

Impact of Basal Testosterone on Assisted Reproductive Technique (ART) Outcomes in Women with Normal Ovarian Reserve

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Abstract

Basal androgens influence follicular development and ovarian responsiveness, yet the optimal testosterone range for maximizing assisted reproductive technique (ART) outcomes remains uncertain. This prospective observational study evaluated the relationship between basal serum testosterone and ovarian, embryological, and clinical outcomes in women with normal ovarian reserve undergoing their first IVF/ICSI cycle using a GnRH antagonist protocol. A total of 103 women aged 25–35 years who underwent good-quality embryo transfer were analyzed. Basal testosterone levels were measured on cycle days 2–4 and categorized into quartiles. Significant differences were observed in ovarian and embryological outcomes across testosterone quartiles, particularly in the number of mature oocytes and grade 1 embryos, with the best outcomes seen in women with mid-range testosterone levels (0.18–0.31 ng/ml). However, clinical pregnancy rates did not differ significantly between groups, and testosterone was not an independent predictor of pregnancy after adjustment for confounding variables. Testosterone levels demonstrated moderate predictive ability for identifying poor and excessive ovarian response. These findings suggest that maintaining testosterone within an optimal physiological range may improve ovarian stimulation outcomes and embryo quality, thereby supporting individualized stimulation strategies in ART.

INTRODUCTION

Despite the advances in the field of ART, a considerable subset of patients known as “poor responders” consistently experience suboptimal response (1). Early identification of these patients enables clinicians to design more effective treatment strategies and to predict clinical outcomes better. Androgens in females play a crucial role in ovarian follicular development. The disturbances in androgen levels can significantly contribute to unexpectedly poor embryological and clinical outcomes (2)(3)(4). A decreased level of testosterone is associated with a weaker ovarian response and a reduced chance of delivering a pregnancy (5–7). However, hyperandrogenemia seen in obese and PCOS females is associated with resistance to ovulation induction, lower clinical pregnancy rates, and live birth rates (8, 9). Thus, the concentration of androgens needs to be at an optimal level to maximize the reproductive outcomes. Serum testosterone is the primary measurable form of androgen. Some studies have found that basal testosterone levels are specifically correlated with embryological outcomes but not with clinical outcomes, while other studies have not demonstrated a consistent association (10). Most of these studies have not been able to define an optimal testosterone range within which embryological and clinical outcomes are maximized.

The present study aimed to evaluate the impact of serum total testosterone levels on ovarian response and embryological outcomes in women undergoing assisted reproductive techniques (ART). Additionally, the study sought to assess the association between serum testosterone levels and clinical pregnancy rates and to determine whether testosterone levels could serve as predictors of poor or excessive ovarian response in women with normal ovarian reserve. Finally, the study aimed to identify an optimal

testosterone range that maximizes laboratory outcomes and supports individualized ovarian stimulation strategies.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Reproductive Medicine & Surgery at Kasturba Hospital, Manipal, a tertiary care center in South India. The study was carried out over one and a half years, from November 2023 to June 2025. Ethical clearance was obtained from the Institutional Ethics Committee, Kasturba Medical College, Manipal (IEC No. 251; registration no. ECR/146/Inst/KA/2013/RR-19). The study was registered with the Clinical Trials Registry - India (CTRI/2023/11/059971). All methods were performed in accordance with the relevant guidelines and regulations.

All patients between the age group of 25–35 years, undergoing controlled ovarian stimulation cycles with the antagonist protocol, were included. Informed consent was obtained from all participants involved in the study. Only the first embryo transfers were considered. The patients had at least one good-quality embryo transferred. The patients having a previous poor response, mullerian anomalies, or acquired disorders like polyps, submucosal myoma, and intrauterine adhesions, and male factor infertility were excluded. Considering a 95% confidence interval, a margin of error of 4, and a standard deviation of testosterone levels of 20.7, the calculated sample size was 103.

Treatment protocol

Stimulation and oocyte pickup

All participants included were undergoing GnRH antagonist protocols. Before starting exogenous gonadotropin stimulation on the second day of the menstrual cycle, baseline concentrations of AMH, FSH, testosterone, AFC, estradiol, and progesterone were measured. Patients received daily subcutaneous recombinant FSH injections at doses ranging from 150 to 300 IU, adjusted based on age, BMI, AFC, AMH levels, and follicular development. After four days of stimulation, a flexible protocol was followed for starting the GnRH antagonist. Cetrorelix (0.25 mg) was given subcutaneously when either at least a single follicle measured ≥ 14 mm or there were ≥ 6 follicles of ≥ 11 mm, or when serum estradiol concentrations were ≥ 400 pg/ml. Trigger was given with recombinant hCG (250 μ g) once leading follicle reached a size exceeding 18 mm. Retrieval of oocyte was performed under transvaginal ultrasound guidance 34–36 hours following the ovulation trigger.

