

A CORRELATIVE INTERACTION BETWEEN THYROID DYSFUNCTION AND SEMEN PARAMETERS IN MALE INFERTILITY: A PROSPECTIVE CASE CONTROL STUDY

M. VENKATESWARA RAO^{1*}, K. V. N. GEETHA DEVI², D. RAJARAJESWARI³, K. ASHALATHA⁴

¹ Department of Anatomy, Narayana Medical College, Nellore, Andhra Pradesh, India.

² Department of Anatomy, ACSR Government Medical College, Dargamitta, Nellore, Andhra Pradesh, India.

³ Department of Biochemistry, Narayana Medical College, Nellore, Andhra Pradesh, India.

⁴ Department of Anatomy, Narayana Medical College, Nellore, Andhra Pradesh, India.

Abstract

The role of thyroid function on sperm quality has not been well studied from a pathological aspect. This study aimed to report the degree of association between the status of thyroid hormones, sperm quality and aetiology in infertile men compared to healthy subjects. A prospective case control investigative study was conducted on 100 infertile males and age matched healthy controls. Semen samples were collected for sperm quality examination, and the serum levels of tetraiodothyronine (T4), triiodothyronine (T3), and thyroid stimulation hormone (TSH) were measured. Out of 100 infertile men, oligozoospermia (32%), asthenozoospermia (48%), and oligo-asthenozoospermia (20%) were found. There was a statistical difference between the group I and group II groups related to sperm count (28.32 ± 14.60 vs $66.50 \pm 10.50 \times 10^6/\text{ml}$), sperm motility (40.1 ± 13.8 vs $64.8 \pm 7.85\%$), and sperm morphology (55.92 ± 5.27 vs $83.50 \pm 5.25\%$, $p < 0.05$). There was a statistical difference among the oligozoospermia, asthenozoospermia, and oligo-asthenozoospermia groups related to T3 (115 ± 0.40 vs 1.29 ± 0.59 vs 1.25 ± 0.32 ng/ml), T4 (7.35 ± 1.42 vs 9.15 ± 1.85 vs 7.85 ± 1.65 µg/dl), and TSH (1.69 ± 0.55 vs 2.12 ± 1.45 vs 1.98 ± 0.4 µIU/ml) ($P < 0.05$). There was a significant inverse correlation of TSH levels with sperm volume ($r = -0.12$, $p = 0.02$), sperm motility ($r = -0.26$, $p = 0.02$), and sperm morphology ($r = -0.304$, $p = 0.02$) observed. T4 levels were significantly correlated with sperm count ($r = -0.278$, $p = 0.02$), and sperm motility ($r = -0.249$, $p = 0.032$). T4 levels were very highly associated with asthenozoospermia. Relative operating curve analysis shows that Sperm motility of $>40.1\%$, T3 levels of <1.29 ng/ml, total T4 levels of <8.42 µg/dl, TSH levels of <1.98 µIU/ml inferred the male infertility.

Conclusion: Although thyroid function screening is not currently recommended as a part of the diagnostic workup of the infertile male, it may be reconsidered in light of the physiopathological background. Studies will be necessary to initiate the trial of a small dose of anti-thyroid drug in asthenozoospermic patients.

Keywords: Male infertility, Thyroid hormones, Oligozoospermia, Asthenozoospermia.

INTRODUCTION

Infertility estimates ~15% of couples globally. Males are found to be solely responsible for 20-30% of infertility cases, and contribute to 50% of all cases (1). According to the Indian Society of Assisted Reproduction, infertility affects 10 to 14% of the Indian population, with higher rates in urban areas (2). In India, nearly 27.5 million couples are actively trying to conceive and suffer from infertility (3). According to the National Health Portal of India, 5% of infertility seen in Andhra Pradesh state and 15% of infertility in Kashmir state (4, 5).

Corresponding author: Dr. M. Venkateswara Rao; mvraosj@yahoo.co.in

© 2022 Venkateswara Rao et al.

This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 License (<https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Previous research shows that a significant proportion of male infertility is idiopathic, and the underlying molecular mechanisms is still unknown (6). The significant factors of male infertility are low sperm concentration (oligospermia), poor sperm motility (asthenospermia), and abnormal sperm morphology (teratospermia), and other less associated male infertility factors are semen volume and seminal markers of epididymal, prostatic, and seminal vesicle function (7, 8). Although several causes of male infertility have been identified (9), the exact aetiology and pathogenesis of male infertility remain unknown (10, 11). Infertility aetiology varies from region to region, population to population, and even locality to locality within the same population (12).

Although the effects of hyperthyroidism and hypothyroidism on female reproduction are well established, the effects of thyroid disorders on male infertility have not been extensively studied, most likely because attention in thyrotoxic males is usually focused on other manifestations of the disease, and fertility status is frequently not evaluated (13,14). Thyroid hormones interfere with both androgen biosynthesis and spermatogenesis, either directly on Leydig and Sertoli cells or indirectly by modulating gonadotropin secretion (15). Thyroid hormones act on various cells of the male reproductive system via genomic and nongenomic mechanisms to regulate testicular testosterone secretion and the concentration of seminal plasma components such as calcium, fructose, magnesium, zinc, etc.

The optimal concentration of intratesticular testosterone promotes spermatogenesis, while other seminal properties improve sperm motility and viability and keep the sperm volume stable (16). Thyrotoxicosis can cause oligozoospermia, asthenozoospermia, and/or teratozoospermia, as well as decreased sperm volume (17). Hypothyroidism has also been associated with a decrease in serum testosterone levels. Hence, it is associated to erectile dysfunction, delayed ejaculation, hypoactive sexual desire, and poor sperm quality (18).

Hence, developing an effective preventive therapeutic intervention for male infertility necessitates a thorough understanding of the relationship between thyroid function and sperm quality. Extensive research is required to reveal the role of thyroid function and its disorders in the maintenance and deterioration of male infertility and to draw more reliable conclusions that can be used in clinical practise.

