a minor abdominal trauma due to lose of consciousness.

Suspect: Epileptic attack with abdominal trauma, suggesting abdominal bleeding. Since the patient arrived by ambulance she was unconscious. Therefore, a diagnostic laparotomy was carried out under general anaesthesia to find the origin of bleeding.

Diagnosis: Hemorrhagic stroke related to misdiagnosed eclamptic attack. The diagnosis was made by the gynaecologist, called to carry out a simultaneous emergency caesarean section.

Our report emphasizes the inability for a doctor to formulate a proper differential diagnosis in all systems, even more so in case of atypical symptoms, with a high-risk of diagnostic errors. This overlap of symptoms is present in all fields. Therefore, the obstetrics-gynecology ER presented particular difficulties in

being the referral site only when considering female gender or the state of pre-existing pregnancy; in contrast, the general ER reported rare and specific gynaecological complications.

Although during the course of university studies, all future doctors study emergencies in different specialist fields, without continuous re-training, the diagnostic aptitude may be lost, especially for rare diseases. Moreover, in cases of rare diseases or atypical symptoms, misdiagnosis may even more likely.

Other studies have tried to understand and reduce ER diagnostic and clinical errors, even if no specific strategies have been reported yet (1,2). Thus, we would like to share this "a to z" summary of cases to focus on some basic, but frequently forgotten points.

1) In a road traffic accident involving a pregnant woman, the woman must always be evaluated and treated first, even before a pregnancy assessment. The foetus may be saved thanks only to appropriate care given to woman.

2) Septic diseases during pregnancy can be severe and rapidly progressive and any system or body location may be involved.

3) Pre-eclampsia and eclampsia may affect second and third trimester and puerperium. All doctors should pay attention to blood pressure.

4) Extra uterine pregnancy may present with atypical symptoms and remain a life-threatening emergency.

We understand that our report does not analyse a specific approach or scheme to reduce these risks. Our aim was to focus attention on the need for continuous training and implementation of the skills of doctors working in a first aid position, the various ERs, not only in a specific speciality. Regular attendance in the general ER and the possibility to follow additional lessons, given by all physicians working in the field of emergency, on all life-threatening events, with both typical and atypical presentations, would help specialists to obtain adequate and continuous training. Moreover, the acquisition of skills by young doctors doing on-call shifts alongside colleagues of greater experience may encourage the sharing of a wealth of unwritten knowledge acquired over time and prevent the situation of "not written, not known".

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Second-trimester spontaneous uterine rupture: a rare case of diagnostic nuances and multidisciplinary management

To the Editor,

Uterine rupture, characterized by the complete separation of all uterine layers, poses significant risk to both the mother and fetus (1). This condition is predominantly observed in the third trimester of pregnancy, with earlier occurrences being exceptionally rare (2). The incidence of uterine rupture is approximately 0.7 per 10,000 deliveries in unscarred uteri and 5.1 per 10,000 in scarred uteri (3). Second-trimester ruptures are typically associated with induced pregnancy terminations in scarred uteri, trauma, or complications, such as placenta accreta spectrum. Spontaneous rupture before labor in the second trimester is an extremely uncommon event (4). Notably, 80% of uterine ruptures occur between 28 and 36 weeks of gestation, with mid-trimester ruptures reported at an incidence of 1 per 5,000 deliveries (5).

Identifying risk factors, including a history of uterine rupture, previous surgery including vertical hysterotomy, and labor is important for anticipating and managing this condition. Diagnosing uterine rupture is challenging and often overlooked without a high index of suspicion. We aim to highlight the importance of early recognition and illustrate how delays in diagnosing uterine rupture can result life-threatening outcomes.

A 25-year-old, G9P2144 at 25 weeks and 6 days, presented to the emergency department with severe, diffuse abdominal pain that began 24 hours prior and progressively worsened. The pain was non-contraction-like, exacerbated by movement and respiration, and accompanied by an episode of loss of consciousness reported by the emergency medical services. She was not postictal and denied vaginal bleeding, contractions, loss of fluid, or gastrointestinal symptoms.

