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The Impact of The Hormone Profile of Reproductive Technology Outcomes in Women Undergoing Gonadotropin-Releasing Hormone

Athraa Zaid Hussein, Asmaa Mohammed Saud

Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq.

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Abstract

Hormones greatly influence women's health. A variety of factors can bring on women's hormonal imbalances. The understanding of the origins and diagnosis of female infertility has been viewed as being highly dependent on hormonal abnormalities. The current study was designed to monitor hormonal changes during ovarian stimulation in an assisted reproductive technology (ART) setting. A total of 84 samples were collected: 43 from infertile women and 41 from healthy controls. Participants were selected after clinical and laboratory evaluations confirmed their respective conditions. Patients and controls ranged in age from 15 to 45 years. The mean age was 27.60 ± 4.90 years for the patient group and 27.85 ± 4.90 years for the control group. Serum concentrations of hormones pre-stimulation, such as prolactin (PRL), thyroid-stimulating hormone (TSH), anti-mullerian hormone (AMH), estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and progesterone (Proge) before ovarian stimulation were assessed during the ovarian stimulation protocol. The findings showed that the outcomes of FSH, AMH, TSH, PRL, and Proge were statistically significant ($p < 0.05$) between infertile patients and healthy controls. In the process of examining the impact of the level of hormones on the female groups of ages, the age was grouped into four categories; the first category was those whose age was below 30 years, the second category was those whose age was between 30 to 35 years old, the third category was those whose age was between 36 and 40 years old and the fourth category was those whose age was over 40 years old. E2 and FSH were only statistically significant ($p < 0.01$) in all age groups of patients and controls. Besides the level of serum hormones following ovarian stimulation, the level of hormones in infertile and fertile women was also determined to obtain the median level of hormones before and after stimulation, according to age group. The outcome of E2 was significant ($p < 0.01$). The article concludes that the variation in hormonal levels of FSH and E2 is a significant measure of infertility and ovarian response to stimulation. Age has a profound influence on these variances, and the need for hormonal assessment to be individualized in the context of fertility management.

Keywords: Hormones, Female infertility, hormone profile, Gonadotropin-releasing hormone, In Vitro-Fertilization.

*Email: athraazaid443@gmail.com



أثر ملف الهرمونات على نتائج التكنولوجيا الإنجابية لدى النساء اللاتي يخضعن لعلاج الهرمون المطلق لموجهة الغدد التناسلية.

عذراء زيد حسين*، أسماء محمد سعود

قسم التقنيات الاحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق.

الخلاصة

تؤثر الهرمونات بشكل كبير على صحة المرأة. ويمكن لمجموعة متنوعة من العوامل أن تسبب اختلالات هرمونية لدى المرأة. ويُنظر إلى فهم أصول وتشخيص العقم عند النساء على أنه يعتمد بشكل كبير على الاضطرابات الهرمونية. تهدف هذه الدراسة إلى إجراء مراقبة هرمونية أثناء تحفيز المبيض في سياق علاج تقنيات الإنجاب المساعدة (ART). أُجريت الدراسة على أربع وثمانين عينة، ثلاث وأربعون منها من نساء يعانين من العقم وواحدة وأربعون من نساء سليمات، بعد تشخيص حالتهن من خلال الفحوصات السريرية والمخبرية. تراوحت أعمار المريضات ونساء المجموعة الضابطة بين 15 و45 عامًا، وكان متوسطهن 4.90 ± 27.60 مقابل 4.9 ± 27.85 . خلال بروتوكول تحفيز المبيض، تم قياس مستويات الهرمونات في المصل قبل التحفيز، بما في ذلك البرولاكتين (PRL)، وهرمون تحفيز الغدة الدرقية (TSH)، وهرمون مضاد مولر (AMH)، وإستراديول (E2)، وهرمون ملوتين (LH)، وهرمون تحفيز الجريبات (FSH)، والبروجسترون قبل تحفيز المبيض. وقد وجد أن نتائج AMH وFSH وTSH وPRL وProg كانت ذات دلالة إحصائية ($p < 0.05$) لدى المريضات المصابات بالعقم مقارنةً بالضوابط الصحية. وعند تحليل تأثير المستويات الهرمونية على الفئات العمرية للإناث، تم تقسيم العمر إلى أربع مجموعات: المجموعة الأولى كانت أقل من 30 عامًا، والمجموعة الثانية كانت بين 30-35 عامًا، والمجموعة الثالثة كانت بين 36-40 عامًا، والمجموعة الرابعة كانت أكثر من 40 عامًا. وقد وجد أن هرموني E2 وFSH فقط كانا ذو دلالة إحصائية ($p < 0.01$) في هذه الفئات العمرية. كما تم اختبار مستوى الهرمونات في المصل بعد تحفيز المبيض لدراسة المتوسط قبل وبعد التحفيز عبر الفئات العمرية؛ وكانت النتيجة بالنسبة لـ E2 ذات دلالة إحصائية ($p < 0.01$). توصلت الدراسة إلى أن تقلبات مستويات الهرمونات، وتحديدًا هرموني FSH وE2، تُعد مؤشرات حاسمة لتقييم العقم واستجابة المبيض للتحفيز. تتأثر هذه الاختلافات بشكل كبير بالعمر، مما يؤكد ضرورة إجراء تقييم هرموني فردي في إدارة الخصوبة.