Hence, the current study investigated the relationship between thyroid hormone levels and sperm quality in a population of men recruited from an infertility clinic to detect associations between thyroid hormone levels and sperm quality and also with aetiology.

METHODS

A prospective case control investigative clinical Study conducted from September 2019 to September 2022 at the department of Anatomy, Narayana Medical College, Nellore, Andhra Pradesh.

Sampling Technique and Sample Size

The current study was carried out by a random sampling technique selecting the infertile males those were attending to the fertility clinic.

The sample size calculation was done by formula,:

$$\text{Sample size (n)} = Z^2 PQ / d^2$$

Where, Z^2 value is fixed at 95% of confidence interval was 1.96,.

Prevalence (P) = 7%, i.e., 0.07 (based on previous studies)

Q = 1-P.

Error (d) is fixed at 5%, i.e., 0.05.

$$\begin{aligned} \text{The sample size (n)} &= (1.96*1.96) *(0.07) *(0.93) / (0.05*0.05) \\ &= 0.2501 / 0.0025 = 100.04 \Rightarrow 100. \end{aligned}$$

Therefore, the sample size = 100.

A total of 100 group I (male infertility) cases, and an age matched 100 group II subjects (fertile male) were enrolled in the study. The Institutional Ethics Committee, Narayana Medical College, Nellore, India; an approval has been taken for the study protocol. A written informed consent form was taken from all the study subjects.

The study groups:

Group I (case) – Infertile males who fits into the inclusion criteria.

Group II (control) – Age matched fertile males without any co-morbidities.

Inclusion criteria

- Males with >24 years of age having no children for more than 2 years; even with unprotected sex and without achieving pregnancy.
- Physically and Mentally healthy males.
- Males without any congenital anomalies and co-morbidities.

Exclusion criteria

- Males with prostate cancer.
- Males with erectile dysfunction.
- Males with venereal diseases.
- Males with HIV.
- Psychologically unhealthy males.
- Males with co-morbidities like diabetes (both I & II), renal pathology.
- Patients with congenital anomalies of genital organ like hypospadias.
- Klinefelters syndrome.
- Hypogonadotropic hypogonadism.
- Y-chromosome microdeletion or abnormality.
- Obstructive azoospermia.
- History of treatment with Cytotoxic drugs, irradiation, or sulfasalazopyrine.
- Patients who refuse to participate in the study.

Data collection:

Interviews for medical and nutritional history were part of the participant's examination.

Semen analysis:

After the detailed explanation about the procedure, the subjects were instructed to refrain from ejaculating for at least 72 hours before providing a sample of sperm. Masturbation in a clean wide mouth plastic container provided by the laboratory yielded the entire sample. The container was labelled with the patient's details, and the time and date of collection. The sample was liquefied for at least 20 minutes, but no more than 1 hour before performing a routine semen analysis, which included volume, pH, sperm concentration, sperm motility, progressive motility, and sperm morphology measurements. A fructose biochemical test was also performed. Sperm count performed using a Neubauer counting chamber and stained with Field's stain to check the sperm morphology.

Motile sperm was defined according to WHO grade as 'A' grade sperm (rapidly progressive with a velocity >25 $\mu\text{m/s}$ at 37°C) and 'B' grade sperm (slow/sluggish progressive with a velocity >5 $\mu\text{m/s}$ but <25 $\mu\text{m/s}$) (19).

Method for sperm motility: Morphological evaluation was carried out using an Olympus microscope and oil immersion. A coverslip was covered after placing a drop of sperm on the glass slide and examined under high power (40 ×) field. Motile spermatozoa were counted out of the 100. The percentages of motile and nonmotile spermatozoa were recorded.

Method of Sperm count: Semen was diluted 1:20 with formalin (0.1 ml semen and 1.9 ml formalin) and added the Neubauer++ chamber with diluted semen and allowed to settle for 15 minutes. The chamber was placed under the microscope and spermatozoa counted in 4 large corners squares using either 20 × or 40 × objective.

A drop of semen was placed on a glass slide, and prepared a smear, and stained the smear with field stain. Approximately 200 spermatozoa were counted under oil immersion. The percentage of normal & abnormal spermatozoa was recorded (20).

Sperm count per ml was calculated as follows:

Sperms counted (N) × correction factor for dilution (20)

= ----- × 1000

Number of squares counted (4) × volume of one square (0.1)

= N × 50000/ml.

On the same day of the sperm sample was collected, a blood sample was drawn using an aseptic technique after a 12-hour overnight fast. Sera were extracted from the blood samples. Total T₃, T₄, and TSH levels were assessed using Chemiluminescence Immunoassay (CLIA) assay.

Statistics:

The data was entered into a MS-Excel, and the statistics performed using the statistical package for Social Science Program (SPSS, IBM, US) for Windows Version 25.0. The data values for categorical variables were expressed as numbers and percentages, and the chi-square test were used to test the association between the groups. The data values for the continuous variables were expressed as mean and standard deviation, and to test mean differences between the two groups, the student's t-test was used. One-way analysis of variance (ANOVA) test was used to test mean differences among three or more groups. Pearson's correlation analysis was used to test the relationships between the parameters. All p-values less than 0.05 are considered statistically significant.

RESULTS

The Group I consists 100 men with a mean age of 32.4 ± 4.5 years (range: 25- 40 years), and the group II comprised of 100 men with a mean age of 31.5 ± 4.8 years (range: 25 to 40 years).

In the group I, sperm counts varied from 9 to 60 million/ml with a mean of 28.32 ± 14.60, the percentage of sperm motility varied from 15 to 70 with a mean of 40.1 ± 13.8, and the percentage of normal sperm morphology varied from 40 to 70 with a mean of 55.92 ± 5.27. Whereas in the group II, the percentage of sperm counts varied from varied from 45 to 80 million/ml with a mean of 66.5 ± 10.5, the percentage of sperm motility with a mean of 64.8 ± 7.85, and the percentage of normal sperm morphology with a mean of 83.5 ± 5.25 with a statistical difference in sperm quality between the two groups (p<0.05) (Table 1).

