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THE EFFECT MEDICATION USED IN THE COVID-19 PROTOCOL ON HORMONE LEVELS (FSH, LH, TESTOSTERONE) AND SPERM PARAMETERS IN MALE RATS

Nawras Jassim Mohmmed[1], Ali Khalaf Ali[1], Mustafa S. Chaji[2]
1- Department of Biology, College of Science, University of Misan, Maysan, Iraq
2- Department of Anatomy and Histology, Collage of Veterinary Medicine, University of Basrah, Basrah, Iraq
Mostafa.saddam@uobasrah.edu.iq

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Abstract:
The medications employed in the COVID-19 treatment regimen may contribute to the detrimental effects of the coronavirus disease (COVID-19) on the male reproductive system. This study aims to evaluate the effect of drugs used in the treatment protocol for COVID-19 on the male reproductive system of rats by measuring the level of sex hormones (LH, FSH Testosterone) and also through changes in sperm parameters (concentration, motility, dead and abnormal sperm). A total of 30 adult male rats were used, and they were split into two groups, each with 15 rats. The protocol group received the medication used in the therapeutic protocol for Covid-19, while the control group received saline solution. Five rats from each group were euthanized at the end of the first and second weeks of treatment as well as at the end of the fifth week after treatment had ended. Blood samples were collected to obtain a serum to test hormones levels, and the epididymis was also removed for sperm examination. The results of the study showed a significant increase in LH hormone in the first and second weeks of the experiment and a decrease in the fifth week, while the FSH hormone decreased in the fifth week. While testosterone hormone decreased in the second and fifth weeks, as well as decreased sperm concentration and motility, and increased the percentage of dead and abnormal sperm in the protocol group, we deduce from these findings that medicines used to treat Covid-19 have a deleterious impact on male rat sperm parameters and sex hormones levels.

Keywords: COVID-19 Protocol; LH; FSH; Testosterone; Sperm parameter

1-INTRODUCTION

Numerous studies have revealed that COVID-19 may have an impact on the male reproductive system either directly or indirectly. Many investigations have identified morphological alterations in the testicles, including interstitial congestion, microthrombosis, a reduction in Sertoli, Leydig, and germ cells, and atrophic seminal tubules with orchitis. These changes can raise the chance of male infertility. Men who are infected exhibit changes in the characteristics of their sperm as well as a malfunction in the hypothalamus-pituitary-testicular axis. (Rago & Perr. 2023).In COVID-19 treatment protocols, a variety of medications have been used, including antivirals like Remidsvir, antibiotics like Azthromycin, corticosteroids, including dexamethasone as an anti-inflammatory, and heparin as an anticoagulant, in addition to dietary supplements like vitamins C and D and minerals like zinc.(COVID-19 Treatment Guidelines.,2021and COVID-19 rapid guideline). Through several previous studies conducted separately, animal models showed that these treatments affect male fertility and spermatogenesis and also affect sex hormones) Fan.,et al. ,2020 & Abeer,.2015 & El-Sayed,et al., 2017& Sadeghzadeh, et al.,2019& Hanafy, & Khalil.2015). a literature analysis also found a link between the toxicity of the reproductive system and the medications used to treat Covid-19,) Guo et al.,2021).Diagnosing male infertility mostly relies on semen. Semen analysis should be accompanied by measurement of serum hormone levels of at least the pituitary-produced gonadotrophins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in addition to testosteron,(Tüttelmann et al.,2018). Follicle stimulating hormone (FSH) is a glycoprotein synthesized and secreted by the anterior pituitary (Mullen et al.,2013), and is essential for sperm production because it plays a crucial role in the differentiation of sperm cells into sperm. Since germ cells lack testosterone receptors, this hormone works by binding to the Sertoli cells in the testicles that are in charge of feeding germ cells (Hameed et al., 2011). Luteinizing hormone (LH), a glycoprotein also known as interstitial cell stimulating hormone (ICSH), stimulates spermatogenesis. by stimulating the release of the testicular lipid hormone testosterone through its impact on the leydig cells found in the testicular interstitial cells (Robert., 2010 & Jimoh et al .,2012). The primary male hormone responsible for regulating sexual differentiation and fertility is testosterone. Additionally, it affects spermatogenesis. Testosterone is produced in the interstitial cells, distributed in the seminiferous tubules, and interacts with receptors in Sertoli cells located in the cytoplasm and nucleus of these cells. Its main purpose is to support spermatogenesis (Nassar and Leslie ., 2018 & Dutta et al., 2019). This study aims to assess the impact of medications employed in the COVID-19 treatment protocol on sex hormones (FSH, LH, and testosterone) and sperm parameters (sperm concentration, motility, and the percentage of dead and abnormal sperm).

