



## Effect of Hydrocortisone on Spermatogenesis in Male Rats

Maha Kh. Aljuboury<sup>1</sup> and Hassan I. Mohamed<sup>2\*</sup>

<sup>1</sup>Department of Biology, College of Science, University of Mosul, Mosul, Iraq.

<sup>2</sup>Department of Animal production, College of Agriculture, University of Telafer, Mosul, Iraq.



### Abstract

**T**HE STUDY investigates the effect of a month's treatment with hydrocortisone acetate on rat fertility and sperm activity. The study included 24 adult male rats divided into 3 groups. The first group (control) received DW, while the second received 2.5 mg/kg hydrocortisone. The third group received 5 mg/kg hydrocortisone. The injection period lasted 30 days, with an injection rate of 3 times weekly. The animals were anesthetized at the end of the treatment period. The sperm motility was calculated. Several readings taken and each reading was the same estimate of the sperm movement rate. The sperm concentration was calculated, and the live was classified as dead or mutilated. Blood was also taken to examine the testosterone concentration. The results showed that giving hydrocortisone had a dose-dependent effect on the number of sperm and their forward movement compared to the control group. The results also revealed a drop in testosterone levels in rats treated with low and high dosages of hydrocortisone. The findings also indicated an increase in the number of dead and malformed sperm. The study concluded that hydrocortisone at the levels used in the study has a direct negative effect on sperm and their activity, which is reflected in the animal's fertility. The present study concludes that hydrocortisone, even at modest doses, may have abnormal effects on spermatogenesis in the testes. In this present study, we recommend an extensive study on the impact of hydrocortisone drug to investigate the causes of infection and extend its infertility.

**Keywords:** Hydrocortisone, Testis, Sertoli cells, Testosterone.

### Introduction

Hydrocortisone is a solid and intense white and high-melted in water and alcohol and this property is to the glucocorticoids. Glucocorticoids group is a steroid hormonal group the most important cortisone hormone, and used to treat adrenal insufficiency and allergies, and it is also used to treat arthritis [1,32]. It works by lowering the inflammatory response in the body, reducing pain, itching, and swelling [1,2]. It is produced by the adrenal cortex and is one of the glucocorticoid hormones. An increase in hydrocortisone in the body causes tissue breakdown or catabolism, premature aging, and a decrease in the body's natural defense reaction [3].

Hydrocortisone is widely used to treat a variety of diseases as it improves the physical condition of patients with prostate cancer [4], and it also helps in the treatment of acute liver failure by increasing the rate of nuclear ribonucleic acid (RNA) synthesis, and increasing hepatic enzyme synthesis [5], in addition to its use to treat cerebral edema, especially in patients with brain tumors undergoing radiation and chemotherapy [6,7]. In mice, dexamethasone treatment has been linked to reduced androgen and sperm levels [8]. Mice exposed to mild chronic stress showed signs of decreased male spermatogenesis due to increased apoptosis and germ cell cycle arrest with activation of glucocorticoid receptor signaling in the testes [9].

\*Corresponding author: Hassan Mohamed, E-mail: hsaanft144@gmail.com.; Tel.: +964 751 515 4730

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Patients experiencing stress, severe illness, or surgical procedures—including mild, moderate, and major surgery are advised to take hydrocortisone liberally [10-12]. It is also used to treat adrenal hyperplasia, as an adjuvant in malignancies of some psychiatric disorders, some blood diseases, and as an anti-inflammatory in diseases of adrenal insufficiency [13]. Additional research has investigated the effects of synthetic hydrocortisone on the reproductive system and testicles [14], female reproduction, and mammalian testis [15].

The testicle is the main male reproductive organ responsible for producing sperm, and sperm formation is affected by the function of Sertoli cells [16; 13]. Previous studies have shown that using hydrocortisone in high doses for a long time causes an effect on high blood pressure, which reduces the blood supply to the testicles, as well as causes obesity and decreased sexual desire [17].

To investigate the direct effect of hydrocortisone on the testicle and its function, this study was conducted.

## **Material and Methods**

### *Place of Study*

The study was conducted at the University of Mosul\College of Veterinary Medicine \Animal House.