Embryo Transfer

Depending on semen parameters, fertilization was done via normal insemination (IVF) or ICSI. The patients underwent either a cleavage-stage embryo transfer (2 embryos) or a one-blastocyst-stage transfer. A preference was given to transferring a single, top-quality embryo. A high-quality Day 3 embryo

was defined as having $\leq 10\%$ fragmentation, at least eight cells, uniform blastomeres, and no multinucleation. The blastocyst quality was based on blastocoele expansion, number, and density of inner cell mass and trophectoderm (11). Blastocysts with grade $>3BB$ were considered good quality. A "freeze-all" strategy with embryo transfer at a later stage was used in cases of high serum progesterone on trigger day (≥ 1.5 ng/ml), inadequate endometrial thickness (≤ 6 mm), intrauterine fluid, or possibility of hyperstimulation syndrome, such as retrieval of > 20 oocytes. All the patients underwent frozen embryo transfer by an HRT cycle.

In HRT cycles, injection of leuprolide was given on the 21st day of the cycle, 1 month prior to the embryo transfer. Oral contraceptive pills were given along with it for 7 days. Patients were assessed on the second day of the next cycle by transvaginal ultrasound to see for latency of the ovaries and endometrium. Oestradiol valerate was started orally at a dose of 6mg/day until the embryo transfer day. A transvaginal ultrasound was performed after one week to assess the endometrial thickness and vascularity. When the thickness of the endometrium was 7mm, progesterone gel was started intravaginally. If the endometrial thickness was less than 7mm after 2 weeks of oestrogen therapy, the dose was augmented up to 12mg/day, or other routes of oestrogen administration were used. For cleavage-stage embryo transfer, progesterone gel was given for 3 days, and for blastocyst transfer, progesterone gel was given for 5 days. Post-embryo transfers, luteal phase support was given with oral progesterone 20mg twice a day, micronized progesterone gel vaginally 90mg twice a day, and injectable aqueous progesterone intramuscularly once in 3 days. Luteal phase support was sustained till 12 weeks of gestation.

Pregnancy was initially evaluated by serum hCG testing 2 weeks after embryo transfer, and clinical pregnancy was established by the appearance of a gestational sac on transvaginal USG, after 6 weeks of embryo transfer.

The primary outcome was the rate of clinical pregnancy per initiated cycle. Secondary outcomes included biochemical pregnancy rate, total gonadotropin dose, and ovarian response parameters (number of oocytes retrieved, number of mature oocytes, number of embryos obtained, and number of high-quality embryos).

Hormone measurement

During the stimulation phase, 2–3 ml of blood was taken on cycle days 2–4 in the morning to measure the hormones and basal AMH. Serum levels of AMH, testosterone, LH, and FSH were quantified using an electrochemiluminescence immunoassay. Detection range for detection of testosterone, LH, FSH, and AMH were approximately 0.025–15 ng/ml, 0.1–200 mIU/ml, 0.1–200 mIU/ml, and 0.01–23 ng/ml, respectively, with inter-assay variation kept below 5%.

STATISTICAL ANALYSIS

Normally distributed quantitative variables were expressed as mean $\pm$ SD, while skewed variables were presented as median (Q1, Q3). Categorical variables were summarized as frequencies and percentages. Comparisons between testosterone quartiles were performed using the Kruskal-Wallis or one-way ANOVA test as appropriate. Spearman's correlation coefficient was used to evaluate associations between testosterone levels and other variables. Differences in demographic and clinical parameters between the groups were analyzed using the Student's t-test or the Mann-Whitney U test for continuous data, and the Pearson χ^2 test for categorical data. Binary logistic regression was conducted to identify factors influencing clinical pregnancy rates. Receiver operating characteristic (ROC) curves were constructed to assess the predictive capacity of serum testosterone for clinical pregnancy, as well as for poor and excessive ovarian response. Additionally, a logistic regression-based nomogram was developed to further evaluate the predictive utility of testosterone for clinical pregnancy. Statistical analyses were conducted using IBM SPSS software (version 26.0), and a p-value < 0.05 was considered statistically significant.

ETHICAL CONSIDERATION

Ethical clearance was obtained from the Institutional Ethics Committee, Kasturba Medical College, Manipal (IEC No. 251; registration no. ECR/146/Inst/KA/2013/RR-19). The study was registered with the Clinical Trials Registry - India (CTRI/2023/11/059971).