Introduction

Infertility, which is the inability to achieve a viable pregnancy after one year of having unprotected sex, is experienced by one out of every six couples in the world. Female disorders cause the infertility of couples in about half of the cases [1]. It causes a significant impact on the population as couples can experience melancholy, anxiety, discomfort, low self-esteem, and feelings of guilt and blame throughout the process [2]. The causes of infertility may include ovulatory, endometriosis, chromosome abnormality, fallopian tube, unexplained infertility, and sperm causes [3]. Poor ovarian reserve, along with the quality and number of primordial follicles in the female ovary, is probably one of the causes of the increased occurrence of infertility in women. It remains unclear how menstrual cycle variables and changes in menstrual flow duration affect AMH and reproductive hormones. Women with varied menstrual patterns and cycles may have increased AMH and abnormalities in reproductive hormones compared to the general population [4]. The brain begins the secretion of hormones in the hypothalamus, which releases gonadotropin-releasing hormone (GnRH). This hormone stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in pulses by the pituitary gland. When the cycle starts, the levels of estrogen drop, triggering the release of GnRH, which stimulates the production of FSH and LH, leading to ovarian stimulation and the formation of eggs. When the egg is formed, the

hormone estrogen is released, and its level in the blood gradually increases. At this stage, an egg develops at a faster rate than the others and secretes more of the estrogen hormone. The increased levels of this hormone reduce the secretion of FSH, encourage the increase in the LH and FSH surge, and suppress the secretion of granulosa cells [5]. Luteinizing hormone (LH) plays a crucial role in the optimal development of follicles and oocyte maturation, as it stimulates the growth of theca cells, thereby increasing androgen production and accelerating estrogen production [6]. The study aimed to compare the parameters of the main hormones (FSH, AMH, TSH, PRL, Progesterone, and Estradiol) in infertile women with the healthy controls, investigate the response of the hormones to the ovarian stimulation, and determine the effects of age on the variations of these hormones relating to female fertility.

Material and methods

Study subjects

This study was conducted in the laboratory of Dr. Atyaf Center, which is a privately owned reproductive health facility in Al-Jadriya, Baghdad, between January 2024 and July 2024, and a total of 43 female patients who are 15-45 years old after being confirmed to have experienced infertility through medical and clinical tests conducted by the specialized physicians of the institution. The study involved a random selection of 41 healthy women, aged between 15 and 45 years, who exhibited no hormonal imbalances, while their husbands had issues with semen tests regarding sperm count, motility, and morphology. The specialists, together with laboratory testing, proved the safety of these women in menopause, hormonal abnormalities, or any diseases associated with infertility. The study was commenced after obtaining clearance from the College of Science Ethics Committee at the University of Baghdad (Ref.: CSEC/0225/0030).

Studies' inclusion criteria for infertility

Individuals who fulfill the diagnostic criteria for infertility in obstetrics and gynecology. The individual undergoing the medical examination exhibits abnormal reproductive function and sexual life.

Exclusion criteria for excluded subjects

Individuals with hematological disorders and those with malignant neoplasms.

