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# Effect Of Ghrelin on Epididymal Sperm Analysis and Antioxidant System in Hyperthyroidic Adult Male Rats

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## Abstract

The study's objective is to evaluate the enhanced effect of Ghrelin (GRE) on sperm analysis and the antioxidant system in male rats after inducing hyperthyroidism by L–thyroxin. The rats (50 males) were split into five groups. The first group consisted of 10 male rats that were given normal saline for 30 days S.C and set as a control group. While the second group (positive group) was 10 male rats with hyperthyroidism were given normal saline for 30 days S.C, the third group was 10 male rats with hyperthyroidism were given Ghrelin at a dose of (0.5nmol/100µl saline) for 30 days S.C, and the fourth group was male 10 rats with hyperthyroidism were given Ghrelin (1nmol/100µl saline) for 30 days S.C. The fifth group consisted of 10 hyperthyroid male rats that were given Ghrelin at a dose of (2nmol/100µl saline) for 30 days S.C. The results show a significant ($P \leq 0.05$) decrease in all parameters of sperm analysis, the number of births and antioxidative system in hyperthyroid group compared to control group. While, all treated groups with GRE (0.5, 1 and 2 nmol) shows enhance effect in sperm analysis, the number of births and antioxidant system compared to the hyperthyroid group.

**Keywords: mice, thyroid gland, hormone.**

## Introduction

The thyroid hormones are a key metabolic regulator of testicular development and function, and it has the potential to affect spermatogenesis (1). Thyroid hormones affect the testis in a variety of ways and on a variety of cell types, including Leydig and Sertoli cells, as well as germ cells. Thyroid hormones excess or deficiency causes changes in testis function, including semen abnormalities.

Hyperthyroidism has been linked to decreased semen volume, sperm density, motility, morphology and increase oxidative stress whilst hypothyroidism has been linked to Disturbances in sperm morphology (2). Hyperthyroid rats had delayed spermatogenesis with maturation arrest, no pachytene spermatocytes, smaller seminiferous tubule diameters, reduced mitochondrial activity, and lower lipid levels (3). When compared control groups with hyperthyroid states, hyperthyroid individuals had reduced sperm motility, asthenozoospermia, oligozoospermia, and teratozoospermia (4). Furthermore, a recent study discovered a link between free T3 and ejaculate volume and seminal fructose levels positively (5). hyperthyroidism is bound to overstimulate metabolic state and upregulate excess free radical generation, causing oxidative damage and lipid peroxidation in various tissues (6,7).

Ghrelin is a peptide hormone with 28 amino acids produced mostly by secretory granules in the stomach's submucosal layer's X/A-like endocrine cells (8 ,9). Ghrelin may act as an autocrine or paracrine regulator of spermatogenesis. Ghrelin suppressed mRNA expression of the gene encoding stem cell factor (SCF), a critical signal for germ cell generation and a possible regulator of Leydig cell development, in adult rats (5). Chronic Ghrelin therapy improves the antioxidant defense mechanism in the rat testis by increasing glutathione peroxidase activity (GPX) and lowering malondialdehyde (MDA) levels (10). In this study we evaluated the effect of Ghrelin on sperm analysis and the antioxidant system.

## Materials and Methods

The present investigation was done at University of Basrah / Veterinary Medicine College. The study comprises two experiments a total of fifty adult male rats (12 weeks old, weighing (250-300g) were used male rats were kept for adaptation period of one month.

**Experimental design:** The rats (50 males) were split into five groups. The first group consisted of 10 male rats that were given normal saline for 30 days sub cutaneous (S.C) and set as a control group. While the second group (positive group) was male 10 rats with hyperthyroidism were given normal saline for 30 days S.C, the third group was 10 hypothyroidic male rats were given Ghrelin at a dose of (0.5nmol/100µl saline) for 30 days S.C, and the fourth group was 10 hypothyroidic male rats were given Ghrelin (1nmol/100µl saline) for 30 days S.C. The fifth group consisted 10 hypothyroidic male rats that were given Ghrelin at a dosage of (2nmol/100µl saline) for 30 days S.C.