RESULTS

A total of 162 patients were initially enrolled for IVF during the study period. After excluding 38 patients (13 with AMH < 1.2 , 11 with AFC < 5 , and 14 who underwent a GnRH agonist flare protocol), 124 patients (76.54%) underwent controlled ovarian stimulation using the antagonist protocol. Out of them, 110 patients underwent embryo transfer, and out of them, 103 patients who underwent good-quality embryo transfer were included in the final analysis.

Basic demographic characteristics of the population are shown in Table 1. The median value of testosterone is 0.20 (25–75th percentile: 0.09–0.29) ng/ml.

Table 1

Demographic and clinical characteristics of the cohort overall, between non-pregnant and pregnant patients, respectively

Parameter	Overall (n-103) (Median, Q1, Q3)	Non-Pregnant (n-70) (Median, Q1, Q3)	Pregnant (n- 33) (Median, Q1, Q3)	P- value
Age (years)	32.00 (29.00, 34.00)	32.00 (29.00, 35.00)	31.00 (31.00, 33.00)	0.848
BMI (kg/m ²)	24.18 (22.15, 26.99)	24.00 (22.27, 26.48)	24.20 (22.10, 29.10)	0.724
AMH	2.89 (1.84, 4.63)	2.80 (1.59, 4.57)	3.55 (2.36, 5.06)	0.095
AFCs	15.00 (11.00, 20.00)	14.00 (10.00, 20.00)	17.00 (12.00, 24.00)	0.058
Dose of gonadotropins (IU)	2700.00 (2250.00, 3300.00)	2712.50 (2324.25, 3300.00)	2475.00 (2100.00, 3000.00)	0.093
Oocytes	10.00 (6.00, 14.00)	9.00 (6.00, 14.00)	11.00 (9.00, 14.00)	0.210
M2 oocytes	8.00 (5.00, 12.00)	7.50 (5.00, 11.75)	10.00 (6.00, 12.00)	0.200
Testosterone (ng/ml)	0.20 (0.09, 0.29)	0.21 (0.09, 0.32)	0.16 (0.09, 0.26)	0.243
Estradiol	43.30 (34.25, 52.45)	43.15 (32.73, 52.65)	44.40 (37.40, 52.00)	0.447
FSH	6.30 (5.25, 7.70)	6.35 (5.33, 7.67)	6.30 (5.20, 7.70)	0.764
LH	5.80 (4.15, 7.65)	5.45 (4.10, 7.55)	6.10 (4.50, 7.90)	0.275
Progesterone	0.20 (0.10, 0.30)	0.20 (0.10, 0.30)	0.20 (0.10, 0.30)	0.463
Embryos formed	5.00 (4.00, 6.00)	4.00 (4.00, 6.00)	6.00 (6.00, 8.75)	0.006*
Day 3 embryos	4.0 (4.0, 6.0)	4.0 (4.0, 5.0)	5.0 (4.0, 7.0)	0.075
Day 5 embryos	0.0 (0.0, 2.0)	0.0 (0.0, 2.0)	2.0 (0.0, 3.0)	0.088
Grade 1 embryos formed	3.00 (2.00, 5.00)	2.00 (2.00, 5.00)	4.00 (2.00, 6.00)	0.068
Fresh vs Frozen embryo transfer				
Fresh embryo transfer	16/103 (15.5%)	9/70 (12.85%)	7/33 (21.21%)	0.42
Frozen embryo	87/103 (84.4%)	61/70 (87.14%)	26/33 (78.78%)	

Parameter	Overall (n-103) (Median, Q1, Q3)	Non-Pregnant (n-70) (Median, Q1, Q3)	Pregnant (n- 33) (Median, Q1, Q3)	P- value
transfer				
Day of embryo transferred				
Day 3 embryo transfer	80/103 (77.67%)	57/70 (81.4%)	23/33 (69.7%)	0.28
Day 5 embryo transfer	23/103 (22.33%)	13/70 (18.6%)	10/33 (30.3%)	

Mann–Whitney U test (also called the Wilcoxon rank-sum test) was used to compare the characteristics between pregnant and non-pregnant patients

BMI, Body Mass Index; FSH, Follicle Stimulating Hormone; AMH, Anti Mullerian Hormone; AFC- Antral Follicle Count

**- p value statistically significant*

The basal testosterone levels (ng/ml) were categorized into quartiles: Q1 (0.03–0.17), Q2 (0.18–0.31), Q3 (0.32–0.46), and Q4 (0.47–0.61), and the embryological and clinical outcomes were compared in these quartiles.