Out of 100 infertile male, oligozoospermia was observed in 32 cases (32%, 95% confidence interval [CI], 27.5% - 35.5%), asthenozoospermia in 48 cases (48%, 95% confidence interval [CI], 41.5% - 50.5%), and oligo-asthenozoospermia in 20 men respectively (20%, 95% confidence interval [CI], 16.85% - 22.8%).

Total T₃ of oligozoospermic men ranged from 0.60 to 1.61 with mean of 1.15 ± 0.4, total T₄ varied from 5.5 to 9.1 with a mean of 7.35 ± 1.42, and TSH (μIU/ml) ranged from 0.89 to 2.65 with a mean of 1.69 ± 0.55.

Whereas in asthenozoospermic men total T₃ ranged from 0.72 to 2.65 ng/ml with a mean of 1.29 ± 0.59, total T₄ varied from 6.98 to 12.8 with the mean of 9.15 ± 1.85, and TSH (μIU/ml) ranged from 1.1 to 6.75 with a mean of 2.12 ± 1.45. Only total T₄ was significantly higher in asthenozoospermic men compared to those with normal sperm profiles (P<0.05), but it was still well within the normal limits.

Total T₃ (ng/ml) of men with oligo-asthenozoospermia ranged from 1.12 to 1.70 with a mean of 1.25 ± 0.32, total T₄ varied from 5.5 to 10.5 with the mean of 7.85 ± 1.65, and TSH ranged from 1.60 to 2.20 with a mean of 1.88 ± 0.4.

After adjusting for potential confounders, the study revealed no significant differences in sperm volume, concentration, progressive sperm motility, or morphology between the euthyroid and SCH groups (p>0.05) (Table 2) (Table 3).

Table 1 Comparison of semen and thyroid profile of normal and abnormal subjects

Characteristics		Group I (n=100)	Group II (n=100)	P value
Age (Years)	Mean $\pm$ SD	32.4 $\pm$ 4.5	31.5 $\pm$ 4.8	
Age (yr)	21-25	10%	10%	0.15
	26-30	56%	58%	
	31-35	26%	23%	
	36-40	8%	9%	
Duration of active married life (yr)	2-3	35	34	0.26
	3.1-5	33	30	
	5.1-7	20	18	
	7.1-9	8	10	
	9.1-15	4	8	
Semen analysis : Quantity (c.c.)	<1	16	2	0.0001*
	2	57	63	
	2.1-3	16	15	
	>3	12	20	
Semen analysis : Sperm density (million/ml)	>15	48	100	-
	10.1 – 15	29	0	-
	<10	23	0	-
Semen analysis : Sperm motility	Actively motile	32	100	-
	Sluggishly motile	40	0	-
	Non-motile	28	0	-
Semen analysis : Frequency of motile sperm %	>50	22	100	-
	20.1-50	48	0	-
	5-20	30	0	-
Semen analysis : Pus cell/HPF	>10	16	-	-
	4-10	29	-	-
	2-3	55	10	-

Continuation of table 1

Sperm count (10 ⁶ /ml)	Mean ± SD	28.32 ± 14.60	66.5 ± 10.5	<0.0001*
Sperm motility (%)	Mean ± SD	40.1 ± 13.8	64.8 ±7.85	0.0045*0.03
Normal morphology (%)	Mean ± SD	55.92 ± 5.27	83.5 ± 5.25	8*
Total T3 (ng/ml) (0.45 – 1.95)	Mean ± SD	1.29 ± 0.54	1.15 ± 0.55	0.25
Total T4 (µg/dl) (4.2 – 9.8)	Mean ± SD	8.42 ± 1.82*	7.2 ± 3.2	0.02*
TSH (µIU/ml) (0.40 – 5.55)	Mean ± SD	1.98 ± 1.3	2.11 ± 1.2	0.14

*p<0.05

Table 2 Thyroid status of infertile men

	Euthyroid (n=69)	Subclinical hypothyroidism (n=31)	P value
Clinical parameters			
Suspected hypothyroidism	19	21	-
Obstructive sleep apnoea	8	4	-
Bipolar affective disorders	6	6	-
Headache	3	1	-
Constipation	6	5	-
Speech problems	1	2	-
Semen parameters			
Oligospermia	8%	9%	0.40
Asthenozoospermia	7%	6%	
Oligo-asthenoteratospermia	24%	20%	
Total motile sperm count	27.5 ± 19.7 x 10 ⁶	26.8 ± 18.4 x 10 ⁶	0.10
Semen volume, mL	2.9 [1.0–3.2]	2.6 [1.2–3.6]	0.45
Sperm motility, %	42.5 [32.9–37.5]	37.2 [30.3–36.1]	0.20
Sperm morphology, %	47.5 [40.2–60.5]	30.5 [28.5–55.5]	0.12
Thyroid profile	1.38 ± 0.44	1.45 ± 0.65	0.25
Total T3(ng/ml)	9.2 ± 1.90	8.66 ± 1.7	0.02*
Total T4 (µg/dl)			
TSH (µIU/ml)	1.89 ± 1.1	2.32 ± 1.5	0.043*

Table 3 Comparison of thyroid profile, and risk factors in group I and group II subjects

		Group I			Group II
		Oligozoospermia (n=32)	Asthenozoospermia (n=48)	Oligo-asthenozoospermia (n=20)	
Total T3(ng/ml)	Mean ± SD	1.15 ± 0.4	1.29 ± 0.59	1.25 ± 0.32	1.2 ± 0.40
	Mean ± SD	7.35 ± 1.42	9.15 ± 1.85*	7.85 ± 1.65	7.2 ± 2.15
	Mean ± SD	1.69 ± 0.55	2.12 ± 1.45	1.88 ± 0.40	2.1 ± 1.20
BMI-	Normal	19	22	11	71
	Overweight	8	14	7	21
	Obese	5	12	2	8
Varicocele		7	14	6	0
	Mild	5	4	4	7
	Moderate	3	2	2	2
Smoker	Heavy	2	1	1	2
	Light Smokers	6	4	4	8
	Moderate Smokers	4	2	2	3
	Heavy Smokers	2	2	2	2

Mild alcohol: consuming 40g or less; Moderate alcohol: consuming 40-80g; and Heavy alcohol: consuming more than 80g per day. Light Smokers: 01-10 cigarettes/day; Moderate Smokers: 11-20 cigarettes /day; and Heavy Smokers: >21 cigarettes /day.

