



## Evaluation of FSH, LH ,Testosterone , Prolactine, TSH and T4 hormones levels in different subgroups of infertile males.

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### Abstract

Gonadotropins (FSH, LH) and testosterone are the prime regulators of germ cell development. Abnormal spermatogenesis is often associated with altered serum gonadotropins and testosterone. the potential relationship between other hormones, including thyroid hormones, and semen quality are still not completely understood. Thus in the present study an attempt has been made to report the degree of associations between thyroid hormones and semen quality. FSH, LH .T. PRL.T4 and TSH.hormones levels were estimated in 39 infertile men of whom 5 were azoospermia, 13 were oligozoospermic, 21 were with asthenozoospermia . Results showed statistically significant differences ( $P \leq 0.05$ ) in the mean FSH, Testosteron (T.), PRL. And T4. Levels in all the infertile males studied when compared with the fertile controls ( $n=39$ ). However, there is no significant difference in the mean levels of LH and TSH between the infertile and fertile men. Present study found that though men with abnormal semen profile had slightly higher in T4 concentrations in asthenozoospermia group as compared to those with normal semen profile. This study also show positive correlation between T4 and T. ( $R^2=0.408$ ) and negative correlation between T4 and FSH was ( $R^2=-0.515$ ), T4 and LH was ( $R^2=-0.345$ ).

**Keywords:** Gonadotropins, Thyroid hormone, spermatogenesis, male infertility.

### Introduction

Male infertility is a problem of the reproductive system, and the word infertility itself means no fertile, and that would be equivalent to sterility [1], sterility means that a man is totally unable to have a child [2]. The World Health Organization (WHO) and the American Society for reproduction Medicine Practice Committee defines infertility as no conception after at least 12 months of unprotected sexual intercourse [3] .

In the post pubertal male, the gonadotropins follicle stimulating hormone (FSH), and luteinizing hormone (LH), regulate spermatogenesis and steroidogenesis, respectively. LH and FSH are produced by the anterior pituitary. The production of these two hormones is stimulated by gonadotropin releasing hormone (GnRH) made by the hypothalamus. It is largely known how LH, FSH and testosterone (T.) regulate spermatogenesis. Testosterone is required for successful completion of the spermatogenesis process. Without it, conversion of round spermatids to spermatozoa during spermiogenesis is impaired [4,5]. It should be noted that follicle-stimulating hormone (FSH) plays important role in this conversion, as well as differentiation of spermatogonia into spermatocytes [6]. Since the germ cells have no receptors for testosterone and FSH, these hormones must act through the Sertoli cells, which are responsible for nurturing the germ cells. Testosterone is produced by the Leydig cells after receiving the signal from luteinizing hormone (LH) for its synthesis. This pathway is collectively known as the hypothalamus-pituitary-gonadal axis (HPG axis). PRL hormone secreted from anterior pituitary, has detrimental effect on male fertility when it exceeds its physiological level [7].

Thyroid hormones have a central role in controlling basal metabolic rate, growth, as well as the development and differentiation of many cells in the body [8], research is now actively being pursued to understand the primary effects of thyroid hormones on spermatogenesis. For this reason, the potential of thyroid hormone in the



modulation of male reproductive function was not determined. However, in the past two decades, clinical studies have demonstrated that thyroid hormone plays an important role in testicular development and function. It is now established that T4, T3 regulates the maturation and growth of testis, controlling Sertoli cell and Leydig cell proliferation and differentiation during testicular development in rats and other mammal species [9,10]. The efficiency of spermatogenesis, reflected by daily sperm production in adulthood, correlates to the total number of functional Sertoli cells established during prepubertal life [11]. Furthermore, changes in thyroid hormone levels during early testis development have been shown to affect testicular maturation and reproduction later in life [12].

**Material And Method**

**Collection of Semen Samples**

Semen was collected from seventy eight men aged (26-35) years old, about 39 suffered from infertility men and 39 healthy fertile men as control, by masturbation into a sterile plastic specimen cup at the hospital. Subjects were instructed to abstain from ejaculation for at least 48 hours prior to performing a routine the semen sample. The sample was liquefied for at least 20 minutes, but no longer than 1 hour prior to performing a routine semen analysis, which included measurements of volume, sperm count, sperm motility, and sperm morphology. The samples were collected in Fertility Center Laboratories / AL-Sadder Medical City at AL-Najaf province

**Sperm morphology:**

Ideally, a good sperm should have a regular oval head, with a connecting mid-piece and a long straight tail. If too many sperms are abnormally shaped (round heads; pin heads; very large heads; double heads; absent tails) this may mean the sperm are abnormal and will not be able to fertilize the egg.