**Sperm Motility Analysis:** The left caudal epididymis was immediately submerged in 2 mL of normal saline and sliced into small pieces with a surgical micro-scissor to liberate the spermatozoa from the epididymal tubules (11). The epididymal semen suspension was immediately incubated at 37°C for further investigation. A glass slide with 5µL of the suspension was placed on it and a computer assisted semen analysis was used to analyze it (CA.SA; Genex Laboratory / Florida - USA) at the artificial insemination and frozen semen straws production unit, Faculty of Veterinary Medicine, University of Basrah to assess general and progressive motilities. The value of progressive motility was just the straightforward spermatozoa; the value of general motility was the entire motile spermatozoa.

**Sperm Viability and Morphology Analysis:** Sperm viability and normal morphology values were determined using eosin-nigrosin stain (EN) as previously described by (12). Briefly, a 10 µL drop of epididymal semen suspension was mixed with 20 µL of EN, and the mixture was smeared on a microscopic a slide and left to dry on slide warmer at 45℃. Then, the slides were examined under a phase-contrast microscope at ×400 for sperm viability. The percentages of alive and dead sperms were calculated. The stained sperm with pink color considered dead and unstained sperm being lived. Moreover, for sperm normal morphology, only the normal sperm in terms of head, mid-piece and tail considered to be normal regardless the color of the sperm at ×1000 with oil emersion, and 200 sperm were counted for each test. The percentages of normal and abnormal shaped sperms were calculated.

**Epididymal Sperms Concentration:** To make a 1:100 dilution factor, 1 µl of epididymal semen suspension was mixed with 99 µl of formal saline. Yokoi *et al.,* (13) used a Neubauer hemocytometer to measure total sperm concentration.

**Calculating Concentration**: Each of the nine squares on the grid, including the central counting area of 25 large square, has an area of 1 square mm, and the cover glass rests $0.1mm^3$ or 0.1 microliters. You can thus multiply the average number of sperm over each central counting area by 10,000 to obtain the number of sperm per ml of diluted sample.

**Antioxidant system evaluation:** Measurements of MDA, Glutathione Peroxidase (GPX) catalase (CAT): all determined by ELISA kit Elabascience Biotechnology Inc. China

## Results

**Effect of Ghrelin on Epididymal Sperm Analysis and Number of Births in Hypothyroidic Adult Male Rats.**

**General Motility Percentage:** Table (1) shows that there was a significant (P≤0.05) decrease in all parameters of semen characteristics (percentage of general sperm motility, Progressive Motility Percentage, Morphologically Normal Sperms Percentage, Sperm Concentration**,** Viability of Sperm Percentage and number of births)

In the hyperthyroidism group compared to control group. While, all treated groups with Ghrelin (0.5, 1 and 2 nmol) show a significant (P≤0.05) increase in all parameters of semen quality (percentage of general sperm motility, Progressive Motility Percentage, Morphologically Normal Sperms Percentage, Sperm Concentration**,** Viability of Sperm Percentage and number of births) as compared to the hyperthyroidism group.

**Effect of Ghrelin on Testicular Tissue (MDA, CAT, GSH and GSH) in Hyperthyroidism Adult Male Rats:** Table (2) shows that there was a significant (P≤0.05) increase in serum MDA concentration of hyperthyroidism group as compared to the control group, while a significant (P≤0.05) decrease in serum MDA concentration were noticed in all groups with hyperthyroidism treated with GRE (0.5, 1 and 2 nmol) as compared to the hyperthyroidism group.