2. MATERIALS AND METHODS

2.1 Experimental animals

Thirty male rats, aged 4 to 8 weeks and weighing between 230 and 310 grams, were purchased from the college of veterinary medicine at Basra University. Rats were kept in a cage with a regulated climate (25 ± 2°C, 50 ± 5% relative humidity), a 12-hour light/dark cycle, and unrestricted access to food and drink. Pathogens are not present in these rats. Two weeks before the start of the experiment, allow the rat to adjust, and then dose it with medication

2.2.Groups:

Table (1): the animals were divided into two groups:

Control group **15-male rat**	**Protocol group** **15-male rat**
received normal saline orally for 14-day	administered medication used in the COVID-19 Protocol (COVID-19 Treatment Guidelines. 2021), once a day for 14-day 1-Remidsvir (IP) injection for five day ,(10 ml/Kg) 2-Dexamethasone injection (IM), (0.6 ml/Kg). 3-Heparin injection (SC) ,(4.8 ml/Kg). 4-Azthromycin (5.2 ml/Kg) orally. 5- Supplement → Zink (4.8 mg/Kg) orally. → Vitamin C (800 mg/Kg) orally. → Vitamin D (0.12mg/kg) orally.

Based on the surface area technique, the doses were estimated using the human doses and converted to the animal dose,(Nair & Jacob., 2016).

2-3-Sample collection

Five rats from each group were euthanized by chloroform at the end of the first and second weeks after giving the treatment and at the end of the fifth week from the beginning of the experiment after stopping the treatment**.** In order to get serum for hormone measurements, including LH, FSH, and testosterone, blood samples were taken from the heart and placed in tubes that aid in clotting, and it was measured by using a device (ELISA) , and then the epididymis was taken to examine sperm parameter According to (Jaâ.,2015).Sperm concentration and motility were measured by a computer-assisted sperm analysis (CASA) according (Adamkovicova et al ., 2016). dead and abnormal sperm were calculated according to (Björndahl et al., 2003).

2-4-Statistical analysis

Software (SPSS) was used to assess the data's mean and standard deviation using a one-way ANOVA (analysis of variance) procedure, utilizing the LSD test for statistical differences and at the ($p \leq 0.05$) threshold of significance (Nwaigwe & Chrysogonus. 2021).

3- RESULTS

3-1-LH serum levels

The study's findings indicated that there were significant increase ($p \leq 0.05$) in the LH serum level in the protocol group during the first, second weeks. Where the LH serum level in the first week of the control group was (0.004 ± 0.005), the protocol group (0.03 ± 0.004), While In the second week, the LH serum level of the control group was (0.004 ±0.005), the protocol group (0.03 ± 0.01), In the fifth week, the LH serum level significant decreased ($p \leq 0.05$) in the protocol group, the LH serum level of the control group was (0.004 ± 0.005),the protocol group (0.00 ± 0.00), as shown in table (2).

3-2-FSH serum level

The study's findings indicated that there were no significant differences ($p > 0.05$) during the first and second weeks compared to the control group, where the FSH serum level in the first week of the control group was (0.012± 0.01), the protocol group (0.01 ± 0.01), While the FSH serum level of the control group in the second week was (0.012 ± 0.01), the protocol group had (0.00 ± 0.00), In the fifth week, the result of the study showed that there were significant decreases ($p \leq 0.05$) in FSH serum level in the protocol group compared to the control group The FSH serum level of the control group was (0.012 ± 0.01), the protocol group was (0.004 ± 0.005), as shown in table (2).

