Saudi Journal of Biological Sciences 28 (2021) 2558–2565

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Effect of Filgrastim on adult male rats' fertility and reproductive performance

Sara Mohamed Saber [a], Nouf Bader Alduweesh [b,c], Heba Ali Abd El-Rahman [c,*], Amel Ramadan Omar [c]

[a] Department of Histopathology, National Organization for Drug Control and Research, (NODCAR), Egypt
[b] Department of Zoology, Faculty of Science, Kuwait University, Kuwait
[c] Department of Zoology, Faculty of Science, Cairo University, Egypt

ARTICLE INFO

Article history:
Received 25 August 2020
Revised 24 January 2021
Accepted 27 January 2021
Available online 11 February 2021

Keywords:
Filgrastim
Rat
Testis
Sperms
Reproductive hormones
Oxidative stress
Histology
Caspase-3

ABSTRACT

Filgrastim is a recombinant protein used in treatment neutropenia caused by myelosuppressive medications for patients with non-myeloid cancer. However, its effect in male fertility is not clear. So, the current work aims to clarify the effect of Filgrastim on the reproductive state in Wistar rats. Eighteen (18) male Wistar rats were divided into three groups (6/each). Group (I) where the rats were injected with 0.5 ml/kg/day of distilled water and served as Control Group. The Group (II) animals received intraperitoneal injection of therapeutic dose of 30.83 mcg/kg/day of Filgrastim for one week. The Group (III) rats received the same dose by the same route of Filgrastim for two weeks. Sera of blood samples were processed for serum follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (TS). Semen analysis and resazurine reduction test (RRT) were performed. Assaying for malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) was done. The testes were retrieved for histopathological and immunohistochemical studies for caspase-3 detection. Our results revealed that filgrastim affects sperm morphology, significantly decreased the RRT and the reproductive hormones level, elevated the oxidative stress status and induced several histopathological changes in testes with an increased in immunoexpression of caspase-3 in testes tissues. The results of this work demonstrated that Filgrastim may had a deleterious effect on male fertility.

1. Introduction

Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic glycoprotein predominantly secreted by stromal, endothelial cells, macrophages, fibroblast and monocytes. It is highly combined with G-CSF receptors. It induces the proliferation and differentiation of cells in the granulocyte origin (Crobu et al., 2014).

Exogenous G-CSF is used for patients with acute myelogenous leukemia, solid and hematologic malignancies and bone marrow/peripheral stem cell transplantation after chemotherapy so as to increase the rate of neutrophil recovery. In spite of the common use of G-CSF, the risk of undesirable effect has to be thought out in the use of such medication (Liongue et al., 2009).

Previous studies on G-CSF associated adverse effects with healthy persons and persons with chronic neutropenia, cancer, splenic rupture flares of underlying autoimmune disorders, bone pain, vascular events and lung injury. G-CSF administration may cause some complications for the healthy individuals, such as splenic rupture and acute myeloid leukemia from one to five years (D'Souza et al., 2008).

Filgrastim or recombinant Met-G-CSF is a single chain protein composed of 175 amino acids and two disulfide bonds. It is one of the three G-CSF currently used to decrease the frequency of febril neutropenia (FN) when used as prophylaxis after chemotherapy (Cooper et al., 2011).

The human life is affected by several factors like reproductive, maternal health and fetal and child health as well (Shishehgar et al., 2014). Thus, reproductive health has recently helped change the areas researchers are interested in. Infertility is the inability to give birth to a baby after one year or more of regulars exist mating and it is considered a fundamental defect of the venereal system (Hafeez et al., 2011). It is very hard to define the causes of male

* Corresponding author.
E-mail address: hebashalaby@sci.cu.edu.eg (H.A. Abd El-Rahman).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.01.060
1319-562X/

infertility due to cultural restrictions. A number of conditions leading to reproductive dysfunction including varicocele, accessory gland inflammation, immunological factors, malignancies, genetic abnormalities, endocrine disturbances, and congenital deformation that have been reported. But unknown causes scored about 30–40% of male infertility (Benbella et al., 2018). Environmental causes, like occupation and other several chemical agents, such as organophosphate compound, pollutants, manufacturing output and medication, may have a role in investigating the male infertility reasons. Numerous studies have evaluated and recorded the effects of medications on male reproductive system. The administration of drugs at specific dose for a particular period of time can cause infertility by affecting sperm count, movement ability and external shape, endocrine system, and the different types of cells inside the seminiferous tubules.

The present study mainly focuses on the potential effect of Filgrastim on the testicular and spermatogenic processes that are essential for reproductive success in male Wistar rats.