Protocol for ovarian stimulation

All subjects received either the pituitary down-regulated Gonadotropin-releasing hormone agonist (GnRH-a) protocol or the GnRH-ant treatment, which included daily injections of recombinant Follicle Stimulating Hormone (r-FSH), namely Gonal-F or Puregon, for ovarian stimulation. The initial daily dosage of r-FSH (112.5–300U) was established based on the patient's age, anti-Müllerian hormone (AMH) levels, baseline antral follicle count (AFC), body mass index (BMI), and baseline blood FSH concentration. Measurement of the size of the follicles and the level of hormones was performed during ovarian stimulation, leading to dosage adjustments. It is essential that transvaginal ultrasound (TVUS) aids in determining the degree of ovarian response and serum levels of steroid hormones, which can be adjusted by modifying the dose of gonadotropins (Gn), thereby improving oocyte maturation. Human chorionic gonadotropin (hCG) or GnRH-a was administered together with human chorionic gonadotropin (hCG), and cumulus-oocyte complexes were collected through transvaginal ultrasound-guided needle aspiration 36 to 37 hours later, depending on the size of the follicles (at least two dominant follicles of 18 mm or more in diameter) and serum estradiol levels being above 300 pg/ml. Following ovum retrieval, fertilization of oocytes occurs either in standard in vitro fertilization (IVF), otherwise known as traditional methods, or intracytoplasmic sperm injection (ICSI) when indicated [7].

Laboratory technique

Each subject had 10 milliliters of blood taken from a vein in the forearm. The blood was put into single-use, EDTA-free test tubes. The blood was kept at room temperature for 20 minutes, after which the serum was isolated by centrifugation at 3000 rpm for 10 minutes. The serum was then extracted with the help of a micropipette. The separated serum was divided into three disposable tubes to prevent the sample from undergoing multiple freeze-thaw cycles, thereby ensuring accurate results. The levels of the hormone in serum were measured using the electrochemiluminescence assay (ECLIA). ECLIA immunoassay is supposed to be used on Cobas immunoassay analysers, which employ the biotin double-antibody sandwich technique. The ELISA was used to assess the amount of FSH, AMH, TSH, PRL, LH, Proge, and E2.

Assessing the levels of hormone profiles

The hormone profile level was assessed by adhering to the procedures outlined in the test kit and by the manufacturer's guidelines for the ELISA device [8].

Statistical analysis

Analysis of the data was carried out using the SPSS 26 statistical package (Statistical Package for the Social Sciences, version 26). Normality of the data was tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Serum hormone levels were presented as median (IQR), while age and BMI were expressed as mean $\pm$ SD. Comparisons between two groups for non-normally distributed data were performed using the Mann–Whitney U test, while comparisons among more than two groups were analyzed by the Kruskal–Wallis H test. For normally distributed data, differences between two groups were assessed using the independent t-test. A p-value < 0.05 was considered statistically significant, and $p < 0.01$ very significant.

Results

1-Baseline characteristic

The results in Table 1 showed that the mean level of age and BMI in 43 patients compared to 41 healthy controls was 27.60 ± 4.90 versus 27.85 ± 4.91 years old and 34.86 ± 4.60 versus 29.5854 ± 5.70 kg/m², respectively. The age differences were not significant, whereas the BMI differences were statistically significant ($p \leq 0.05$).

Table 1: Baseline characteristics in patients with healthy controls.

Group	N	Age (year) Mean $\pm$ S.D	BMI (Kg/m2) Mean $\pm$ S.D
Patients	43	27.60 ± 4.90	34.86 ± 4.60
Controls	41	27.85 ± 4.91	29.5854 ± 5.70
p-value		0.1 NS	0.04*

P-value= Probability value, N=Frequency, NS= Non-significant, *= Significant (≤ 0.05)

2-Hormone profile

The median hormone levels of eighty-four individuals (43 infertile women and 41 healthy women) are presented in Table 2, including PRL, TSH, AMH, E2, LH, FSH, and Proge. The median of prolactin in patients compared to controls was 15 versus 21.0 ng/mL, respectively; TSH was 1.95 versus 2.36 MIU/mL; for AMH, 0.44 versus 3.11 ng/mL; FSH was 7.55 versus 0.2 mUL/mL; and Proge was 0.2 versus 5.50 ng/mL. The median of PRL, TSH, AMH, FSH, and Proge was statistically significant ($P < 0.01$), while E2 and LH were not significant ($P > 0.05$).

Table 2: Hormone profile of infertile women and healthy controls.

Group	UNIT	Patients(N=43) Median (IQR 25%-75%)	Controls (N=41) Median (IQR 25%-75%)	P-value
PRL	ng/mL	15(12-18)	21.0(15.28-28.0)	<0.01**
TSH	MIU/mL	1.95(1.46-2.88)	2.36(1.49-2.99)	<0.01**
AMH	ng/mL	0.44(0.3-0.62)	3.11(1.79-5.48)	<0.01**
E2	pg/mL	33(22-57.70)	37(22.25-44.0)	0.93NS
LH	mUL/ml	3.4(2.35-5.45)	3.31(2.57-6.60)	0.38NS
FSH	mUL/ml	7.55(6.22-9.12)	0.2(0.1-0.3)	<0.01**
Progesterone	ng/mL	0.2(0.1-0.3)	5.50(5.0-6.5)	<0.01**

P-value= Probability value, N=Frequency, NS= Non-significant, **= Highly significant ($P < 0.01$), IQR: Interquartile range, 25%: represents the lower quartile, 75%: represents the upper quartile.