3-3-Testosterone serum level

The study's findings indicated that there were no significant differences ($p>0.05$) in the testosterone serum level in all groups during the first week period, where the testosterone serum level of the control group was (3.56 ± 1.41), the protocol group (4.00 4± 3.73), In the second and fifth weeks period, the result of the study showed that there was a significant decrease ($p \leq 0.05$) in the testosterone serum level in the protocol group compared to the control group where the testosterone serum level of the control group was (3.58 ± 1.33), the protocol group (0.53 ± 0.63), the testosterone serum level of the control group was (3.95 ± 1.44), the protocol group (1.22 ± 1.40), as shown in table (2).

Table (2): The changes in the LH, FSH and Testosterone serum levels of male Rat over the five weeks:

Group	Week	LH (m IU/ml)	FSH (m IU/ml)	Testosterone (ng/ml)
Control	**1st week**	0.004 ± 0.005 **a**	0.012 ± 0.01 **a**	3.56 ± 1.41 **a**
	2nd week	0.004 ± 0.005 **a**	0.012 ± 0.01 **a**	3.58 ± 1.33 **a**
	5th week	0.004 ± 0.005 **a**	0.012 ± 0.01 **a**	3.95 ± 1.44 **a**
Protocol	**1st week**	0.03 ± 0.004 **b**	0.01 ± 0.01 **a**	4.004 ± 3.73 **a**
	2nd week	0.03 ± 0.01 **b**	0.00 ± 0.00 **a**	0.53 ± 0.63 **b**
	5th week	0.00 ± 0.00 **b**	0.004 ± 0.005 **b**	1.22 ±1.40 **b**

3-4- Sperm concentration

The study's findings indicated that there was a significant decrease ($p \leq 0.05$) in the concentration of sperm in the protocol group during the first, second, and fifth weeks, where the concentration of sperm in the first week of the control group was (123.84 ± 31.10), the protocol group was (48.06 ± 10.68), the concentration of sperm in the second week of the control group was (126.42 ± 28.72), the protocol group was (35.02 ± 9.48), While the concentration of sperm in the fifth week of the control group was (130.46 ± 26.87), the protocol group was (35.54 ± 6.56), as shown in the table (3).

3-5-Sperm motility

The study's findings indicated that there was a significant decrease ($p \leq 0.05$) in the motility of sperm in the group over a five-week period: the motility of sperms in the control group in the first week was (70.72 ± 8.96), the protocol group (15.96 ± 2.72), the motility of sperms in the control group in the second week was (71.68 ± 8.16), the protocol group (12.68 ± 4.52), the motility of sperms in the control group in the fifth week was (71.94 ± 9.05), and the protocol group (20.00 ± 2.87) as shown in the table (3).

3-6-Sperm dead

The study's findings indicated that there was a significant increase ($p \leq 0.05$) in the count of dead sperm in all groups during the first, second, and fifth weeks. where the count of dead sperms in the control group was (23.60 ± 4.16), the protocol group (34.52 ± 3.31), the count of dead sperms in the control group in the second week was (22.28 ± 4.69), the protocol group (36.10 ± 3.48), the count of dead sperms in the control group in the fifth week was (22.22 ± 4.39), and the protocol group (31.94 ± 2.45), as shown in the table (3).

3-7-Abnormal sperm

The study's findings indicated that there was a significant increase ($p \leq 0.05$) in the count of abnormal sperm in the protocol group during the first, second, and fifth weeks, where the count of abnormal sperm in the control group was (20.36 ± 1.06), the protocol group (29.20 ± 5.63), in the second week the count of abnormal sperm in the control group was (19.08 ± 1.37), the protocol group (29.90 ± 4.68), in the fifth week the count of abnormal sperm in the control group was (18.76 ± 2.24), the protocol group (26.00 ± 2.91), as shown in the table (3).