$$\text{Percentage of Normal sperms morphology} = \frac{\text{No. of normal sperms}}{\text{Total no. of spermatozoa count}} \times 100$$

**sperm viability:**

Sperm viability was assessed using eosin staining. One drop of prepared semen was mixed with one drop of eosin solution on a microscope slide, covered with cover slip and examined after 30 seconds under the microscope (400 X). The slide had to be assessed immediately. Live spermatozoa were unstained (white), while dead cells are stained red. The percentage sperm viability was estimated below:

$$\text{Sperm viability \%} = \frac{\text{No. of alive spermatozoa}}{\text{Total No. of spermatozoa}} \times 100$$

**Sperm motility:**

Ten Ml of well mixed semen was put on a clean microscope slide and covered with cover slip. Assessment of sperm motility should begin immediately to avoid artifacts caused by either a temperature decrease or dehydration of the preparation. Spermatozoa with pin heads or free tails should not be counted. Percentage of sperm motility was assessed as following:

The total number of spermatozoa in each motility group was divided on the total number of spermatozoa assessed in each field, Sperm analysis was carried out according to the World Health Organization [13] guidelines. Based on the sperm concentration the infertile subjects were classified as follows: Normozoospermia (> 20 million sperm /ml), Oligozoospermia (<20 million sperm/ml) and azoospermia (no



spermatozoa). In proven fertile controls, the sperm count ranged from 20 –120 million sperm /ml.

blood sample obtained from these patients and control, serum separated on the same day that the semen sample was collected and stored for further analysis and assayed for hormonal levels (LH, FSH , PRL, T., TSH andT4) were achieved by using immunoassay methods of TSH, T3, T4 and PRL, (ELISA kits from Monobind ,USA ) The reference values that used were: LH, 0.8-7.6  $\mu$ IU; FSH, 0.7-11.1  $\mu$ IU/ml; and PRL. 2.5-15 ng/ml. T.,3-10 ng/ml ;TSH..25-5 $\mu$ IU/ml and T4 0.52-1.6 ng/ml .

**Statistical analysis**

Statistical analysis was done by using Mega stat and SPSS software, version 12 (SPSS Inc, Illinois, USA) a P-value  $\leq 0.05$  was considered statistically significant.

**Results**

Thirty – nine men (age 26 to 35 years) of infertile couple were divided into two groups on the basis of semen profile as per WHO protocol [13] .

**control** Comprised of 39 men of age group 26 to 35 years having normal semen parameters (Table 1). **Infertile men** Comprised of 39 men of age group 26 to 35 years having abnormal semen parameters (Table 1).

**Table- 1 : Comparison of Semen profile of normal and abnormal subjects (Mean  $\pm$  SD)**

characterestics	Infertile men (Mean $\pm$ SD)	Control (Mean $\pm$ SD)	Pvalue
Sperm count %	32.6 $\pm$ 21.12	62.6 $\pm$ 13.47	1.14E-10
Sperm motility %	24.32 $\pm$ 15.47	76 $\pm$ 9.9	3.45E-28
Sperm morphology %	26 $\pm$ 17.9	69.1 $\pm$ 12.2	6.55E-20

P $\leq 0.05$

Table 1 shows the various semen characteristics of study population. In group A sperm counts with mean of 62.6  $\pm$  13.47, percentage of sperm motility with mean of 76  $\pm$  9.9 and percentage of normal sperm morphology mean of 69.1  $\pm$  12.2 , whereas in group B sperm counts mean of 32.6  $\pm$  21.12 , percentage of sperm motility with mean of 24.3 $\pm$ 15.47 percentage of sperm morphology with mean of 26 $\pm$ 17.9

**Table-2 :Distribution of sperm abnormalities in infertile subjects**

Sperm abnormality	No.(%)
azoospermia	5(15.3)
oligozoospermia	13(33)
asthenozoospermia	21(43.5)

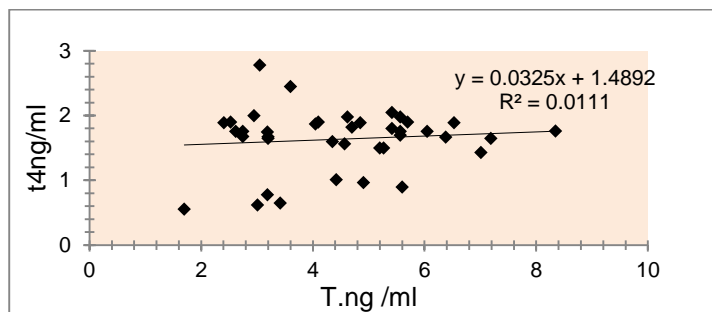
Out of 39 abnormal subjects, azoospermia 5(15.3%) Oligozoospermia was found in 13 (33%) and 43.5% asthenozoospermia in 21.

