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Investigating the Effect of Thyroid Stimulating Hormone (TSH) on Human Sperm Parameters

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ABSTRACT

Thyroid-stimulating hormone (TSH), produced by the pituitary gland, regulates thyroid gland function, and its levels can influence male fertility due to the thyroid's role in sex hormone production. The impact of TSH on semen concentration, sperm count, sperm DNA integrity, and sperm activity has been a subject of extensive research in male infertility. This study aimed to investigate the effects of TSH on these parameters using semen and sperm samples from patients referred to the infertility department of Nobel Laboratory in Isfahan, Iran. The research methodology involved statistical analysis, microscopy, flow cytometry, and sperm staining kits for comprehensive sample evaluation. The findings indicate a clear relationship between TSH levels and semen parameters. Non-normal TSH levels (both elevated and reduced) were associated with decreased or excessively increased semen concentration, reduced sperm count, and abnormal sperm morphology, suggesting a potential negative impact on male fertility. Furthermore, abnormal TSH levels correlated with increased sperm DNA fragmentation, highlighting a potential link between thyroid function and sperm genetic integrity. TSH levels were also found to affect sperm activity, with non-normal levels potentially leading to decreased sperm motility and viability. Overall, this study provides valuable insights into the relationship between TSH and male reproductive health, emphasizing the importance of monitoring TSH levels in male patients with infertility issues and highlighting the potential implications of thyroid dysfunction on sperm quality and fertility outcomes. Further research is essential to elucidate the underlying mechanisms and potential therapeutic interventions for thyroid-related male infertility. Our findings suggest that TSH affects sperm and semen, which may be useful for clinicians evaluating male infertility and for developing new treatments.

Introduction

Semen, also known as seminal fluid, is a fluid produced by the male reproductive system containing sperm cells, which are male gametes responsible for fertilizing the female egg during sexual reproduction. Sperm cells are produced in the testes and stored in the epididymis before ejaculation during intercourse [1]. The health of sperm is critically important for male fertility and reproduction, as healthy sperm can effectively swim towards and fertilize the female egg [2]. Factors affecting sperm health include lifestyle choices such as smoking, excessive alcohol consumption, and poor diet, as well as environmental factors like exposure to toxins and pollutants [3]. Maintaining sperm health is essential for couples planning conception, and adopting a healthy lifestyle, including regular exercise, a balanced diet, and avoiding harmful substances, is recommended to improve sperm quality and increase the chances of successful fertilization [4]. Regular check-ups with a healthcare provider can also help monitor and address potential issues related to sperm health.
Infertility affects approximately 15% of couples worldwide. While males are solely responsible for 20-30% of infertility cases, environmental factors, nutrition, and female fertility issues also play a role in 50% of cases [5]. A significant portion of male infertility is idiopathic, meaning its cause, mechanism, or origin is unknown, and the underlying molecular mechanisms remain unclear [6]. Key factors contributing to male infertility include low sperm concentration (oligospermia), poor sperm motility (asthenozoospermia), abnormal sperm morphology (teratozoospermia), absence of sperm in semen (azoospermia), and other less related factors such as semen volume, epididymal, prostate, and seminal vesicle function [7,8]. Although various causes of male infertility have been identified, the exact etiology and pathogenesis often remain elusive [9,10]. The etiology of infertility varies across regions, populations, and even within the same population locally [11].

Table 1-1 Volume (Concentration) of the studied sperm groups

Group Number	Sperm	Volume (Ml)
1,2,3	normal	1.5 – 6
2,3	Mostly abnormal	1 > 0.1
2,3	Mostly normal	6 <

Table 1-2 percentage of nucleotide damage by Tunel method

Group Number	Sperm	Damage (Percentage)
1	Healthy	1-13
2	Relatively healthy	13-20
3	Has minor damage	20-26
4	hurt	26-31
5	Severe injury	31-37

Table 1-3 Percentage of Total Nucleotide Damage by SCSA Acid Detergent Method

Group Number	Sperm	Damage (Percentage)
1	Healthy	1-14.9
2	Relatively healthy	15-25.1
3	Has minor damage	25.2-28.9
4	hurt	29-37.9
5	Severe injury	38-45.9