### *Animals*

The study utilized 24 adult male rats obtained from the College of Veterinary Medicine at the University of Mosul. The rats were 10-12 weeks old and weighed around 20-30 grams. Four animals were placed in each cage, and the animals were kept in laboratory conditions appropriate for laboratory animals, including temperature, humidity, light, and darkness. The animals were also given the necessary water and fodder throughout the trial.

### *Medicines and doses*

Pharaonia Pharmaceutical Company, Egypt, offers ready-to-inject ampoules containing 100 mg/2 ml of hydrocortisone acetate. The rats received intracecal injections at doses of 2.5 mg/kg and 5 mm/kg. The dose for each animal was determined based on its weight, and the medicine was diluted with distilled water.

### *Ethical approval*

Ethical approval was obtained from the University of Mosul, College of Science, Department of Life Sciences, and the controls and instructions followed in the Code of Laboratory Animal Care were followed.

### *Experiment design*

Number of 24 animals were distributed into 3 groups: The first group (control) (8 male) was injected with distilled water only. The second group (8 male) was injected with a dose of 2.5 mg/kg of hydrocortisone. The third group (8 male) was injected with a dose of 5 mg/kg hydrocortisone. The injection period lasted for thirty days, twice a week.

### *Animal anatomy*

The animals were anesthetized at the end of the treatment period and dissected on the 31st day. The belly was sliced from above, the abdominal cavity was opened in the shape of an inverted T, the epididymis was removed, the adipose tissue adhering to it was removed, it was dried with filter paper, and it was immersed in a physiological saline solution for the following calculations. They were prepared in accordance with (Al-Shenawy and Abu Al-Wafa,)[18].

### *Laboratory preparations*

Sperm motility was calculated using method 2.5.1. The epididymis was placed in a glass dish on a hot plate at 37 degrees Celsius, filled with a physiological saline solution, and severed the tail of the epididymis to release the sperm, then took a drop and placed it on the solution. Then a glass slide was placed on it, and sperm movement was monitored under a microscope at 40 times magnification. Multiple readings were collected, and each measurement was the same estimate of the rate of sperm movement [19].

### *Sperm concentration*

Using a special pipette, add a drop of the sperm combination to the 0.5 mark and fill the rest of the bottle with a 1:200 diluted solution. The pipette was inverted to mix the two solutions that were placed inside. Tossed the first two drops and then placed the third on a red blood cell counting slide. Then the sperm were counted. The number of sperms were counted in 25 tiny squares divided into corner squares and a middle square (five middle squares) using the recognized method [19].

$$\text{Sperm count/ml} = (n \times 4000 \times 200) / (80 \times 0.1)$$

#### *Percentage of live and dead sperm and sperm motility*

A drop of the semen mixture was placed on a glass slide on a hot plate set to 37 degrees Celsius. A second drop of the nigrosin-eosin mixture was applied in the same size as the first. Two droplets were combined on a clean glass slide. The slide was then placed in the second layer, on top of the first slide. Drop the mixture along the first slice and let it dry at room temperature before examining it under 40x magnification. The percentage of live and dead sperm is estimated using the following equation: Number of live sperm = 100 (number of live sperm)/(number of total sperm)

Number of dead sperm = 100 (number of dead sperm)/(number of total sperm)

#### *Sperm abnormalities*

The prepared slide was used to count the number of live and dead sperm to calculate the percentage of deformed sperm, and was examined under 40 times magnification, and the percentage of deformed sperm was calculated according to the formula:

Number of abnormal sperm = 100 (number of abnormal sperm)/(total number of sperm)

#### *Collect blood samples to measure testosterone concentration:*

Before killing the animal, blood samples were taken using the heart stab procedure and a 1 ml syringe. The blood samples were deposited straight in a sterile Eppendorf tube, centrifuged at 2000 rpm for 10 minutes to separate the serum, and then frozen at -20 °C. Hormone concentration was determined using the Vidas Mini device and a device-specific kit.

#### *Statistical analysis*

The results were statistically analyzed using the SPSS statistical program, and a one-way ANOVA test for least significant differences (LSD) was used to calculate the significance of the differences at the significance level specified for the test ( $P < 0.05$ ) [20].