Total 30% patients were having smoking habit in the infertile male group. 16 out of 30 smoker had >50% motility while 14 had a motility of <5%. Also 20 patients of the smoking group had <10% of morphologically normal sperm. Oligozoospermia was present in as high in the alcoholics than in the nonalcoholic cases. All three abnormalities like oligo-asthenozoospermia, asthenozoospermia, and oligozoospermia were seen in 32 patients of the alcoholic group.

10 out of 19 obese patients had sperm motility <5%. Totally, 27 patients were found varicocele, among them 14 had asthenozoospermia, 7 had oligozoospermia, and 6 had oligo-asthenozoospermia.

Relative operating curve (ROC) analysis

We evaluated diagnostic accuracies of investigative parameters using ROC curve analysis to predict the accurate value for male infertility occurrence (Table 4). ROC analysis between the group I vs the group II showed a significant increase in sperm count in the group I than the group II, however increase in sperm count was not linear (Figure 1. a). Comparative ROC analysis showed a linear response for cases with >28.32 sperm count vs <28.32 million/ml of Sperm count (Figure 1. b.). ROC analysis did not show a linear response for sperm motility in cases with >40.1, sperm motility vs <40.1% (Figure 1. d). ROC analysis did not show a significant change in normal morphology in the group I compared to the group II (Figure 1.e). Comparative ROC analysis did not show a linear response for normal morphology of >55.92 in the group I, and <55.92 in the group II (Figure 1.f.).

ROC analysis did not show a significant change in total T3 levels in the group I compared to the group II (Figure 2.a.). Comparative ROC analysis did not show a linear response for Total T3 levels in cases with >1.29 Total T3 vs <1.29 Total T3 levels (Figure 2.b). ROC analysis did not show a significant change in total T4 levels in the group I compared to the group II (Figure 2c.). Comparative ROC analysis did not show a linear response for total T4 levels in cases with >8.42 Total T4 vs <8.42 Total T4 levels (Figure 2.d.). ROC analysis did not show a significant change in TSH levels in the group I compared to the group II (Figure 2.e.). Comparative ROC analysis did not show linear response for TSH levels in cases with >1.98 TSH vs <1.98 TSH levels (Figure 2.f.).

Correlation analysis:

Table 5 shows the Pearson correlation analysis of blood thyroid profile with seminal parameters. There were inverse correlations of thyroid profile with sperm count, sperm motility, and normal sperm morphology. There were significant ($p < 0.01$) inverse correlations of serum TSH levels with sperm volume ($r = -0.12$, $p = 0.02$), sperm motility ($r = -0.26$, $p = 0.02$), and sperm morphology ($r = -0.304$, $p = 0.02$). There was a significant correlation seen between Serum T4 levels and sperm count ($r = -0.278$, $p = 0.02$), and serum T4 levels and sperm motility ($r = -0.249$, $p = 0.032$).

Table 4 ROC analysis showing Area under the ROC Curve (AUC), sensitivity and specificity of different parameters

Parameters	AUC	Sensitivity	Specificity
Group I vs group II			
Sperm count	0.7860	87.50	50.80
Total T3	0.5315	50.46	52.31
Normal morphology	0.6390	72.36	42.63
Sperm motility	0.7110	69.85	50
Total T3	0.5030	46.91	54.23
TSH	0.5740	50	57.78
Cases with Sperm motility < 40.1 vs Sperm motility > 40.1			
Sperm count	1.000	93.75	68.95
Total T3	0.5882	57.91	50.09
Normal morphology	0.5579	48.35	60.06
Sperm motility	0.5671	57.91	48.03
Total T3	0.6278	58.54	51.96
TSH	0.5570	62.17	44.27

Table 5 Pearson correlation of Total T3, T4, and TSH levels with semen parameters in infertile men

	Total T3 (ng/ml)		Total T4 (µg/dl)		TSH (µIU/ml)	
	R value	P value	R value	P value	R value	P value
Semen volume (ml)	-0.147	0.22	-0.095	0.430	-0.12	0.02
Sperm count (×10 ⁶ cells/ml)	-0.150	0.32	-0.278	0.020	-0.26	0.22
Sperm motility (%)	-0.091	0.42	-0.249	0.032	-0.26	0.02
Morphology (%)	-0.079	0.45	0.160	0.130	-0.304	0.02

