Original Research Article

The correlation between age and hormonal concentration in infertile females: a cross-sectional study

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ABSTRACT

Background: Infertility is a global health issue with significant social and emotional consequences, affecting approximately 48 million couples and 186 million individuals worldwide. Female infertility commonly results from abnormalities of the uterus, fallopian tubes, ovaries, or endocrine disturbances that disrupt reproductive hormones.
Methods: This cross-sectional study included 110 infertile women who attended the department of obstetrics and gynaecology after at least one year of marriage without conception. Participants were categorized into three age groups: 20–25 years, 26–35 years, and ≥36 years.
Results: Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels showed a significant positive correlation with age (FSH: r=0.44, p<0.0001; LH: r=0.35, p=0.0002), with the highest values observed in women aged ≥36 years, indicating declining ovarian reserve. In contrast, anti-Müllerian hormone (AMH) levels demonstrated a significant negative correlation with age (r=-0.30, p=0.0015), reflecting reduced fertility potential in older women. Thyroid-stimulating hormone (TSH) levels also increased significantly with age (r=0.50, p<0.0001), suggesting a higher prevalence of thyroid dysfunction, which may contribute to ovulatory disturbances and menstrual irregularities.
Conclusions: Advancing age is associated with increased FSH, LH, and TSH levels and decreased AMH levels, highlighting the impact of aging on reproductive function. Comprehensive hormonal evaluation, particularly in women aged ≥36 years, is essential for understanding infertility and optimizing fertility management strategies.

Keywords: Infertility, FSH, LH, AMH, TSH

INTRODUCTION

Infertility poses a global challenge with substantial repercussions for families and communities. Approximately forty-eight million couples and one hundred eighty-six million individuals are impacted by infertility on a global scale. Female infertility arises from disorders within the female reproductive system, including issues with the uterus, fallopian tubes, ovaries, and endocrine system disruptions causing abnormalities in reproductive hormones.[1] Numerous factors are implicated in female infertility. Normal reproductive function in women depends on functional ovaries, uterus, fallopian tubes, and a balanced endocrine system. Dysfunction in any of these areas can compromise fertility.[2]

Infertility is defined as the inability to achieve pregnancy after twelve months of regular, unprotected intercourse. It is a prevalent issue, impacting around 8-12% of couples globally. Primary and secondary infertility are the two main classifications.[3] Primary infertility refers to the incapacity to attain conception at least twelve-month consistent, Unprotected sexual activity, without any previous pregnancies.[4]

The World Health Organization (WHO) carried out comprehensive multinational research to determine the

distribution by gender and factors leading to infertility. The Investigation highlighted the highest prevalent components contributing to female infertility disorder were disorders affecting ovulation 25%, endometriosis 15%, pelvic adhesions 12%, blockage of fallopian tubes 11%, other abnormalities of the fallopian tubes/uterus 11%, and hyperprolactinemia 7%.[5]

The reproductive cycle is a complex process regulated by a combination of hormones including FSH, LH, AMH, and TSH. These hormones work together to control ovulation, fertilization, and implantation. Several studies have demonstrated that hormonal imbalances play a significant role in female infertility. For instance, research by Boivin et al indicated that hormonal disorders are present in 20-30% of infertile women. Similarly, Hall et al found that irregular surges of LH and FSH can interfere with ovulation, contributing to infertility.[6] Additionally, thyroid dysfunction has been linked to infertility, with Krassas et al reporting a higher prevalence of hypothyroidism among infertile women compared to fertile controls.[7]

This hormonal imbalance can disrupt ovulation, fertilization, and implantation, thereby hindering the ability to conceive. According to the American Society for Reproductive Medicine (ASRM), hormonal imbalances are a prevalent cause of infertility in women, affecting approximately 20-30% of couples experiencing infertility.[6]

This study conducted to explore the correlation between various hormones and age of female in infertile women focusing on follicle-stimulating hormone (FSH), luteinizing hormone (LH), anti-Mullerian hormone and thyroid stimulating hormone (TSH). Our study deepens insight into the endocrine mechanisms regulating the female reproductive cycle and their role in infertility. By clarifying these hormonal interactions, it may support the development of more precise diagnostic approaches and targeted therapeutic interventions for affected women.[8] Furthermore, these findings may aid in the early identification, prevention, and management of associated reproductive disorders, thereby promoting better reproductive health outcomes and overall well-being in women.