No significant differences observed in serum MDA concentration in groups treated with GRE (1 and2 nmol) as compared to control group. also, no significant differences observed in serum MDA concentration in group treated with GRE (1 nmol) as compared to groups treated with GRE (0.5 and 2 nmol). also, a significant (P≤0.05) increase in serum

MDA concentration in group treated with GRE (0.5 nmol) as compared to the hyperthyroidism group treated with GRE (2 nmol) and control group. On other hand, there was a significant (P≤0.05) decrease in serum CAT and GPX concentration of hyperthyroidism group as compared to the control group, while a significant (P≤0.05) increase in serum CAT and GPX concentration were noticed in all groups with hyperthyroidism treated with GRE (0.5, 1 and 2 nmol) as compared to the Hyperthyroidism group and control group. also, a significant (P≤0.05) increase in serum CAT and GPX concentration were noticed in group with hyperthyroidism treated with GRE (2 nmol) as compared to the other treated groups (0.5 and 1nmol). also, a significant (P≤0.05) increase in serum CAT and GPX concentration were noticed in group with Hyperthyroidism treated with GRE (1 nmol) as compared to the group with hyperthyroidism treated with GRE (0.5 nmol). Finally, there was not a significant in serum Fructose (FRU) concentration of hyperthyroidism group as compared with the control group, also non a significant in serum FRU concentration were noticed in all groups with hyperthyroidism treated with GRE between them (0.5, 1 and 2 nmol) and as compared to the hyperthyroidism group and control group.

**Table (1): Effect of Ghrelin on Epididymal Sperm Analysis and Mating in Hyperthyroidism Adult Male Rats. (Mean ±SD) (n=10)**

| Parameters / Groups | Motility | | Morphology (%) | Viability (%) | Concentration × $10^6$ | N. B |
|---|---|---|---|---|---|---|
| | General Motility (%) | Progressive Motility (%) | | | | |
| **Control (Normal saline)** | 77.8±10.7a | 74.1±11.2b | 96.3±0.5a | 80.6±6.5b | 3378.5±579.4d | 6.42±0.78b |
| **Hyperthyroidism** | 33.2 ±9.7b | 31.1±3.1c | 86.5±2.6c | 53.4±6.2c | 1730.0±613.9e | 0.00±0.00c |
| **Hyper+(0.5GHR)** | 85.7±3.4 a | 82.2±4.1ab | 90.8±4.8b | 87.9±5.7a | 4664.2±1424.4c | 6.71±0.75b |
| **Hyper+(1GHR)** | 90.7±3.4 a | 83.5±7.4ab | 93.0±3.2b | 91.3±1.2a | 6428.5±734.7b | 8.00±0.00a |
| **Hyper+(2GHR)** | 93.0±2.3 a | 89.8±0.6a | 97.5±0.73a | 92.1±1.5a | 7367.7±600.9a | 8.00±0.00a |
| **LSD** | 15.6 | 13.2 | 3.15 | 5.3 | 931.7 | 0.53 |

**Values expressed in the small letters mean significant differences at the (P≤0.05) level.**

**Table (2): Effect of Ghrelin on Testicular Tissue in MDA, CAT, and GPX in Hyperthyroidism Adult Male Rats. (Mean ±SD) (n=10)**

| Parameter Groups | MDA (nmol/L) | CAT (U/ml) | GPX (µmol/L) | FRU (µmol/L) |
|---|---|---|---|---|
| **Control** | 6.35±0.66c | 460.08±19.01d | 74.67±6.22d | 5.26±0.44a |
| **Hyperthyroidism** | 9.33±0.41a | 223.73±20.13e | 38.03±5.45e | 5.06±0.59a |
| **Hyper+(0.5GRE)** | 7.16±0.57b | 491.66±8.73c | 81.65±7.10c | 5.17±0.34a |
| **Hyper+ (1 GRE)** | 6.58±0.55bc | 550.06±14.98b | 102.37±5.43b | 5.22±0.72a |
| **Hyper+(2 GRE)** | 6.09±0.38c | 587.00±19.93a | 117.05±4.43a | 5.11±0.43a |
| **LSD** | 0.62 | 20.35 | 6.89 | 0.65 |

**Values expressed in the small letters mean significant differences at the (P≤0.05) level.**

## Discussion

Significant reduction in sperm concentration, percentage of motile, a viability and morphologically sperms in hyperthyroidism group compared with control group as shown in table. With these results, we agreement with other authors who found in comparison to control groups with euthyroid states, hyperthyroid individuals had lower sperm motility and morphology (14,2). Hyperthyroid male rats mainly suffer from change in the levels of testosterone and FSH hormones, these alterations in these hormones led to abnormal in epididymal sperm characteristics (15).