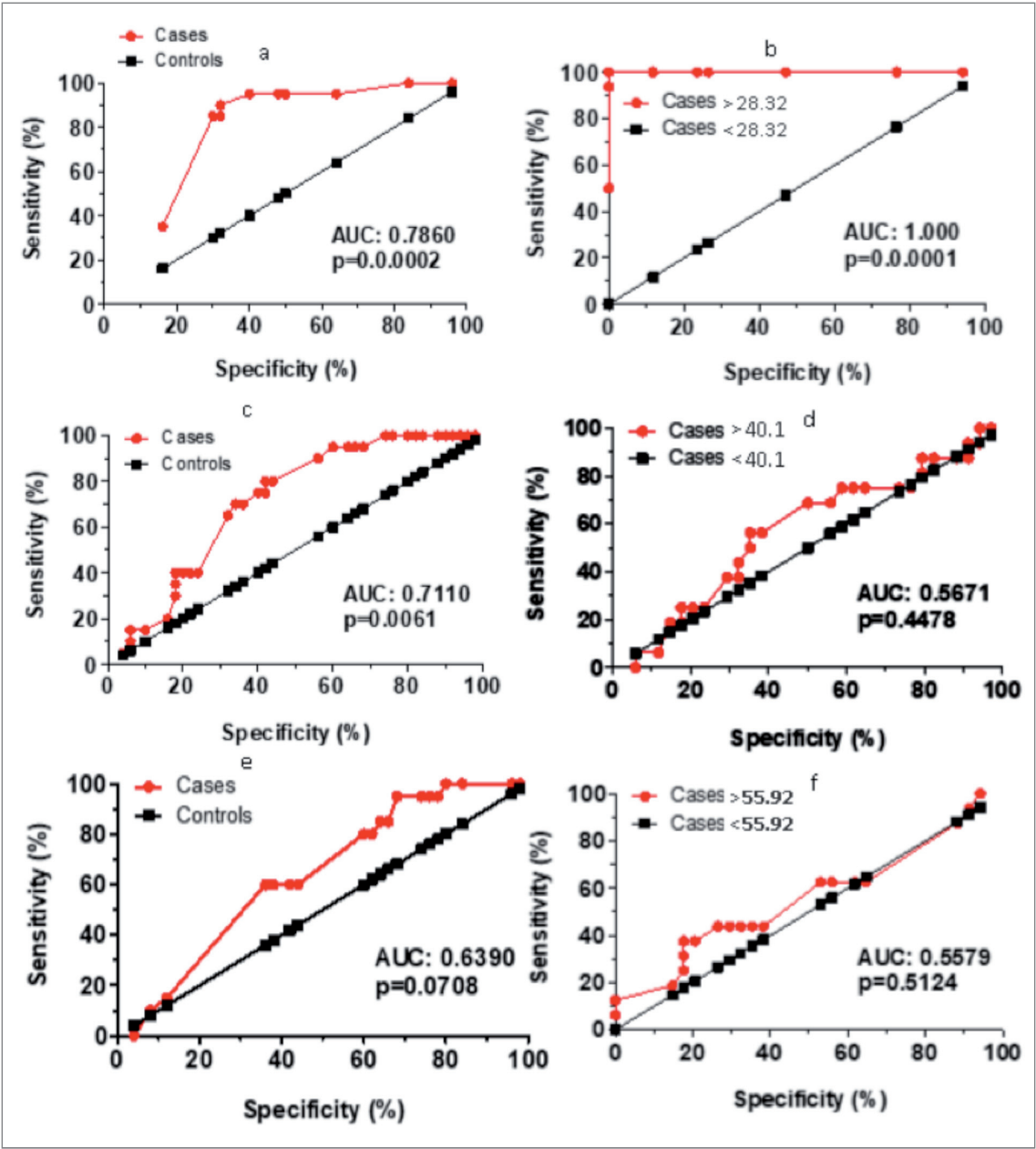


Fig. 1 a. ROC of sperm count between the group I and the group II. b. ROC of sperm count levels between the cases with sperm count <28.32 to >28.32. c. ROC of sperm motility levels between the group I and the group II. d. ROC of sperm motility levels between the cases with sperm motility value >40.1, and <40.1. e. ROC of normal morphology levels between the group I and the group II. f. ROC of Normal morphology between the cases with normal morphology value >55.92 and <55.92.

