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Role of Testosterone in Serum and Follicular Fluid in Sub Fertile Women Undergo Intracytoplasmic Sperm Injection

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ABSTRACT

Background: *Testosterone is the most common and regarded as the main hormone in the testicles among androgen (Tremblay, J. J,.2015) Testosterone synthesis is initiated by luteinizing hormone and cAMP signaling, promoting the transport of cholesterol to the inner mitochondrial membrane with the involvement of the steroidogenic acute regulatory protein (StAR). Cholesterol is then converted into pregnenolone, which further transforms into progesterone and is eventually metabolized into testosterone. This steroidogenesis pathway is similar in both thecae cells and interstitial cells of the ovaries (Cipolla-Neto, J., et al., 2022). Low-quality oocytes with a propensity for lower cleavage rates following fertilization have been associated with elevated androgen levels, more especially testosterone, in follicular fluid (FF) (Costa, L. O. B., et al., 2004). When FF contained immature oocytes as opposed to mature oocytes, testosterone levels were found to be noticeably higher. Pregnancy-associated follicles were found to have a higher estrogen-to-testosterone (E2/T) ratio reported. These results raise the possibility that an early follicular atresia linked to a low estrogen/androgen ratio in FF may reduce the viability of the oocytes and reduce the likelihood of fertilization and pregnancy* ***purpose****: to detect the follicular fluid and blood concentration of the testosterone hormone on subfertility in women undergoing Intracytoplasmic Sperm Injection (ICSI) for fertility treatment.* ***Methods****: This case-control study was carried out on eighty women, from these, forty females were sub-fertile patients defined as the cases, other forty females as a control included fertile females came to fertility center due to males factor of subfertility. A detailed medical history and physical examination were recorded. At the same day of ovum pickup blood and follicular fluid were collected, for hormonal analysis of testosterone.*

Results: *Show significantly higher levels of serum and follicular testosterone in sub-fertile women (cases) than control group ($p < 0.05$), and significant positive correlations between serum and follicular fluid.* ***Additional materials****: notes the number of references, tables, graphs, exhibits, test instruments, appendixes, or other supplemental materials in the paper. Also, the abstract must be written in a single paragraph in English, max 250 words.*

Conclusion: *Testosterone hormone level in blood and follicular fluid were significantly higher in cases group than control and there is a positive correlations between serum and follicular fluid of testosterone.*

Keywords: ***Testosterone*****, Intracytoplasmic Sperm, Sub Fertile Women**.

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INTRODUCTION

Steroid hormones, integral to the endocrine system, play a significant role in various aspects of reproductive processes, including granulosa cell (GC)-oocyte interactions, oocyte maturation, embryonic developmental competence, ovarian response to stimulation drugs, and endometrial receptivity.

These hormonal dynamics are particularly crucial in the context of in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and embryo transfer (ET) treatments. (S. Palomba *et al*.2021)**.** Therefore, gaining a more profound comprehension of the connections between subfertility and irregularities in steroid hormones is imperative for improving pregnancy outcomes in women experiencing subfertility and undergoing IVF/ICSI treatment. (Falkenstein, E., *et al*.2000), steroidogenesis is common between ovaries and adrenal glandsAnd there are five major classes of steroid hormones: an androgen Testosterone, along with its more potent metabolite dihydrotestosterone , progesterone, and estradiol fall under the category of sex-steroids. Cortisol and aldosterone collectively belong to the group of corticosteroids. Testosterone, an androgen, along with its more potent metabolite dihydrotestosterone (DHT), . (Payne AH, Hales DB, 2004) (Miller WL, 2008). The adrenal gland is tasked with the synthesis of androgens, especially Androstenedione is produced through cellular synthesis within the zona reticularis layer(Azhar S, Reaven E, 2002), while in the ovary, granulosa cells primarily secrete progesterone (along with its metabolite 20-α hydroxyprogesterone) and estradiol. Theca cells, also within the ovary, are predominantly responsible for synthesizing androgens.