METHODS

This cross-sectional study was conducted in Department of Obstetrics and Gynaecology, D. Y. Patil Medical Hospital, Kadamwadi, Kolhapur, over 12 months from February 2024 to January 2025. A random sampling method was used to collect the data in infertile females between the age of 20-45 years. Data were collected from 110 infertile women who had been married for more than one year without successful conception. Participants who met the inclusion criteria were enrolled in the study after obtaining informed consent. To study the hormonal profile in relation to age, the cases were divided into three groups based on their age: 20–25 years, 26–35 years, and ≥36 years. Females who are having secondary infertility, unmarried are excluded from the study. The study protocol was approved by the institutional ethical committee.

Depending on the number of tests required, approximately 3–5 ml of venous blood was drawn on the third day of the menstrual cycle (follicular phase) to evaluate the hormone levels of FSH, LH, AMH, and TSH. The blood samples were collected in appropriate tubes, serum separator tubes (SST) with gold or red caps, with or without a gel barrier, for hormone assays; EDTA tubes (lavender cap) for tests requiring plasma; and heparin tubes (green cap) when immediate plasma separation was necessary.

RESULTS

Table 1 illustrates the relationship between age groups and serum hormone levels.

Table 1: Analysis shows the relationships between various hormone levels and age groups.

Hormones	Cases (110)			Mean age 30.92±4.82	Regres-sion (R²)	Correla-tion coefficie-nt (r)	t statis-tics	P value
	Age group (years)			Mean hormone level 11.31±9.29				
	20-25	26-35	≥36					
FSH (mIU/ml)	6.73±1.69	8.23±2.92	11.82±7.16	8.70±4.19	0.189	0.44	5.03	1.96E-06*
LH (mIU/ml)	8.30±3.63	9.02±4.55	12.99±5.81	9.65±4.92	0.123	0.35	3.90	0.0002*
AMH (ng/ml)	5.32±4.65	2.46±2.84	1.52±2.48	2.65±3.22	0.089	-0.30	-3.26	0.0015*
TSH (µIU/ml)	2.39±0.94	3.92±2.78	6.78±3.31	4.25±3.00	0.246	0.50	5.94	3.58E-08*

FSH showed a progressive increase with advancing age, rising from 6.73±1.69 IU/l in the 20–25 years group to 8.23±2.92 IU/l in the 26–35 years group, and further to 11.82±7.16 IU/l in women aged ≥36 years (Figure 1).

Linear regression analysis demonstrated an R² value of 0.189, indicating that 18.9% of the variability in FSH levels can be explained by age (Figure 2). The correlation coefficient (r=0.44) revealed a moderate positive

correlation between age and FSH levels. The t-statistic (t=5.03) and highly significant p value (p=1.96×10⁻⁶) confirm a statistically significant increase in FSH levels with advancing age. Similarly, LH levels increased with age, with mean values of 8.30±3.63 IU/l (20–25 years), 9.02±4.55 IU/l (26–35 years), and 12.99±5.81 IU/l (≥36 years) (Figure 3). The R² value of 0.123 indicates that 12.3% of the variance in LH levels is attributable to age (Figure 4). A moderate positive correlation was observed (r=0.35), though weaker than that for FSH. The association was statistically significant, as evidenced by the t-statistic (t=3.90) and p value (p=0.0002). In contrast to FSH and LH, AMH levels demonstrated a declining trend with increasing age. Mean AMH levels decreased from 5.32±4.65 ng/ml in the 20–25 years' group to 2.46±2.84 ng/ml in the 26–35 years' group, and further to 1.52±2.48 ng/ml in women aged ≥36 years (Figure 5).
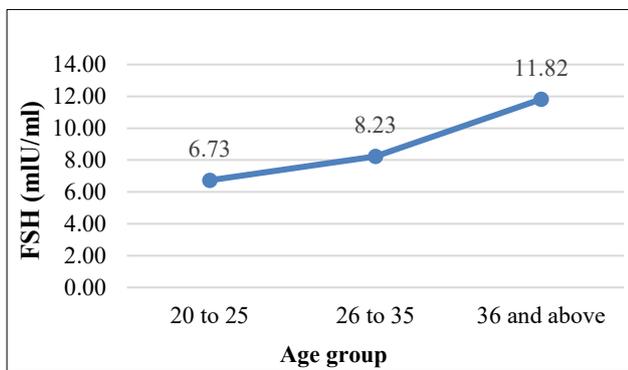