2. Materials and methods

2.1. Drug

Granulocyte-colony stimulating factor (G-CSF) with the trade name Filgrastim was purchased from (Sidco Company, Egypt).

2.2. Animals

Adult male rats belonging to albino strains weighing (180 ± 20) gm were used in the current study. The rats were brought from a closed and randomly bred colony maintained in the animal house of Zoology Department, Cairo University. The experiment protocol was approved by the Cairo University Ethical Commission for Animal Experiments (CU/I/F/78/18). The animals were preserved in ideal experimental conditions; fair time of lightness and darkness at 23 °C and were provided with normal rat food and water *ad libitum*.

2.3. Experimental design

18 Wistar rats were organized into three groups (n = 6). The Group (I) rats were injected with 0.5 ml/kg/day of distilled water as the Control Group that subdivided into two subgroups (n = 3/each), subgroup IA will be keep for 1 week and subgroup IB will be last for 2 weeks. The Group (II) animals received intraperitoneal injection of Therapeutic dose 30.83 mcg/kg/day of Filgrastim for one week (T1). The Group (III) animals received the same dose by the same route of Filgrastim for two weeks (T2). The animal dosage, suit to the Human Equivalent dose (HED) according to Nair and Jacob (2016) during one to two weeks, depend on (Advani et al., 2010). At the end of the experiment, all the rats were euthanized, dissected to expose testes, epididymis, and seminal vesicles of both sides, washed with PBS, dried and weighted.

2.4. Semen collection

The cauda epididymis was removed and kept in a disinfected glass plate. 2 ml physiological saline was then added and minced by using sterilized scissor and incubated for 10 min to allow sperm to swim out of the epididymal tubules (Najafi et al., 2016).

2.5. Sperm morphology screening

Morphology abnormalities were recorded by eosin/nigrosine stained smears. A small micro-liter (10 μl) of semen was put on glass slide and stained with 15 μl of 5% aqueous bluish eosin solution and a drop of nigrosine by precisely blending the semen with stains. A semen smear was made, put on microscopic stag, and examined using higher magnification (400x). We prepared 6 slides per each group and one hundred sperms were examined in each slide (Filler, 1993).

2.6. Semen quality test

Resazurin reduction (RRT) was performed using the method of Reddy and Bordekar (1999) which depends on the ability of metabolically active spermatozoa to reduce the resazurin dye (blue) with maximum absorption at 615 nm, and resorufin (pink) with maximum absorption 580 nm. The ratio of the optical densities reduced to oxidized form (i.e. 580 nm to 615 nm) can be used to evaluate the various grades of semen sample. The highest correlation of RRT ratio was observed with sperm motility, count, morphology.

2.7. Hormones profile

Blood samples were collected by heart puncture from the rats in all groups in clean tube having EDTA, centrifuged at 3000 rpm for 15 min to get sera and then were kept at −20 °C for hormones analysis. Quantitative determination of Testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) level were determined employing enzyme-linked immunosorbent assay immunodiagnostic reagents and ELISA kits specific for rats with CV (%) = SD/meanX100, Intra-Assay: CV < 10%, Inter-Assay: CV < 12%, FSH Assay range 0.5 IU/ 0.1 IU/L, LH Assay range 7 IU/LL −10 IU/L, T Assay range 0.1 ng/ml −3.8 ng/ml, sensitivity 0.1 IU/L for FSH & LH and sensitivity 0.01 ng/mlfor T (SunLong Biotech Co., LTD).

2.8. Oxidative stress markers:

The left testis of each rat in all groups was removed and kept in clean Eppendorf tube at −20 °C for biochemical investigation using reagent kits obtained from Bio Diagnostic (Egypt).

For tissue homogenate preparation, freeze testes homogenized in PBS (1:10 ml) by using blending apparatus. The suspension was centrifuged at 12,000 rpm for 20 min, and the liquid lying above a solid residue was used for assay of MDA using method of Satoh (1978), GSH according to Beutler et al. (1963), CAT as described by Aebi (1984) and SOD employing the method of Zhang et al., (2017).

2.9. Histopathological studies

The right testis was taken, fixed in 10% neutral buffered formalin solution, put in serial dilution of ethanolic solutions to remove water, transferred into xylene and then fixed firmly and deeply in paraffin wax, cut into slices using microtome knife at 5 μm thickness, and stained with H&E for histological examination (Kiernan and Horobin, 2009).