Additionally, luteal cells in the ovary contribute to progesterone secretion, along with its metabolite 20α-hydroxyprogesterone. In the testes, Leydig cells serve as the site of testosterone production. This division of labor among different cell types in the ovary and testes highlights the coordinated synthesis of sex hormones essential for reproductive functions. (Payne AH, Hales DB, 2004) As follicular cells mature, steroids produced by them build up in the FF. The corona-cumulus-oocyte complex is close to the FF; thus, it is thought that the oocyte's quality and maturity level are related to the hormonal content of the FF. As so, the hormonal makeup of FF is thought to be associated with the processes of fertilization, embryo development, and implantation rate. (Costa, L. O. B.,*et al*.2004)

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METHODS

Case control type which was held in fertility center of Al-Sader medical city and from fertility center in Al-kafeel Hospital in Karbala city between January, 2022 and February, 2024. It included a total number eighty female with random sampling method, the age of these women ranging between (19-40) years with different ranging BMI, with duration of subfertility between (1-23) years, all are reside at middle Euphrates region in Iraq, from these eighty women, forty female were sub fertile patient defined as the cases, half of these cases have polycystic ovaries whom diagnosed according to history, clinical examination, ultrasound and hormonal level and the other half of cases are non-polycystic ovary. The other forty female (the control group) included fertile female came to fertility center due to male factor of subfertility with matched age and body weight.

Biochemical analysis

Serum and follicular level of steroid hormone Testosterone analysis using enzyme-linked immunosorbent assay "ELISA" system "Bio Tek" is used. Human –type kit from (Elabscience- USA) were applied for detection of Testosterone level in serum and follicular fluid.

Statistical analysis

The data was analyzed using the statistical package for Social Sciences (SPSS) software (version 25 IBM SPSS, Inc., Chicago, Illinois, USA) and Microsoft Excel 2019.

RESULTS

A total of 80 sub fertile women had been included in this study. Half of them due to male factor (controls) and the other half were female factors (cases). The female factors were PCO (N=20) and non PCO (N=20). The mean age of all women was 29.5±6.8 years(19-40) and the mean duration of infertility was 7.16±4.6 years (1-23). Table 1 shows some sociodemographic characteristics in which there is no significant difference between cases and controls regarding age, duration of infertility and body mass index of participants (p value>0.05).

Comparison between cases and controls regarding age, duration and BMI.

Table (1) Comparison between cases and controls regarding age, duration and BMI

Variables	Cases	Controls	P value
Age / years	30.90 ± 6.9	28.12 ± 6.5	0.070
Duration / years	7.32 ± 4.20	7.00 ± 4.90	0.755
BMI Kg/m^2	26.60 ± 5.36	27.89 ± 3.78	0.241

Comparison of Serum hormone Testosterone in cases and controls.

Table (2) compares the serum hormones of the cases and controls. The data compares serum Testosterone, between cases and controls. The serum Testosterone levels is significantly higher in cases as compared controls (p value<0.05).

Table (2) Comparison of Serum hormones in cases and controls.

Variables	Case	Control	P value
S. testosterone ng/dl	1.66±1.16	0.62±0.31	<0.001

Comparison of follicular fluid hormone Testosterone in cases and controls.

Table (3) Comparison of follicular fluid hormone testosterone in cases and controls, FF hormone testosterone presented in table (3) shows significantly higher levels in cases compared to controls with p<0.05

Table (1) Comparison of FF hormones in cases and controls.

Hormone ng/dl	Case	Control	P value
F. testosterone	1.10±0.61	0.36±0.32	<0.001

Comparison between PCO and Non PCO patients in serum hormones.

Regarding cases with female factors due to PCO or non PCO causes, table (4) compare between the two groups and the result were significantly higher level in PCO women compared to non PCO causes.

Table (2) Comparison between PCO and Non PCO patients in serum hormone.

Serum Hormones ng/dl	PCO	Non PCO	P value
Testosterone	2.27±1.28	1.04±0.59	<0.001

FF hormone comparison in PCO and non-PCO patients.

Tables (5) compare PCO and non-PCO that showed causes in FF testosterone which also were significantly higher in PCO patients compared to non PCO.

Table (3) Comparison between PCO and Non PCO patients in FF hormone.

FF Hormones ng/dl	PCO	Non PCO	P value
Testosterone	1.39±0.63	0.81±0.45	0.002

Correlation between serum and follicular hormone among cases.

Tables (6) present statistically significant correlations between serum and FF hormone testosterone among cases; figures (1) demonstrate a positive significant correlation between serum and FF hormones, testosterone among all participants.

Table (6) significant positive correlations between serum and FF hormones among cases.