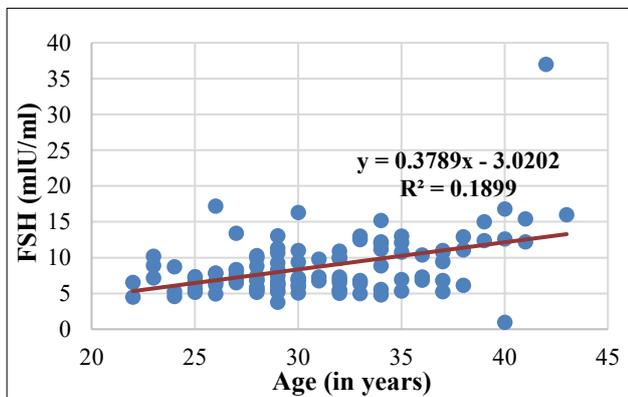**Figure 1: Age group wise average FSH.**

Figure 2: Correlation between age and FSH.

The R² value of 0.089 indicates that 8.9% of the variability in AMH levels is explained by age (Figure 6). A moderate negative correlation was observed (r=–0.30), suggesting that AMH levels decline as age increases. The negative t-statistic (t=–3.26) and statistically significant p value (p=0.0015) confirm a significant inverse association between age and AMH levels. TSH levels also showed a significant upward trend with age, increasing from 2.39±0.94 mIU/l (20–25 years) to 3.92±2.78 mIU/l (26–35 years), and reaching 6.78±3.31 mIU/l in women aged ≥36 years (Figure 7).
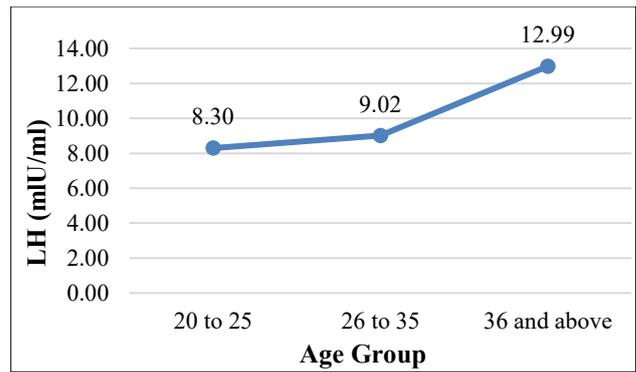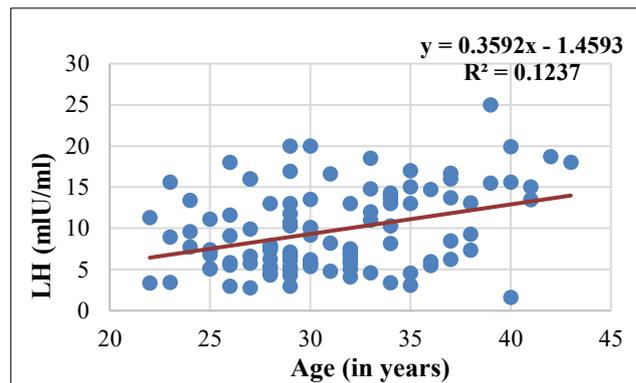