2.10. Immunohistochemical staining

Caspase- 3 Antibody was used to detect apoptotic cells (Sun et al., 2014). Immunohistochemical stain was performed on paraffin embedded sections. These specimens were irrigated with PBS twice for 5 min each. The paraffin sections were put in 3% hydrogen peroxidase in methanol, preserved with a blocking solution for 7 min at room temperature in a moist container and then kept with caspase-3. Rabbit polyclonal caspase 3 with dilution was used (dilution 1:500) (CPP32) Ab-4 IgG (RB-1197-R7, Thermo Fisher Scientific; Fremont, USA) antibody at +4 °C overnight, conserved with

secondary antibody using HRP Envision kit (Dako, Denmark) for twenty minutes, and then incubated with diaminobenzidine for ten minutes. Counterstaining was done by Mayer's hematoxylin, followed by dehydration and clearing in xylene.

2.11. Morphometric study

We have examined 3 slides per each group using Leica Qwin 500 LTD (Cambridge UK) computer assisted image analysis (image pro-plus 5.0 software), calculation of mean area % and mean optical density of caspase-3 immunoexpression (IE) in 10 high power fields (HPF) using an interactive measurements menu in immunostained sections.

2.12. Statistical analysis

The collected data were presented as the mean ± standard error means (SEM). The relationships between different sets of data were examined by performing analysis of variance (ANOVA), Tukey's honest significance test, and post hoc analysis. Significance was set at probability $P < 0.05$ using IBM SPSS version 22 software package (SPSS, IBM, Chicago, IL, USA).

3. Results

3.1. Effect of Filgratism on weights of reproductive organs

The obtained results indicate insignificant differences with slight increment in the absolute and relative right testicular weights, while the absolute weight of left testis from T2 showed a significant reduction in relationship with control. The treatment for one week caused a significant rise in absolute and relative weight of right epididymis related with Control and T2 Groups, although T2 revealed insignificant lessening in right epididymal weight competed with Control Group. As well as, there are significant increase in relative weight of left epididymis when matched with Control and T2 Groups. On the other hand, the both sides of seminal vesicles recorded insignificant raise of absolute and relative weight following treatment with Filgrastim with the two respective doses compared to the Control Group (Table 1).

3.2. Sperm morphology

The morphological analysis of semen samples revealed a significant ($P < 0.05$) lower of spermatozoa with normal morphology in the two treated groups compared with the Control Group (Table 2). The Filgrastim administration induced a significant incident of sperm shape abnormalities head less sperm, bent and twisted tail, tail less sperm and abnormal head appears without hook (Fig. 1). There was a significant difference between two treated groups in all values of sperm abnormalities. Estimation of the semen quality test showed a significant inhibition in the resazurin reduction (RRT) ratio of two treated groups when compared with the untreated group (Table 3) also, in-between two treated groups there was a significant change of RRT value.

3.3. Effects of Filgrastim on sexual hormones

Filgrastim significantly decreased the serum T levels in both treated groups compared with the Control Group ($p < 0.05$). The level of serum FSH and LH were significantly higher in Filgrastim treated groups compared with the Control Group ($p < 0.05$) moreover, group III (T2) revealed a significant change in all hormones level when compared with group II (T1) (Table 3).

3.4. Oxidative stress

The levels of GSH and CAT were significantly decreased in treated groups compared with Control Group. SOD activity increased in the group II (T1) but significantly reduced in the group III (T2) as compared with the Control Group. The G-CSF-exposed groups showed a significant rise in MDA as compared to the Control Group which indicated an increase in lipid peroxidation in sperm membrane furthermore, the treatment for two weeks presented a significant variance in all ROS markers activities in comparison with the other treated group (Table 4).

3.5. Histopathology

The testis of normal rats was characterized by several rounded, well organized seminiferous tubules with regular basement membrane lined by germinal epithelium (germinal cells and Sertoli cells). Germinal cells comprise spermatogonium, spermatocytes, spermatid and spermatozoa with lumen filled with sperms. The interstitial tissue in-between tubules contain Leydig cells and blood vessels (Fig. 2a, b). Histological findings of testicular tissues from the one week treated group revealed moderate to severe pathological changes, where subscapular blood vessels showed congestion and dilation with fibrotic changes in capsule. Some tubules appeared with partial loss of germ cells and irregularity in basement membrane in addition to lack of interstitial tissues. Apoptotic changes in different types of germinal cells in many tubules were observed (Fig. 2c, d). Marked histopathological alteration was detected in testicular tissues from the two weeks treated

Table 1
Filgrastim on the reproductive organ weight.

