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ENDOCRINE FACTORS ASSOCIATED WITH SUCCESSFUL *IN VITRO* FERTILIZATION PREGNANCY

Rihana Dilshad[1], Hina Allah Ditta[2], Madiha Shahzad Lodhi[3*], Rizwana Dilshad[4], Maqsood Ahmad[5], Rabia Nisar[6], Aqsa Afzal[7], Sultan Ahmad[8]

[1]Assistant Professor at Sheikh Zayed Medical College, Rahim Yar Khan, Pakistan. Email: rehanadilshad88@gmail.com
[2]Additional Principal Women Medical Officer at Sheikh Zayed Medical College, Rahim Yar Khan, Pakistan. Email: hina.ditta@gmail.com
[3*]Assistant Professor at the University of Lahore, Pakistan. Email: madeeha.shahzad@imbb.uol.edu.pk
[4]Associate Professor at Swedish College of Pharmacy and Allied Health Sciences, Rahim Yar Khan, Pakistan. Email: rizwanadilshad8@gmail.com
[5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan. Email: dr.maqsoodrao@gmail.com
[6]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The Islamia University of Bahawalpur, Pakistan. Email: rabya.nisar@gmail.com
[7]Faculty of Pharmacy, Department of Pharmaceutics, Bahauddin Zakaria University, Multan, Pakistan. Email: aqsaafzalpharmacist@gmail.com
[8]Assistant Professor, Thoracic surgery, Sheikh Zayed Medical College and Hospital, Rahim Yar Khan, Pakistan. Email: sultanshaheen@yahoo.com

***Correspondent Author**: Madiha Shahzad Lodhi,
*Assistant Professor at the University of Lahore, Pakistan. Email: madeeha.shahzad@imbb.uol.edu.pk

ABSTRACT

Background: Infertility is the term used when a clinical pregnancy cannot be achieved within at least 12 months. Thyroid dysfunction impacts the anti-Mullerian hormone (AMH), a recognized marker of ovarian function. Proper growth, sexual maturation, and reproductive processes rely on gonadotropins, peptide hormones that regulate ovarian and testicular functions.

Objective: The objective of this study is to assess the serum levels of specific endocrine factors, including anti-mullerian hormone, prolactin, gonadotropins, sex-related hormones, and thyroid-stimulating hormones in women undergoing IVF treatment.

Materials and Methods: The study consisted of women who underwent IVF at the Australian Concept Infertility Medical Center, Lahore. This was a cross-sectional study involving 80 women undergoing IVF. The causes of infertility were distributed as follows: female infertility, primary and secondary, tubal factor infertility, polycystic ovary syndrome, endometriosis, and other reasons. Blood samples were collected from patients undergoing IVF on various days to analyze endocrine variables. Enzyme-linked immunosorbent assay (ELISA) kits were used to detect FSH, LH, estrogen, progesterone, AMH, TSH, and prolactin. Statistical analysis was conducted using SPSS.

Results: Mean age of women was 33.42±4.74 years. Among women 76(95%) were nulliparous and only 3(3.8%) women were prim-parous while only 1(1.3%) women present with parity 4. Mean body

mass index of women was 27.20±4.68. 64(80%) women were present with primary and 16(20%) presents with secondary infertility. The most frequent blood group among women was O^{+ve} (57.5%), followed by B^{+ve} (31.25%), AB^{+ve} (6.25%), and A^{+ve} (5%), respectively.Mean FSH level in women was 6.36±2.92. Mean LH level in women was 5.24±2.63. Mean prolactin level in women was 19.71±12.33. Mean serum AMH level among women was 2.49±1.68. The mean AMH level ranges between 0.09 and 19.30. Mean TSH level among women was 2.02±1.29. Mean estrogen and progesterone levels among women were 108.70±80.44 and 2.98±2.19, respectively.
Conclusion: Our findings demonstrated that FSH, LH, prolactin, AMH, and estrogen levels were slightly higher among women with positive pregnancy outcomes. Women with negative pregnancy outcomes had higher values for serum TSH and progesterone levels.