Table 2

Comparison of ovarian response and pregnancy outcomes among women categorized by basal serum testosterone levels in the four quartiles

Testosterone (ng/ml)	First Quartile (0.03–0.17)	Second Quartile (0.18–0.31)	Third Quartile (0.32–0.46)	Fourth Quartile (0.47–0.61)	P value
Characteristics	(N- 48) (Median, Q1,Q3)	(N-34) (Median, Q1, Q3)	(N-12) (Median, Q1, Q3)	(N- 07) (Median, Q1, Q3)	
Age (years)	33.00 (27.50, 36.50)	31.50 (29.00, 33.00)	31.00 (28.75, 32.25)	32.00 (30.00, 34.25)	0.506
BMI (kg/m ²)	24.35 (22.27, 26.82)	23.15 (20.75, 26.48)	24.42 (22.68, 27.42)	27.70 (26.25, 29.15)	0.098
AMH	2.70 (1.73, 4.00)	3.60 (1.94, 5.39)	2.87 (2.03, 4.55)	4.49 (2.89, 6.70)	0.336
AFC	14.00 (10.00, 18.25)	18.00 (13.25, 23.50)	13.00 (10.00, 17.50)	19.00 (14.50, 23.50)	0.065
Dose of Gonadotropin	3000.00 (2381.25, 3600.00)	2517.50 (2100.00, 2925.00)	2562.50 (2250.00, 3262.50)	2325.00 (1687.50, 2578.00)	0.035*
No. of oocytes	9.00 (6.00, 11.00)	13.50 (8.00, 16.00)	9.00 (7.25, 14.50)	7.00 (3.50, 16.00)	0.084
M2 Oocytes	7.00 (5.00, 11.00)	12.00 (7.00, 14.00)	8.00 (5.75, 12.50)	5.00 (3.00, 9.50)	0.008*
Number of Embryo	5.00 (4.00, 6.00)	6.00 (4.00, 7.75)	6.00 (5.50, 7.50)	3.00 (2.00, 5.00)	0.325
Embryo Grade1	2.00 (1.00, 4.00)	4.50 (2.00, 7.00)	5.00 (2.00, 5.25)	2.00 (1.50, 2.50)	0.003
Beta hCG + rate	19/48 (39.5%)	13/34 (38.2%)	4/12 (33.33%)	2/7 (28.5%)	0.797
Clinical pregnancy rate	18/48 (37.5%)	12/34 (35.3%)	2/12 (16.7%)	1/ 7 (14.3%)	0.378

The Kruskal-Wallis test was used to compare the values among the quartiles

BMI, Body Mass Index; FSH, Follicle Stimulating Hormone; AMH, Anti-Mullerian Hormone; AFC- Antral Follicle Count

*- *p* value statistically significant as compared to other quartiles

The total gonadotropin dose required for stimulation showed a decreasing trend with rising testosterone levels. There were significant differences in the number of M2 oocytes ($p = 0.008$) and grade 1 embryos ($p = 0.003$) across the serum testosterone quartiles (Table 2). Notably, patients in the second quartile (0.18–0.31 ng/ml) had the highest median values for these embryological parameters, suggesting an optimal range for improved oocyte maturation and embryo quality. Although the total number of oocytes showed a trend toward higher values in this group ($p = 0.084$), this did not reach statistical significance. Importantly, clinical pregnancy rates did not significantly differ among the quartiles ($p = 0.378$), indicating that while testosterone levels may enhance embryological outcomes, they do not directly influence clinical pregnancy rates. Thus, maintaining basal testosterone within the second quartile range appears beneficial for maximizing laboratory outcomes in ART cycles, even though it may not translate into improved pregnancy rates.

A multivariate logistic regression analysis was performed to assess the impact of testosterone on clinical pregnancy rates after adjusting for confounders (age, BMI, AMH, AFC). It was seen that testosterone quartiles were not statistically significant independent predictors of clinical pregnancy (Table 3). Only AFC (antral follicle count) showed a significant positive association (OR- 1.08, p value < 0.05). Although Q2 had good embryological outcomes descriptively, testosterone quartile alone does not have an independent predictive effect on clinical pregnancy when adjusted for age, BMI, AMH, and AFC.