On physical examination, the patient appeared uncomfortable, with diffuse abdominal tenderness, a positive Murphy's sign and bilateral costovertebral angle tenderness. There were no findings of acute abdomen, such as rigidity, guarding, or rebound tenderness. The patient was unable to engage in a thorough history, until pain was better managed, revealing a complex medical background (Figure 1). Outside records were unavailable initially given that she had been receiving prenatal care outside the facility. Medical history included a cardiac history of atrial fibrillation and supraventricular tachycardia, having undergone four cardiac ablations and cardioversion during a previous pregnancy. Surgical history was only significant for a laparoscopic appendectomy and umbilical hernia repair. Obstetrically, she experienced preterm labor at 28 weeks gestation with twins, managed with a vaginal birth (baby A) and a subsequent cesarean section via a classical incision (baby B) in August 2022. During the current pregnancy, a cerclage had been placed at approximately 13 weeks gestation. Since placement, the patient had multiple presentations with similar symptoms. During these admissions, ultrasounds were completed, and no pain-related pathology was noted; a low-lying placenta was observed.

Upon initial assessment, the cardiotocography was appropriate for gestational age. However, during triage, a prolonged deceleration lasting four minutes was observed, which resolved spontaneously without intervention. The tocodynamometer showed no uterine contractions throughout this period. The patient, experiencing severe pain, intermittently refused further fetal monitoring; subsequent tracings remained within normal parameters.

The patient was tachycardic at 130 beats per minute (bpm). Vital signs including blood pressure, temperature, oxygen saturation, and respiratory rate were all within normal limits. Laboratory findings revealed an initial white blood cell count

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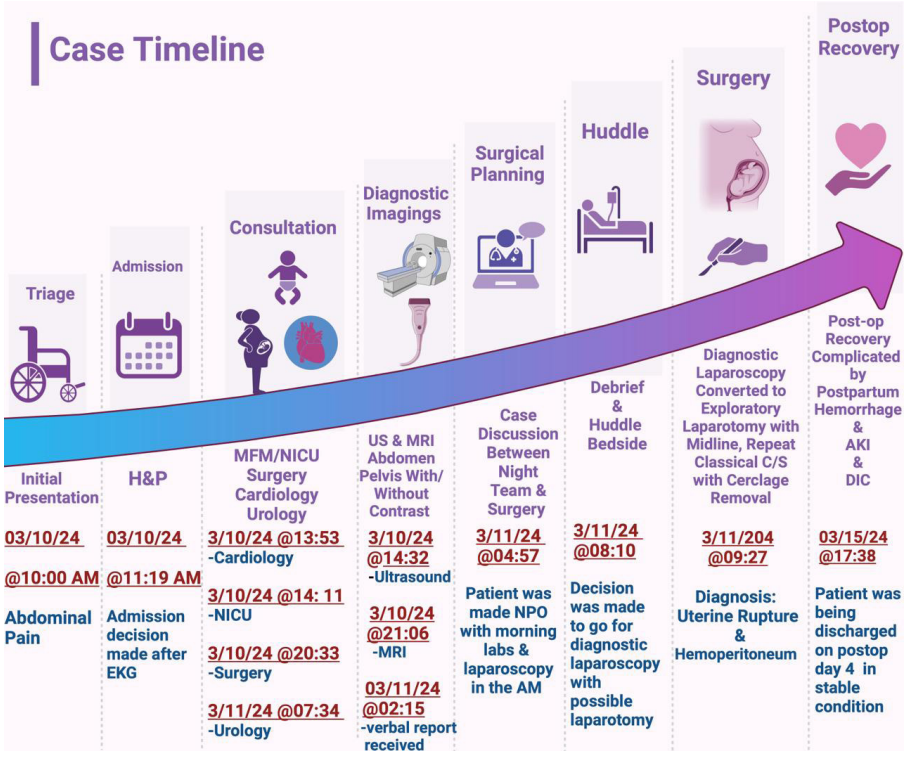