Thyroid-stimulating hormone (TSH) plays a crucial role in controlling brain and organ development in infants and metabolic activities in adults [25]. Upon TSH stimulation, the thyroid gland secretes triiodothyronine (T3) and thyroxine (T4), which are primary thyroid hormones essential for regulating basal metabolic rate, growth, and the development and differentiation of many cells in the body [12]. Research on the impact of thyroid hormones on male infertility has emerged only in the last decade. Previously thought not to affect spermatogenesis and male fertility, these hormones are now evidenced to play a significant role . Thyroid hormones influence the testes in multiple ways, affecting various cell types including Leydig, Sertoli, and germ cells. Deficiency or excessive levels of thyroid hormones lead to changes in testicular function, including semen abnormalities [30]. Often, hyperthyroidism is associated with decreased semen volume, reduced sperm density, motility, and morphology, while hypothyroidism is linked to reduced sperm morphology. Therefore, thyroid function tests should be part of the diagnostic procedures for infertile men [12,13].

The identification of thyroid hormone receptors on Sertoli cells (sperm-nurturing cells in the testes) indicates an important regulatory role in sperm production. Thus, thyroid dysfunction can affect spermatogenesis and male fertility. Studies on human subjects and animal models investigating the effect of thyroid hormones on male fertility and infertility, along with updated reviews on the role of thyroid hormones in spermatogenesis and male fertility, are currently revealing more insights into this field [14]. However, the effects of thyroid disorders on male infertility have not been extensively studied, likely because attention in male thyrotoxicosis usually focuses on other manifestations of the disease, and consequently, fertility status is often not evaluated [15,16]. Thyroid hormones are involved in androgen biosynthesis and spermatogenesis, either directly by acting on Leydig cells (the primary source of testosterone or androgens in men) and Sertoli cells, or indirectly by modulating gonadotropin secretion (hormones that stimulate the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland) [17]. Thyroid hormones play a role in the differentiation, growth, and function of many organs [18]. Indeed, prepubertal hypothyroidism may cause male infertility. However, the role of thyroid dysfunction in post-pubertal men is controversial. Andrologists usually evaluate thyroid function during the assessment of infertile men due to evidence that sperm motility may decrease in some patients with overt hypothyroidism [19].

Table 1-4 normal range of TSH hormone

Group No	TSH	Amount (mIU/L)
1	High	5.0 <
2	normal	0/5 – 5/0
3	Low	5/0 >

The figure illustrates the correlations between TSH secretion and various sperm parameters, including concentration, motility, morphology (assessed through spermogram and Kruger's staining), and DNA damage (evaluated using SCSA and TUNEL tests). It visually represents the relationships between TSH levels and sperm quality indicators.

Table 1-5: Relationship between TSH secretion rate and sperm concentration

Group Number	Group Variety	TSH Concentration (mIU/L)	Sperm Concentration (ML)
1	32	4.1 – 9	6 - 14
2	26	3.9 – 4.1	1.2 - 7
3	10	0.1 – 0.5	0.3 - 6

The current decline in the fertile population leads to an ascending trend in the age of society, moving towards an aging population, which will be evident in the near future. Achieving more precise causes and better pathogenesis in male infertility will aid in developing novel methods for effective preventive therapeutic interventions, improved treatment, especially in conventional hormonal therapy for infertile men in treatment centers, and the discovery of suitable drugs depending on the type of infertility for each individual[20].

This study aims to investigate the effects of TSH on human sperm concentration, count, motility, and DNA fragmentation using spermogram and SCSA/TUNEL methods in the andrology department of Nobel Laboratory in Isfahan.

2. Materials and Methods

This study employed a set of up-to-date methods to investigate the effect of TSH on semen parameters and sperm DNA integrity. The research involved the analysis of semen and sperm samples from patients referred to the infertility department of Nobel Laboratory in IsfahanThe methodological approach included the use of statistical methods, microscopy, flow cytometry, and sperm staining kits for a comprehensive analysis of the samples.

Specific methods mentioned in the thesis include:

Spermogram Analysis: For evaluating sperm concentration, count, and motility.

