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Impact of maternal age, thyroid function, and vitamin D status on *in vitro* fertilization outcomes

R. H. Jawad, D. Masheta

University of Babylon, Hilla City, Iraq

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Department of Pharmaceutics, College of Pharmacy, University of Babylon, Hilla City, Iraq. E-mail: pha768.rafal.hussien@student.uobabylon.edu.iq, phar.dhafir.qahtan@uobabylon.edu.iq

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In vitro fertilization (IVF) is one of the most effective assisted reproductive technologies for the treatment of infertility. However, despite substantial technological advancements, IVF success rates remain limited, and implantation failure continues to represent a significant clinical challenge. Hormonal balance plays a crucial role in female reproductive physiology. The present study aimed to evaluate the association between prolactin, thyroid function, reproductive hormones, anthropometric parameters, and IVF success. The cross-sectional study included 101 women undergoing IVF treatment in Tiba IVF Center, Hilla city, Iraq, from November 2025 to March 2026, comprising 60 successful IVF cases and 41 failed IVF cases. Serum TSH and 25-hydroxyvitamin D levels were measured. The results indicated that the mean age was significantly higher in the failed IVF group compared with the successful IVF group (30.33 ± 6.00 vs 28.19 ± 4.91 years). Similarly, mean serum TSH levels were significantly elevated in the failed IVF group (2.8 ± 1.1 µIU/mL) compared with the successful group (1.9 ± 0.9 µIU/mL). In contrast, serum vitamin D levels showed no statistically significant difference between successful and failed IVF groups (15.46 ± 11.27 vs 17.69 ± 11.83 ng/mL). Also, BMI, height, or weight shown to have no significant association. The study indicated that maternal age and serum TSH levels were significantly associated with IVF outcome, whereas vitamin D levels and anthropometric measurements were not.

Keywords: *in vitro* fertilization; thyroid stimulating hormone; vitamin D; age; infertility; implantation; assisted reproductive technology.

Introduction

Infertility is a major global health problem affecting approximately 10–15% of reproductive-aged couples worldwide and represents a significant medical, psychological, and social burden (Cox & Thoma, 2022). Five million persons suffer from infertility in the United States. The infertility rate reaches 14% in Europe. Based on a 1996 household health survey in Indonesia, it was shown that there were 3.5 million married couples suffering from infertility. In Indonesia, specialists confirm that the infertility rate has reached 15–20% of about 50 million couples (Sirait et al., 2023).

Infertility occurs because the male sperm fails to fertilise the female ovum. According to declaration of the World Health Organization (WHO), it is announced that there are cases of infertility in 8–10% of couples, which account about 50 to 80 million couples around the world (Lepore & Petruzziello, 2021). Assisted technologies in fertility mainly IVF, have developed the management of infertility and supported millions of couples to achieve good pregnancy outcomes. About eight million children were conceived by Assisted Reproductive technology (ART) in 2020 worldwide (Goisis et al., 2020).

Furthermore, IVF has become a treatment option for couples with unexplained infertility to increase pregnancy rates compared with many other treatment approaches (Mahabbat et al., 2024). Also, IVF enables embryo cryopreservation and preimplantation genetic testing to improve reproductive planning and reduce the risk of transmitting genetic disorders to babies (Bentov & Schenker, 2025). Moreover, AI used in ART can assess sperm quality, embryonic development, and predict pregnancy outcomes, which supports the clinical decisions for improving treatment efficiency. These emerging technologies will be used to enhance the precision and success rate of IVF procedures in the future (Kaveh et al., 2025). ART refers to initiation of procedures *in vitro* with multiple steps, termed the ART cycle. These steps involve ovarian stimulation; surgical removal of the ovule from the ovary; the ova are fertilized with sperm in the laboratory. After that, the embryo is returned to the female reproductive tract. ART technique can depend on donor oocyst, sperm, and gestational carriers. IVF and ICSI procedure were included in this reproductive approach. In addition, there are distinct indications for either fresh or frozen embryo transfer mechanism (Graham et al., 2023). Despite significant advances in IVF techniques, implantation failure remains a major challenge, and the overall success rate per cycle remains relatively low, typically ranging between 30% and 40% depending on patient characteristics (Bulletti et al. 2024). Successful implantation depends on complex interactions between embryo quality and endometrial receptivity. These processes are influenced by multiple factors, including maternal age, hormonal balance, genetic integrity of the embryo, and uterine environment (Lessey & Young, 2019).