Figure 1. Chronological clinical course of maternal-fetal management: from initial assessment to postoperative recovery

of 24.7 $10^3/\mu$L, which up trended to 29.1 $10^3/\mu$L. Hemoglobin levels decreased from 9.4 g/dL to 7 g/dL. Lactate levels initially measured at 4.1 mmol/L, decreased to 2.8 mmol/L, following aggressive fluid resuscitation. Hyperkalemia at 5.8 mEq/L was noted in the setting of acute kidney injury, with a creatinine level of 1.2 mg/dL. Abdominal ultrasound showed concern for ascites, sludge in the gallbladder and right-sided nephrolithiasis. Magnetic resonance imaging (MRI) of the abdomen and pelvis identified hemoperitoneum, concerning a ruptured, hemorrhagic ovarian cyst, and a moderate-sized umbilical hernia (Figure 2).

Consultations were obtained from general surgery, urology, maternal-fetal medicine (MFM) and cardiology. Cardiology noted sinus tachycardia secondary to pain, dehydration, anemia and concern for infection. They diagnosed vasovagal syncope secondary to pain. The patient's hyperkalemia resolved. General surgery reviewed imaging, and a mutual discussion determined the best next course of action which was a co-scrubbed diagnostic laparoscopy. The patient was given betamethasone, intravenous (IV) Zosyn, one unit of fresh frozen platelets (FFP) and packed red blood cells (pRBC's).

During the diagnostic laparoscopy, a large organized haematoma was observed in the midline, which significantly restricted the visual assessment of the abdominal cavity. Consequently, the procedure was escalated to an exploratory laparotomy. Upon entry, the hematoma was evacuated and a uterine rupture at the site of the previous classical hysterotomy incision was immediately identified with the placenta anterior and visible at the site of the dehiscence. The decision was made to proceed with delivery. No other abnormalities or bleeding was noted. Cerclage was removed. The APGAR scores were 2 at 1 minute, 3 at 5 minutes, and 5 at 10 minutes. The hysterotomy was closed with Vicryl 0, in two layers, with the second being a baseball stitch. Disseminated intravascular coagulation (DIC) panel was completed intraoperatively and DIC was diagnosed with a fibrinogen of 121,000 mg/dL. The total quantitative blood loss was 3 liters. The patient received 1 unit of FFP and 5 units of pRBC intraoperatively.

Pain complicated the postoperative period. Palliative care and pain management were consulted, and pain improved with oral analgesia. The patient met all postoperative milestones and was discharged on postoperative day 4. The neonate stayed in the neonatal intensive care unit and was then transferred to another facility for evaluation and management of ventriculomegaly and intraventricular hemorrhage secondary to prematurity.

This case emphasizes the diagnostic challenges in second-trimester uterine rupture, highlighting the importance of vigilant monitoring and prompt intervention. The patient's multiple emergency department visits for similar pain-related complaints subsequent to the 14th week of gestation indicate that the initial phases of uterine dehiscence might have occurred well before the final diagnosis of complete uterine rupture. This prolonged onset is particularly noteworthy given the patient's gestational

