Original Article

Correlation between anti-Mullerian hormone, luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, estradiol, and vitamin D: a retrospective study in women with secondary infertility

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Abstract

Background: This study aims to evaluate the correlation between anti-Müllerian hormone, luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, Estradiol, and vitamin D in women experiencing secondary infertility.

Materials and Methods: In this retrospective study, we enrolled 97 women with secondary infertility who underwent treatment between 2012 and 2022. Information regarding each couple, including names (confidential and coded), ages, causes of infertility, number of children and abortions, and duration of infertility were collected. Laboratory test results were gathered, which included gonadotropin and estradiol hormone levels measured on the second to third day of menstruation, as well as anti-Müllerian hormone, thyroid-stimulating hormone, and vitamin D levels. The data were analyzed using SPSS software (version 26).

Results: The mean ages of females and males were 33/5±4/4 and 36/5±5/6 years, respectively. There was no significant correlation between vitamin D and estradiol and other hormone levels. In addition, there was a significant positive correlation between the follicle-stimulating and luteinizing hormones ($P = .021$). The luteinizing hormone also showed a significant negative correlation with the thyroid-stimulating hormone ($P=.019$). Conversely, anti-Müllerian hormone correlated negatively with follicle-stimulating hormone ($P < 0.01$). The age of females had a negative significant correlation with anti-Müllerian hormone ($P=0.002$).

Conclusion: According to our study, as women age, their anti-Müllerian hormones decrease, and their follicle-stimulating hormone levels increase. In addition, the study found a correlation between luteinizing hormone and thyroid-stimulating hormone, indicating a potential interplay between thyroid function and reproductive hormones. The findings emphasize the complex relationships between age, hormonal levels, and secondary infertility.

Keywords: Secondary infertility, Vitamin D, Reproductive hormones, Gonadotropins, AMH, TSH, Estradiol

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Introduction

Infertility is recognized as a disease by the World Health Organization[1,2]. As defined in the latest international glossary on infertility and fertility care, it refers to the inability to achieve a clinical pregnancy after 12 months of regular, unprotected sexual intercourse[3]. Infertility is categorized into primary and secondary forms. Primary infertility occurs when an individual has never achieved a pregnancy, while secondary infertility occurs when at least one prior pregnancy has been achieved[4].

Various factors can contribute to infertility, including elevated levels of prolactin hormone, abnormal body weight (under or overweight), advanced age, hormonal imbalances such as hypothalamic and thyroid disorders, substance abuse, genetic mutations, and others[(5)]. Ovarian reserve, which reflects the number of remaining oocytes in the ovary, can be indirectly assessed using hormones like inhibin B, follicle-stimulating hormone (FSH), and anti-Mullerian hormone (AMH)[6]. AMH, produced by granulosa cells in ovarian follicles, is a reliable biomarker for ovarian reserve. Its levels remain consistent throughout the menstrual cycle and offer high specificity in detecting inadequate ovarian response[7,8]. Thyroid disorders can also impact ovarian function and reserve, with thyroid diseases being a prevalent endocrine issue among women of reproductive age, potentially leading to menstrual irregularities, ovulation problems, and infertility[9]. The luteinizing hormone (LH) surge plays a crucial role in meiotic processes within the ovary, triggering oocyte release from prophase arrest and resumption of meiosis[10]. The ovary comprises two distinct cellular components activated separately by LH and FSH to produce ovarian steroids essential for follicular development and estrogen production[11].

Additionally, vitamin D regulates reproductive functions in both men and women, with deficiencies often found in reproductive-aged women[12,13]. It is suggested that vitamin D levels may impact the initial implantation of embryos by modulating immune cells such as natural killer cells, dendritic cells, macrophages, and T cells within the uterine and decidual tissues[14]. Vitamin D receptors and 1,25(OH)2D3 play a role in regulating the transcription of HOXA10, which is a key gene associated with implantation. Additionally, vitamin D directly influences the production of AMH, contributing to the prolonged maintenance of ovarian reserve in patients with higher serum concentrations of this vitamin[(15)]. Research indicates that serum vitamin D levels are typically higher in healthy women compared to those with PCOS, who often experience obesity, insulin resistance, hyperandrogenemia, and amenorrhea[(16)].

Hormones are key players in female fertility, influencing the menstrual cycle, follicle growth, ovulation, implantation, and pregnancy. Hormonal imbalances significantly impact female fertility and necessitate proper diagnosis for effective treatment[(17)]. Despite numerous studies investigating the relationships between AMH, LH, FSH, TSH, estradiol, and vitamin D in infertile women, the specific interactions among these factors in women with secondary infertility have not been extensively studied. Given that fewer studies examine the relationship between hormones and secondary infertility compared to primary infertility, we aimed to assess the correlation among levels of AMH, LH, FSH, estradiol, vitamin D, and TSH. This study focuses specifically on women dealing with secondary infertility, highlighting the intricate relationships between hormonal levels and secondary infertility.

