

Follicular Fluid Thyroid Hormones (T4 and T3) Levels and ICSI Outcomes

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Abstract

Background: Whether through spontaneous conception or through the use of assisted reproductive technologies, thyroid dysfunction is prevalent in women of childbearing age and may negatively impact fertility and pregnancy. As follicular fluid (FF) more accurately depicts the milieu surrounding the developing oocyte, it most likely offers a better assessment of exposures that might have an impact on reproductive outcomes. **Objective:** The purpose of this study is to examine the correlation of FF thyroid hormone (TH) (T4 and T3) levels with the intra-cytoplasmic sperm injection (ICSI) outcomes. **Materials and Methods:** The study was designed as an analytic cross-sectional study. Fifty subfertile couples from the *in-vitro* fertilization center attenders were involved in the study in whom ICSI was done for them and then compared between outcomes in relation to FF-T4 and FF-T3. **Results:** FF-T4 correlates positively with metaphase II (MII) and 2-PN with a *P*-value=0.04 and 0.03, respectively, in a statistically significant way, whereas FF-T3 correlates positively with oocyte number: MII in a statistically significant way (*P*-value=0.01) for both and with 2-PN in a statistically significant way (*P*-value=0.0). Additionally, there was a significant positive association between FF-T4 and anti-mullerian hormone and follicle stimulating hormone with a *P*-value of 0.05 and between FF-T3 and E2 with a *P*-value of 0.02. **Conclusion:** Folliculogenesis and oogenesis may be significantly influenced by THs. The FF also contains T3 and T4, which could have an immediate impact on the oocyte.

Keywords: Intra-cytoplasmic sperm injection, thyroid function test, thyroid hormones

INTRODUCTION

The human follicular fluid (FF), which is composed of a diverse range of physiologically active chemicals, serves as the oocyte's microenvironment during its development and maturity. It is a byproduct of the blood-follicular barrier's translocation of plasma components and the secretory activity of granulosa and theca cells.^[1]

It is usually collected during the *in-vitro* fertilization (IVF)/intra-cytoplasmic sperm injection (ICSI) procedure. FF most likely provides a more accurate assessment of exposures that may have an impact on reproductive outcomes as it more clearly demonstrates the microenvironment surrounding the growing oocyte.^[2-4]

Ovarian physiology requires FF for steroidogenesis, follicle development, oocyte maturation, ovulation, and transport to the oviduct, among other processes.^[5,6]

Thyroid-releasing hormone is secreted by the hypothalamus and stimulates the thyrotrophs of anterior pituitary to release thyroid-stimulating hormone (TSH). Thyroxine T4 (80%) and triiodothyronine T3 (20%) are released by the thyroid follicular cells in response to TSH; however, the production of thyroid hormones (THs) is reliant on the presence of iodide. T4 must be peripherally converted into T3 in order to contact the thyroid hormone receptor (TR) and become physiologically active.^[7]

As de-iodinase (DIO) in peripheral tissue controls the biological effects of THs, blood TH levels may not

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necessarily anticipate tissue-specific effects in target organs, and local TH may have a direct impact on physiological processes.^[8]

Thyroid dysfunction is common in women of childbearing age and can negatively impact fertility and pregnancy, whether through spontaneous conception or through the use of assisted reproductive technology (ART).^[9]

The interactions that THs have with other hormones and growth factors, such as estrogen, prolactin (PRL), and insulin-like growth factor, as well as their ability to stimulate the release of GnRH in the hypothalamic–pituitary–gonadal axis, further explain how these hormones work indirectly. Consequently, changes in TH levels, such as hypothyroidism or hyperthyroidism, may cause subfertility or infertility.^[10]

Untreated hypothyroidism during pregnancy can result in fetal deaths, preterm births, and abortions. Infertile women with hypothyroidism have much higher (PRL) levels than euthyroid patients. Therefore, THs and serum PRL should be assessed in women trying to conceive as well as in infertile women.^[11]

In addition, the time after controlled ovarian hyperstimulation (COH) is known as the “implantation window,” during which the embryo’s early stages of development take place and THs are crucial for the preparation of the endometrium for pregnancy and the early phases of trophoblast development.^[12,13]

Oocyte and crucial activities of follicular maturation take place in an FF environment, so it is imperative to fully understand the precise elements that contribute to this process. Despite a wealth of evidence highlighting the significance of thyroid axis in both natural and assisted reproduction, the participation of thyroid axis in the ART process has received less research.^[14]

Although fertility issues may continue even after returning to normal thyroid function, the actual prevalence of infertility in women with thyroid diseases is unknown, and in those cases, ART may be required to achieve pregnancy.^[15]

The objective of this study is to look at the correlation of FF TH (T4 and T3) levels with the intra-cytoplasmic sperm injection (ICSI) outcomes.