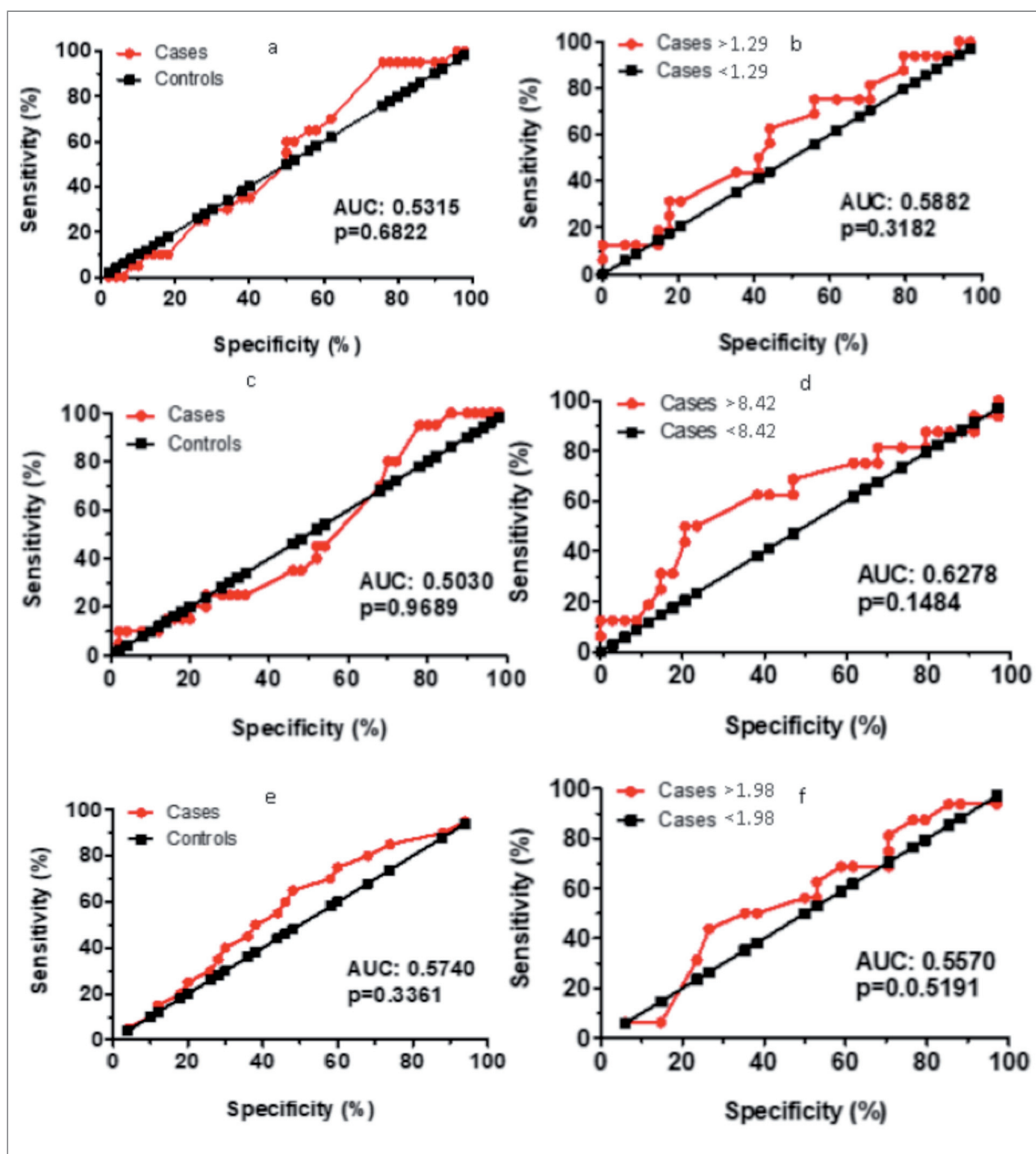


Fig. 2 a. ROC of total T3 levels between the group I and the group II. b. ROC of total T3 levels between the cases with total T3 value >1.29 and <1.29. c. ROC of total T4 levels between the group I and the group II. d. ROC of total T4 levels between the cases with total T4 value >8.42 and <8.42 .e. ROC of TSH between the group I and the group II. f. ROC of TSH between the cases with TSH value >1.98 and <1.98.

DISCUSSION

Although several causes of male infertility have been identified, the exact aetiology and pathogenesis of approximately half of all male infertility cases remains unknown.

Oligozoospermia was found in 32 (32%, 95% confidence interval [CI], 27.5% - 35.5%), asthenozoospermia in 48 (48%, 95% confidence interval [CI], 41.5%- 50.5%), and oligo-asthenozoospermia in 20 men in our study of 100 abnormal subjects (20%, 95% confidence interval [CI], 16.85% - 22.8%). Sharma et al., were also observed the similar results (21).

In the current study, it was observed that the majority of subjects were from urban areas, possibly due to pollution or lifestyle-related factors such as stress and other factors that may affects male fertility. In this study, the age range for presentation was 24 to 39 years, with a mean age of 32.4 ± 4.5 years.

In the current study, 35% had 2 to 3 years of active married life, and 33% had 4 - 5 years of active married life, with a mean of 5.1years. After 6 years of active married life, this was reduced to 5% in patients with 2 years of active married life. Hence, the shorter the duration of male infertility, had the better the prognosis.

It was observed that 16 of 30 smokers had a motility of more than 50%, while 14 had a motility of less than 5%. Sperm motility was less than 5% in 8 percent of light smokers, 4% of moderate smokers, and 4 percent of heavy smokers. This study was comparable to that of Zhang et al., (22) and Tandel and Patel (23).

Zukerman et al., also reported that Oligo-asthenozoospermia, asthenozoospermia, and oligozoospermia in 32% of alcoholics (24). Villalta's study showed that Oligo-asthenozoospermia, asthenozoospermia, and oligozoospermia in 15% of smokers and 72% in non-alcoholics (25).

In our study, 10 of 19 obese patients had 5% sperm motility. While 4 out of 29 overweight patients and 4 out of 52 normal weight patients had 50% sperm motility, normal weight patients had 50% sperm motility. Korte et al., observed that men with a high BMI (>25) have a low number of normal-motile sperm cells (26).

Infection of the male reproductive tract is represented by the presence of >4 pus cells in the sperm. A pus cell count of 4-10 pus cells/HPF indicated a moderate infection in 29% of patients, and >10 pus cells/HPF indicated a severe infection in 16% of patients in our study. According to Saxena, 20% of cases had severe infections (27).

In our study, 27% of the cases had varicocele, 14% had asthenozoospermia, 7% had oligospermia, and 6% had oligo-asthenozoospermia. Ali study showed 36% asthenozoospermia, 25% oligospermia, and 21% teratozoospermia (28, 29).

Thyroid hormone levels have been shown to correlate positively with serum testosterone levels. This condition is associated to a number of male reproductive disorders, including hypoactive sexual behaviour, erectile dysfunction, delayed ejaculation, and poor sperm quality (30). Various studies have described an association between the hypothyroidism and male infertility, but it is unclear that hypothyroidism may directly affects the sperm quality (31-34).

In our current study, 31% of the subjects had a subclinical hypothyroidism. Subclinical thyroid dysfunction was associated with an altered sperm count but not sperm motility or morphology. This finding is consistent with the findings of other studies (35).

There were inverse correlations of thyroid profile with sperm count, sperm motility, and normal sperm morphology. There were significant ($p < 0.01$) inverse correlations of serum TSH levels with sperm volume ($r = -0.12$, $p = 0.02$), sperm motility ($r = -0.26$, $p = 0.02$), and sperm morphology ($r = -0.304$, $p = 0.02$). Similarly, serum T4 levels were significantly ($p < 0.01$) correlated with sperm count ($r = -0.278$, $p = 0.02$), and sperm motility ($r = -0.249$, $p = 0.032$).

Both hyperthyroidism and hypothyroidism affects the testicular function and neuroendocrine regulation of reproductive functions via the hypothalamic-pituitary-thyroid (HPT) and hypothalamic-pituitary-gonadal (HPG) axis crosstalk. TSH is critical in the regulation of the HPT axis. The HPG axis normally regulates testicular function. Thyroid hormones work biologically by binding to two nuclear thyroid hormone receptors (TRs): TR and THR. Thyroid hormones are also important in the initiation of Leydig cell differentiation before puberty (36).