Figure 3: Age group wise average LH.
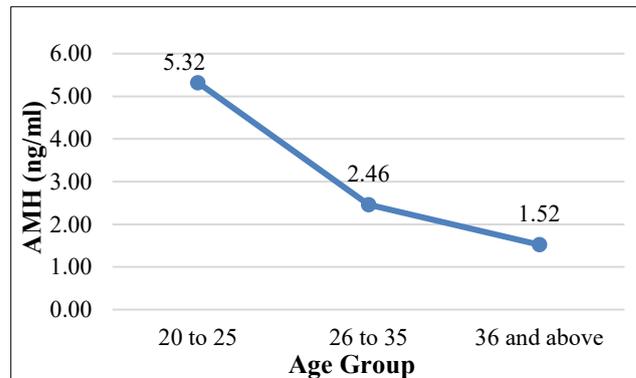

Figure 4: Correlation between age and LH.
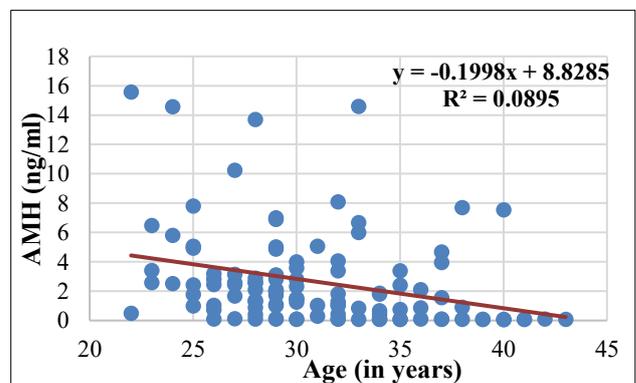

Figure 5: Age group wise average AMH.

Figure 6: Correlation between age and AMH.

Regression analysis revealed an R² value of 0.246, indicating that 24.6% of the variance in TSH levels is explained by age (Figure 8). A strong positive correlation was observed (r=0.50). The high t-statistic (t=5.94) and extremely significant p value (p=3.58×10⁻⁸) confirm a strong association between advancing age and increased TSH levels.
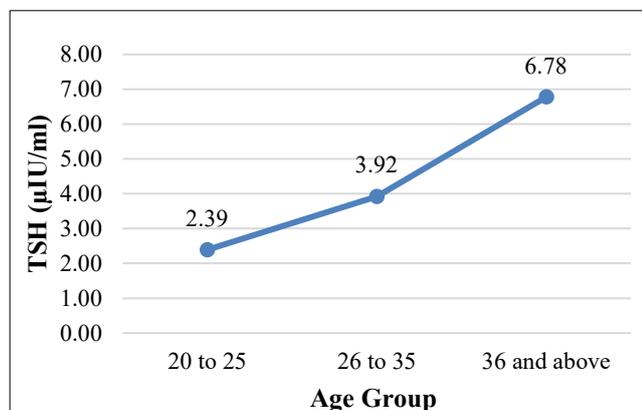

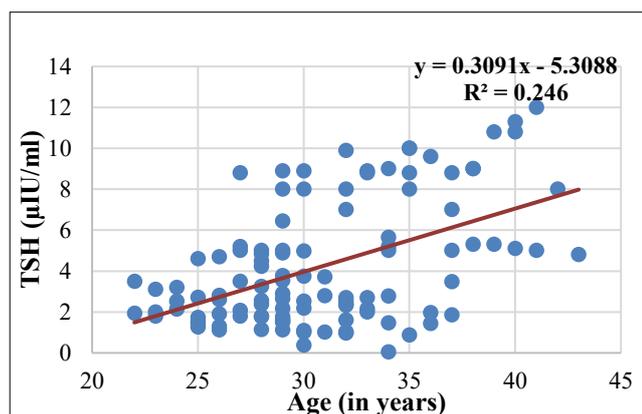**Figure 7: Age group wise average TSH.**

Figure 8: Correlation between age and TSH.

DISCUSSION

FSH, LH, TSH, and AMH are key regulators of female reproductive function through their coordinated action within the hypothalamic–pituitary–ovarian (HPO) axis. Together, they maintain menstrual cycle, follicular maturation, ovulation, and endometrial preparation for implantation.

FSH promotes follicular growth by stimulating granulosa cell proliferation and estrogen synthesis. Estrogen and inhibin B provide negative feedback to the pituitary to regulate FSH secretion. When ovarian reserve declines, reduced feedback leads to compensatory elevation of basal FSH, reflecting diminished ovarian function. LH works synergistically with FSH by stimulating androgen production in theca cells and triggering ovulation through the mid-cycle LH surge, followed by progesterone secretion from the corpus luteum. Disruption in LH

dynamics can impair ovulation and luteal support. AMH, produced by granulosa cells of small follicles, reflects the remaining follicular pool and regulates early follicular recruitment. Its relatively stable levels throughout the cycle make it a reliable marker of ovarian reserve. Declining AMH indicates progressive follicular depletion, particularly with advancing age. TSH regulates thyroid function, which indirectly influences reproductive physiology by modulating GnRH secretion, prolactin levels, and ovarian responsiveness. Thyroid dysfunction can disturb ovulation and menstrual regularity, contributing to infertility. In infertile women, advancing age is typically associated with reduced AMH and elevated FSH, reflecting diminished ovarian reserve and weakened ovarian feedback. LH and TSH alterations may further compromise ovulatory efficiency. Collectively, these hormonal changes represent adaptive endocrine responses to declining follicular quantity and quality.