Table 3
Multivariate logistic regression analysis for the factors impacting clinical pregnancy rate (adjusted for age, BMI, AMH, AFC):

Variable	Odds Ratio	95% CI Lower	95% CI Upper	P-value
Quartile Q1	-	-	-	-
Quartile Q2	0.77	0.29	2.08	0.61
Quartile Q3	0.35	0.07	1.82	0.21
Quartile Q4	0.18	0.02	1.90	0.15
Age	1.00	0.89	1.13	0.94
BMI	1.02	0.93	1.12	0.68
AMH	0.88	0.71	1.09	0.24
AFC	1.08	1.01	1.16	0.03

Binary Logistic Regression test was used to determine the factors having an independent impact on the clinical pregnancy

Q1 is considered the reference quartile, and all other quartiles are compared with it

BMI, Body Mass Index; AMH, Anti-Mullerian Hormone; AFC- Antral Follicle Count

ROC curve analyses were done to assess the predictive ability of testosterone further (Fig. 1). The area under the curves for predicting clinical pregnancy rate was 0.43 (< 0.6), indicating that basal serum testosterone alone had poor performance in predicting clinical pregnancy rate. The ROC curve to determine the ability of testosterone to predict poor ovarian response (< 4 oocytes) and excessive ovarian response (> 15 oocytes) showed an area under the curve of > 0.6 , indicating that testosterone performed well in predicting both poor and excessive ovarian responses (p -value < 0.05) (Fig. 2).

SUBGROUP ANALYSIS IN PCOS PATIENTS

A subgroup analysis was done considering PCOS patients. The testosterone levels were higher among the PCOS females, but the difference was not statistically significant (p value = 0.079) (Supplementary Table 1).

The ovarian reserve markers, including AMH and AFC, were significantly higher among PCOS patients ($p < 0.01$). Consequently, these patients were given a significantly lower total dose of gonadotropins for stimulation ($p < 0.01$). Additionally, the number of oocytes retrieved and the number of M2 oocytes were both significantly higher in the PCOS group (p -value < 0.05). However, the number of good-quality embryos formed, and the clinical pregnancy rates were comparable between the two groups (Supplementary Table 2). The quartile analysis revealed similar findings as seen overall, with the embryological outcomes being best in Q2. The clinical pregnancy rates were also best in Q2 as compared to those seen in Q1, but the trend of decreasing pregnancy rates with increasing testosterone levels was similar (Supplementary Table 3).

DISCUSSION

In the current study, although serum testosterone levels did not show a statistically significant independent impact on clinical pregnancy rates after adjusting for confounding factors, they clearly influenced key embryological outcomes, including the number of oocytes retrieved, M2 oocytes, and grade 1 embryos. These findings suggest that while testosterone may not directly determine pregnancy success, it plays a crucial role in optimizing ovarian response and enhancing overall laboratory outcomes. Therefore, maintaining serum testosterone levels within an adequate range (0.18–0.32 ng/ml) can be recommended to improve embryo quality and quantity, ultimately supporting better cumulative reproductive potential. Furthermore, testosterone levels effectively identified patients at risk for poor oocyte yield (< 4 oocytes) and excessive ovarian response (> 15 oocytes) in normal ovarian reserve patients.

These observations align with the study by Sun et al. (7), which demonstrated that testosterone levels were strong predictors of ovarian response and could guide FSH dosage, although they did not predict pregnancy outcomes. Similarly, Khalaf et al. (12) reported that basal testosterone levels were useful for

estimating gonadotropin requirements and ovarian response but were insufficient as a standalone marker for predicting overall outcomes.

In the present study, the total gonadotropin dose decreased with higher testosterone levels (Table 2). Elevated androgen levels enhance follicular sensitivity to FSH, thereby reducing the amount of gonadotropin required for stimulation (13). This finding corroborates the results of Lu et al., who observed that patients with lower testosterone levels (< 1.115 nmol/L) required higher total gonadotropin doses (10). The most favorable embryological outcomes were seen within the testosterone range of 0.18–0.32 ng/ml. This may be attributed to the dual role of androgens in folliculogenesis: directly via androgen receptors and indirectly through aromatization to estrogen (13). Granulosa and theca cells in early-stage follicles express high levels of androgen receptors, which enhance FSH receptor expression and stimulate cyclic adenosine monophosphate (cAMP) production, promoting follicular cell proliferation and differentiation. Additionally, androgen receptor activation increases the expression of insulin-like growth factor (IGF) and its receptor genes, facilitating follicular recruitment and development (14). However, at higher androgen concentrations, these hormones are converted to potent 5 α -reduced forms that cannot be aromatized to estrogen, leading to suppressed aromatase activity and inhibition of FSH and LH receptor induction. Consequently, follicles become hyperandrogenic and undergo atresia (15).