### **Results**

The effects of hydrocortisone acetate on sperm parameters were analyzed using one-way ANOVA followed by LSD test. Results showed a significant difference in the total number of sperm,  $p < 0.01$ ), sperm density,  $p < 0.05$ ), and forward movement of sperm,  $p < 0.05$ ) among the Control, Low dose, and High dose groups. the differences were significant between

Control and Low dose groups ( $p < 0.05$ ) for all parameters. There was also a significant difference between Control and High dose groups for total number of sperm ( $p < 0.01$ ). However, there were no significant differences observed between the Low dose and High dose groups for any parameter.

Additionally, the comparison of testosterone levels between the Low dose and High dose groups showed a significant difference ( $p < 0.05$ ). (Table 1).

#### *Live sperm, dead sperm, and Teratozoospermia*

The effects of hydrocortisone acetate on sperm parameters were analyzed using one-way ANOVA followed by LSD test. Results showed a significant difference in the percentage of live sperm,  $p < 0.05$ ), percentage of dead sperm,  $p < 0.05$ ), and percentage of teratozoospermia,  $p < 0.05$ ) among the Control, Low dose, and High dose groups. the differences were significant between Control and Low dose groups ( $p < 0.05$ ) for all parameters. There was also a significant difference between Control and High dose groups for percentage of live sperm ( $p < 0.05$ ). However, there were no significant differences observed between the Low dose and High dose groups for any parameter.” (Table 2).

### **Discussion**

The current study emphasizes the need of exploring and investigating the impact of hormone medicines on fertility and reproduction in people and animals. According to the current study, hydrocortisone medication reduces the overall number of sperm as well as their density, which may be due to sperm production issues. This is mostly due to the interplay between testosterone and follicle-stimulating hormone, which is essential for the production of immature testis [21; 22], Hydrocortisone also had an effect on sperm motility when compared to control, indicating a defect in the process of normal sperm development and maturation, as corticosteroids cause problems with male gamete formation and usually cause damage to testicular tissue, reducing or stopping sperm formation in cases of primary testicular dysfunction [23].

Hydrocortisone injections also reduced testosterone levels in the blood due to pituitary gland suppression, as hydrocortisone inhibits the secretion of ACTH from the

pituitary gland, which is required for the production of DHEA and pregnenolone from the adrenal glands, two precursor hormones required for testosterone production [24]. Cortisol is a stress hormone that, when secreted excessively, can decrease testosterone production. Hydrocortisone can cause testosterone to bind more to proteins in the blood, reducing its availability for usage by the body [5, 25].

Research suggests that decreased testosterone levels may cause a shift in the activity of Leydig cells, leading to an increase in abnormally shaped dead sperm [26]. According to one study, some men who take hydrocortisone develop oligospermia or azoospermia due to a decrease in sperm production rate [26].

Previous research [27; 28] demonstrated that after treatment with hydrocortisone, sperm lost their usual shapes and exhibited the characteristics of dead cells, resulting in severe weakening, as they are harmful to Leydig cells, sperm, and Sertoli [29].

There is a hypothesis that the level of masculinity and femininity in male children of stressed mothers is related to increased production of maternal corticosteroids, implying that corticosteroids have a direct effect on masculinity [30, 31].

## Conclusion

The present study concludes that hydrocortisone, even at modest doses, may have abnormal effects on spermatogenesis in the testes, impairing male gonadal function. As a result, systematic preventive measures must be implemented in order to limit the dangers connected with hydrocortisone, which is utilized as a therapeutic medicine.

## Recommendation

In this present study, we recommend an extensive study on the impact of hydrocortisone drug to investigate the causes of infection and extend its infertility and its impact on the body condition as a whole.

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**TABLE 1. Effect of hydrocortisone acetate on total number of sperm, density of sperm, forward movement of sperm.**

Group/ Slandered	*Total number of sperm	*Sperm density	*Forward movement of sperm	*Testosterone (ng/ml)
Control	2817151.0±92088.685 <sup>A</sup>	52.68±1.22287 <sup>A</sup>	40.12±8.11877 <sup>A</sup>	1.97±0.043 <sup>A</sup>
Low dose of hydrocortisone acetate	1231887.4±39217.199 <sup>B</sup>	31.48±0.62081 <sup>B</sup>	21.50±1.24177 <sup>B</sup>	1.15±0.28 <sup>A</sup>
High dose of hydrocortisone acetate	674713.80±23431.790 <sup>C</sup>	20.48±0.69742 <sup>C</sup>	5.960±0.55191 <sup>C</sup>	0.79±0.15 <sup>A</sup>

\*Mean ±SE According to Duncan's test, the number followed by vertically distinct letters indicates that there are significant differences between them when possible (P<0.05).