Thyroid problems (either excess or deficiency) are linked to controversial effects on male reproductive physiology, according to evidence from clinical and experimental research in male rats and humans (15). Deformed sperm heads can result from morphological abnormalities in the development or proper shaping of the sperm head, which generally reduces the sperm's ability to fertilize a mature oocyte. Defects in various sections of the tail cause morphological malformations in the tail area. The fraction of morphologically normal sperm was decreased in both hyperthyroid and hypothyroid males (16). aside from that, because to its high polyunsaturated fatty acid content, the sperm head membrane is extremely vulnerable to oxidative stress (17).

Changes in permeability of the cell membrane or mitochondrial membrane potential are caused by oxidative stress, which results in the release of apoptosis-inducing substances such cytochrome C from the mitochondria (18), Sperm viability and motility are reduced due to mitochondrial dysfunction.

Noblanc *et al.,* (19), For three key reasons, spermatozoa are particularly vulnerable to oxidative damage: first, post testicular spermatozoa are quiet cells with compressed DNA that are unable to participate in any transcriptional activation when exposed to oxidative stress. Second, due to the loss of most of their cytoplasm and subcellular organelles, spermatozoa are also protein synthesis quiet. Third, spermatozoa are especially susceptible to oxidative harm because their membranes contain large levels of lipids that are easily oxidized.

In hyperthyroid rats, increased ROS production owing to lipid peroxidation and an impaired antioxidant defense system has been linked to lower sperm count and motility (20,21). Likewise, Sahoo *et al.,* (22) found that T3-induced hyperthyroidism resulted in a decrease in sperm counts, exfoliation of germ cells into the lumen of seminiferous tubules, disorganization of the germinal epithelium, and an increase in interstitial space in the testicular section. In the testes of sexually mature hyperthyroid rats, there was a substantial halt in germ cell development and

signs of increased germ cell death (23). In addition, Sahoo *et al.,* (24) found that hyperthyroidism generated in rats by oral administration of L-T4 for 30 days resulted in a reduction in epididymal sperm counts and the percentage of viable sperms when compared to the control group.

According to the researchers, oxidative stress caused by hyperthyroidism in the testis can reduce Leydig cell steroidogenic capability and the germinal epithelium's ability to distinguish normal spermatozoa by activating sertoli cells, impairing male fertility (25). Catalase activity and total antioxidant capacity were shown to be substantially linked with sperm motility and morphology by (26). The foregoing ideas are consistent with the findings of the current investigation, which revealed a decrease in sperm concentration, viability, morphology, and motility, as well as a decrease in GPX and CAT activities.

Symptoms of reduced libido, elevated estrogens (gynecomastia), and erectile dysfunction, including sexual problems, are frequent in hyperthyroid males. Hyperthyroidism has also been linked to aberrant sperm characteristics, such as sperm motility and morphology (27). On the other hand, the results of hyperthyroidism groups treated with GRE reveal significant degrees of improvement in the above cited parameters compared with hyperthyroidism non treated. These findings are comparable to those of Ramírez *et al*., (28) found the administration of GRE causes an improvement in the sperm motility, sperm viability and sperm concentration. This may be due to the role of GRE in reducing the toxic effects of hyperthyroidism as well as the role of GRE as an antioxidant in the removal of free radicals resulting from hyperthyroidism effect (29) who found the GRE injection led to a significant degrees of improvement in epididymal sperm viability, sperm morphology, sperm concentration and observed In the cyclophosphamide (CP) group, the rapid progressive motility of spermatozoa, which is required for sperm fertility, was shown to be drastically reduced.