- Kruger Test: For assessing sperm morphology; is a precise microscopic examination of sperm within a semen analysis. Its purpose is to evaluate sperm morphology, focusing on the exact shape and dimensions of individual sperm. This assessment is vital for determining male fertility and uncovering potential factors contributing to infertility, as it meticulously categorizes sperm as either normal or abnormal against stringent standards.
- DNA Fragmentation Assessment (SCSA/TUNEL methods): For evaluating the integrity of sperm DNA.
- TSH Hormone Level Assessment: To determine the levels of Thyroid Stimulating Hormone.

Table 2-1 TUNEL protocol and components of the TUNEL kit

TUNEL sample preparation and washing method:
a) Take 500 microliters of fresh semen sample and pour into microtube b) b) Add 500 microliters (equal amount to semen) of wash buffer (PBS or Phosphate Buffer Saline) c) c) Pipette and shake microtube d) d) Centrifuge at 3000 RPM for 5 minutes and then pour off supernatant and keep plate with small amount of liquid (about 100 microliters) then finger tap
Stabilization and fixing of the TUNEL specimen:
a) Add an equal amount (500 microliters) of fixative solution (e.g., 4% paraformaldehyde) to the sperm sample. b) Keep the sample in the fixative solution for 15-30 minutes at room temperature. c) Wash the fixed sperm sample with PBS buffer to remove excess fixative. d) Add 0.1% Triton X-100 permeabilization solution to the fixed sperm sample to allow the TUNEL reaction mixture to access the DNA. e) Incubate the sample in the permeabilization solution for 10 minutes at 37 degrees Celsius. f) Gently wash the permeabilized sperm sample with PBS buffer to remove excess solution.
Components of the TUNEL kit:
a) Terminal deoxynucleotidyl transferase (TdT) enzyme: This enzyme catalyzes the addition of labeled nucleotides to the free 3'-OH ends of fragmented DNA. b) Nucleotide mixture: This mixture contains labeled nucleotides such as fluorescein-12-dUTP or fluorochrome-conjugated dUTP, which are incorporated into the fragmented DNA. c) Reaction buffer: This buffer provides the necessary conditions for TdT enzyme activity and DNA labeling. d) roteinase K or other protein digestion reagents: These reagents may be included to remove proteins and improve TdT enzyme access to the DNA.
TUNEL reaction:
a) Preparation of TUNEL reaction mixture: This is prepared by combining the TdT enzyme and the dUTP nucleotide mixture according to the protocol. b) Add the TUNEL reaction mixture to the sperm sample that was permeabilized in the previous step. c) Incubate the sample in the TUNEL reaction mixture for 1 hour at 37 degrees Celsius. d) Wash the TUNEL-labeled sperm sample with a PBS buffer to remove excess TUNEL reaction mixture.
Flow cytometry analysis:
a) a) Prepare the flow cytometer according to the device instructions and optimize the sperm analysis settings. b) b) Place the labeled sperm sample from the previous step into the flow cytometer tube. c) c) Obtain the percentage and graph flow cytometry data by running the sample through the flow cytometer. d) d) Finally, analyze the obtained data using appropriate software to determine the percentage of TUNEL positive or negative sperm.

Table 2-2 SCSA (Sperm Chromatin Structure) Protocol with Flow Cytometry

1. Sample preparation: According to the spermogram sample report and the set-up performed, we collect the fresh semen sample and allow it to liquefy. We dilute the sample with 0.1% TNE X-100 (TNE X-10) permeable buffer solution in a ratio of 10:1 in a centrifuge tube. (For example, we add 900 microliters of buffer to 100 microliters of normal sample)
2. Unfolding the DNA strand of the sample with acid: Add Detergent to the sample for 30 seconds, which causes the DNA strand of the sperm cells to open.
3. Staining: DNA dye (Acridine Orange) is added to the sample, which neutralizes the acid and binds to the DNA of sperm cells, denaturing the damaged DNA.
4. Flow cytometric analysis: The stained sample is placed in a flow cytometer, which analyzes the fluorescence emitted by the stained sperm cells. The flow cytometer measures the DNA fragmentation index (DFI) or other relevant parameters.

5. Data Interpretation: We analyze flow cytometry data, including percentages and graphs, to determine the level of DNA fragmentation in the sperm sample. This information provides insight into sperm quality and fertility potential.