Table (3): The changes in the concentration, motility ,dead sperm, and abnormal sperm of male Rat over the five weeks:

Group	Week	Concentration of sperms10^5	Motility of sperms %	Dead of sperms%	Abnormal of sperms%
Control	**1st week**	123.84 ± 31.10 **a**	70.72 ± 8.96 **a**	23.60 ± 4.16 **a**	20.36 ± 1.06 **a**
	2nd week	126.42 ± 28.72 **a**	71.68 ± 8.16 **a**	22.28 ± 4.69 **a**	19.08 ± 1.37 **a**
	5th week	130.46 ± 26.87 **a**	71.94 ± 9.05 **a**	22.22 ± 4.39 **a**	18.76 ± 2.24 **a**
Protocol	**1st week**	48.06 ± 10.68 **b**	15.96 ± 2.72 **b**	34.52 ± 3.31 **b**	29.20 ± 5.63 **b**
	2nd week	35.02 ± 9.48 **b**	12.68 ± 4.52 **b**	36.10 ± 3.48 **b**	29.90 ± 4.68 **b**
	5th week	35.54 ± 6.56 **b**	20.00 ± 2.87 **b**	31.94 ± 2.45 **b**	26.00 ± 2.91 **b**

- Values show mean and standard deviation. The same small letter indicates no significance betweengroups, while a distinct small letter indicates significance ($p \leq 0.05$) between groups.