Although the biochemical and clinical pregnancy rates (37.5%) were highest in Q1 (0.03–0.17 ng/ml) and decreased with higher testosterone levels, these differences were not statistically significant across quartiles. Nomogram curve analysis also indicated an inverse relationship between testosterone levels and clinical pregnancy rates. This contrasts with the findings of Wan et al. (13) and Sun et al. (7), who reported higher clinical pregnancy and cumulative pregnancy rates at higher testosterone levels. The discrepancy may be explained by disruption of the adiponectin signaling pathway in the endometrium in cases of hyperandrogenemia and hyperinsulinemia, particularly among obese PCOS patients. This altered hormonal environment may impair endometrial function and negatively impact implantation (16). In our study, the highest percentage of PCOS patients was observed in Q4, correlating with higher testosterone levels and lower pregnancy rates. Wei et al. (17) similarly reported reduced endometrial expression of insulin-like growth factor binding protein 1 (IGFBP-1) and leukemia inhibitory factor (LIF) in such patients, which may contribute to lower clinical and ongoing pregnancy rates.

Moreover, in the present study, testosterone levels were useful for predicting poor or excessive ovarian responses in patients with normal ovarian reserve, though they did not predict pregnancy outcomes, as demonstrated by ROC and logistic regression analyses. Sun et al. (7) also found testosterone levels to be reliable predictors of ovarian response and the number of large follicles on hCG day in non-PCOS women undergoing IVF, but not of pregnancy outcomes. In contrast, Guo et al. (5) reported that a multivariate model combining age, AFC, FSH, FSH/LH ratio, and testosterone had excellent predictive value for both poor ovarian response and clinical pregnancy, surpassing the performance of testosterone alone.

In the current study, the testosterone levels were not significantly higher among the PCOS females (p value = 0.079). Most of the patients having PCOS in the present study were PCOS category D, having irregular cycles, and PCO morphology on ultrasound. The evidence of clinical and biochemical hyperandrogenism was not seen in the majority.

Subgroup analysis among PCOS patients in our study showed similar findings: embryological outcomes were significantly better in Q2 (0.18–0.32 ng/ml), while clinical pregnancy rates remained comparable across quartiles. Also, in PCOS females in PCOS patients, the conversion of oocytes to good-quality embryos was lower. Similar findings were observed in a study by Heidenberg et al., where patients with PCOS had a greater no. of oocytes as compared to patients with hypothalamic hypogonadism, but the no. of day 5 embryos formed was similar (14). However, the number of PCOS patients was small, limiting definitive conclusions.

One of the major strengths of this study is that it assessed testosterone's role in an unselected population, rather than focusing solely on poor responders or non-PCOS women. The use of multivariate logistic regression allowed adjustment for confounders when evaluating factors influencing clinical pregnancy rates. Furthermore, subgroup analysis in PCOS patients offered additional insights. Nonetheless, certain limitations must be acknowledged. The study's single-center design and relatively small sample size may limit generalizability. Additionally, testosterone levels were measured using electrochemiluminescence immunoassay (ECLISA) rather than tandem mass spectrometry, the latter being considered the gold standard. Only total serum testosterone was assessed, whereas free testosterone more accurately reflects bioavailable androgen levels. Finally, most participants had normal baseline testosterone levels, and levels were not reassessed during the ART cycle. Nevertheless, significant changes in testosterone levels are unlikely over the short duration of ovarian stimulation.

CONCLUSION

Serum testosterone levels significantly impact ovarian and embryological outcomes, though not directly the clinical pregnancy rates. Maintaining an optimal testosterone range (0.18–0.32 ng/ml) improves oocyte yield and embryo quality, helping to maximize IVF success and guide individualized stimulation protocols. Additionally, testosterone levels can serve as a useful marker to predict oocyte yield in patients with normal ovarian reserve.

DECLARATIONS

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CRedit Authorship Contribution Statement:

A.A.- Conceptualization, Methodology, Writing- Original draft preparation. A.M.- Software, Writing - Review & Editing, and Data curation. V.P.- Supervision and Validation. P.A.- Resources. P.K.- Supervision and Validation. All authors read and approved the final manuscript.

Data availability statement:

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request

This study was conducted and reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for observational studies.

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Declaration of Interests-

There are no conflicts of interests to declare.