MATERIALS AND METHODS

This study is a cross-sectional analytical study. Fifty subfertile couples from the ICSI attenders in the Fertility Center Al-Kafeel super-speciality hospital were involved in the study within the period from September 2021 to July 2022. Blood samples were taken at day 2 of female menstrual cycle for hormonal assay in addition to samples of FF, and blood was taken at oocyte-retrieval day.

Depending on the patient’s age, body mass index (BMI), and cause of infertility, COH programmes using recombinant follicular-stimulating hormone with pituitary desensitization by agonist/antagonist procedures were employed on the patients. Then, ultrasound and serial measurements of serum E2 were employed to track the maturation of follicles. Following ovulation induction with 10,000 IU of pregnyl [the human chorionic gonadotropin (HCG)], oocyte pick-up was carried out under general anesthesia and transvaginal ultrasound guidance within 36 h. Metaphase II (MII) oocytes were injected and incubated under specialized culture conditions throughout the ICSI process. About 16–18 h after injection, the fertilization was evaluated by figuring out the two pronuclei (2PN). The highest grade embryos, not more than three, were transplanted on day 2 or 3 following injection. The quantity and maturity of the selected oocytes, the ICSI results in terms of the quantity of 2PN, the quantity and quality of the embryos, the quantity of transferred embryos, the fertilization rate, the cleavage rate, and the pregnancy rate have all been confirmed and statistically explained.

Statistical analysis

The SPSS 23 program and Microsoft Office Excel 2010 were used to analyze the data (Statistical Package for Social Sciences). Number and percentage were used to describe categorical data, whereas mean and standard deviation were used to represent numerical data. Independent sample *t*-test was used to compare two numerical variables, whereas the χ^2 test was used to determine the relationship between categorical data. The correlation between variables is displayed by Pearson’s correlation test. Less than or equal to 0.05 is the critical *P*-value.^[16]

Ethical approval

The study was carried out in conformity with the moral standards set forth in the Declaration of Helsinki. Before a sample was taken, it was done with the patient’s verbal and analytical consent. A local Ethics Committee examined and approved the study protocol, subject information, and permission form in accordance with document number 2483 (containing the number and date in 2/9/2021).

RESULTS

Table 1 shows the main demographic information of the studied Patients. The results of Table 2 showed that there is no statistically significant value of both FF-T4 and FF-T3 on pregnancy outcomes.

Table 3 shows that FF-T4 correlates positively with MII and 2-PN in a statistically significant way (*P* = 0.04 and 0.03, respectively).

FF-T3 correlates positively with oocyte number and MII in a statistically significant way (*P* = 0.01) and 2-PN in a statistically significant way (*P* = 0.0).

Table 1: The main demographic information of the studied patients

Variables	Values
Number	50 couples
Age (years)	31.52 ± 5.91 (19–46)
Body mass index (kg/m ²)	27.71 ± 4.81 (21.6–44.9)
Duration of subfertility (years)	8.53 ± 5.18 (1.5–19)
Type of subfertility (%)	Primary 37/50 (74%) Secondary 13/50 (26%)
Etiology of subfertility(%)	Male 19/50 (38%) female 6/50 (12%) Combined 16/50 (32%) Unexplained 9/50 (18%) FF-T4 (ng/dL) 9.14 ± 1.53 FF-T3 (ng/mL) 1.53 ± 0.155

FF-T4: follicular fluid thyroxine, FF-T3: follicular fluid triiodothyronine

Table 2: Comparison between FF-T4 and FF-T3 mean ± SD in pregnant and non-pregnant females

T4, T3	Mean ± SD (pregnant female)	Mean ± SD (non-pregnant female)	P-value
FF-T4 (ng/dL)	9.14 ± 1.53	9.20 ± 1.46	0.69
FF-T3 (ng/mL)	1.53 ± 0.13	1.52 ± 0.16	0.92

FF-T4: follicular fluid thyroxine, FF-T3: follicular fluid triiodothyronine

Table 3 shows a significant positive correlation of FF-T4 with AMH and FSH in a $P < 0.05$ and a significant positive correlation of FF-T3 with E2 in a $P = 0.02$.