3- Effect of hormone level on fertility according to female age

3.1- Estradiol (E2) levels across age groups

Across all age categories, notable differences in E2 levels were observed between infertile patients and healthy controls (Table 3). Although the interquartile ranges varied, the median E2 in patients over 30 was 32.00, which was statistically significant ($p = 0.04$) when compared to the same median value in controls. Infertile women in the 30- to 35-year-old age range had a lower median 24.50 than controls 42.00, with a wide interquartile range, which again produced significant differences ($p = 0.04$). Unexpectedly, the controls showed wider and higher interquartile ranges (particularly <40: median 92.00 in controls vs. 36.00 in patients), supporting a consistent and significant disparity ($p = 0.04$), even in the 36–40 and <40 age groups, where E2 levels in patients appeared similar or slightly higher. These results point to a general hormonal imbalance in infertile women's ovarian follicular response or E2 production.

Table 3: E2 levels across age groups in patients and controls.

Age group	Patients (Median, IQR 25%-75%)	Controls (Median, IQR 25%-75%)	Statistical Significance
>30 y	32.00 (29-0000)	32.00 (11.50-43.00)	Significant ($p=0.04^*$)
30–35 y	24.50 (11.5–48.25)	42.00 (21.75-4886.25)	Significant ($p=0.04^*$)
36–40 y	40.50 (27.75-88.25)	38.00 (29.50-40.50)	Significant ($p=0.04^*$)
<40 y	36.00 (25.25-69)	92.00 (42.00-223.75)	Significant ($p=0.04^*$)

E2: Estradiol, NS: Non-significant, $p < 0.05$: Statistically significant, $p < 0.01$: Highly significant, P-value=Probability, IQR: Interquartile range, 25%: represents the lower quartile, 75%: represents the upper quartile.

3.2- Luteinizing Hormone (LH) levels across age groups

Table 4 shows that Luteinizing hormone (LH) levels exhibited no statistically significant changes between infertile patients and healthy controls across all age categories ($p = 0.3$). In women aged over 30, the median LH level in patients was 3.27, whereas in controls it was 4.60. In the 30–35 age range, patients exhibited a median of 2.63 compared to 3.00 in controls. Although LH levels in infertile women below the age of 40 years (median 6.14) increased modestly as compared to controls (3.20), this difference was not found to be significant. These data indicate that LH concentration, by itself, may not be a differentiating

variable in infertile status, especially when measured regardless of the cycle phase and ovulation timing.

Table 4: LH levels across age groups in patients and controls

Age group	Patients (Median, IQR 25%-75%)	Controls (Median, IQR 25%-75%)	Statistical Significance
>30 y	3.27 (2.7-000)	4.60 (1.95-7.05)	NS (p=0.3)
30–35 y	2.63 (2.01-5.26)	3.00 (0.73-5.90)	NS (p=0.3)
36–40 y	4.07 (2.63-5.27)	6.30 (2.89-8.15)	NS (p=0.3)
<40 y	6.14 (4.32-6.57)	3.20 (2.87-0000)	NS (p=0.3)

LH: Luteinizing Hormone, NS: Non-significant, $p > 0.05$: Not significant, P-value=Probability, IQR: Interquartile range, 25%: represents the lower quartile, 75%: represents the upper quartile.

3.3- Follicle-stimulating hormone (FSH) levels across age groups

Statistical analysis revealed that infertile women, as opposed to controls, had significantly higher FSH levels across all age groups ($p < 0.01$). Table 5 illustrates this point; the median FSH level in the control group was 5.30, and in the patient group it was 8.96 among women aged 30 and over. In the age groups of 30-35 (6.80 vs. 6.70) and those under 40, this pattern persisted consistently. Because increased FSH is usually linked with decreased follicular responsiveness and compensatory pituitary stimulation, infertile people's high FSH levels probably show impaired ovarian reserve. It appears that this hormone, particularly during the middle to late stages of puberty, is a reliable indicator of fertility status.

Table 5: FSH levels across age groups in patients and controls.