Methods

In this retrospective research study, 97 women experiencing secondary infertility were enrolled. These participants sought treatment at the infertility center of Amir-Al-Momenin Hospital in Semnan City between 2012 and 2022. Women over the age of 40, those with endometriosis or polycystic ovary syndrome (PCOS), and women whose partners had abnormal semen parameters were excluded from the study.

A couple of pieces of information, including name (confidential and coded), ages, causes of infertility, number of children, number of abortions, and duration of infertility, were collected. Additionally, laboratory test results were gathered, including gonadotropin and estradiol levels measured on the second to third day of menstruation and levels of AMH, TSH, and vitamin D. All hormonal analyses were done in two laboratories approved by the university. The collected data were analyzed using SPSS software (version 26). After assessing the normality of the data with the One-Sample Kolmogorov-Smirnov Test, Spearman's correlation was employed to examine relationships between the parameters. A p-value of less than 0.05 was considered statistically significant.

The study was approved by the ethics committee of Semnan University of Medical Sciences (ethics code: IR.SEMUMS.REC.1402.008).

Results

The mean ages of females and males were 33.5 ± 4.4 years and 36.5 ± 5.6 years, respectively. The minimum and maximum sperm counts in males were 15 and 238 million/mL (mean: 74.9 ± 47.1). The duration of secondary infertility ranged from 1 to 17 years, with an average of 3.8 ± 2.8 years. Among the participants, 49.5% had no children, 44.3% had one child, and 6.2% had two children. Additionally, 41.2% of the women had no history of abortion, while 40.2%, 14.4%, 1.1%, and 3.1% reported histories of one, two, three, and four abortions, respectively. The causes of secondary infertility included male factors (12.5%), female factors (35.5%), combined factors (14.6%), and unexplained infertility (37.5%).

Table 1. The averages of different parameters.

Parameters	Mean	Std. deviation
BMI of female	26.2	3.3
Vit D (ng/mL)	29.6	15.4
FSH (IU/L)	8.6	13.3
LH (IU/L)	6.1	6.7
Estradiol (pg/mL)	62.5	43.9
AMH (ng/mL)	2.9	2.4
TSH (μIU/mL)	2.4	2.0

Table 2. Correlations between parameters.

		Vit D	FSH	LH	Estradiol	AMH	TSH
Vit D	CC	1.0	-0.1	-0.05	0.1	-0.6	-0.1
	Sig	--	0.3	0.6	0.2	0.5	0.1
FSH	CC	-0.1	1.0	0.2*	-0.1	-0.3**	0.02
	Sig	0.3	--	0.02	0.2	0.0	0.7
LH	CC	-0.05	0.2*	1.0	0.1	0.09	-0.2*
	Sig	0.6	0.02	--	0.3	0.3	0.01
Estradiol	CC	0.1	-0.1	0.1	1.0	0.06	0.05
	Sig	0.2	0.2	0.3	--	0.6	0.7
AMH	CC	0-0.08	-0.3**	0.09	0.02	1.0	0.1
	Sig	0.4	0.0	0.3	0.8	--	0.3
TSH	CC	0-0.1	0.02	-0.2*	0.05	0.09	1.0
	Sig	0.1	0.7	0.01	0.7	0.3	--

CC: Correlation coefficient, Sig: Sig (2- tailed), *Correlation is significant at the 0.05 level (2–tailed), **Correlation is significant at the 0.01 level (2–tailed).

The average values for BMI, vitamin D, and reproductive hormones are provided in Table 1.

As depicted in Table 2, there was no significant correlation between Vitamin D and other hormone levels, nor between Estradiol and other hormones. A positive significant correlation was observed between FSH and LH (P=0.021), while LH showed a negative significant correlation with TSH (P=0.019). In addition, AMH correlated negatively with FSH (P<0.01).

As shown in Table 3, female age had a significant negative correlation with AMH (P = 0.002). No significant correlation was found between female BMI and other parameters.

Discussion

This study aims to explore the relationships between AMH, LH, FSH, TSH, estradiol, and vitamin D in women with secondary infertility. Our results indicated no significant correlation between vitamin D, estradiol, and other hormone levels. Additionally, we found a significant positive correlation between FSH and LH,

Table 3. Correlation of female age and BMI with hormone levels.

		Age	BMI	Vit D	FSH	LH	Estradiol	AMH	TSH
Age	CC	1.0	0.1	0.1	0.1	-0.07	0.1	-0.3**	-0.03
	Sig	--	0.07	0.08	0.1	0.4	0.2	0.002	0.7
BMI	CC	0.1	1.0	0.1	-0.05	0.07	0.06	.0.1	0.06
	Sig	0.07	--	0.2	0.6	0.4	0.6	0.1	0.5

**Correlation is significant at the 0.01 level (2–tailed).

while LH exhibited a significant negative correlation with TSH. Conversely, AMH showed a negative correlation with FSH. Furthermore, female age had a significant negative correlation with AMH, while no significant correlation was observed between female BMI and other parameters.