T3 binds to TRs on postpubertal Sertoli cells, inhibiting the synthesis of aromatase and androgen-binding protein, as well as modulating testosterone conversion to 17-estradiol and testosterone concentration in the seminiferous tubules. Thyroid hormones promote testosterone steroidogenesis in adult Leydig cells by activating the steroidogenic acute regulatory (StAR) protein, which is involved in cholesterol transport into the mitochondria (31).

Many conditions may influence the sperm quality of males. Oxidative stress is well-known to be harmful to sperm quality, primarily by causing DNA damage in sperm cells. Thyroid hormone physiological ranges regulate metabolism, including cell oxygen consumption, and both hyperthyroidism and hypothyroidism are associated with oxidative damage.

A low sperm volume was reported in a small series of subclinical hypothyroidism ($n=31$); in our study, no subjects were overtly hypothyroid/hyperthyroid, though a few had lower TSH levels (32, 33).

In our study, the mean T3, T4, and TSH levels in infertile male subjects were 1.29 ± 0.54 ng/ml, 8.42 ± 1.82 g/dl, and 1.98 ± 1.3 IU/ml, respectively. Within the normal range of TSH values, the relationship of TSH with the sperm parameters was more pronounced in our study. In our study, 31% of the subjects had subclinical hypothyroidism and lower sperm counts. In other studies, 24-25% of hypothyroid subjects had lower sperm counts, with no significant difference in sperm volume (34-38).

In the current study, total T3 of oligozoospermic men with a mean of 115 ± 0.4 ng/ml, total T4 with a mean of 7.35 ± 1.42 µg/dl, and TSH with a mean of 1.69 ± 0.55 IU/ml, whereas in asthenozoospermic men total T3 with mean of 1.29 ± 0.59 ng/ml, total T4 with the mean of 9.15 ± 1.85 µg/dl, and TSH ranged with mean of 2.12 ± 1.45 IU/ml.

Clyde et al. (39) studied three young thyrotoxic males and observed that two had marked oligozoospermia with a decreased motility and the third had borderline low sperm counts with a decreased motility. Kidd et al. (40) studied Grave's disease patients and observed that four of the five had total sperm counts less than 40 million/ml and only one had sperm density less than 24 million/ml.

Furthermore, total T4 was found to be significantly higher in men with asthenozoospermia, but no relationship between T3 and/or TSH and oligozoospermia, asthenozoospermia, or oligoasthenozoospermia was found, which is consistent with Kumar et al. (19).

Our findings are consistent with the findings of Lotti et al. (18), who observed that euthyroid subjects ($n=145$) had lower semen volumes compared to those with subclinical hyperthyroidism ($n=6$) and higher than those with subclinical hypothyroidism ($n=12$); however, no relationship between the semen parameters and TSH was found. Thyroid function abnormalities appear to have the most significant effect on sperm parameters, namely impaired motility and/or morphology (41).

Hence, the most noticeable effect of thyroid function abnormalities on sperm parameters was impaired motility and/or morphology. Based on our findings, we concluded that the relationship between TSH and sperm volume/count was stronger.

CONCLUSION

Smoking, obesity, and infection all reduce fertility by lowering sperm count, motility, and changing the morphology of sperm. We observed a significant relationship between TSH and sperm volume and motility. These findings suggest that normal thyroid function is a component of good sperm quality.

Acknowledgements: Authors acknowledged to all the subjects for their active participation in the study. Authors acknowledged to laboratory staff for their sample collection, and analysis of investigations.

Conflict of interests: Authors declare that there is no conflict of interest

REFERENCES

1. Agarwal A., Mulgund A., Hamada A., Chyatte M.R. A unique view on male infertility around the globe. *Reprod Biol Endocrinol* 2015; 13:37.
2. Mewara H., Mewara J. A critical review on aetiology of Vandhytwa through Scientific approach. *International Journal of Research and Analytical Reviews* 2020:2348-1269.
3. Lal N. India's Hidden Infertility Struggles. *The diplomat*. 2018.
4. Zahid. (2016, aug 5). National Health Portal. Retrieved may 21, 2020, from Infertility: <https://www.nhp.gov.in/disease/reproductive-system/infertility>
5. Fourth National Family Health Survey of TFR, Department of Health and Family Welfare, Ministry of Health and Family Welfare, Government of India 2017
6. Wu W., Tang Q., Gu H., Chen Y., Xia Y., Sha J., Wang X. Infection and Infertility. In *Genital Infections and Infertility* 2016 Jun 29. IntechOpen.
7. Harris I.D., Fronczak C., Roth L., Meacham R.B. Fertility and the aging male. *Reviews in urology* 2011;13(4):e184.
8. Iwamoto T., Nozawa S., Yoshiike M. Semen quality of Asian men. *Reprod Med Biol* 2007. 6(4),185-193.
9. Ji B.T., Shu X.O., Linet M.S., Zheng W., Wacholder S., Gao Y.T., Ying D.M., Jin F. Paternal cigarette smoking and the risk of childhood cancer among offspring of nonsmoking mothers. *J Natl Cancer Inst*. 1997; 89: 238-244.
10. Kumar N., Singh A.K. Trends of male factor infertility, an important cause of infertility: A review of literature. *Journal of human reproductive sciences*. 2015 Oct;8(4):191.
11. Stojanov M., Baud D., Greub G., Vulliamoz N. Male infertility: the intracellular bacterial hypothesis. *New microbes and new infections*. 2018; 26:37-41.
12. Ganguly S., Unisa S. Trends of Infertility and Childlessness in India: Findings from NFHS Data. *Facts Views Vis Obgyn*. 2010;2(2):131-138.
13. Alahmar A., Dutta S., Sengupta P. Thyroid hormones in male reproduction and infertility. *Asian Pacific Journal of Reproduction*. 2019;8(5):203.
14. Sengupta P., Dutta S. Thyroid disorders and semen quality. *Biomedical and Pharmacology Journal*. 2018;11(1):01-10.
15. Castañeda Cortés, D.C., Langlois V.S., Fernandino J.I. Crossover of the hypothalamic pituitary-adrenal/interrenal, -thyroid, and -gonadal axes in testicular development. *Front. Endocrinol*. 2014; 5:139.
16. Jimoh O.A., Oyeyemi W.A., Okin-Aminu H.O., Oyeyemi B.F. Reproductive characteristics, semen quality, seminal oxidative status, steroid hormones, sperm production efficiency of rabbits fed herbal supplements. *Theriogenology*. 2021;168:41-9.
17. Trummer H., Ramschak-Schwarzer S., Haas J., Habermann H., Pummer K., Leb G. Thyroid hormones and thyroid antibodies in infertile males. *Fertility and sterility*. 2001;76(2):254-7.
18. Lotti F., Maggi M. Sexual dysfunction and male infertility. *Nature Reviews Urology*. 2018; 15(5):287-307.
19. (World Health Organization Laboratory Manual for the Examination of Human Semen and SpermCervical Mucus Interactions. New York: Cambridge University Press; 1999).
20. Kruger T.F., Acosta A.A., Simmons K.F., Swanson R.J., Matta J.F., Oehninger S. Predictive value of abnormal sperm morphology in in-vitro fertilization. *Fertil Steril*. 1988;49:112-7.
21. Kumar Sharma M., Parchwani D., Maheria P., Upadhyah A. Relationship between thyroid profile and semen quality. *Natl J Community Med*. 2012;3:250-8.
22. Zhang J.P., Meng Q.Y., Wang Q., Zhang L.J., Mao Y.L., Sun Z.X. Effect of smoking on semen quality of infertile men in Shandong, China. *Asian J Androl*. 2000;2 (2): 143-6.
23. Tandel H.K., Patel Y.K. Analysis of factors affecting the semen pathology in male infertility. *Trop J Path Micro* 2019;4(1):14-21.
24. Zukerman Z., Rodriguez-Rigau L.J., Smith K.D., Steinberger E. Frequency distribution of sperm counts in fertile and infertile males. *FertilSteril*. 1977;28 (12): 1310-3.