**Table-3: Comparison of hormones profile in normal subjects and men with various sperm abnormalities**

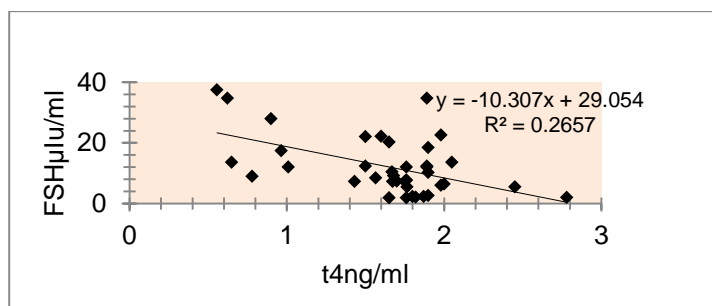
groups	FSH $\mu$ IU/ml	LH Miu/ml	PRL. g/ml	T.ng/ml	TSH $\mu$ IU/ml	T4ng/ml
control	4.99 $\pm$ 1.75	4.610 $\pm$ 2.015	7.465 $\pm$ 3.32	7.42 $\pm$ 2.13	2.24 $\pm$ 1.5	0.939 $\pm$ 0.29
Infertile men	12.206 $\pm$ 9.45*	5.07 $\pm$ 3.97	11.4 $\pm$ 3.82*	4.48 $\pm$ 1.5*	2.05 $\pm$ 1.89	1.63 $\pm$ 0.47*
Oligozoospermia	15.88 $\pm$ 6.04	3.63 $\pm$ 2.30	13 $\pm$ 2.16	4.87 $\pm$ 0.78	1.98 $\pm$ 2.02	1.47 $\pm$ 0.41
azoospermia	28.5 $\pm$ 10.27	13.38 $\pm$ 2.24	15.89 $\pm$ 1.21	4.5 $\pm$ 1.5	2.39 $\pm$ 2.5	1.06 $\pm$ 0.63
Asthenozoospermia	5.73 $\pm$ 3.56	3.98 $\pm$ 2.44	9.81 $\pm$ 3.7	4.37 $\pm$ 1.7	2.02 $\pm$ 1.72	1.82 $\pm$ .37

\*P $\leq$ 0.05

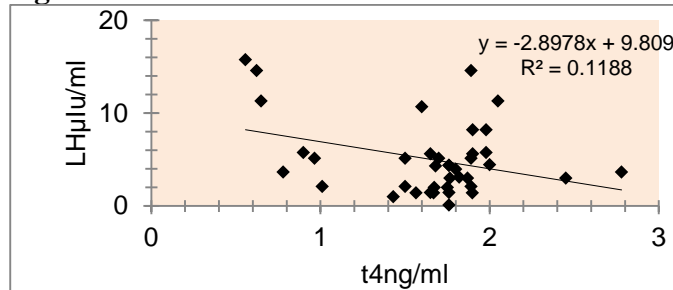
(Table 3) revealed FSH of oligozoospermia men with mean of 15.88 $\pm$ 6.04 Miu/ml, LH with the mean of 3.63 $\pm$ 2.30 Miu/ml, PRL. With the mean of 13 $\pm$ 2.16 ng/ml , T. with the mean of 4.87 $\pm$ 0.78 ng/ml, TSH (Miu/ml) with the mean of 1.98 $\pm$ 2.02 and T4 with mean of 1.47 $\pm$ 0.41 ng/ml. FSH of azoospermia men with mean of 28.5 $\pm$ 10.27 Miu/ml, LH with the mean of 13.38 $\pm$ 2.24 Miu/ml , PRL. With the mean of 15.89 $\pm$ 1.21 ng/ml ,in asthenozoospermia there is no elevated in FSH ,LH and PRL. ,there is slightly increasing T4 but none of the TSH showed any significant difference when compared with men having normal semen profile, whereas T. not revealed differences among infertile group but significant differences with control (P  $\leq$ 0.05). fig. 1 show positive correlation between T4 and T. hormones R<sup>2</sup>=0.011 (P= 0.408) . Fig. 2 revealed -ve correlation between T4 and FSH. Hormones (P= -0.515). Fig. 3 show negative correlation between T4 and LH hormones R<sup>2</sup>=0.265 (P= -0.345).