3. Results

This study revealed a clear relationship between TSH levels and semen parameters. Non-normal TSH levels were associated with a decrease or excessive increase in semen concentration, reduced sperm count, and abnormal sperm morphology, indicating a potential negative impact on male fertility.

High TSH levels were consistently linked to a decrease in semen concentration. For instance, a study by Young et al. [21] found that men with subclinical hypothyroidism (characterized by high TSH but normal thyroid hormone levels) had significantly lower semen concentration compared to men with normal TSH levels. Similarly, elevated TSH levels were associated with reduced sperm count. A meta-analysis by Li et al. [22] reported that men with subclinical hypothyroidism had 20% fewer sperm than those with normal TSH levels.

Beyond count and concentration, TSH may also impact sperm DNA integrity. A study by Agarwal et al. [23] observed higher levels of sperm DNA fragmentation in men with subclinical hypothyroidism compared to those with normal TSH levels. This suggests that high TSH levels might contribute to male infertility by damaging sperm DNA. Interestingly, a decrease in TSH levels below the normal range also led to increased DNA fragmentation in sperm. Furthermore, non-normal TSH levels negatively affected sperm morphology, and both higher or lower than normal TSH levels potentially led to decreased sperm motility and viability.

Collectively, these findings strongly suggest that TSH plays a role in male infertility. The correlation between TSH secretion and sperm concentration (Table 1-4), sperm count and motility (Table 2-4), sperm morphology using spermogram (Table 3-4) and Kruger staining (Table 4-4), and sperm DNA damage using SCSA and TUNEL tests (Table 5-4) are further detailed in the respective tables.

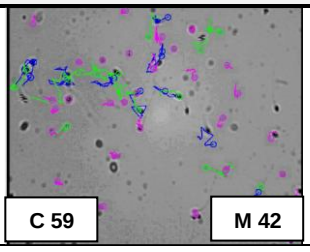
Group Number	Group Variety	TSH Concentration (mIU/L)	Sperm Count (Million)	Sperm Motility (Percent)	Image Of Analyze
1	32	4.1 – 9	42 - 80	21 - 60	

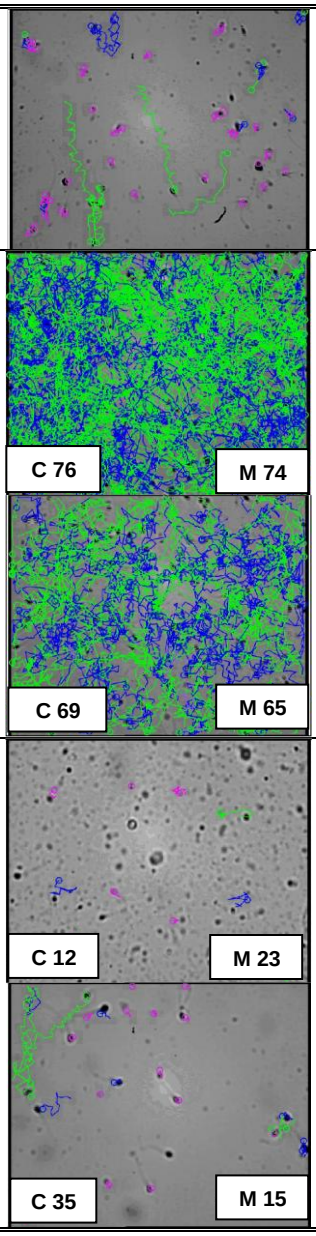
Figure 3-1: Correlation between TSH secretion rate and sperm count and motility					
2	26	3.9 – 4.1	55 - 76	41 - 99	
3	10	0.1 – 0.5	Azoospermia - 37	0-37	

Figure 3-2: Correlation of TSH secretion with sperm morphology using spermogram method