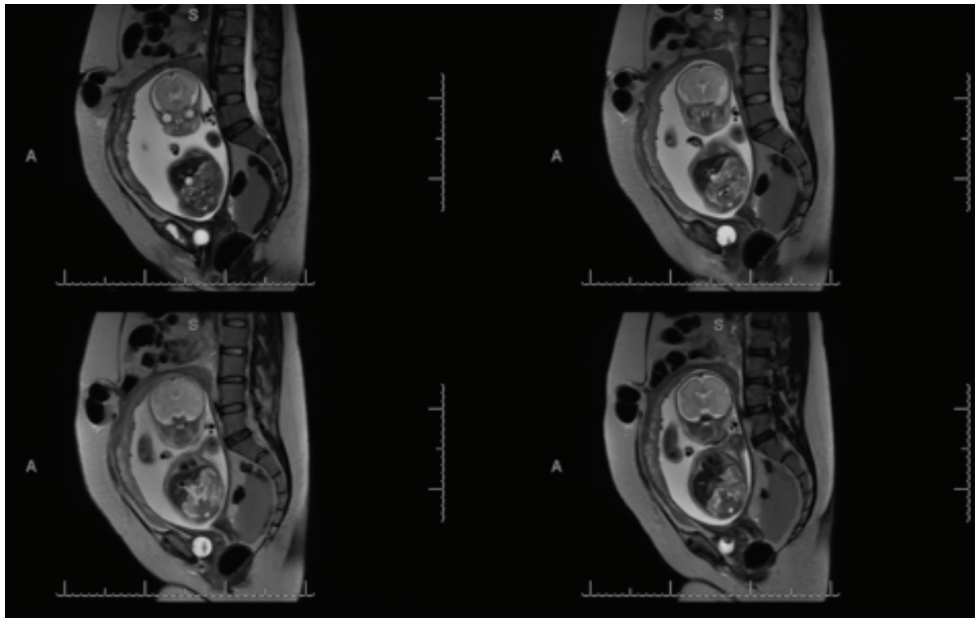

Figure 2. Non-contrast sagittal magnetic resonance imaging of the abdomen and pelvis demonstrating hemoperitoneum, ruptured hemorrhagic ovarian cyst, and umbilical hernia

age and the absence of labor contractions, which are more typical indicators of uterine distress.

Another point of interest is that following cerclage placement, the patient received hydromorphone 0.5 mg and 1 mg IV for pain control. Typically, patients with cerclage do not require IV opioids. We speculate that there is a possibility that the cerclage may have contributed to the rupture due to a combination of increased intrauterine pressure, and contractions, in the setting of the previous classical cesarean section.

Imaging, such as a focused assessment with sonography for trauma scan or MRI, should not be delayed in similar cases. In our case, imaging revealed hemoperitoneum but was unable to diagnose uterine rupture. Despite its rarity in the second trimester, this case emphasizes the need for vigilance and early recognition of symptoms, even in the absence of traditional signs, like vaginal bleeding, non-reassuring fetal heart tones or contractions.

Our management highlights multidisciplinary collaboration involving obstetrics, general surgery, cardiology, and MFM, underscoring the complexity and coordination required in such critical scenarios. Surgical intervention was vital in this case, emphasizing the role of timely surgical exploration once uterine rupture is suspected. This becomes particularly challenging in the absence of classic symptoms and signs, where delays in diagnosis and intervention can profoundly affect patient outcomes. Moreover, the onset of DIC in this patient highlights the systemic impact of uterine rupture, necessitating meticulous management, including blood products and monitoring for complications. The resolution of DIC and the patient's recovery were facilitated by comprehensive postoperative care and effective pain management strategies.

In conclusion, this report illustrates the importance of early recognition, prompt diagnostic evaluation, and decisive surgical management in uterine rupture. Clinicians should maintain a heightened awareness of this potentially life-threatening complication to optimize outcomes. Futher future research and reports will be important to refine diagnostic strategies and management protocols for uterine rupture.

Acknowledgements: *Figure 1 reprinted from "General Timeline for Prosthesis Fitting", by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates.*

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A new technique for stress urinary incontinence without using vaginal mesh

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Abstract

Stress urinary incontinence (SUI) is a fairly common disease among women. Synthetic meshes are frequently used in midurethral sling procedures due to the high long-term success rates. Because of the publications about vaginal mesh complications in recent years, urogynecologists are turning to techniques without mesh. The purpose of this video is to show that SUI can be treated without mesh complications by utilizing the meshless urethropexy technique. A 50-year-old woman applied to our urogynecology department with complaints of incontinence. Physical examination, stress test, Q-tip test, urine test and transperineal ultrasound performed. Post-void residual urine measured. The patient completed incontinence questionnaires: urogenital distress inventory-6, incontinence impact questionnaire-7. After discussing results SUI was diagnosed. Treatment options were offered to the patient. Due to mesh complications concern the patient preferred this approach and underwent urethropexy. The steps of meshless urethropexy technique was demonstrated in this video. SUI can be treated with this approach without worrying about mesh complications, but long-term results are needed. (J Turk Ger Gynecol Assoc. 2024; 25: 277-9)

Keywords: Incontinence, midurethral sling, stress urinary incontinence, urethropexy

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Introduction

Urinary incontinence is a common and significant health issue among women, with prevalence reported to vary between 25-50% (1). Although various surgical techniques have been described for stress urinary incontinence (SUI), midurethral sling (MUS) procedures using synthetic mesh are the most commonly used procedure due to their high long-term success rates. Nilsson et al. (2) reported a long-term objective cure rate of 90% and a subjective cure rate of 77% for retropubic MUS.