Keywords: AMH; FSH; TSH; LH; Estrogen; Progesterone; Prolactin; *In vitro* fertilization.

INTRODUCTION

Infertility is a widespread issue globally, characterized by the inability to conceive after 12 months of regular sexual contact without using contraceptive measures, according to the World Health Organization (WHO) (Brassard *et al*., 2008). A significant contributor to female infertility lies in issues with ovulation, often linked to imbalances in hormones within the hypothalamic-pituitary-gonadal (HPG) axis. Stress-induced hormonal fluctuations, such as increased cortisol and prolactin release, further impact the HPG axis and may exacerbate infertility (Chen *et al*., 2016). When dealing with infertility, *in vitro* fertilization (IVF) represents a set of techniques aimed at improving fertility, boosting the likelihood of a successful pregnancy, and reducing the risk of genetic abnormalities in children (Kollmann *et al*., 2016). The IVF procedure includes collecting viable eggs from a woman's ovaries, fertilizing them with sperm in a lab setting, and then transferring the resulting embryos into the woman's uterus. IVF is a complex and time-consuming process that typically spans approximately three weeks, potentially extending further if additional stages are required (Man *et al*., 2021).

IVF boasts the highest success rates among various assisted reproductive technologies and allows couples to use their own eggs and sperm. Additionally, IVF procedures may involve the use of donor eggs, sperm, or embryos to address specific fertility challenges (Xu *et al*., 2021). In some cases, where it is necessary to use a gestational carrier—a woman who agrees to carry a child by having an embryo implanted in her uterus—low success rates in IVF may be attributed to an increased prevalence of numerical chromosomal abnormalities in embryos (Ghobara *et al*., 2017). The female menstrual cycle is intricately governed by hormones such as gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and progesterone. These hormones are vital for follicular development, corpus luteum formation, and ovulation induction (Dong *et al*., 2017). The expression of LH receptor gene mRNA dynamics can influence cellular LH responsiveness during follicular development, thereby affecting the fate of the follicle and the quality of the oocyte (Sadruddin *et al*., 2020). Progesterone plays a crucial role in endometrial maturation, implantation, and sustaining pregnancy, resulting in significant increases in pregnancy and live birth rates when administered during the luteal phase following infertility treatment (Barbakadze *et al*., 2015). Estrogen influences oocyte maturation, embryo quality, and fertilization, with serum estradiol levels predicting pregnancy after IVF, in combination with free FSH and age (Mulders *et al*., 2003).

Anti-Müllerian Hormone (AMH) functions as a novel marker that inhibits Müllerian duct formation and was initially discovered in Sertoli cells of the fetal testis. It is produced by granulosa cells surrounding preantral and antral follicles in the ovary, contributing to reducing the sensitivity of preantral and small antral follicles to FSH (Merseburger & Hupe, 2016). As follicular development progresses, AMH expression decreases, and atretic follicles stop producing AMH altogether (Tesarik *et al*., 2003). Reproductive-aged women experience a gradual decline in AMH levels throughout their reproductive years, with complete disappearance at menopause. Regardless of whether polycystic ovary syndrome (PCOS) is present or not, there appears to be no correlation between Body Mass Index (BMI) and circulating AMH levels in women (Wang *et al*., 2020). Ovarian activity regulation involves a feedback loop consisting of GnRH, FSH, and LH (Liu *et al*., 2012). The prediction of pregnancy success in infertility treatment is associated with measuring serum FSH levels during the early follicular phase, where low FSH and high LH levels are associated with increased pregnancy success (Zong *et al*., 2020). Gonadotropins, such as FSH and LH, stimulate the ovaries to produce multiple follicles, enhancing the chances of pregnancy through various methods like IVF, intrauterine insemination, or natural intercourse. However, these approaches also increase the likelihood of multiple pregnancies (Chi *et al*., 2018).