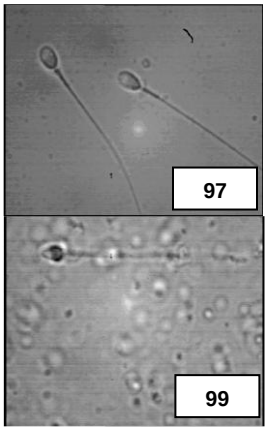
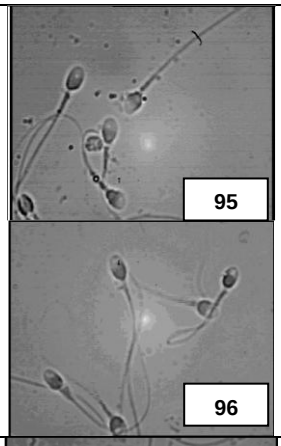
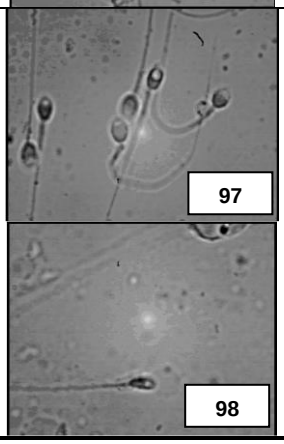
Group Number	Group Variety	(mIU/L)TSH Concentration	Sperm Morphology	Image Of Spermogram Analysis
1	32	4.1 – 9	96 - 100	
2	21	3.9 – 4.1	94 - 96	
3	10	0.1 – 0.5	97- 99	

Figure 3-3: Correlation of TSH secretion with sperm morphology using Kruger's staining test

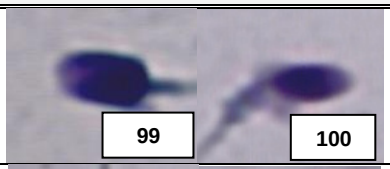
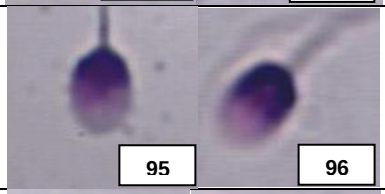
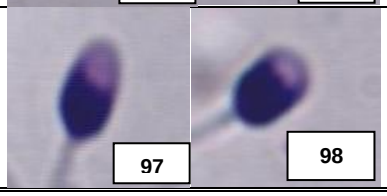
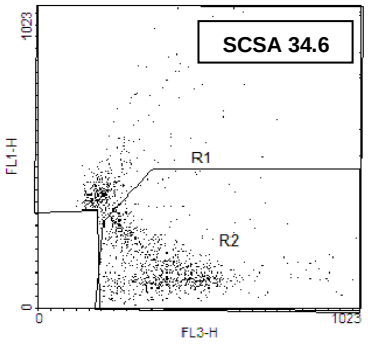
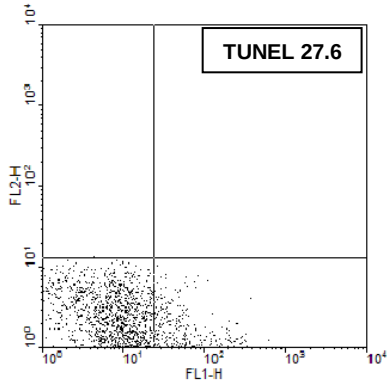
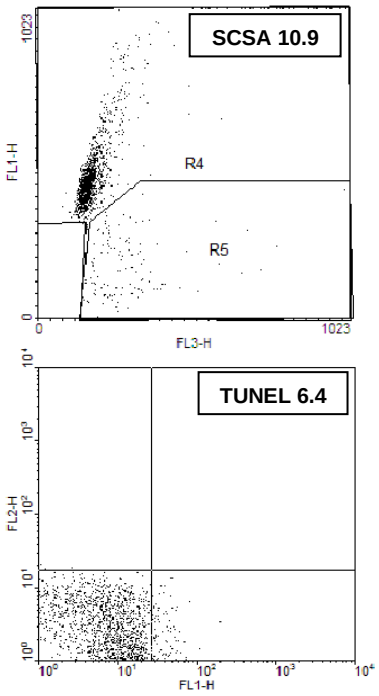
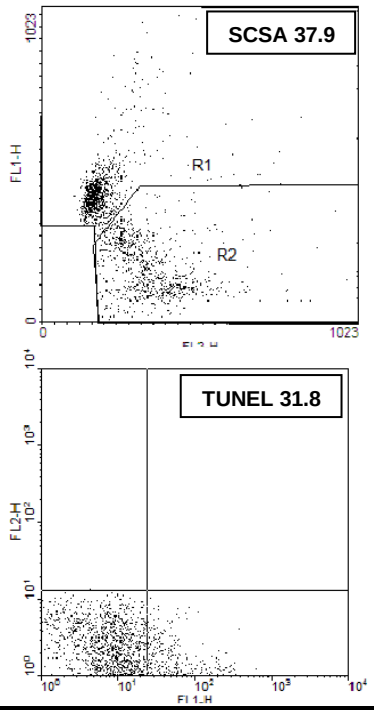
Group Number	Group Variety	TSH Concentration (mIU/L)	Sperm Morphology by Kruger	Image Of Kruger Analysis
1	32	4.1 – 9	96 - 100	
2	26	3.9 – 4.1	94 - 96	
3	10	0.1 – 0.5	97- 99	