The present study demonstrates significant age-related variations in reproductive hormone levels among infertile women. Regression analysis (Table 1) revealed correlations between advancing age and key hormonal parameters. Serum FSH levels showed a moderate positive correlation with age (r=0.44, p=1.96×10⁻⁶), with the highest mean values observed in women aged ≥36 years. This rise in FSH with advancing age reflects declining ovarian reserve and reduced follicular responsiveness, which are well-recognized physiological changes associated with reproductive aging. Similarly, LH concentrations increased progressively with age, showing a statistically significant positive correlation (r=0.35, p=0.0002). Elevated LH levels in older women may indicate altered hypothalamic–pituitary–ovarian axis regulation, contributing to ovulatory dysfunction and compromised fertility potential. In contrast, AMH levels demonstrated a significant negative correlation with age (r=−0.30, p=0.0015), with the lowest concentrations detected in women aged ≥36 years. Since AMH is a reliable marker of ovarian reserve, its decline with advancing age further supports the concept of follicular depletion as a central mechanism underlying age-related infertility. TSH levels also showed a moderate positive correlation with age (r=0.50, p=3.58×10⁻⁸), with higher mean values in the older age group. Thyroid dysfunction, even in subclinical forms, may adversely affect menstrual regularity, ovulation, and overall reproductive outcomes. The observed increase in TSH with age suggests a possible contributory role of thyroid imbalance in infertility among older women.

These findings are consistent with previous studies. Investigations conducted by Chioma and Emine reported comparable variations in FSH, LH, AMH, and TSH levels among women with primary infertility.[9] Sudha and Reddy observed that although most infertile women had hormone levels within standard reference ranges, a subset exhibited hormonal disturbances, emphasizing that even subtle endocrine variations may influence fertility outcomes.[10] Female infertility is multifactorial, involving ovulatory

dysfunction, tubal obstruction, uterine abnormalities, endocrine disturbances, infections, obesity, stress, and socioeconomic influences. Prior research by Scott et al and Ban et al has demonstrated a strong association between hormonal imbalance and infertility.[11,12] Their findings, particularly the elevation of gonadotropins and altered ovarian reserve markers in infertile women compared to controls, align with the results of the present study.

The relationship between age and hormonal profile in infertility is complex and influenced by both physiological aging and duration of infertility. Advancing age is associated with progressive follicular depletion, diminished ovarian reserve, and compensatory elevation of FSH. Prolonged infertility may further exacerbate these endocrine alterations due to sustained ovarian insufficiency and cumulative reproductive stress. Therefore, age remains a critical determinant of hormonal dynamics and reproductive potential in infertile women. Overall, the findings underscore the importance of early hormonal evaluation and timely intervention, particularly in women of advanced reproductive age, to improve fertility outcomes.

CONCLUSION

This study demonstrates a significant association between advancing age and alterations in key reproductive hormones among infertile women. Increasing age was positively correlated with elevated levels of FSH, LH, and TSH, whereas AMH levels showed a declining trend. These hormonal shifts reflect age-related changes in ovarian reserve and endocrine function, reinforcing the well-established impact of reproductive aging on fertility potential. The findings emphasize the multifaceted relationship between age, hormonal balance, and infertility. As ovarian reserve diminishes over time, compensatory changes in gonadotropins and possible thyroid dysfunction may further compromise reproductive capacity. Recognizing these endocrine patterns is essential for accurate evaluation and targeted management of infertile women. Comprehensive hormonal profiling, particularly in women above 36 years of age or those with prolonged infertility, can facilitate early identification of diminished ovarian reserve and associated endocrine disturbances. Such an approach supports individualized treatment planning, enhances clinical decision-making, and may improve overall fertility outcomes. Overall, this study contributes to a better understanding of age-related hormonal dynamics in female infertility and underscores the need for timely assessment and intervention in reproductive health care.

ACKNOWLEDGEMENTS

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