The Ghrelin receptor was found mostly in Leydig cells and to a lesser amount in Sertoli cells, confirming GRE function in spermatogenesis regulation. Furthermore, research have revealed that GRE delays the beginning of puberty in male rats and modulates the proliferation of Leydig cells (10). Chronic Ghrelin treatment improves the antioxidant defense mechanism in the rat testis by increasing GPx activity and lowering MDA levels, according to evidence (30).

Antioxidants have been shown in several studies to minimize lipid peroxidation and protect cells and tissues from the damage caused by oxidative stress (31, 32). Ghrelin is said to protect spermatozoa from oxidative damage caused by testicular ischemia/reperfusion (33). Likewise, (30) discovered that giving Ghrelin as an antioxidant lowers MDA levels in the testes.

The injection of Ghrelin to hyperthyroidic rats corrected for the hyperthyroidism-induced declines in epididymal sperm count, motility, and viability in this research. According to our findings, Ghrelin reduced hyperthyroidism-induced oxidative stress by limiting a rise in MDA levels and lipid peroxidation, resulting in an increase in sperm parameters as compared to the hyperthyroidic group. Higher spermatozoa motility and viability would, in turn, boost the fertility rate. Furthermore, Ghrelin treatment dramatically improved the antioxidant system in male rats.

(34) found that Ghrelin can raise serum antioxidant in rats during hypoxia, which is compatible with these data.

A considerable increase in testicular MDA concentrations was seen in hyperthyroid rats, associated with a reduction in CAT and GPX, according to the current study. In rat testis, altered thyroid function has been found to affect a variety of oxidative stress and enzymatic antioxidant defense (24, 35, 36, 37). These findings suggest that thyroxine administration resulted in a significant oxidative damage to the testis. the increased synthesis of MDA in hyperthyroidism has been attributed to a number of factors. One is reliant on the production of reactive oxygen species in excess (ROS). Thyroid hormones are recognized to be important in cell mitochondrial respiration. Hyperthyroidism can enhance mitochondrial respiration by altering the quantity and activity of mitochondrial respiratory chain components, resulting in the production of superoxide radicals as a result of increased cellular metabolism (38, 39). Superoxide radicals in turn may produce hydroxyl radicals, which subsequently initiate the process of lipid peroxidation (40).

Lipid peroxidation does not depend only on the capacity for generation of ROS, but also on the level of polyunsaturated fatty acids (PUSFAs) present in membrane phospholipids. the testis and spermatozoa have been considered to be highly susceptible to lipid peroxidation in the presence of increased ROS level, due to abundance of PUSFA in their membranes (41).

SOD, CAT, and GSH are antioxidant enzymes that serve as the first line of defense against ROS in the body. Superoxide radicals are dissimulated by SOD to hydrogen peroxides, which are then reduced to water by the cells' CAT and GPx enzymes (42). Increased oxidative stress surpasses cellular antioxidant enzyme activity, which may explain the decrease in SOD and CAT activities in the testis in response to hyperthyroidism (22, 36, 14) According to Sahoo et al., (24), the decrease in CAT activities in hyperthyroid rats' testicular tissue may impede the testis' ability to neutralize superoxide radicals and hydrogen peroxide created as a result of the hypermetabolic state caused by hyperthyroidism. In the current investigation, L-T4-induced hyperthyroidism results in a considerable reduction in GSH levels. This finding agrees with the prior study (43). All mammalian tissue contains GSH and is involved in a variety of biological activities in cells, including protein synthesis, DNA repair, and protection against oxidative damage (44). The reduction in GPX level in hyperthyroid testis has been considered to represent an adaptive mechanism in response to hyperthyroidism's increased generation of ROS. This result agreement with Choudhury et al., (45), did a study on rat testis after generating hyperthyroidism and observed that, while there is a negative influence on testicular glutathione peroxidase levels. Choudhury et al., (45) discovered a reduction in the content of reduced glutathione GSH, a "vital antioxidant molecule in Sertoli and spermatogenic cells." This theory was supported by the findings of the current investigation, which showed a rise in MDA and a reduction in GPX levels in hyperthyroidism. In contrast, according to Chattopadhyay et al. (46), the drop in testicular GSH concentration can be linked to an increase in GPX production in response to hyperthyroidism.