Thyroid dysfunction and autoimmune thyroiditis present risks for infertility and preterm birth in women of reproductive age. Hypothyroidism in women is linked to an elevated risk of infertility and menstrual irregularities due to impaired estrogen metabolism, hyperprolactinemia, and disruptions in gonadotropin-releasing hormone production (Kuroda *et al*., 2015). Subclinical hypothyroidism, characterized by high TSH levels and normal FT4 levels, is observed in about 20% of infertile women. Elevated TSH levels in infertile women are linked to reduced ovarian reserve, impacting fertility (Mantzavinos *et al*., 2017). The presence of prolactin receptors on endometrial cells during IVF cycles enhances the likelihood of successful blastocyst implantation (Ugwu *et al*., 2022). Estradiol, a hormone produced in the uterus, plays various roles in women's health, including fostering the necessary endometrial thickness for a healthy pregnancy. Increased endometrial thickness correlates with a higher live birth rate, with estradiol secretion relying on a healthy ovarian reserve indicated by AMH, FSH, and LH levels (Hu *et al*., 2021; Zong *et al*., 2020). Ovarian reserve, vital for establishing pregnancy, is evaluated through studies examining AMH, FSH, and LH. The interaction between estradiol and progesterone can be disrupted by decreased ovarian reserve and increased premature progesterone during ovulation (Christ *et al*., 2019). We investigate the association between AMH, gonadotropic hormones (FSH and LH), sex-associated hormones, and TSH with the outcome of IVF. By examining the levels and interactions of these hormones, the study aims to identify potential correlations and predictive factors related to the success of IVF procedures.

METHODOLOGY

Study Design: A cross-sectional study design was used in this research to explore the correlation between hormonal markers and outcomes of IVF in women receiving fertility treatment.

Participants: The research involved 80 women between the ages of 30 and 40 undergoing IVF at a reputable fertility clinic. Inclusion criteria included a background of IVF, either primary or secondary infertility, and a history of recurrent miscarriages. Exclusion criteria included a history of specific surgical procedures and the lack of histopathologic diagnoses.

Sampling: Non-probability and convenience sampling techniques were used to recruit 80 participants from the Australian Concept Infertility Medical Center in Lahore. The sample was screened at the University of Lahore.

Ethical Considerations: Approval from the Ethics Committee of the Institute of Molecular Biology and Biotechnology (IMBB) at the University of Lahore was secured for this study. All participants provided informed consent, guaranteeing confidentiality and compliance with ethical standards.

Data Collection: Comprehensive data on patient characteristics, including age, medical history, and causes of infertility, were collected through medical records. Blood samples were drawn from participants during IVF procedures for hormonal analysis.

Hormonal Analysis: Blood samples underwent processing to determine hormonal markers. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were utilized to measure levels of gonadotropic hormones (FSH and LH), sex hormones (estrogen and progesterone), TSH, AMH, and prolactin. ELISA procedures were performed using a microwell plate according to the manufacturer's guidelines. Standard solutions, samples, and reagents were prepared as instructed by the kits. The optical density (OD value) of each well was assessed at 450 nm using a microplate reader.

Statistical Analysis: Quantitative variables were summarized using descriptive statistics, indicating the mean ± SD. The data's normality was evaluated, and group comparisons were carried out using appropriate statistical tests, such as the Student's t-test (or the Mann-Whitney test) and ANOVA. The statistical analysis was conducted using SPSS version 26.

RESULTS

In this study, the mean age of women was 33.42±4.74 years. The minimum and maximum ages of the women were 26 and 46 years, respectively (Table 1). The mean body mass index of women was 27.20±4.68. The minimum and maximum BMIs of women were 16 and 35. Among women 76(95%) were nulliparous and only 3(3.8%) women were prim-parous while only 1(1.3%) woman present with parity 4. In this study, 64(80%) women present with primary and 16(20%) presents with secondary infertility. The most frequent blood group among women was O^{+ve} (57.5%) followed by B^{+ve} (31.25%), AB^{+ve} (6.25%) and A^{+ve} respectively (5%). There were 40(50%) women with positive and 40(50%) with negative pregnancy outcome.