Among these factors, maternal age is widely recognized as one of the most important determinants of reproductive success. The age-related decline in fertility is primarily attributed to a progressive decrease in ovarian reserve and deterioration in oocyte quality. Women are born with a finite number of primordial follicles, which decline continuously throughout life due to apoptosis and follicular atresia. In addition, advancing age is associated with increased chromosomal abnormalities in oocytes, which reduce fertilization rates and increase embryo implantation failure (Franasiak et al., 2014). Female age is an essential factor which must be evaluated when describing Recurrent Implantation Failure (RIF). It is important to known that the pregnancy rates decrease with increasing maternal age. Women aged above 35 years require more transfer cycles for implantation process in compare to younger ones. Therefore, it is meaningless to describe RIF without identifiying the maternal age. The more general definition of RIF by Coughlan is clinical pregnancy failure after a minimum of four healthy embryo transfers across three cycles in women under 40 years old (Ma et al., 2023).

Human anthropometric factors (such as weight, height, body mass index (BMI), etc.) have significant effects on the reproductive outcomes in females. Furthermore, obesity has a strong impact on the hormonal levels and ovarian function, and may lead to reduction in fertility (Łuszczki et al. 2025). Recent studies suggest that women's height has a minor association with IVF success rate, mainly live birth rates. Furthermore, the strongest determinants of successful IVF include maternal age, embryonic quality, ovarian reserve, in addition to uterine factors. Therefore, height may contribute slightly to prognostic

models and should not be measured as a primary factor in predicting IVF successful rate (Vaegter et al., 2017). Body mass index is described as an important predictor of pregnancy outcomes in women undergoing IVF treatment. A study suggests that increased BMI was linked to lower cumulative live birth rates and high risk of adverse obstetric outcomes (Kluge et al., 2023). The negative effects of increased obesity on IVF outcomes may be related to impaired oocyte quality, reduced endometrial receptivity, inflammation, and metabolic dysfunction (Boddeti et al., 2025). In addition, a systematic review and meta-analysis found that weight loss interventions leading to improved metabolic parameters and decrease in the body weight did not conclusively lead to substantial improvement in IVF success outcomes. Weight management should be encouraged mainly for overall health benefits for couples undergoing ART and pregnancy rather than as a guaranteed strategy to increase IVF success rate (Jeong et al., 2024).

In addition to age-related factors, endocrine function plays a critical role in reproductive success. Thyroid hormones are essential regulators of metabolism, growth, and development and are also involved in reproductive physiology. Thyroid-stimulating hormone (TSH), secreted by the anterior pituitary gland, regulates thyroid hormone production and plays a key role in maintaining normal thyroid function. Thyroid hormone receptors have been identified in ovarian tissue, endometrium, and placenta, suggesting a direct role in follicular development, implantation, and early pregnancy maintenance (May-Panloup et al., 2016). Thyroid dysfunction, particularly hypothyroidism and subclinical hypothyroidism, has been associated with infertility, implantation failure, and miscarriage. Elevated TSH levels may impair ovarian function, reduce oocyte quality, and disrupt endometrial receptivity (Brown et al., 2023). Even mild elevations in TSH levels within the normal reference range have been associated with reduced pregnancy rates in women undergoing IVF (Zhang et al., 2023). For this reason, the American Thyroid Association recommends maintaining TSH levels below 2.5 μIU/mL in women attempting pregnancy (Alexander et al., 2017). Thyroid hormones have appeared as a clinically relevant biomarker. Growing evidence from systematic studies has shown that thyroid disorders may negatively impact on ART outcomes by reducing the count of retrieved oocytes, decreasing the quality of embryos, lowering implantation and pregnancy rates, in addition to increasing the possibility of adverse reproductive outcomes. Furthermore, normal thyroid function before fertility treatment is related to higher ratio of successful IVF outcomes. (Teixeira et al., 2023).