25. Villalta J., Balleasca J.L., Nicolas J.M., Martinez de Osaba, M.J., Antunez E., Pimentel C. Testicular function in asymptomatic chronic alcoholics (relation to ethanol intake). *Alcohol Clin Exp Res.* 1997;21:128.
26. Kort H.I., Massey J.B., Elsner C.W., Mitchell-Leef D., Shapiro D.B., Witt M.A., Roudebush W.E. Impact of body mass index values on sperm quantity and quality. *J Androl.* 2006;27(3):450-2.
27. Saxena S.C. Proceedings of the all India obstetrics and gynaecological congress 1973.
28. Al-Ali B.M., Marszalek M., Shamloul R., Pummer K., Trummer H. Clinical parameters and semen analysis in 716 Austrian patients with varicocele. *Urology.* 2010; 75: 1069-1073
29. Gunes, S., Agarwal A., Henkel R., Mahmutoglu A.M., Sharma R., Esteves S.C., Aljowair A., Emirzeoglu, D., Alkhani A., Pelegrini, L. et al. Association between promoter methylation of MLH1 and MSH2 and reactive oxygen species in oligozoospermic men- A pilot study. *Andrologia* 2018, 50, e12903.
30. Singh, R., Hamada A.J., Agarwal A. Thyroid hormones in male reproduction and fertility. *Open Reprod. Sci. J.* 2011, 3, 98-104.
31. Alahmar A.T. Role of Oxidative Stress in Male Infertility: An Updated Review. *J Hum Reprod Sci.* 2019, 12(1),4-18.
32. Abalovich M., Levalle O., Hermes R., Scaglia H., Aranda C., Zylbersztejn C., Oneto A., Aquilano D., Gutierrez S. Hypothalamic-pituitary-testicular axis and seminal parameters in hyperthyroid males. *Thyroid* 1999, 9, 857-863.
33. Corrales Hernández J.J., Miralles García, J.M., García Díez, L.C. Primary hypothyroidism and human spermatogenesis. *Arch. Androl.* 1990, 25, 21-27.
34. Nikoobakht M.R., Aloosh, M., Nikoobakht N., Mehri A.R., Biniyaz F., Karjalainen M.A. The role of hypothyroidism in male infertility and erectile dysfunction. *Urol J.* 2012; 9: 405-409.
35. Krassas, G.E.; Papadopoulou, F.; Tziomalos, K.; Zeginiadou, T.; Pontikides, N. Hypothyroidism has an adverse effect on human spermatogenesis: A prospective, controlled study. *Thyroid* 2008; 18:1255-1259.
36. LeFevre, M.L. Screening for thyroid dysfunction: U.S. Preventive Services Task Force recommendation statement. *Ann. Intern. Med.* 2015;162:641-650.
37. Wagner M.S., Wajner, S.M., Maia A.L. Is there a role for thyroid hormone on spermatogenesis? *Microsc. Res. Tech.* 2009;72:796-808.
38. Corrales Hernández J.J., Miralles García, J.M.; García Díez, L.C. Primary hypothyroidism and human spermatogenesis. *Arch. Androl.* 1990; 25:21-27
39. Clyde H.R., Walsh P.C., English R.W. Elevated plasma testosterone and gonadotropin levels in infertile males with hyperthyroidism. *Fertility and Sterility.* 1976;27(6):662-6.
40. Kidd G.S., Glass A.R., Vigersky R.A. The hypothalamic-pituitary-testicular axis in thyrotoxicosis. *The Journal of Clinical Endocrinology & Metabolism.* 1979;48(5):798-802.
41. La Vignera S., Vita R. Thyroid dysfunction and semen quality. *Int J Immunopathol Pharmacol.* 2018;32:2058738418775241

Received: July,18, 2022

Accepted: August, 26, 2022