However, vaginal mesh can lead to serious complications that negatively impact patients' quality of life. Cohen et al. (3) found that the incidence of one or more complications within 30 days after the MUS procedure was 4%. Ulrich et al. (4) reported 7% mesh extrusion/erosion, 26% de novo urgency, 25% dyspareunia, and 13% intermittent inguinal pain at the 10-year follow-up.

In recent years, attention has focused on complications associated with synthetic mesh in surgery. Following the U.S. Food and Drug Administration report in 2008, warnings regarding mesh complications were issued to hospitals in Canada in 2014 (5).

This case was selected from a cohort of 21 cases that we have performed since 2019 and in this video article, we aim to present our original technique, which does not use any mesh in the suburethral region.

Case Report

A 50-year-old G5P1 patient presented to the urogynecology department with complaints of urinary incontinence.

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Preoperative evaluation showed a positive stress test, a Q-tip test angle of 60°, and a post-void residual urine volume of 20 cc. Using transperineal ultrasound, anterior (α angle) and posterior (β angles) urethral angles were evaluated both at rest and during the Valsalva maneuver ($\Delta\alpha = 50°$, $\Delta\beta = 20°$). Urine culture was negative, and the patient had no comorbidities. Based on the diagnosis of SUI, urethropexy was indicated.

In the lithotomy position, the following steps (Video 1) were performed:

Step 1: A 1 cm^2 islet was created on the vaginal mucosa in the midurethral region. The edges of this islet were dissected from the adjacent mucosa (Figure 1), and the area was de-epithelialized with electrocautery.

Step 2: In the mid-urethral region, a first no: 1 prolene suture (yellow) and a second no: 1 prolene suture (turquoise) were used to create a handmade hammock (Figure 1). The first suture was placed in a reversed "U" shape, while the second suture was placed in a "U" shape.

Step 3: Bilateral tunnels were opened under the symphysis pubis using scissors. Prolene sutures were passed through the retropubic area with the help of guides and retrieved from the skin 2.5 cm lateral to the midline on both sides over the mons pubis. Bladder walls were inspected via simultaneous cystoscopy. After confirming the integrity of the bladder walls, the guides were removed.

Step 4: A polypropylene mesh was placed into the mons pubis 2 cm below the skin using a guide (Figure 2). Approximately 5 cm of mesh was used, and the excess was trimmed (Figure 3).

Step 5: Prolene sutures on both sides were secured with small hemoclips placed 1 cm from the ends of the mesh, preventing slippage. The sutures were then tied with 3-4 knots on the hemoclips, and the incisions were closed.

The procedure was completed in 30 minutes. On postoperative day 1, post-void residual urine was measured at 40 cc, and the patient was discharged the same day. Follow-up visits were scheduled for the first, third, sixth, and twelfth months, during which no complications were observed. At the 12-month follow-up, the stress test was negative, the Q-tip test showed 10°, and the 1-hour pad test was dry. The difference in anterior (α angle) and posterior (β angle) urethral angles during rest and the Valsalva maneuver was remeasured via transperineal ultrasound ($\Delta\alpha = 10°$, $\Delta\beta = 10°$). The patient remained asymptomatic with no signs of incontinence or complications. Written informed consent was obtained from the patient for publication of this video article and any accompanying images.