Figure 3-4: Correlation of TSH secretion with sperm DNA damage using SCSA and TUNEL tests

Group Number	Group Variety	TSH Concentration (mIU/L)	Sperm DNA Fragmentation by SCSA	Image Of Analyze
1	32	4.1 – 9	18.4 – 27.6	
			Sperm DNA Fragmentation by TUNEL	
			25.3 – 37.7	

2	26	3.9 – 4.1	Sperm DNA Fragmentation by SCSA	
			1 – 14.9 and 15.1 – 24.9	
			Sperm DNA Fragmentation by TUNEL	
			1 – 11.7 and 13 – 18.9	

3	10	0.1 – 0.5	Sperm DNA Fragmentation by SCSA	
			37.9 – 45.8	
			Sperm DNA Fragmentation by TUNEL	
			29.1 – 37.3	

4. Conclusion

Overall, this study offers valuable insights into the relationship between thyroid-stimulating hormone and male reproductive health. The results underscore the importance of monitoring TSH levels in male patients experiencing infertility problems and highlight the potential consequences of thyroid dysfunction on sperm quality and fertility outcomes [72]. Our research provides evidence that TSH influences sperm and semen parameters, which could be beneficial for clinicians assessing male infertility and for the development of new treatments for this condition.

5. Discussion

The findings of this study demonstrate a significant association between TSH levels and various parameters of male fertility, including semen concentration, sperm count, morphology, motility, and DNA integrity. The observation that both elevated and reduced TSH levels are linked to adverse effects on sperm quality aligns with existing literature suggesting a role for thyroid hormones in male reproductive health.

Specifically, the correlation between high TSH levels and decreased semen concentration and sperm count, as supported by studies like Young et al. and Li et al. [21,22], reinforces the idea that thyroid hormone imbalances can directly impact spermatogenesis and sperm production. The finding that TSH affects sperm DNA integrity, with abnormal levels leading to increased DNA fragmentation, is particularly significant. This suggests a potential mechanism through which thyroid dysfunction could contribute to male infertility, as DNA integrity is crucial for successful fertilization and embryonic development.

Furthermore, the impact of TSH on sperm activity, leading to reduced motility and viability, highlights another critical aspect of sperm function affected by thyroid health. Impaired sperm motility is a common cause of male infertility, as it reduces the sperm's ability to reach and fertilize the egg.

While the study provides valuable insights, it's important to consider certain aspects. The discussion of the underlying mechanisms by which TSH exerts these effects could be expanded. Although the thesis mentions the role of thyroid hormones in regulating metabolism and energy production, which are essential for sperm movement, a more detailed explanation of direct molecular pathways or cellular interactions would strengthen the argument.

The study also reiterates the need for comprehensive diagnostic approaches in male infertility, advocating for the inclusion of thyroid function tests. This is particularly relevant given the findings that even subclinical thyroid disorders can impact sperm parameters.

In conclusion, the study significantly contributes to the understanding of the complex interplay between thyroid function and male fertility. The consistent negative impact of non-normal TSH levels on multiple sperm parameters underscores the importance of hormonal balance for reproductive health. Further research is warranted to delve deeper into the specific molecular and cellular mechanisms involved, as well as to develop targeted therapeutic strategies for thyroid-related male infertility. The long-term implications of an aging fertile population also emphasize the urgency of such research.

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