Table-1: Characteristics of Study Participants

n	80
Age (Years)	33.42±4.74 [26-46]
BMI	27.20±4.68 [16-35]
Parity	
0 (Nulliparous)	76(95%)
1 (Prim-parous)	3(3.8%)
4	1(1.3%)
Type of Infertility	
Primary	64(80%)
Secondary	16(20%)
Blood Groups	
A (Positive)	4(5%)
B (Positive)	25(31.25%)
AB (Positive)	5(6.25%)
O (Positive)	46(57.5%)
Pregnancy Outcome (IVF)	
Positive	40(50%)
Negative	40(50%)

Mean FSH level in women was 6.36±2.92 (Table 2). The minimum and maximum FSH levels among women were 2.26 and 16.20. Mean LH level in women was 5.24±2.63. Minimum and maximum levels of LH were 1.77 and 13.02, respectively. Mean prolactin level in women was 19.71±12.33. The minimum and maximum prolactin levels were 0.25 and 56.11, respectively. Mean serum AMH level among women was 2.49±1.68. Minimum and maximum AMH levels were 0.09 and 6.01 (Figure 1). Mean TSH level among women was 2.02±1.29. The minimum and maximum TSH levels among women were 0.36 and 58.58. Mean estrogen and progesterone levels among women were 108.70±80.44 and 2.98±2.19, respectively (Figure 2).

Table 2: Descriptive statistics for Biomarkers.

	FSH	**LH**	**Prolactin**	**AMH**	**TSH**	**Estrogen**	**Progesterone**
n	80	80	80	80	80	80	80
Mean	6.36	5.24	19.71	2.49	2.02	108.70	2.98
SD	2.92	2.63	12.33	1.68	1.29	80.44	2.19
Minimum	2.26	1.77	0.25	0.09	0.36	11.70	0.12
Maximum	16.20	13.02	56.11	6.01	9.33	523.20	9.67