Vitamin D has also emerged as an important factor in reproductive health. Vitamin D is a steroid hormone that regulates calcium homeostasis and immune function and exerts its biological effects through the vitamin D receptor (VDR), which is expressed in reproductive tissues including the ovary, endometrium, and placenta (Eller et al., 2023). Vitamin D has been shown to regulate genes involved in implantation, modulate immune responses, and improve endometrial receptivity.Several studies have suggested that vitamin D deficiency may be associated with reduced IVF success rates (Zhou et al., 2022). However, other studies have reported conflicting results, and the role of vitamin D in IVF outcomes remains controversial (Eller et al., 2023). Vitamin D deficiency is particularly common in Middle Eastern populations due to limited sun exposure and lifestyle factors, making it an important area of investigation (Meng et al., 2023). Moreover, vitamin D sufficiency increases the chance of pregnancy and positive outcomes after ART. Indeed, the plasma level of vitamin D reflects its concentration in follicular fluid, which is an important marker for ovarian vitamin D reserve. Observational and meta-analysis studies found that sufficient vitamin D in women undergoing IVF is accompanied by an increase in clinical and biochemical pregnancies in addition to increasing the number of live births. Recent evidence shown that in women above 36 the normal level of vitamin D improves reproductive outcomes (Baldini et al., 2024). Given the importance of maternal age, thyroid function, and vitamin D status in reproductive physiology, evaluating their association with IVF outcomes may help improve patient selection and treatment strategies. Therefore, the aim of the present study was to investigate the association between maternal age, anthropometric measurement, serum TSH levels, and vitamin D status with IVF outcomes in women undergoing assisted reproductive treatment.

Materials and methods

Ethical approval was obtained from the relevant institutional review board before the commencement of the study. All participants provided informed consent prior to the commencement of the study, and the study procedures were performed in accordance with ethical standards and the Declaration of Helsinki. Ethical approval for the study was obtained from the Ethics Committee of Babylon University, College of Pharmacy (A-0051 in 9/10/2025). All data obtained from the patients was maintained in complete secrecy.

This cross-sectional study was performed on females undergoing an IVF procedure at Tiba IVF center, Hilla City, Iraq. The total number of subjects included in the study was 101 women, and they were classified into two groups according to IVF outcomes. Group with successful IVF (n = 60), the women had positive clinical pregnancies, which were confirmed by ultrasound to visualize the gestational sac and heartbeats. Failed IVF group (n = 41) included all women who did not become pregnant after the embryonic transfer. All recruited women in this study underwent IVF protocols based on clinical guidelines specific to the fertility center.

The inclusion criteria included women of reproductive age between (18–40 years). These women are undergoing IVF treatment with the availability of complete laboratory and clinical data. The exclusion criteria included women who had thyroid disease and were indicated to receive the thyroid hormone. Furthermore, women with known metabolic and endocrine diseases such as diabetes mellitus and hyperprolactinemia, respectively, were excluded to avoid confounding effects on hormonal measurements. Finally, subjects with incomplete clinical and laboratory data were also excluded.

Age was analyzed as a continuous variable and expressed as mean ± standard deviation (SD). The distribution of age data was assessed for normality prior to statistical comparison. Differences in age between the successful IVF group and the failed IVF group were evaluated using the independent samples t-test when the assumption of normal distribution was satisfied. When the distribution deviated from normality, the non-parametric Mann–Whitney U test was applied as an alternative method to compare the two groups. A P-value of less than 0.05 was considered statistically significant. Age distribution of participants was categorized into four groups (<25, 25–30, 31–35, and >35 years) to evaluate the relationship between maternal age and IVF outcomes. The percentage of patients within each age category was calculated separately for the failed IVF and successful IVF groups. The results were visually presented using a bar chart with error bars representing the standard deviation (SD) for each age group.

Anthropometric measurements were obtained for all participants. Body weight was measured using a calibrated digital scale, and height was measured using a standard stadiometer. Body mass index (BMI) was calculated using the formula: BMI = weight (kg) / height (m^2).

Under aseptic conditions, the blood samples were collected from the subjects' veins prior to IVF induction. The blood was drawn into plain tubes, the amount of the sample approximately 3–5 mL. After being collected, the blood samples were left at room temperature for 20–30 minutes to clot. Then, the clotted blood was centrifuged at 3000 rpm for 10 minutes for serum separation. Serum samples are either sent directly for analysis or frozen at –70 ºC until hormone analysis was performed.

Serum TSH and 25-hydroxyvitamin D concentrations were measured using the Vidas® Blue automated immunoassay analyzer (BioMérieux, France), which is based on the Enzyme-Linked Fluorescent Assay (ELFA) technique. This method combines the principles of enzyme immunoassay with fluorescent detection, providing high sensitivity and specificity for hormone quantification.