Correlations		r	P value
S. testosterone ng/ml	F. testosterone ng/ml	0.649	<0.001

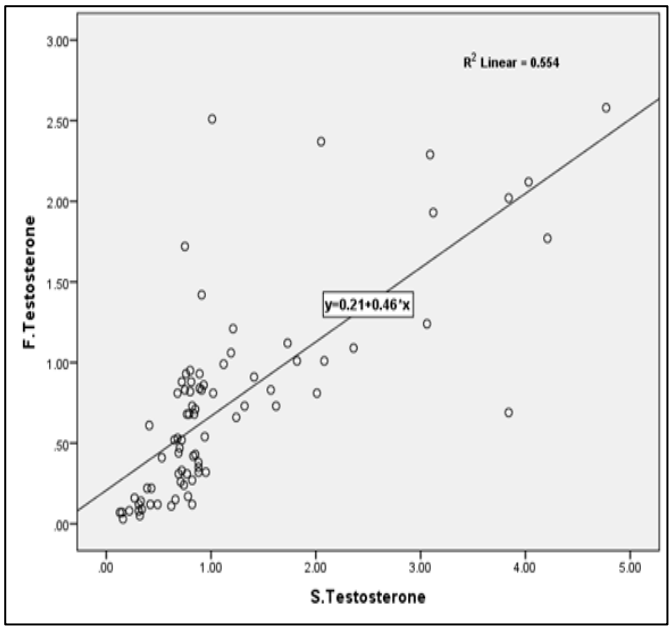

Figure (1) Correlation between serum and FF testosterone.

Correlation between serum and follicular hormones among controls.

Table (7) Show statistically significant correlations between serum and FF hormone Testosterone among controls.

Table (4) significant positive correlations between serum and FF hormones among controls.

Correlations		r	P value
S.testosterone ng/ml	F. testosterone ng/ml	0.633	<0.001

DISCUSSION

Regarding some sociodemographic characteristics table (1) age, duration of subfertility and body mass index, in which there is no significant different between case and controls between all participants, case and control (P value > 0.05).

The results of study in the table (2) and (3) show a significantly higher levels of serum and follicular steroid hormones Testosterone in sub fertile women (cases) than control group. Walters et al.'s 2019 study of steroids hormones in follicular fluid revealed that testosterone hormone has a negative predictive value for the number of oocytes recovered and pregnancy outcome in women undergoing IVF. On the other hand, Hossein Rashidi et al. (2009) discovered that serum testosterone concentration can predict successful IVF cycles; hence, better pregnancy rates should be anticipated with androgen levels regulation. But in 2018 Abide Yayla et al. found that testosterone (T) levels had no predictive value for ovarian response or intracytoplasmic sperm injection (ICSI) cycle outcome.

The results of the study in the table (4) and (5) show a significantly higher levels of serum and follicular steroid hormones Testosterone in infertile patients with polycystic ovarian syndrome, testosterone levels are mainly elevated in infertile women could be due polycystic ovarian syndrome in which both hormones are elevated.

Stener-Victorin *et al*. 2010, Nehir *et al.* 2016) found significant relationship between females with PCO who are sub fertile and high serum and comparison of follicular androgen, estrogen, sex steroid precursors, and glucuronidated androgen metabolites with non PCOS controls, this reflects the role of steroids hormones in folliculogenesis and oocytes quality that associated with pathophysiology of PCO.

Another prospective study conducted in 2020 by Hassan, et al. looked at the follicular testosterone correlation between PCO patients and control groups receiving IVF/ICS. The results showed that PCO patients have much higher serum and follicular testosterone hormone levels than controls. These findings support the present investigation on PCOS, which linked low rates of fertilization and implantation, lower rates of oocyte quality, and a higher rate of miscarriage in patients receiving IVF/ICSI treatment (Rosenfield, R.L. and Ehrmann, 2016) (Li et al. 2019). While mounting data shows that increased androgen levels are a key component in the development of PCOS (Lerchbaum et al. 2014), some researchers have not discovered any relationship between testosterone hormone levels and fertilization. Others proposed that the best growth of follicles depends on a specific number of follicular androgens (Revelli et al. 2009). Because increasing evidence indicates that higher steroid hormones, especially androgen levels, are a fundamental factor in the pathogenesis of PCOS (Lerchbaum et al. 2014).

The results shown in table (6) and (7) and figure (1) show a significant positive correlations between serum and follicular fluid steroid hormones Testosterone between cases and controls. Therefore, it could be a useful predictor of oocyte maturation as well as fertilization rate for sub fertile women undergoing ICSI. This study's findings support those of Teissier et al. (2000), who proposed that serum and follicular steroid content, follicular size, and oocyte maturity have a correlation that may affect the outcome of oocyte fertilization during ICSI.

CONCLUSION

1. Testosterone hormone level in blood and follicular fluid were significantly high in cases (sub fertile women), while significantly lower in control (male factor sub fertile).

2.There is a significant correlation between serum and follicular fluid Testosterone .

3.Study the effect of weight reduction on level of these hormones in sub fertile

4.Follow up of sub fertile women who undergo (ICSI) to evaluate the role of steroid

hormones ((Aldosterone, Cortisol and Testosterone) in predicting the outcome of ICSI in sub fertile women.

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