Age group	Patients (Median, IQR 25%-75%)	Controls (Median, IQR 25%-75%)	Statistical Significance
>30 y	8.96 (8.73-0000)	5.30 (5.00-5.96)	Highly significant ($p < 0.01^{**}$)
30–35 y	6.44 (5-9)	5.85 (0.86-7.00)	Highly significant ($p < 0.01^{**}$)
36–40 y	6.80 (5.34-8.53)	6.70 (5.60-7.31)	Highly significant ($p < 0.01^{**}$)
<40 y	9.29 (6.75-11.57)	6.74 (5.10-0000)	Highly significant ($p < 0.01^{**}$)

FSH: Follicle Stimulating Hormone, $p < 0.01$: Highly significant, NS: Non-significant, P-value=Probability, IQR: Interquartile range, 25%: represents the lower quartile, 75%: represents the upper quartile.

3.4- Progesterone levels across age groups

In any of the age categories that were evaluated, no statistically significant differences in serum progesterone levels were observed between infertile patients and healthy controls ($p = 0.10$). The median level of 0.20 was observed in both cohorts of the >30 group, with minimal variation. In the 30–35 age group, patients reported a lower median level of 0.10 compared to controls at 0.20, while the 36–40 age group had a marginally higher median of 0.30 vs. 0.20. The pattern was once again reversed among women under the age of 40, with a difference of 0.20 versus 0.10. Nevertheless, these discrepancies were not statistically significant. The absence of significant findings may be attributed to the fact that a single-point measurement

may not be adequate to assess luteal function or ovulatory status, given the dynamic nature of progesterone throughout the menstrual cycle.

Table 6: Progesterone levels across age groups in patients and controls.

Age group	Patients (Median, IQR 25%-75%)	Controls (Median, IQR 25%-75%)	Statistical Significance
>30 y	0.20 (0.2-0.2)	0.20 (0.1-0.3)	NS (p=0.10)
30–35 y	0.10 (0.1-0.2)	0.20 (0.1-0.2)	NS (p=0.10)
36–40 y	0.30 (0.1-0.5)	0.20 (0.15-0.35)	NS (p=0.10)
<40 y	0.20 (0.17-0.32)	0.10 (0.1-0.25)	NS (p=0.10)

P4: Progesterone, NS: Non-significant, $p > 0.05$: Not significant, P-value=Probability, IQR: Interquartile range, 25%: represents the lower quartile, 75%: represents the upper quartile.

4- Age-related changes in reproductive hormones before and after stimulation in infertile and healthy females

4.1- Estradiol (E2) levels before and after stimulation across age groups

Table 7 shows that both patients and controls experienced a considerable increase in E2 levels following ovarian stimulation. The median E2 increased from 32 pg/mL before stimulation to 1004.5 pg/mL after stimulation ($p = 0.001$) in patients in the >30 years group, and from 32 pg/mL to 1072 pg/mL in controls ($p = 0.001$). While controls increased from 34 pg/mL to 1082 pg/mL, patients in the 30- to 35-year-old group increased from 24.5 pg/mL to 667 pg/mL (both $p = 0.001$). Patient levels in women between the ages of 36 and 40 climbed from 40.5 pg/mL to 595 pg/mL, but controls' levels increased from 38 pg/mL to 1049 pg/mL ($p = 0.001$). Patients' levels climbed from 36 pg/mL to 623.5 pg/mL in those under 40, whereas controls' levels went from 92 pg/mL to 1302.5 pg/mL ($p = 0.001$).

Table 7: E2 level before and after Ovarian stimulation in patients and controls.

Age Group	Patients Before Stimulation (Median, IQR 25%-75%)	Controls Before Stimulation (Median, IQR 25%-75%)	Patients After Stimulation (Median, IQR 25%-75%)	Controls After Stimulation (Median, IQR 25%-75%)	P-value before and after patient stimulation	P-value before and after controls stimulation
>30 years	32(29- 0000)	32.00(11.50-43.00)	1004.5(834.25-1096)	1072(338-1724)	0.001**	0.001**
30–35 years	24.5(11.5-48.25)	34.00(21.75-486.25)	667(541.25-1269.8)	1082(131-1482)	0.001**	0.001**
36–40 years	40.5(27.75-88.25)	38.00(29.50-40.50)	595(393.5-947)	1049(947-947)	0.001**	0.001**
<40 years	36(25.25-69)	92.00(42.00-223.75)	623.5(405.75-915.75)	1302.5(867.75-1686.25)	0.001**	0.001**

E2: Estradiol, NS: Non-significant, $p < 0.05$: Statistically significant, $p < 0.01$: Highly significant, P-value=Probability, IQR: Interquartile range, 25%: represents the lower quartile, 75%: represents the upper quartile.