A competitive immunoassay technique was used for measurement of serum 25-hydroxyvitamin D. The enzyme-labeled competitive vitamin D antigen assay was used to bind to specific antibodies. Fluorescence was determined following incubation and washing, and

its intensity was inversely related to the sample's vitamin D level. Hormone concentrations are automatically measured by the Vidas® Blue analyzer using calibration curves created with criteria supplied by the manufacturer. To check the assay's accuracy and dependability, quality control samples were routinely examined. Vitamin D levels were determined in nanograms per milliliter (ng/mL), and serum TSH levels were measured in micro-international units per milliliter (μIU/mL).

Statistical Package for the Social Sciences (SPSS) software (version 26, IBM Corp., USA) was used to perform the statistical analysis. The normality of data was tested before the analysis was achieved. Continuous data were expressed as mean ± standard deviation (SD). An independent sample t-test was applied to compare normally distributed data between successful and failed IVF groups, while abnormally distributed variables were tested by the Mann–Whitney U test. Categorical variables, for example vitamin D status categories, were expressed as percentages and compared between groups. A P-value result less than 0.05 was defined as statistically significant.

Results

Women with failed IVF outcomes had a significantly higher maternal age compared with those who achieved successful IVF outcomes (30.33 ± 6.00 vs. 28.19 ± 4.91 years, Table 1). Although the independent t-test demonstrated a borderline non-significant difference (P = 0.059), the Mann–Whitney U test revealed a statistically significant difference between the two groups (P = 0.028), indicating that maternal age was significantly associated with IVF outcome when assessed using a non-parametric approach. These findings suggest that increasing maternal age may negatively influence the likelihood of successful IVF treatment and supports the well-established relationship between advancing reproductive age and reduced fertility potential. Figure 1 show the distribution of IVF outcomes according to maternal age groups.

Table 1
Comparison of maternal age
between failed and successful IVF outcome groups

Parameter	Failed IVF (mean ± SD)	Successful IVF (mean ± SD)	t-test P-value	Mann-Whitney P-value
Age, years	30.33 ± 6.00	28.19 ± 4.91	0.059	0.028

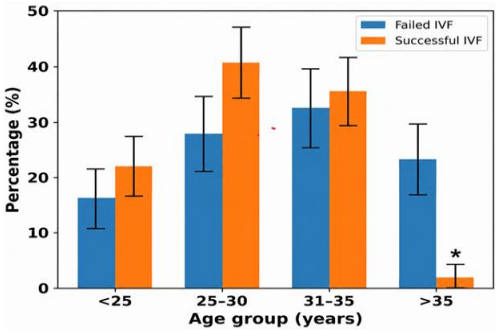

Fig. 1. Distribution of IVF outcomes according to maternal age groups: the sign (*) indicate significant difference

Anthropometric characteristics of the study population are presented in Table 2. The mean BMI in the successful IVF group was 30.7 ± 5.6 kg/m², compared with 30.1 ± 4.1 kg/m² in the failed IVF group. Statistical analysis showed no significant difference between the two groups (P = 0.486). Similarly, there was no statistically significant difference in height between the successful and failed IVF groups (158.5 ± 5.7 vs 158.7 ± 6.0 cm, respectively; P = 0.847). Body weight also did not differ significantly between the two groups. The mean body weight was 76.1 ± 12.2 kg in the successful IVF group and 74.9 ± 12.1 kg in the failed IVF group (P = 0.647). These findings indicate that anthropometric measurements including BMI, height, and weight were not significantly associated with IVF outcome in this study population.

Table 2
Comparison of anthropometric measurements (body mass index, BMI, height, and weight) between successful and failed IVF groups

Parameter	Failed IVF (mean ± SD)	Successful IVF (mean ± SD)	t-test P-value	Mann-Whitney P-value
BMI, kg/m²	30.1 ± 4.1	30.7 ± 5.6	0.486	0.486
Height, cm	158.7 ± 6.0	158.5 ± 5.7	0.847	0.847
Weight, kg	74.9 ± 12.1	76.1 ± 12.2	0.647	0.647

Serum thyroid-stimulating hormone (TSH) levels were higher in women who achieved successful IVF outcomes compared with those who experienced IVF failure (2.80 ± 1.53 vs. 2.25 ± 1.61 μIU/mL) as shown in Table 3. While the independent t-test did not demonstrate a statistically significant difference between the groups (P = 0.083), the Mann–Whitney U test revealed a significant difference (P = 0.0228). These findings suggest that variations in thyroid function, even within the clinically acceptable range, may be associated with IVF success. The significant non-parametric result indicates that TSH levels may have a potential influence on reproductive outcomes and warrant further investigation as a predictor of IVF treatment success.