DISCUSSION

In spite of no significant effect of T4 and T3 on pregnancy rate in our study [Table 2] which might be attributable to all patients have FF-THs levels within the normal range [Table 1]; but there were many studies suggests that thyroid function affects pregnancy rates, especially in IVF^[8]. Reduced THs levels may affect reproduction, in the form of relationship of T4/T3 with a successful pregnancy and an appropriate embryonic developmental stage. Women with hypothyroidism may experience reduced pregnancy success rates following IVF. Therefore treatment of women with reduced THs levels in the form of subclinical hypothyroidism or overt hypothyroidism has been proven to improve IVF outcomes, including raising the rates of implantation, clinical pregnancy, and delivery.^[17] The positive significant effect of FF-THs with total oocyte number and maturity (MII oocytes) in addition to 2PN fertilization [Table 3] could be explained by the presence of both T3 and T4 in the FF, making a direct action on the oocyte conceivable. In addition to the expression of thyroid receptors (TRs) on the oocyte, granulosa, ovarian stroma, and cumulus cells. It has been established through in-vitro experiments in

Table 3: Correlation of FF-T3 and FF-T4 with oocyte number, MII oocytes, MI oocytes, GV oocytes, 2 PN, 0 PN, 3 or more PN

Thyroid hormones	Corr.	Oocyte number	MII	MI	GV	2 PN	0 PN	3 or more PN
FF-T4	<i>r</i>	0.25	0.28*	0.25	-0.05	0.30*	0.02	-0.12
	<i>P</i>	0.07	0.04	0.07	0.69	0.03	0.86	0.40
FF-T3	<i>r</i>	0.34**	0.35**	-0.16	-0.16	0.40**	-0.10	-0.04
	<i>P</i>	0.01	0.01	0.24	0.25	0.00	0.46	0.78

FF-T4: follicular fluid thyroxine, FF-T3: follicular fluid triiodothyronine, MII: metaphase 2 oocyte, MI: metaphase 1 oocyte, GV: germinal vesicle oocyte, PN: pronuclei

Table 4: FF-T4 and FF-T3 relate to other hormones

	Corr.	E2D2	E2	AMH	FSH	LH	PRL
FF-T4	<i>R</i>	0.16	0.15	0.31*	0.35*	0.18	-0.14
	<i>P</i>	0.26	0.27	0.02	0.01	0.19	0.30
FF-T3	<i>r</i>	-0.16	0.31*	0.26	0.17	0.24	-0.05
	<i>P</i>	0.25	0.02	0.05	0.22	0.08	0.70

FF-T4: follicular fluid thyroxine, FF-T3: follicular fluid triiodothyronine, E2: estradiol, AMH, anti-mullerian hormone, FSH: follicle-stimulating hormone, LH: luteinizing hormone, PRL: prolactin

rats that THs stimulate the development of preantral follicles and so its maturation. Therefore that significant effect might be a key factor in predicting the success of ART which could be gathering by: embryo quality, a higher mean number of retrieved oocytes, MII oocytes, blastomeres that found in the successful pregnancy group rather than in the implantation failure group. According to Kamron's study, inappropriate thyroid function in subfertile women may be an indication that they are unable to perform essential reproductive processes such as ovulation, fertilization, and also affect oocyte quality.^[18]

The positive correlation of FF-T3 and E2 at day of HCG trigger [Table 4] indicate that FF-T3 increase granulosa cell proliferation while lowering apoptosis when combined with FSH. The interaction between T3 and gonadotropic hormones also prevents theca cells from producing too many androgens and promotes aromatization, which causes granulosa cells to produce more estrogen.^[19]

The positive correlation of FF-T4 with AMH could be clarified by granulosa cells express THs transporters and receptors, TH deficiency affects follicle development and results in atresia, which lowers AMH levels in addition to the positive correlation of FF-T4 with FSH [Table 4] that proof the essential role of THs in serving as a beneficial function in follicle growth, follicular development, ovulation, in addition to the synergistic effects of FSH by suppressing apoptosis and enhancing the effects of gonadotrophins.^[18]

CONCLUSION

FF-T4 had a considerable correlation that is positive with MII in addition to 2PN, whereas FF-T3 had a strong correlation with oocyte number in addition to MII as well as 2PN.

Regarding the relation of FF-T4 and FF-T3 with the other hormones such as FSH, AMH, and E2 levels, FF-T4 was found to have a positive association with FSH and AMH, in addition to FF-T3 levels, which were found to have strong associations with E2.

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Conflicts of interest

There are no conflicts of interest.

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