4.2- Luteinizing hormone (LH) levels before and after stimulation across age groups

LH levels exhibited no statistically significant alterations following stimulation Table 8. In patients over 30 years old, the median LH level reduced from 3.27 mIU/mL to 1.65 mIU/mL ($p = 0.1$), whereas in controls, it diminished from 4.60 mIU/mL to 3.07 mIU/mL ($p = 0.1$). In the 30–35 years cohort, patient levels diminished marginally from 2.63 to 2.14 mIU/mL ($p = 0.9$), while controls declined from 3.15 to 1.93 mIU/mL ($p = 0.3$). In women aged 36–40

years, patient levels rose from 4.07 to 5.17 mIU/mL, whereas control levels fell from 6.30 to 1.94 mIU/mL ($p = 0.052$ for controls). In the under-40 years cohort, patients' levels decreased from 6.14 to 4.14 mIU/mL, whereas controls decreased from 3.20 to 2.35 mIU/mL ($p > 0.05$ for both).

Table 8: LH level before and after Ovarian stimulation in patients and controls.

Age Group	Patients Before Stimulation (Median, IQR 25%-75%)	Controls Before Stimulation (Median, IQR 25%-75%)	Patients After Stimulation (Median, IQR 25%-75%)	Controls After Stimulation (Median, IQR 25%-75%)	P-value before and after patient stimulation	P-value before and after controls stimulation
>30 years	3.27(2.7-0000)	4.60(1.95-7.05)	1.65(0.3-3.75)	3.07(1.3-4.5)	0.1	0.1
30–35 years	2.63(2.01-5.26)	3.15(0.73-5.90)	2.14(1.16-3.61)	1.93(0.85-4.07)	0.9	0.3
36–40 years	4.07(2.63-5.27)	6.30(2.89-8.15)	5.17(1.05-7.74)	1.94(0.74-2.35)	0.1	0.052
<40 years	6.14(4.32-6.57)	3.20(2.87-0000)	4.14(2.5-7.25)	2.35(1.97-2.95)	0.7	0.1

LH: Luteinizing Hormone, NS: Non-significant, $p > 0.05$: Not significant, P-value=Probability, IQR: Interquartile range, 25%: represents the lower quartile, 75%: represents the upper quartile.

4.3- Follicle-stimulating hormone (FSH) levels before and after stimulation across age groups

FSH is assessed only at baseline (day 2–3 of the menstrual cycle) and is not re-evaluated post-stimulation; however, it was incorporated into the statistical analysis to facilitate a thorough assessment of reproductive hormones, enable baseline comparisons between patients and controls, and verify its anticipated stability in contrast to the fluctuating levels of estradiol. In Table 9, for patients over 30 years of age, the median was 9.2 mIU/mL both before and during stimulation ($p = 0.1$); however, in the control group, it persisted at 5.3 mIU/mL ($p = 0.1$). In the 30–35 years cohort, patients exhibited a level of 7.2 mIU/mL both pre- and post-stimulation, whereas controls maintained a level of 5.7 mIU/mL (both $p > 0.05$). In women aged 36–40 years, patient levels were maintained at 7.3 mIU/mL, whereas those of the controls were at 6.7 mIU/mL ($p = 0.1$). In the cohort aged under 40 years, patients exhibited a level of 9.29 mIU/mL both before and during stimulation, whereas controls maintained a level of 6.48 mIU/mL ($p = 0.1$).

Table 9: FSH level before and after ovarian stimulation in patients and controls.

Age Group	Patients Before Stimulation (Median, IQR 25%-75%)	Controls Before Stimulation (Median, IQR 25%-75%)	Patients After Stimulation (Median, IQR 25%-75%)	Controls After Stimulation (Median, IQR 25%-75%)	P-value before and after patient stimulation	P-value before and after controls stimulation
>30 years	9.2(8.73-0000)	5.30(5.00-5.96)	9.2(8.73-0000)	5.30(5.00-5.96)	0.1	0.1
30–35 years	7.2(5-9)	5.70(0.86-7.00)	7.2(5-9)	5.70(0.86-7.00)	0.3	0.1
36–40 years	7.3(5.34-8.53)	6.70(5.60-7.31)	7.3(5.34-8.53)	6.70(5.60-7.31)	0.1	0.1
<40 years	9.29(6.75-11.57)	6.48(5.10-0000)	9.29(6.75-11.57)	6.48(5.10-0000)	0.1	0.1

FSH: Follicle-stimulating hormone, NS: Non-significant, $p > 0.05$: Not significant, P-value=Probability, IQR: Interquartile range, 25%: represents the lower quartile, 75%: represents the upper quartile.