**Figure 1: correlation between T4 and T. hormones P= 0.408.**



**Figure 2: correlation between T4 and FSH hormones P= -0.515.**



**Figure 3: correlation between T4 and LH hormones P= -0.345.**

## DISCUSSION

In current study was revealed highly significant in sperm quality of infertile men and control, table 1 agree with study [14], semen analysis revealed occurrence of 15.3% azoospermia, 33% oligozoospermia and 43.5% asthenozoospermia in table 2, agree with [15] this result may belong to the disturbance in proper function of a complex system of organs and local balance between androgen and estrogen which is important for spermatogenesis [16].

In the present study table 3 shows significantly ( $P \leq 0.05$ ) higher the serum levels of FSH in the infertile men, when compared with the levels in proven fertile controls, consistent with those in several previous studies [17,18] higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage, and was shown to be associated with azoospermia and severe oligozoospermia [19]. Also in table 3 show elevated levels of LH in azoospermic males when compared to normal fertile men were also reported [20]. FSH, LH and testosterone evaluation is useful in the management of male infertility [21].

In the current study finding the significant difference in the mean serum testosterone levels between fertile and infertile men Significant decrease in testosterone value, the elevated levels of FSH in the presence of low testosterone levels correlate with primary hypogonadism. T. value in this study within normal range but lower than control, is required for successful completion of the spermatogenesis process. This agree with [14].

Mean PRL level in table 3, observed in azoospermia and oligospermia was higher, this result similar to [22], Although the functional significance of PRL. To male reproduction has not been unequivocally established, the hormone has been associated primarily with male infertility. Acute hyperprolactinemia is known to suppress testosterone synthesis and male fertility through It is well known that hyperprolactinemia decreases libido and causes oligozoospermia [23]. On the other hand, hyperprolactinemia is caused by or associated with, a variety of pathogenic stages: pituitary adenoma, hypothalamic disorders, hypogonadism and hypothyroidism, and is detected in patients with infertility [24] This might indicates some disturbance in the spermatogenesis process.

In the current study T4 was within normal limits in both the study group, but its level was significantly increased in men with abnormal semen parameters, observed that T4 was greater in men with asthenozoospermia and oligospermia agree with a few studies [25]. [26] investigated three young with high value of T4, found that two patients presented marked oligospermia with decreased motility, whereas the third patient had a borderline low sperm count with decreased motility However, such abnormalities were corrected when the patients were treated for thyroid disease.



Therefore, [26] concluded that male infertility is more common than previously thought in males with hyperthyroidism, possibly in correlation with elevated levels of testosterone, LH and FSH. [27] investigated five patients with increased T4 and found that all had low total sperm counts. In addition, forward progressive sperm motility in these patients was significantly reduced compared with normal males [28]. More recently, [29] investigated the effect of hyperthyroidism on spermatogenesis in 21 hyperthyroid patients: nine patients (43%) had low total sperm count and (61.9%) displayed progressive motility abnormalities several experimental and clinical studies have demonstrated. [7] found the excess T4 induces abnormalities in sperm motility as well as T4 effect on Oxidative stress has been identified as one of the very important factors that affect fertility status, and has been extensively studied in recent years. Sperm, like any other aerobic cells, are constantly facing the “oxygen-paradox”. Oxygen is essential to sustain life as physiological levels of ROS are necessary to maintain normal cell function and all that is true for sperm as well. However, excessive production of ROS (oxidative stress) is well known to be detrimental to sperm by adversely affecting the quality of sperm DNA. The main function of thyroid hormone within physiological ranges is to regulate and enhance metabolic reaction and oxygen consumption of different cells of the body. ROS which are the by-products of tissue metabolism are normally treated by physiological antioxidants. The role of thyroid in regulating oxidative stress in male reproductive organs is recently being explored. positive correlation between T4 and testosterone as in fig 1 agree with [30] reported Administration of excess T4 to mature male rats resulted in a decrease in total lipids, cholesterol, and phospholipids in testes, and rats rendered thyrotoxic by T4 administration were increased amounts of T. this may explain present result about positive correlation of T4 and T. In this study.

[31] found, the administration of T4 to intact rats resulted in decreased serum gonadotropin (Gn) levels this explain negative correlation between FSH, LH and T4 fig. 2 and fig.3 respectively. TSH was not found to be significantly different in this study agree with [32].

## **Conclusion**

In the present study, the hormonal profile of the subjects 52 characterized as oligozoospermic, azoospermic and asthenozoospermic after their semen analysis showed an elevated level of FSH and abnormal levels of LH and testosterone in oligozoospermic, whereas in azoospermic group suffered from high levels in FSH, and PRL. In asthenozoospermic group there is some men had elevated T4 hormone.

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