Table 3
Comparison of thyroid-stimulating hormone levels
between failed and successful IVF outcome groups

Parameter	Failed IVF, mean ± SD	Successful IVF, mean ± SD	t-test P-value	Mann-Whitney P-value
TSH, μIU/mL	2.25 ± 1.61	2.80 ± 1.53	0.083	0.023

The mean serum vitamin D level was slightly higher in women with failed IVF outcomes than in those with successful IVF outcomes (17.69 ± 11.83 vs. 15.46 ± 11.27 ng/mL) as shown in Table 4. However, neither the independent t-test nor the Mann–Whitney U test demonstrated a statistically significant difference between the two groups (p = 0.331 and p = 0.431, respectively). These findings indicate that vitamin D status was not significantly associated with IVF treatment outcome in the present study. Although vitamin D has been proposed to influence female reproductive function, the current results suggest that serum vitamin D concentrations did not have a measurable impact on the likelihood of achieving a successful IVF outcome within the studied population.

Table 4
Comparison of serum vitamin D levels
between failed and successful IVF outcome groups

Parameter	Failed IVF, mean ± SD	Successful IVF, mean ± SD	t-test P-value	Mann–Whitney P-value
Vitamin D, ng/mL	17.7 ± 11.8	15.5 ± 11.3	0.331	0.431

The distribution of vitamin D status showed that deficiency (<20 ng/mL) was the most prevalent category in both groups, affecting 63.0% of women with failed IVF outcomes and 77.4% of those with successful outcomes as shown in Table 5. Vitamin D insufficiency (20–29 ng/mL) was observed in 19.6% and 13.2% of participants in the failed and successful IVF groups, respectively, while sufficient vitamin D levels (≥30 ng/mL) were identified in 17.4% of women with failed IVF outcomes compared with 9.4% of those who achieved successful IVF outcomes. Overall, vitamin D deficiency was highly prevalent regardless of IVF outcome, and the observed distribution pattern suggests that vitamin D status alone may not be a major determinant of IVF success in the studied population. However, statistical testing is required to confirm whether these differences are significant.

Table 5
Distribution of vitamin D status according to IVF outcome

Vitamin D status	Failed IVF, n (%)	Successful IVF, n (%)
Deficient (<20 ng/mL)	29 (63.0%)	41 (77.4%)
Insufficient (20–29 ng/mL)	9 (19.6%)	7 (13.2%)
Sufficient (≥30 ng/mL)	8 (17.4%)	5 (9.4%)

Discussion

This study examined the association between serum thyroid-stimulating hormone (TSH), maternal age, and vitamin D concentration with *in vitro* fertilization (IVF) outcomes. The major findings of the study demonstrated that maternal age and serum TSH levels were considerably higher in women who failed to become pregnant compared with women who achieved successful clinical pregnancy. On the other hand, there is no significant association between serum vitamin D levels and IVF outcome. These findings show the importance of maternal age and thyroid function in the determination of IVF success rate. However, vitamin D may not play a significant predictive role in this study population.

Maternal age is universally recognized as one of the most important predictors of reproductive success, particularly in assisted reproductive technologies. In the present study, the mean age of women in the failed IVF group was significantly higher than that in the successful IVF group. This finding is consistent with numerous previous studies demonstrating a progressive decline in IVF success rates with increasing maternal age.

The age-related decline in fertility is primarily attributed to deterioration in both the quantity and quality of oocytes. Women are born with a finite ovarian reserve, which decreases progressively due to follicular atresia and apoptosis. In addition to quantitative decline, aging is associated with reduced oocyte competence and increased chromosomal abnormalities resulting from meiotic errors (Franasiak et al., 2014). These abnormalities contribute to reduced fertilization rates, impaired embryo development, and implantation failure. Maternal age related to reduction of life birth rate is due to decreased oocyte quality. Aneuploidy of embryos increases in women over 35 years old with corresponding decrease in implantation rate and increase in miscarriage frequency (Xia et al., 2024).