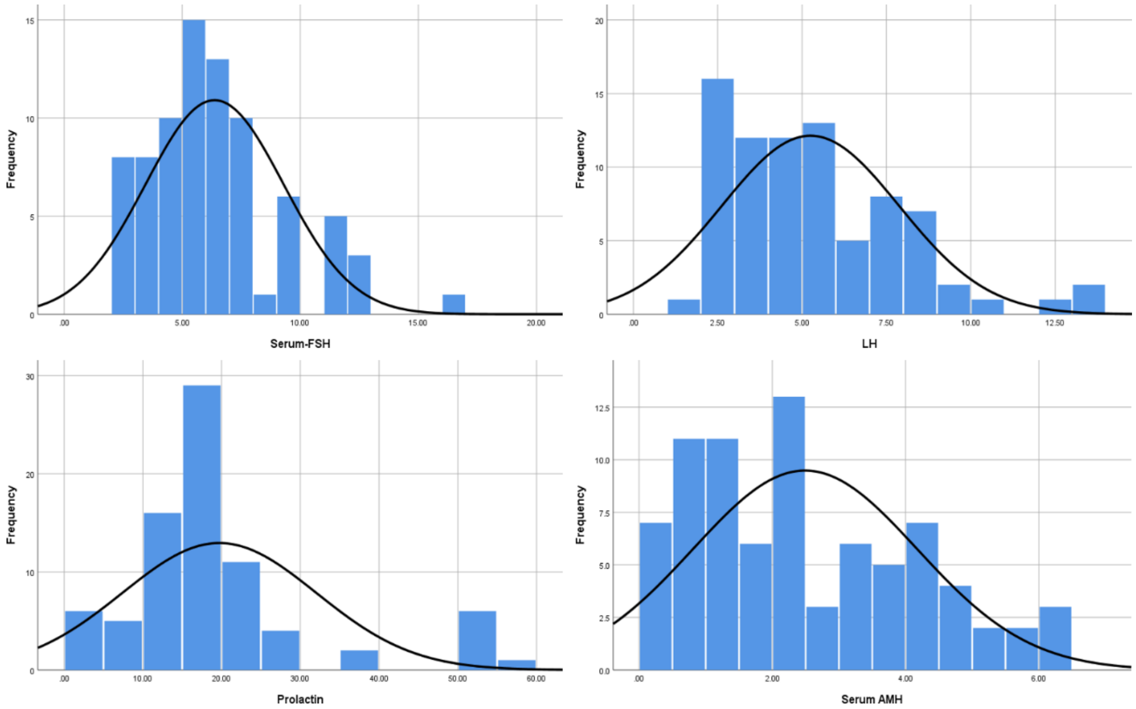

Figure 1: Histogram for Serum FSH, LH, prolactin and AMH.

Above histogram shows FSH distribution among women. Mean AMH level ranges between 0.09 to 19.30.

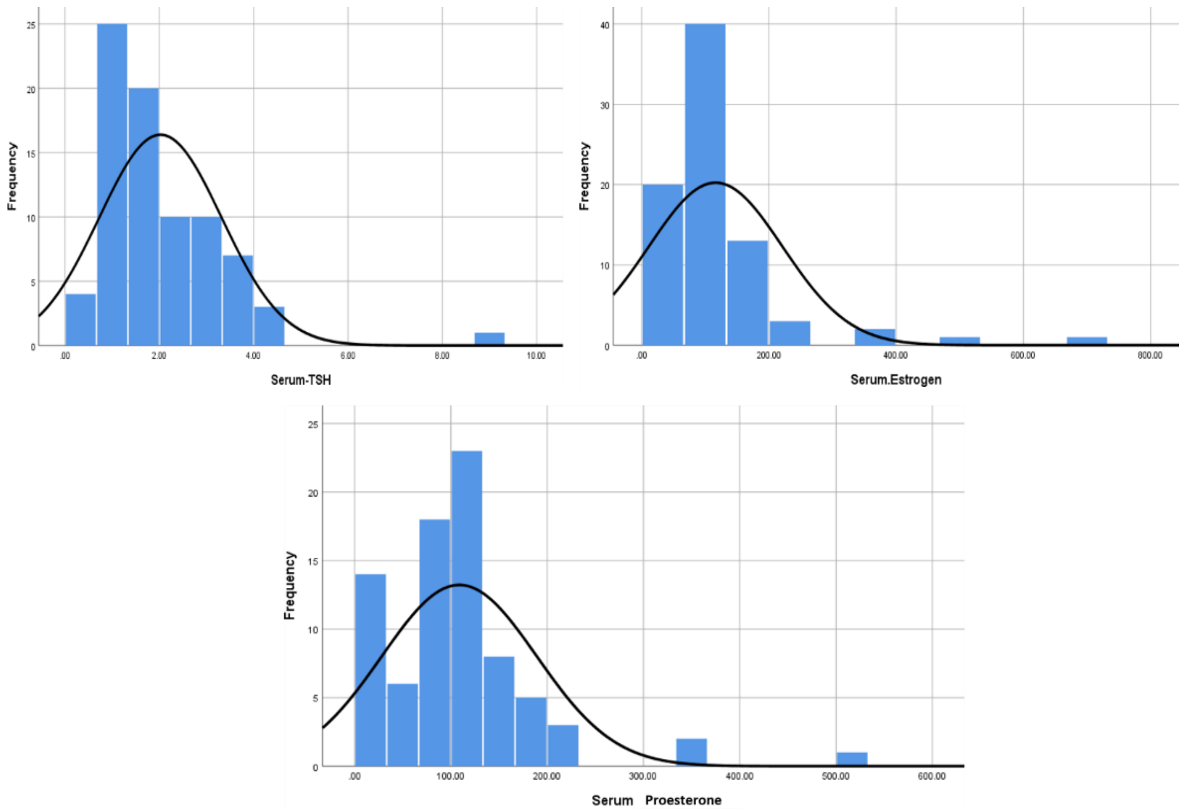

Figure 2: Histogram for Serum TSH, estrogen, and progesterone.