In a cohort study involving 90 women aged 20 to 43 with a history of primary and secondary infertility, FSH and AMH levels were measured during the menstrual cycle. The study concluded that there is a negative correlation between AMH and age and a positive correlation between AMH and the number of antral follicles. Notably, a significant negative correlation between AMH and FSH was reported, which aligns with our findings[18]. Similarly, in a 2024 study conducted by Das et al., a strong negative correlation was reported between FSH and AMH and between age and AMH[(19)]. In a study by Robertson et al., it has been proposed that elevated FSH may suppress AMH directly or indirectly through oocyte-specific growth factors, leading to a marked decrease in AMH per follicle[20]. In a 2019 study by Errutia and colleagues, the role of AMH as a marker of FSH function in male and female fertility was compared. They concluded that the administration of FSH in women results in a decrease in serum AMH, likely due to the increased recruitment of small antral follicles to larger stages, which results in less AMH[21].

In our study, no significant correlation was found between vitamin D and other hormones in women with secondary infertility. Supporting our findings, BEZİRGANOĞLU ALTUNTAŞ et al. investigated the relationship between serum vitamin D levels and FSH, AFC, and AMH in a retrospective study of 197 infertile women, reporting no significant correlations among these markers[(22)]. Another study on women with reduced ovarian reserve indicated no significant relationship between vitamin D levels and AFC, AMH, or FSH, despite administering 300,000 units of vitamin D to vitamin D-deficient women improved calcium, FSH, AFC, and AMH levels[23]. Further research examined the relationship between vitamin D and age, BMI, duration of infertility, FSH, LH, duration of ovulation stimulation, number of follicles, diameter of the largest follicle, and endometrial thickness in infertile women with unexplained infertility. This study observed only a negative correlation between LH levels and vitamin D, with no significant correlations found for other markers[24].

In contrast, another study involving 351 healthy women of reproductive age with an average age of 24 years reported significant relationships between both free and total levels of vitamin D with LH, testosterone, the ratio of LH to FSH, androstenedione, and AMH[25]. Additionally, research by Rafique et al. demonstrated that infertile women with low vitamin D levels also exhibited decreased levels of FSH and LH; they concluded that patients lacking sufficient vitamin D were at a higher risk for secondary infertility[26]. In another study, it was found that vitamin D deficiency and insufficiency are common among infertile women, and the live birth rate following assisted reproductive technique is related to serum vitamin D level[27]. In a systematic meta-analysis conducted in 2023 by Meng et al., the combined results revealed that infertile women treated with vitamin D exhibited a significantly higher clinical pregnancy rate compared to the control group. The improvement in clinical pregnancy rates within the intervention group was influenced by several factors, including the patients' clinical vitamin D levels, the type of medication used, the duration and frequency of administration, and the daily dosage of vitamin D supplements. This study concluded that consuming vitamin D supplements for a duration of 30 to 60 days can lead to improved pregnancy outcomes[28].

According to the literature, thyroid dysfunction is frequently associated with female infertility[29,30]. A cohort study from 2007 to 2012 involving 7,943 individuals demonstrated a significant relationship between vitamin D deficiency and hypothyroidism[31]. In our study, while there was no significant correlation between vitamin D and TSH, TSH exhibited a significant correlation with LH. Sharma et al. also reported significant correlations of TSH with both FSH and LH[32]. Additionally, a 2024 study conducted by Aljaff N found a significant increase in LH, testosterone, TSH, insulin, and glucose levels in the blood serum of patients with secondary infertility and obesity compared to the control group[33].

In the present study, Spearman's correlation analysis indicated no significant correlation between estradiol and LH, FSH, AMH, and TSH hormones. In contrast, a study by Sun et al. conducted in 2018 found a significant positive correlation between estradiol and

AMH[34]. Additionally, another study reported no significant correlation between estradiol and TSH[35]. Furthermore, our study revealed a significant negative correlation between female age and AMH levels, aligning with other research findings[36, 37]. The 2020 study by Racoubian et al. indicated that circulating AMH levels are inversely related to age and serve as predictors of the LH/FSH ratio[36]. The present study did not find significant correlations between female BMI and other parameters, indicating that body mass index may not be a relevant factor in the hormonal profiles of women with secondary infertility. It should be considered that we excluded patients with PCOS from the study, although there are several studies indicating the relation between BMI and hormonal status in PCOS women[38-40].

Conclusion

Our results show a significant negative correlation between female age and AMH levels and between FSH and AMH, indicating that as women age, their AMH levels decrease and FSH increase, consistent with existing literature on ovarian reserve. In addition, the study found a correlation between LH and TSH, indicating a potential interplay between thyroid function and reproductive hormones. No significant correlations were observed between vitamin D and estradiol levels with other hormones, suggesting that these factors may not directly affect the hormonal profile of women with secondary infertility in this sample. Overall, the findings emphasize the complex relationships between age, hormonal levels, and secondary infertility, highlighting areas for further investigation into the hormonal dynamics affecting reproductive health.

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Conflict of interest

The authors further declare that they have no conflict of interest.

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