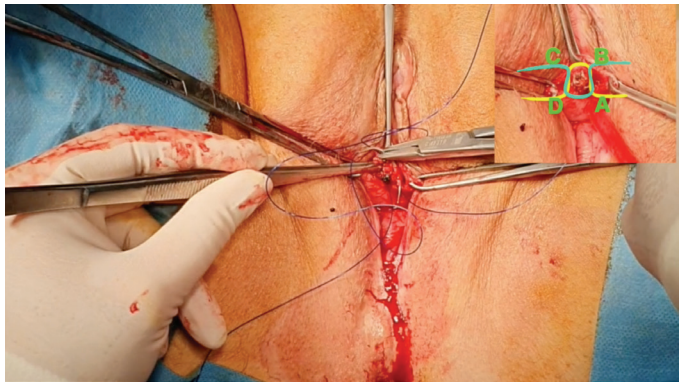

Figure 1. Suburethral mucosal island

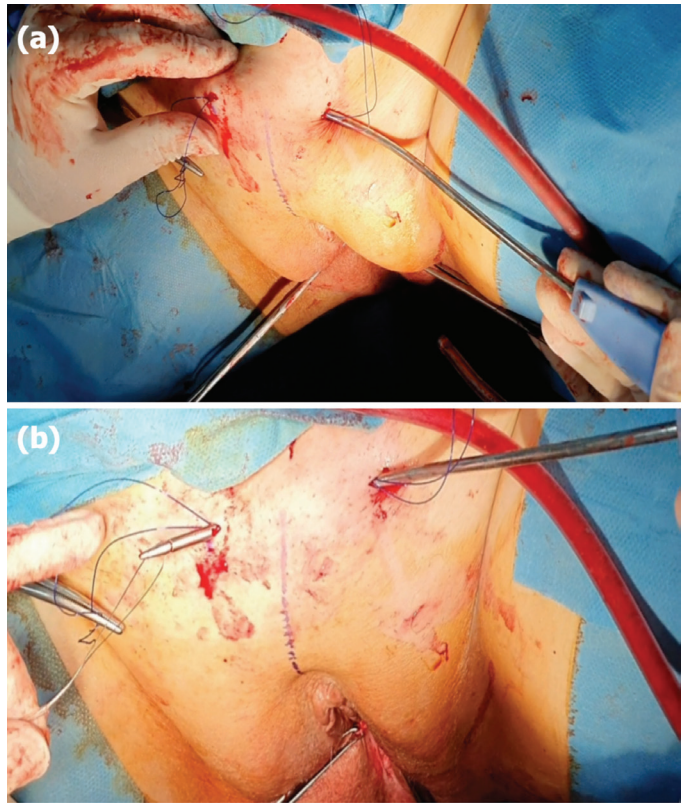

Figure 2. Insertion of polypropylene mesh with the help of guide

Conclusion

This urethropexy technique can be considered in the surgical treatment of SUI, although long-term results are still needed. While no complications were observed in our case, rare complications similar to those seen in retropubic sling procedures, such as bladder or urethral injuries and bleeding, may still occur with this technique. This approach is most suitable for uncomplicated cases of pure SUI without a history of previous SUI surgery.