4.4- Progesterone Hormone (Prog) levels before and after stimulation across age groups

The results showed that Proge levels exhibited no significant variation following stimulation Table 10. In patients over 30 years old, the median Proge remained at 0.2 ng/mL both before and after stimulation ($p = 0.1$), whereas in controls, it rose from 0.20 to 0.40 ng/mL ($p = 0.1$). In the 30–35 years, patients exhibited a marginal increase from 0.10 to 0.15 ng/mL ($p = 0.9$), whereas controls rose from 0.20 to 0.65 ng/mL ($p = 0.3$). In women aged 36–40 years, patients exhibited a decrease from 0.30 to 0.20 ng/mL ($p = 0.5$), but controls maintained a constant level of 0.20 ng/mL ($p = 0.9$). In the cohort under 40 years, patient levels declined from 0.20 to 0.10 ng/mL, whereas control levels rose from 0.10 to 0.15 ng/mL ($p > 0.05$ for both).

Table 10: Proge level before and after ovarian stimulation in patients and controls.

Age Group	Patients Before Stimulation (Median, IQR 25%-75%)	Controls Before Stimulation (Median, IQR 25%-75%)	Patients After Stimulation (Median, IQR 25%-75%)	Controls After Stimulation (Median, IQR 25%-75%)	P-value before and after patient stimulation	P-value before and after controls stimulation
>30 years	0.2(0.2-0.2)	0.20(0.10-0.30)	0.2(0.1-1.65)	0.4(0.1-0.8)	0.1	0.1
30–35 years	0.1(0.1-0.2)	0.20(0.10-0.20)	0.15(0.1-0.4)	0.65(0.3-0.95)	0.9	0.3
36–40 years	0.3(0.1-0.5)	0.20(0.15-0.35)	0.2(0.15-0.5)	0.2(0.1-0.3)	0.5	0.9
<40 years	0.2(0.17-0.32)	0.10(0.10-0.25)	0.1(0.1-0.22)	0.15(0.1-0.27)	0.3	0.3

Proge: Progesterone hormone, NS: Non-significant, $p > 0.05$: Not significant, P-value=Probability, IQR: Interquartile range, 25%: represents the lower quartile, 75%: represents the upper quartile.

Discussion

Hormonal imbalance may be seen as a minor issue of infertility in females, as it can be rectified with medication [9]. Sex hormones facilitate follicular development and ovulation, hence supporting regular pregnancies in women. The abnormal secretion of sex hormones is going to cause abnormal ovulation, resulting in abnormal menstrual cycles and infertility [10]. It is essential to evaluate the combined impact of age and body mass index on the likelihood of delivering a live baby following in vitro fertilization. The samples on which the study was conducted show that there is no significant relationship between age and infertility in females. Women of reproductive age experience a progressive depletion of their ovarian reserve of oocytes until they are used up during the menopause period. This, however, is not usually followed in the expected pattern. This, in turn, requires screening of ovarian reserve (OR) [11], in line with current research. Obesity is a recognized cause of menstruation and ovulatory issues, and infertility. The correlation between body mass index and infertility was found to be statistically significant ($p = 0.04$). A recent global evaluation conducted by the World Health Organization (WHO) shows that obesity has increased threefold since 1975. The number of women in the reproductive age group becoming obese all over the world is on the rise, and it is predicted that 20 percent of women will be obese by the year 2025 [12]. The results were consistent with an alternative study, which revealed that an increased risk of infertility was associated with an increased BMI [13].