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Figures

ROC Curve: Testosterone vs Clinical Pregnancy

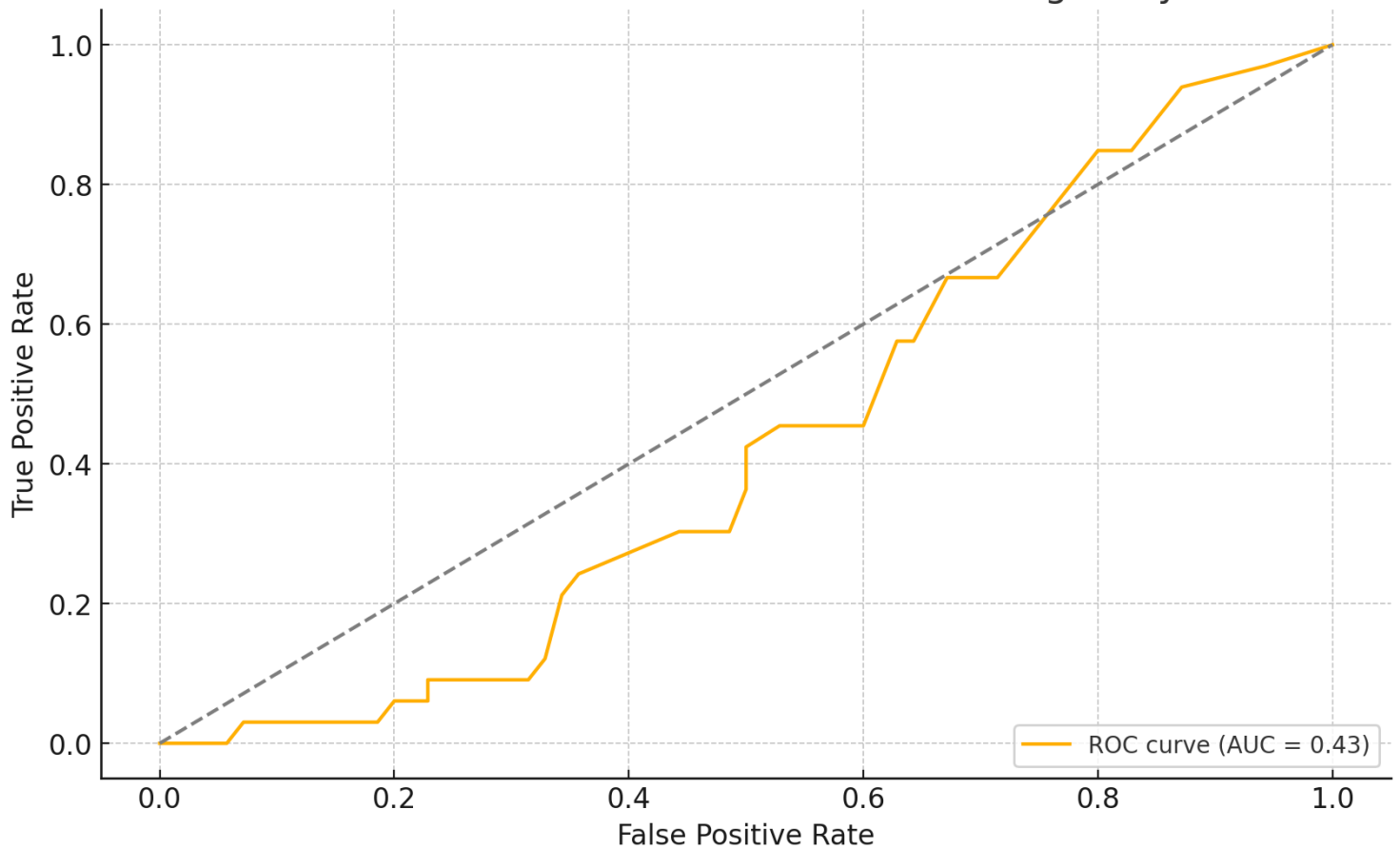


Figure 1

Receiver operating characteristic (ROC) curve of basal serum testosterone level in the prediction of cumulative live birth

X-axis: False Positive Rate (FPR) = 1 – Specificity, Y-axis: True Positive Rate (TPR) = Sensitivity, Dotted line: line of no discrimination. Poor predictive power for clinical pregnancy.

Receiver operating characteristic (ROC) curve showing the predictive ability of basal serum testosterone levels for clinical pregnancy in women undergoing ART. The area under the curve (AUC) was 0.43, indicating poor predictive performance of testosterone for clinical pregnancy outcomes. The dotted diagonal line represents the line of no discrimination. The X-axis represents the false positive rate (1 – specificity), and the Y-axis represents the true positive rate (sensitivity).