The histogram above shows the TSH level among women. TSH level ranges between 0.36 to 9.33. The histogram above shows the mean serum estrogen level among women, which ranges between 0.12 and 9.67. The mean serum progesterone level among women ranges between 11.70 and 523.20. FSH, LH, and prolactin levels were not normally distributed. Therefore, we applied the Mann-Whitney U test to compare FSH, LH, and prolactin levels among women with positive and negative pregnancy outcomes (Table 3). FSH and LH levels were slightly elevated in women with positive pregnancy outcomes, although this difference did not reach statistical significance. Conversely, prolactin levels were marginally higher in women who did not achieve pregnancy. The mean AMH level exhibited a significant disparity between women with positive and negative pregnancy outcomes, with those experiencing a successful pregnancy showing higher serum AMH levels. In contrast, the mean TSH level did not show a notable difference between women with positive and negative pregnancy outcomes. However, women without a successful pregnancy tended to have higher serum TSH levels than those with positive outcomes. There were no significant differences observed in estrogen and progesterone levels between women with positive and negative pregnancy outcomes. However, women who achieved a positive pregnancy had higher estrogen levels, while those with negative outcomes had higher progesterone levels (Figure 3).

Table 3: Comparison of Biomarkers in relation to Pregnancy Outcome

	Pregnancy Outcome		***p-value***[(a)]
	Positive	**Negative**	
	40	**40**	
	Mean±SD	**Mean±SD**	
FSH	6.68±2.67	7.45±3.84	***0.817***
LH	5.36±3.06	4.39±1.46	***0.773***
Prolactin	17.93±13.34	17.96±13.25	***0.729***
Serum AMH	2.71±1.52	1.78±1.89	***0.043***
Serum TSH	1.60±0.98	1.71±0.70	***0.739***
Estrogen	116.14±89.93	82.99±42.96	***0.184***
Progesterone	2.41±1.36	3.93±2.33	***0.273***