On other hand, all of these measures improved significantly after exogenous

treatment of GRE to these hyperthyroid rats. GRE acts as a free radical scavenger and is an endogenous antioxidant. In preadipocyte cell lines, GRE has been shown to exhibit antioxidative characteristics by enhancing antioxidant enzyme activities and lowering lipid peroxidation (47,48). GRE injection led to decrease the MDA this result agreement with Salimnejad et al. (29), discovered GRE injections reduced oxidative stress and testicular injury by enhancing antioxidant activity and lowering lipid peroxidation. This performance is most likely due to GRE's antioxidant properties. It appears that GRE, possibly through its antioxidant properties, protects testicular germ cells from the negative effects of hyperthyroidism, speeds up testicular regenerative processes in this condition, and increases the rate of total sperm motility and the percentage of sperm forward progressive movement, which could be attributed to an increase in intracellular Ca+2 level following GRE binding to its specific receptor (30, 49).

(50) reported that GRE enhances testicular antioxidant enzymes activities (CAT and GSH) and reduces lipid peroxidation and consequently attenuates testicular injury in diabetic rats. Also, rat testicular damage following experimentally induced cryptorchidism and testicular ischemia/reperfusion was attenuated by GRE through its antioxidant (51, 52) Thus, the treatment of hyperthyroidism male rats by exogenous GRE administration might be related to the antioxidant protective effects of this peptide on testes and an increase in semen parameters, as seen in the current work.

**Conclusions:** Ghrelin peptide hormone produce by stomach and it has been shown to have enhance effects on the sperm analysis and number of births which may be due the Ghrelin has antioxidant properties.

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## تأثير المحسن الكريلين على الحيوانات المنوية ونظام مضادات الأكسدة في ذكور الجرذان البالغة المصابة بفرط نشاط الغدة الدرقية

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### الخلاصة

الهدف من الدراسة هو تقييم التأثير المعزز للكريلين على الحيوانات المنوية والنظام المضاد للأكسدة في ذكور الجرذان بعد إحداث فرط نشاط الغدة الدرقية بواسطة الثايروكسين تم تقسيم الجرذان (50 ذكور) إلى خمسة مجاميع. تتكون كل مجموعة من 10 ذكور جرذان المجموعة الاولى تم إعطاؤهم محلول ملحي طبيعي لمدة 30 يومًا وتم تعيينهم كمجموعة سيطرة. بينما المجموعة الثانية (المجموعة الإيجابية) تتكون من 10 ذكور جرذان مصابة بفرط نشاط الغدة الدرقية تم إعطاؤهم محلول ملحي طبيعي لمدة 30 يومًا، المجموعة الثالثة تتكون من 10 ذكور جرذان مصابة بفرط نشاط الغدة الدرقية أعطيت كريلين بجرعة (0.5 نانومول / 100 ميكرولتر ملحي) لمدة 30 يومًا. المجموعة الرابعة تتكون من 10 ذكور جرذان مصابة بفرط نشاط الغدة الدرقية أعطيت كريلين

(1 نانومول / 100 مايكرولتر من محلول ملحي) لمدة 30 يومًا. وتألفت المجموعة الخامسة من 10 ذكور جرذان تعاني من فرط نشاط الغدة الدرقية تم إعطاؤهم كريلين بجرعة (2 نانومول / 100 ميكرولتر ملحي) لمدة 30 يومًا. أظهرت النتائج انخفاض معنوي في جميع معاملات تحليل الحيوانات المنوية وعدد المواليد والمضادات للأكسدة في مجموعة فرط نشاط الغدة الدرقية مقارنة بمجموعة السيطرة. بينما أظهرت جميع المجاميع المعالجة بـالكرلين تأثيرًا ايجابيا في تحليل الحيوانات المنوية، وعدد الولادات ونظام مضادات الأكسدة مقارنة بمجموعة فرط نشاط الغدة الدرقية.

الكلمات المفتاحية: الفئران ،الغدة الدرقية، الهرمون.