**TABLE 2. Effect of hydrocortisone acetate on percentage of live sperm, dead sperm, teratozoospermia**

Group/standard	*% Live sperm	*% Dead sperm	*% Teratozoospermia
Control	72.940±1.087 <sup>A</sup>	16.06±1.07079 <sup>A</sup>	11.00±0.47645 <sup>A</sup>
Low dose of hydrocortisone acetate	37.300±2.053 <sup>B</sup>	28.32±1.26665 <sup>B</sup>	34.38±1.01015 <sup>B</sup>
High dose of hydrocortisone acetate	21.320±3.224 <sup>C</sup>	37.88±2.4084 <sup>C</sup>	40.80±1.21285 <sup>C</sup>

\*Mean ±SE According to Duncan's test; the number followed by vertically distinct letters indicates that there are significant differences between them when possible (P<0.05).

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## تأثير الهيدروكورتيزون على تكوين الحيوانات المنوية في ذكور الجرذان

مها خلف الجبوري<sup>1</sup> و حسان اكرار محمد<sup>2</sup>

<sup>1</sup> قسم علوم الحياة - كلية العلوم - جامعة الموصل - الموصل - العراق .  
<sup>2</sup> قسم الانتاج الحيواني - كلية الزراعة - جامعة تلغفر - الموصل - العراق

### المستخلص

هدفت الدراسة للبحث تأثير استخدام عقار الهيدروكورتيزون لمدة شهر على خصوبة ونشاط الحيوانات المنوية في ذكور الجرذان. استخدمت الدراسة ذكور الجرذان البالغة بعدد 24 من الجرذان، تم توزيع الجرذان على 3 مجاميع: المجموعة الأولى (السيطرة) حقنت بالماء المقطر فقط والمجموعة الثانية تم حقنهم بجرعة 2.5 ملغم/كغم من الهيدروكورتيزون والمجموعة الثالثة تم حقنهم بجرعة 5 ملغم/كغم من الهيدروكورتيزون، استمرت مدة الحقن 30 يوماً وبمعدل 3 مرات بالأسبوع. بعد انتهاء مدة المعاملة تم تخدير الحيوانات ومن ثم التشريح. تم حساب حركة الحيوانات المنوية وتركيز الحيوانات المنوية، وتم تصنيف الحيوانات المنوية (الحية المشوهة)، وقياس تركيز هرمون التستوستيرون في الدم. أظهرت النتائج أن إعطاء الهيدروكورتيزون كان له تأثير على عدد وحركة الحيوانات المنوية بالاعتماد على تركيز الجرعة المعطاة إذ كانت عالية أو منخفضة مقارنة بمجموعة السيطرة، وكذلك أظهرت النتائج انخفاض معنوي في تركيز هرمون التستوستيرون في ذكور الجرذان، كما اشارت النتائج الى زيادة عدد الحيوانات المنوية المشوهة والميتة مقارنة بمجموعة السيطرة. استنتجت الدراسة إلى أن عقار الهيدروكورتيزون على المستويات المستخدمة في الدراسة لها تأثير سلبي مباشر على الحيوانات المنوية ونشاطها، والذي ينعكس في خصوبة الحيوان. تختتم الدراسة الحالية أن الهيدروكورتيزون عند اعطائه بجرعات قليلة قد يكون له أثار غير طبيعية على الحيوانات المنوية في الخصيتين.

في هذه الدراسة نوصي بدراسة واسعة النطاق حول تأثير عقار الهيدروكورتيزون على الجسم والخصى وعلاقتة بالعقم بوجه الخصوص.

**الكلمات المفتاحية:** الهيدروكورتيزون، الخصى، خلايا سرتولي، التستوستيرون.