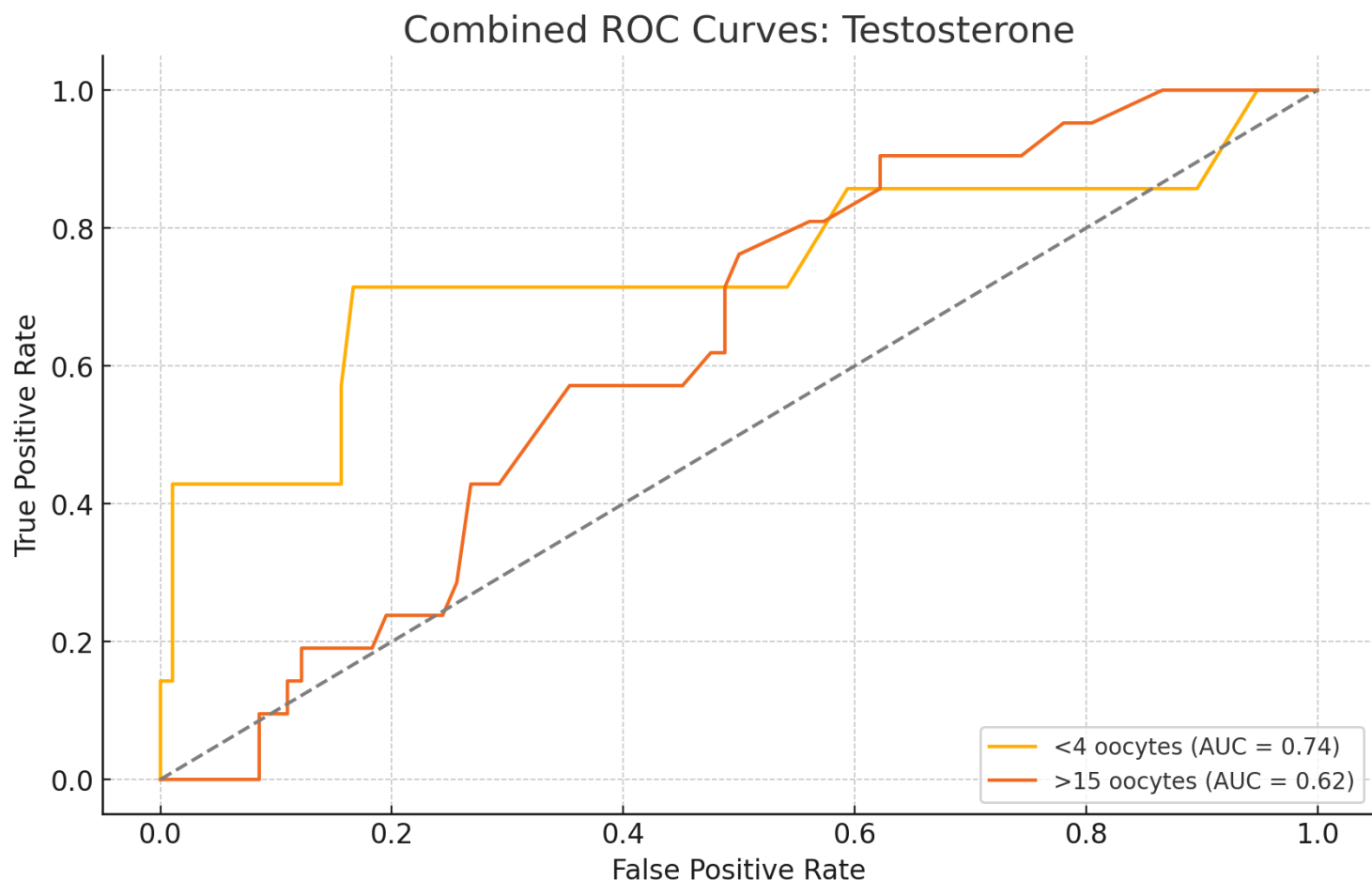


Figure 2

Combined ROC curve analysis to determine the ability of testosterone to predict poor (<4 oocytes) and excessive (>15 oocytes) ovarian response

X-axis: False Positive Rate (FPR) = 1 – Specificity, Y-axis: True Positive Rate (TPR) = Sensitivity, Dotted line: line of no discrimination. Serum testosterone significantly predicted poor ovarian response (<4 oocytes) with an AUC of 0.74, and excessive response (>15 oocytes) with an AUC of 0.62.

Combined ROC curve analysis evaluating the ability of basal serum testosterone levels to predict poor ovarian response (<4 oocytes retrieved) and excessive ovarian response (>15 oocytes retrieved) in women undergoing ART. Testosterone demonstrated moderate predictive ability for poor ovarian response (AUC = 0.74) and excessive ovarian response (AUC = 0.62). The dotted diagonal line represents the line of no discrimination. The X-axis represents the false positive rate (1 – specificity), and the Y-axis represents the true positive rate (sensitivity).

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