Mitochondrial dysfunction also plays a central role in reproductive aging. Mitochondria provide the energy required for oocyte maturation, fertilization, and early embryonic development. With advancing age, mitochondrial DNA mutations accumulate, resulting in reduced ATP production and impaired embryo viability (May-Panloup et al., 2016).

Furthermore, maternal age may influence endometrial receptivity. Successful implantation requires synchronized communication between the embryo and endometrium. Aging is associated with altered endometrial gene expression, impaired vascularization, and reduced hormonal responsiveness, all of which may contribute to implantation failure (Lessey & Young, 2019).

Although the mean age difference between groups in the present study appears relatively modest, even small increases in maternal age may significantly affect reproductive outcomes. This observation emphasizes the importance of early fertility evaluation and intervention in women planning pregnancy.

The present study found no significant association between BMI and IVF outcome. Although obesity has been associated with infertility, its effect on IVF outcome remains controversial. Luke et al. (2010) reported that BMI influences ovarian response but may not directly affect pregnancy success. These findings suggest that BMI alone may not be a reliable predictor of IVF outcome.

One of the most significant findings of the present study was the higher serum TSH levels observed in women who experienced IVF failure compared with those who achieved successful pregnancy (2.8 ± 1.1 vs 1.9 ± 0.9 µIU/mL, $P = 0.02$). This result suggests that thyroid function plays an important role in determining IVF success.

Thyroid hormones are essential regulators of reproductive physiology. Thyroid hormone receptors are expressed in ovarian granulosa cells, endometrial tissue, and trophoblasts, indicating direct involvement in folliculogenesis, fertilization, and implantation (May-Panloup et al., 2016; Brown et al., 2023). Elevated TSH levels may indicate subclinical hypothyroidism, which has been associated with impaired reproductive outcomes. Subclinical hypothyroidism may affect IVF success through several mechanisms. First, thyroid hormones regulate ovarian function and follicular development. Reduced thyroid hormone availability may impair oocyte maturation, resulting in reduced fertilization potential. Second, thyroid hormones play an essential role in endometrial receptivity. Thyroid hormone receptors present in the endometrium regulate genes involved in implantation. Thyroid dysfunction may impair endometrial development and reduce implantation success (Brown et al., 2023). Third, thyroid hormones influence immune regulation during implantation. Successful embryo implantation requires immune tolerance. Thyroid dysfunction may disrupt immune balance and increase inflammatory responses, contributing to implantation failure.

The findings of the present study are consistent with the results of Zhang et al. (2023), who reported that elevated TSH levels were associated with reduced IVF success rates. Similarly, another study demonstrated that women with higher TSH levels had reduced fertility outcomes (Busnelli et al., 2022). Importantly, the mean TSH level in the failed IVF group in the present study exceeded the recommended threshold of 2.5 µIU/mL suggested by the American Thyroid Association for women attempting pregnancy (Alexander et al., 2017). In contrast, the successful IVF group had TSH levels within the optimal range. These findings support the importance of thyroid function screening and management prior to IVF treatment.

Vitamin D has been proposed as an important regulator of reproductive physiology due to the presence of vitamin D receptors in ovarian tissue, endometrium, and placenta. In the present study, serum vitamin D levels did not differ significantly between successful and failed IVF groups ($P = 0.431$). In addition, vitamin D deficiency was highly prevalent in both groups. These findings are consistent with those of another study, which reported no significant association between vitamin D levels and IVF outcomes (Eller et al., 2023). However, other studies have reported positive associations. Many studies have demonstrated that higher vitamin D levels were associated with improved IVF success. The conflicting results may be explained by differences in ethnicity, lifestyle, and geographic location. Vitamin D deficiency is particularly common in Middle Eastern populations due to limited sun exposure, the culture of modesty in clothing, and dietary factors (Meng et al., 2023). It is also possible that vitamin D plays a supportive rather than primary role in reproductive success.

Conclusion

The results of our study showed a significant association between the maternal age, and serum TSH with IVF outcome, whereas vitamin D levels and anthropometrics measurements showed no association. These study findings determined the importance of thyroid function and maternal age in predicting successful IVF rates.

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