Note: (a) Mann Whitney U Test

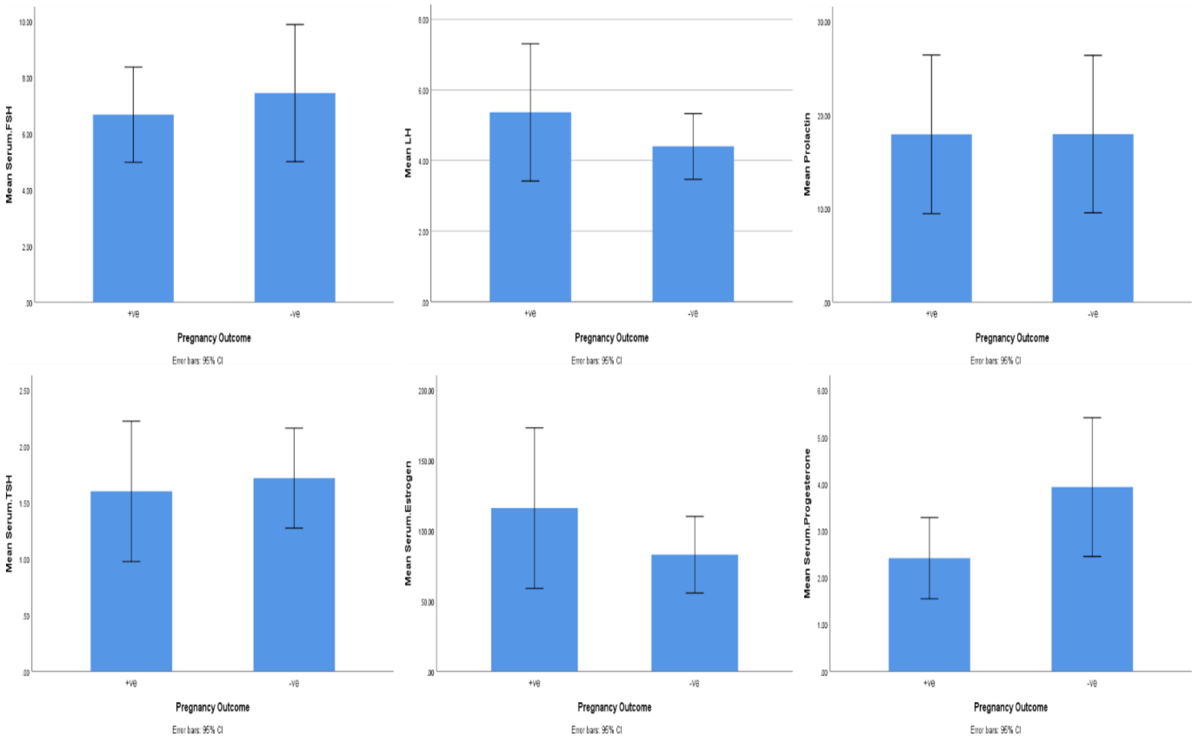

Figure 3: Error bar graph for Biomarkers in relation to Pregnancy.

PREGNANCY OUTCOME:

- Positive: Successful IVF
- Negative: Unsuccessful IVF

DISCUSSION

Infertility is an increasing health concern affecting 20% of couples in their reproductive years. Consequently, treatments like IVF and other assisted reproductive technologies have become more viable options for addressing infertility (Agarwal *et al*., 2015). In a retrospective 9-month study conducted by Puska *et al*. (2003), which focused on women undergoing IVF, participants were categorized based on their BMI (<18.50, 18.50–24.99, 25.00–29.99, and ≥30.00 kg/m^2) (Puska *et al*., 2003). Our study found a mean body mass index of 27.20±4.68 among women, ranging from 16 to 35. Our results, in line with Bellver *et al*. (2013), indicated that higher BMI in women was associated with lower conception rates, increased abortion rates (AR), and a higher likelihood of other reproductive complications (Bellver *et al*., 2013).

We did not identify any statistically significant associations between nulliparity and infertility classification or time to pregnancy concerning the risk of infant mortality. When specifically examining first-time mothers, the estimates closely mirrored those considering three births, with notable significance when disregarding all births (Nohr *et al*., 2006). The study showed that 76% of women were non-birthing, 3.8% were first-time mothers, and 1.3% were at parity 4. Various environmental factors, lifestyle exposures, and genetic predispositions related to female infertility may also impact fetal growth (Sharma *et al*., 2016). Despite blood type O's link with ovarian reserve, our study revealed the most frequent blood group among women was O^{+ve} (57.5%), followed by B^{+ve} (31.25%), AB^{+ve} (6.25%), and A^{+ve} (5%). Besides the recognized association between blood type and ovarian reserve, Levi *et al*. (2001) presented evidence reinforcing the importance of blood type concerning IVF cycle outcomes. Individuals carrying the B antigen, such as those with blood types B or AB, demonstrated a twofold higher risk of achieving live births compared to individuals with blood types O or A (Broekmans *et al*., 2006).

Our study revealed non-normally distributed levels of FSH, LH, and Prolactin. To compare these levels among women with positive and negative pregnancy outcomes, we employed the Mann-Whitney U test. While FSH and LH levels were slightly elevated in women with positive pregnancy outcomes, the differences were not statistically significant. Conversely, Prolactin levels exhibited a marginal increase in women with negative pregnancy outcomes.

Prolactin release plays a crucial role in maintaining endometrial receptivity and creating a favorable environment for implantation of transplanted blastocysts in IVF cycles (Rajinder *et al*., 2012). Elevated prolactin levels can lead to disruptions in the luteal phase, abnormal implantation, and impaired embryo development. This occurs because prolactin inhibits the growth of luteinizing granulosa cells and interferes with corpus luteum function. Numerous studies have documented the presence of hyperprolactinemia in the context of IVF (Zhong *et al*., 2012). In alignment with the earlier work of Doldi *et al*. (2000), our findings suggested that high serum prolactin levels, as observed in this study, did not exert a significant impact on pregnancy outcomes (Doldi *et al*., 2000).