The levels of serum prolactin, TSH, and AMH show a significant positive correlation with female infertility ($p < 0.01$). Clinically significant prolactin (PRL) levels may render a person infertile by suppressing ovulation, and may cause intermittent ovulation disruption that, however, in milder forms, decreases the chances of conception [14]. The current results suggest that there is a high correlation between infertility and the level of TSH ($p < 0.01$). The development of the human placenta, embryo, and implantation are all significant factors in the outcome of pregnancy, and the levels of thyroid hormone are an aspect that should be considered when addressing infertility in women, according to the American Thyroid Association and the American Society for Reproductive Medicine [15]. This study found that E2 and LH levels are not significantly correlated with infertility ($P > 0.05$), which agrees with another study that reported no statistically significant difference in E2 levels between fertile and non-fertile individuals. Besides, there was no statistically significant difference in the E2 levels in the case of abortion rate [16]. The level of LH during ovarian stimulation has been studied in several published research articles to determine its effects on the outcome of clinical practice. However, the findings did not reveal a relationship between LH levels during GnRH antagonist cycles and pregnancy outcomes [17]. No significant difference was found between LH in the follicular phase between the observation and the control groups [10]. A strong association was introduced between the FSH and progesterone hormones and infertility ($p < 0.01$). FSH mediates the production and secretion of estrogen, which, in turn, promotes the growth and maturation of ovarian follicles. Sequential FSH administration to induce hyperstimulation (superovulation) in the ovaries, therefore, remains the most cost-efficient option for most ARTs [18]. Progesterone plays a crucial role in human reproduction, exhibiting a wide range of biological activities. Progesterone increases the suitability of the endometrium for embryo implantation during the covert period of the menstrual cycle, both *in vivo* and *in vitro* [19]. The effect of female age on E2 and FSH hormone was statistically significant ($P < 0.05$) on the hormone levels. The estrogen levels in the circulating fluids of healthy individuals exhibit high levels of variability throughout the lifespan. E2 levels increase with age and during breast development in puberty, and fluctuate during the menstrual cycle [20]. FSH shows the follicular count, whereas the age is a qualitative evaluation of ovarian reserve. The statistics indicate that females under 35 years of age tend to have good-quality oocytes in most cases, and FSH is an indicator of the number of follicles associated with the rate of pregnancy [21]. The level of LH and progesterone is not significantly related to female age ($P > 0.05$). LH isoforms are evolving towards less bioactive forms as people age. Old age causes reduced gonadotropin activity, which causes reduced steroidogenesis and impaired ovarian functions [22]. In addition, different words have been used to explain the decline in production and patterns of secretion of hormones as people grow older, whereby menopause is specifically used to refer to the sudden decline of the production of estrogen and progesterone in middle-aged women as a result of the termination of the ovarian activity [23]. In this research, it was found that there was a dramatic rise in E2 levels after ovarian stimulation in both infertile women and healthy controls across all age groups. This significant rise is a sign of the natural response of granulosa cells to the stimulation of FSH that enhances the secretion of estradiol in the process of follicular growth. Such findings align with prior research that demonstrates high estradiol dynamics during controlled ovarian stimulation (COS) are associated with improved embryological and clinical outcomes, particularly in freeze-all methods, without compromising obstetric safety [24]. The level of LH in the work showed no statistically significant changes before and after stimulation in all the age groups. This stability is expected, as the majority of COS regimens (antagonist or long agonist) are designed to suppress early LH spikes and maintain adequate endogenous LH levels to allow follicular growth. Similar findings have been noted in recent reviews, which suggest that the levels of

LH tend to remain constant in response to stimulation and only become supplemented in certain cases of LH deficiency. These findings are consistent with the results and underscore the fact that a high level of LH differences is abnormal in traditional COS [25]. FSH has not been commonly measured during stimulation; hence, no changes in FSH levels were found across the groups, as it is usually measured only at baseline (days 2-3 of the menstrual cycle). The readings were constant and did not significantly influence the results, as the measurement was made at the beginning of the cycle, prior to ovarian stimulation, and no further measurements were taken. Recent recommendations and systematic reviews have reached the same conclusion: baseline FSH is an effective predictor of ovarian response and remains relatively stable during stimulation. Such an agreement lends credence to the fact that FSH is considered more stable compared to estradiol, which has been found to undergo dynamic changes [26]. The progesterone levels depicted insignificant, small changes in the control group and the participating patients. The outcome shows that the applied stimulation regimens worked well to prevent premature luteinization, and this is encouraging news for endometrial receptivity. Although freeze-all methods could help mitigate the negative effects of an unexpected increase in progesterone on the trigger day, other studies have also shown them to have an adverse effect on implantation rates in fresh embryo transfers. The fact that the current results show no statistically significant increase in progesterone is also additional evidence that the strategy is effective in maintaining stable luteal-phase conditions [27].

Conclusion

The present investigation of infertility brought out the effects of BMI on the fertility of females. It demonstrated that PRL, TSH, AMH, E2, LH, FSH, and Proge levels play a vital role in the reproductive wellness of infertile women. Such hormones are very important in assisted reproductive techniques (ART). Moreover, the levels of E2 and FSH turned out to be directly linked with infertility in the female population in diverse age groups. It was also revealed in the study that there was a significant relationship between the level of E2 in the pre-ovarian stimulation period and post-ovarian stimulation period and female infertility in all age groups.

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Conflict of interest

Conflict of interest does not exist.

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