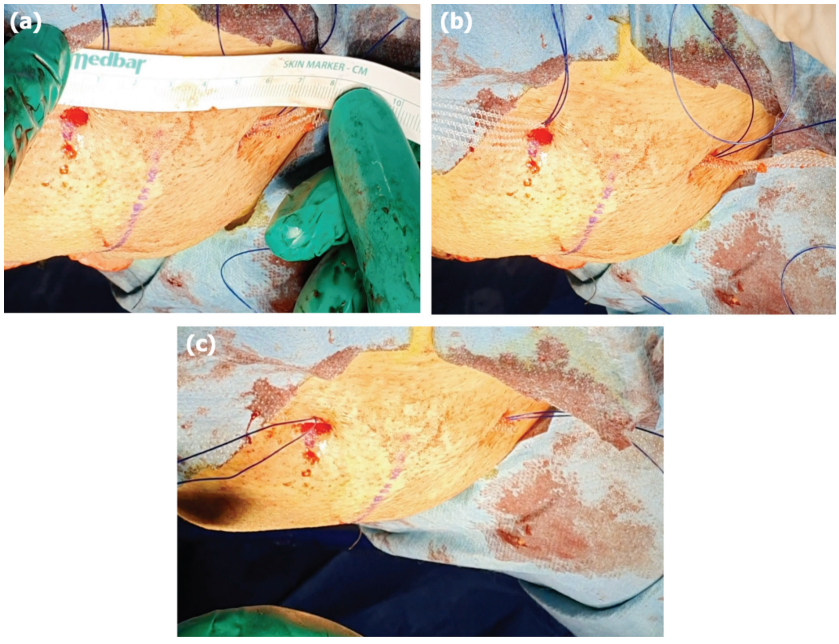

Figure 3. Representation of mesh placed into the mons pubis

Video 1.

https://www.doi.org/10.4274/jtgga.galenos.2023.2022-12-17.video1

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

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2024 Referee Index

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CONGRESS CALENDER

INTERNATIONAL MEETINGS

(for detailed International Meeting please go website: https://www.emedevents.com/obstetrics-and-gynecology)

Feb 20-23, 2025	26th European Congress on Gynaecological Oncology (ESGO), Rome, Italy
March 25-29, 2025	Society for Reproductive Investigation (SRI) 72nd Annual Scientific Meeting, North Carolina, USA
April 24-26, 2025	11th Congress of the Society of Endometriosis and Uterine Disorders (SEUD), Prague, Czech Republic
April 23-27, 2025	XV. Türk Alman Jinekoloji Kongresi, Antalya, Türkiye
April 24-26, 2025	ASCCP 2025 Scientific Meeting, San Diego, USA
May 14-16, 2025	15th European Congress on Menopause and Andropause, Valencia, Spain
May 16-18, 2025	American College of Obstetricians and Gynecologists (ACOG) 2025 Annual Clinical and Scientific Meeting, Minneapolis, USA
May 17-21, 2025	American Society for Reproductive Immunology (ASRI) Annual Meeting 2025, Minnesota, USA
June 17-20, 2025	The Society of Obstetricians and Gynecologists of Canada Annual Clinical Scientific Conference, Whistler, BC, Canada
June 18-21, 2025	International Urogynecological Association (IUGA) 50th Annual Meeting, Barcelona, Spain
June 29-July 02, 2025	European Society of Human Reproduction and Embryology (ESHRE) 41st Annual Meeting, Paris, France
September 14-17, 2025	35th ISUOG World Congess, Cancun, Mexico
October 25-29, 2025	American Society for Reproductive Medicine (ASRM) 81st Annual Meeting, Texas, USA
October 19-22, 2025	ESGE 34th Annual Congress, İstanbul, Türkiye
November 08-11, 2025	The 54th American Association of Gynecologic Laparoscopists (AAGL) Global Congress on Minimally Invasive Gynecologic Surgery (MIGS), Vancouver, BC, Canada
November 27-29, 2024	The 33rd World Congress on Controversies in Obstetrics Gynecology & Infertility (COGI), Rome, Italy

CONGRESS CALENDER

NATIONAL MEETINGS

(for detailed International Meeting please go website: https://www.kongreuzmani.com/2024)

February 20-23, 2025	CİSEF 5. Uluslararası Cinsel Sağlık Kongresi, KKTC
September 11-14, 2025	Uludağ Jinekolojik Endoskopi Kampı, Bursa, Türkiye
February 22-23, 2025	12. İstanbul Kadın Doğum Günleri, İstanbul, Türkiye
September 18-21, 2025	İç Anadolu Kadın Sağlığı Derneği Kongresi, Ankara, Türkiye
May 14-18, 2025	22. Ulusal Jinekoloji ve Obstetrik Kongresi, K.K.T.C.
May 15-19, 2024	4. Uluslararası Pelvik Taban ve Kozmetik Jinekoloji Kongresi, Antalya, Türkiye
October 01-05, 2025	7. Jinekoloji ve Obstetrikte Tartışmalı Konular Kongresi, Antalya, Türkiye
November 06-09, 2025	Uluslararası Jinekoloji ve Obstetri Kongresi (UJOK), Antalya, Türkiye