Due to the significant differences among IVF methods and the potential influence of thyroid hormones on fertilization and early embryo development, our focus was primarily on traditional IVF and its various fertility indications. Although the higher prevalence of thyroid autoimmunity in secondary fertility diminishes after adjusting for age, and there is no observed association in fertile women between elevated-normal TSH levels and fertility levels (Tan *et al*., 2014), further investigation is required to differentiate between primary and secondary fertile women. Our study did not reveal a notable difference in mean TSH levels between women who achieved positive pregnancy outcomes and those with negative outcomes. However, women experiencing negative pregnancy outcomes exhibited higher serum TSH levels compared to those with successful outcomes. Women with negative pregnancy outcomes had higher serum TSH levels compared to those with positive outcomes. Notably, 21% of thyroid-fertile women and 22% of thyroid women undergoing ART had TSH levels greater than 2.5 mIU/L (Dhillon-Smith *et al*., 2020). Considering TSH levels above 2.5 mIU/L as indicative of subclinical hypothyroidism could lead to overdiagnosis, strain on healthcare systems, and an increased prevalence of the condition among infertile women (Reh *et al*., 2010).

Our research revealed a statistically significant disparity in mean AMH levels between women who achieved pregnancy and those who did not, underscoring the predictive value of serum AMH for

successful pregnancies. Recent studies have explored AMH's potential as a prognostic factor for live births, with preliminary findings indicating its relevance; however, the predictive significance of AMH at low or extremely low concentrations remains uncertain (Iliodromiti *et al*., 2014). While some studies suggest that clinical pregnancies can occur with AMH levels as low as 0.1-0.35, others argue against including such patients in IVF due to their unfavorable prognosis (Fraisse *et al*., 2008; Tokura *et al*., 2013). The patient's age influences the intraovarian inhibitory effect of AMH on follicle recruitment, indicating that ovarian reserve, as measured by serum AMH, has a variable association with IVF success depending on the patient's age (Honnma *et al*., 2012).
The primary outputs of granulosa cells, namely estrogen and progesterone, have garnered significant attention as potential indicators of pregnancy. Changes in the secretion of estrogen and progesterone, initiated during the stimulatory phase of IVF cycles, affect the ovarian and uterine environment, influencing implantation. While estrogen and progesterone levels are typically associated with this process, early activities in oocyte development unrelated to fertilization may be crucial for embryo survival in the uterus before implantation (Ceyhan *et al*., 2008). Studies by Van der Linden *et al*. (2011) suggested that follicular concentrations of progesterone are higher following the endogenous LH surge compared to hCG injection, indicating a connection between increasing follicular maturity and enhanced progesterone production (van der Linden *et al*., 2011). Jee *et al*. (2010) observed that granulosa cells associated with mature oocyte-cumulus complexes produce more progesterone (Jee *et al*., 2010), while Elgindy *et al*. (2010) and Moini *et al*. (2010) found elevated serum progesterone levels during laparoscopy in conception IVF cycles (Elgindy *et al*., 2010; Moini *et al*., 2011). Despite these findings, our study did not reveal a statistically significant difference in estrogen or progesterone levels between women who experienced successful pregnancies and those who experienced unsuccessful pregnancies. Successful pregnancies were associated with higher estrogen levels, whereas unsuccessful pregnancies were linked to elevated progesterone levels.

CONCLUSION

This research contributes to the understanding of the intricate interplay between endocrine variables and the success of IVF. The results indicated that women with positive pregnancy outcomes tended to exhibit slightly higher levels of FSH, LH, Prolactin, AMH, and estrogen compared to those with negative consequences. Conversely, women with negative pregnancy outcomes showed higher values for TSH and progesterone levels. These findings suggest that certain hormonal markers may play a role in predicting the success of IVF, with AMH being a particularly notable predictor. The identification of significant associations, particularly the role of AMH in predicting positive pregnancy outcomes, guides future research and clinical practice. Further studies with larger sample sizes and longitudinal designs are warranted to validate and build upon these findings, ultimately enhancing the precision and individualization of infertility treatments.

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