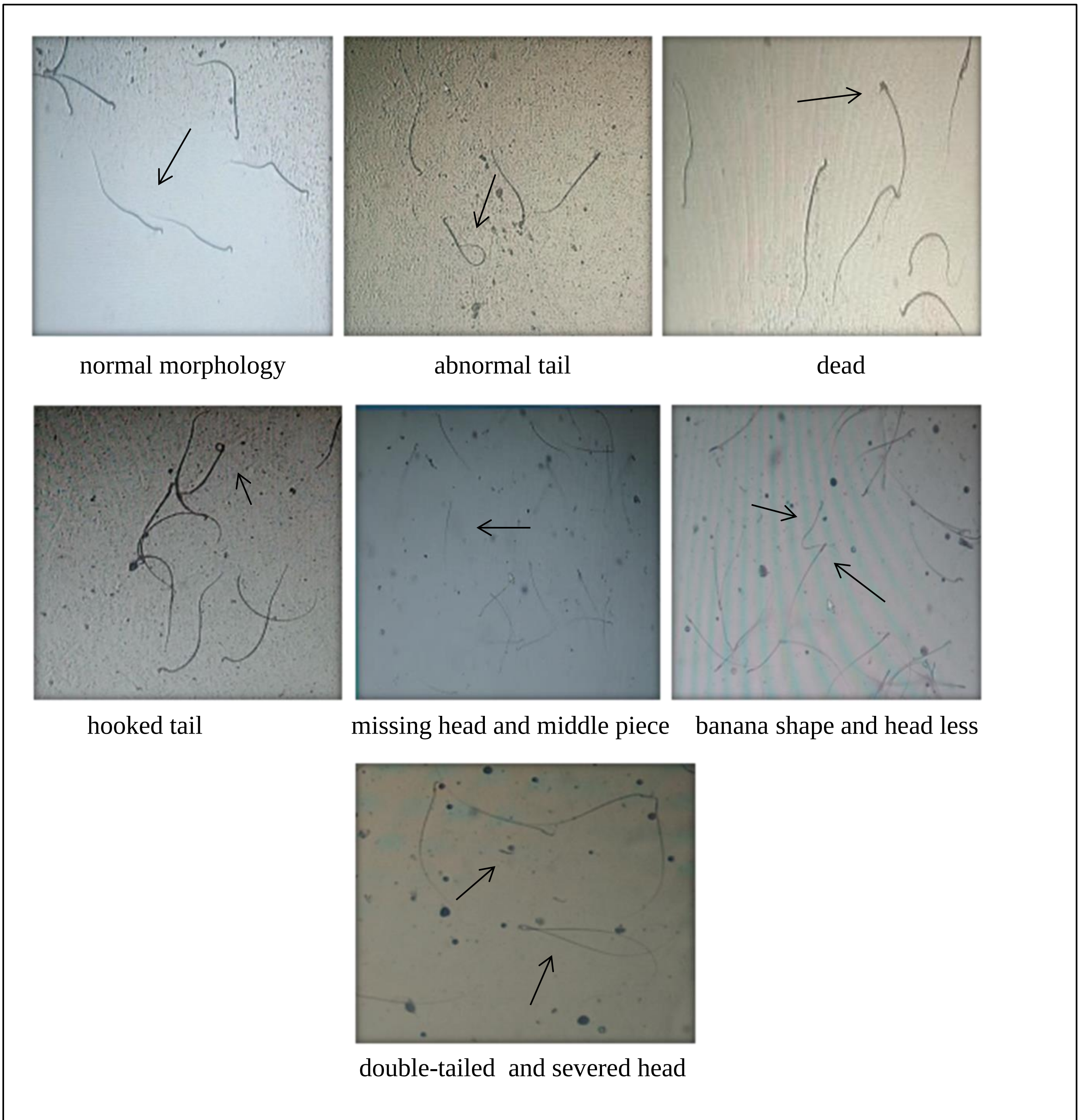

Figure (1): Microphotographs Normal Sperm, And Various Sperm Abnormalities, (Nigrosine And Eosin Stain,400x).

4- DISCUSSION

The results of the current study showed that the medications used in the treatment protocol for Covid-19 cause significant changes in sex hormones in male rats, the level of LH increased during the first and second weeks of treatment and decreased during the fifth week after treatment was stopped. Testosterone did not significantly change during the first week of treatment, although it decreased during the second and fifth weeks, and the hormone FSH did not alter significantly in the first or second week, but it did in the fifth week..

LH production may have increased due to diminished testosterone feedback inhibition.(Elsawah et al., 2020). While decrease in hormone levels could have been caused by damage to Leydig's cells, which generate testosterone. (Abd-Allah et al. 2000, Khaki et al. 2009). These outcomes supported the conclusions stated by (Abeer.,2015) who asserted that taking azithromycin dramatically reduced serum testosterone levels. Also dexamethasone reduces serum levels of testosterone (Sadeghzadeh et al.,2019) this is due to how much transcription of genes encoding testosterone-producing enzymes is significantly reduced as a result,(LaVoie & King.,2009).

Covid-19 protocol causes a decrease in sperm concentration, motility, and increase dead and abnormal sperm, these results coincide with)Sadeghzadeh et al .,2019& El-Sayed et al., 2017 & Fan et al .,2020), sperm motility may be impacted by a stoppage in ATP production as a result of oxidative stress. (Karbalay & Noorafshan.,2011), the earlier studies also demonstrated that dexamethasone's primary target is the mitochondria, as the medication affects the regulation of 72% of the genes involved in the mitochondrial respiratory chain, which lowers ATP levels and slows sperm motility. (Mutsaers & Tofighi.,2012).

The considerable decline in sperm characteristics may be caused by increasing ROS causing oxidative injury to the testicles (Jeje et al., 2017). Lipid peroxidation of the sperm membrane is thought to be the primary mechanism by which ROS cause sperm damage, which may result in infertility (Agarwal et al., 1994).

A key factor in the toxicity of many xenobiotics is oxidative damage, which is indicated by the lipid peroxidation (LPO) marker. MDA is a stable LPO byproduct that can be used to evaluate cumulative LPO indirectly. Due to the abundance of polyunsaturated fatty acids in the plasma membrane and the low levels of cytoplasmic antioxidants, mammalian spermatozoa are vulnerable to LPO, (Aitken et al., 1993).

high levels of LPO can reduce sperm motility, most likely by an abrupt loss of intracellular ATP, which causes axonemal harm , decreasing sperm viability and an increase in abnormal morphology with negatively influencing sperm capacitation and acrosome response (Lenzi et al., 1993). as a result, spermatogenesis and testicular steroidogenesis are affected.

Treatment with dexamethasone and Azthromycin causes a high level of MDA (Jeje et al ., 2020 &El-Sayed et al., 2017). A higher-than-normal MDA level in the testicles indicates lipid peroxidation. As a result, a rise in MDA levels harms sperm function and causes infertility (Hsieh et al., 2006).

5-CONCLUSION

From the current findings, it could be concluded that the covied-19 treatment protocol It affects the balance of male sex hormones and also has negative effects on the features of semen and thus causes a defect in the process of spermatogenesis and that these effects continued for a period after treatment.

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