Groups Parameters	Control	T1	T2
Absolute right testis weight (g)	1.38 ± 0.14	1.3 ± 0.04	1.21 ± 0.06
Relative right testis weight %	0.47 ± 0.07	0.49 ± 0.01	0.50 ± 0.03
Absolute left testis weight (g)	1.42 ± 0.14	1.33 ± 0.03	1.25 ± 0.04[a]
Relative left testis weight %	0.48 ± 0.08	0.51 ± 0.01	0.51 ± 0.02
Absolute right epididymis weight (g)	0.1580.00	0.21 ± 0.01[a,b]	0.15 ± 0.00[b]
Relative right epididymis weight %	0.05 ± 0.00	0.08 ± 0.00[a,b]	0.06 ± 0.00[b]
Absolute left epididymis weight (g)	0.16 ± 0.01	0.22 ± 0.01	0.13 ± 0.01[b]
Relative left epididymis weight %	0.05 ± 0.00	0.08 ± 0.00[a,b]	0.06 ± 0.00[b]
Absolute right seminal vesicle weight (g)	0.3430.03	0.37 ± 0.03	0.36 ± 0.04
Relative right seminal vesicle weight %	0.11 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
Absolute left seminal vesicle weight (g)	0.34 ± 0.02	0.37 ± 0.02	0.36 ± 0.04
Relative left seminal vesicle weight %	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.01

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Tukey's test, the $p \leq 0.05$ statistically significant different; (a) significant compared to control, (b) significant compared between treated groups.

Table 2
Effect of Filgrastim on the sperm morphology.

Groups	Sperms without head	Sperms without tail	Convoluted tail	Abnormal head
Control	1.66 ± 0.210	0 ± 0	2.33 ± 0.210	0.66 ± 0.210
T1	10.5 ± 0.763[a,b]	27.67 ± 2.04[a,b]	28.33 ± 0.760[a,b]	10.33 ± 0.760[a,b]
T2	19 ± 0.577[a,b]	23.33 ± 0.95[a,b]	25.33 ± 0.881[a,b]	8.33 ± 0.666[a,b]

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Tukey's test, the $p < 0.05$ statistically significant different; (a) significant compared to control, (b) significant compared between treated groups.

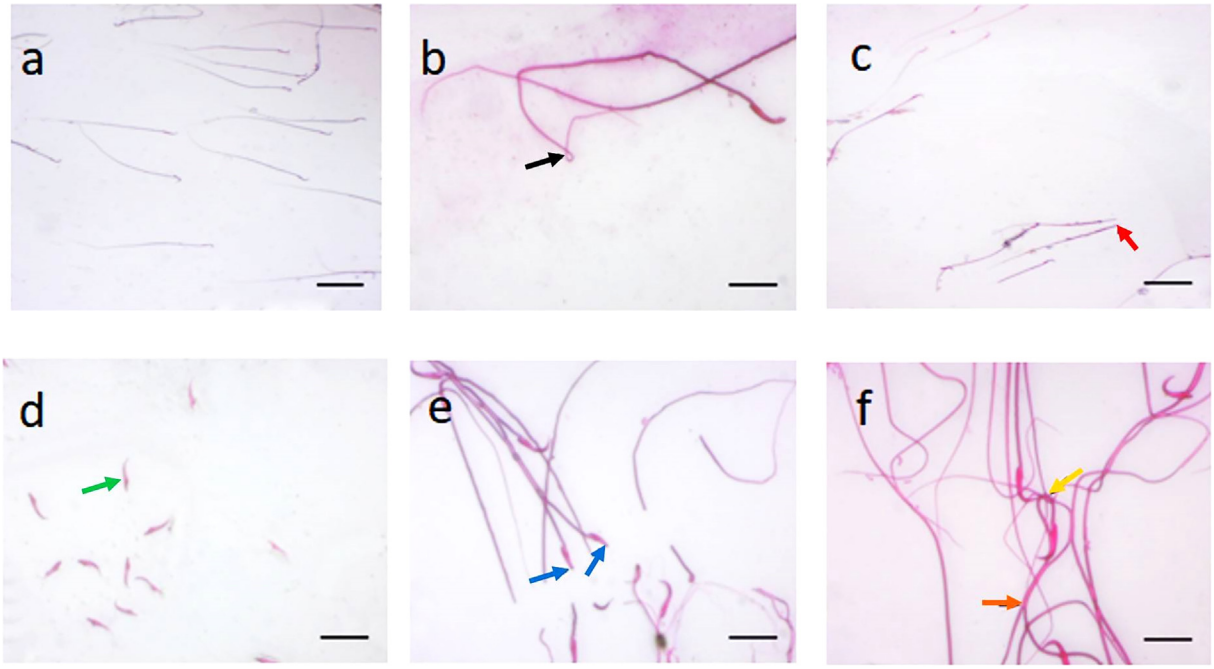

Fig. 1. Microphotographs demonstrating morphologically normal sperm and various sperm defects. (a): Control with normal sperm morphology. (b&c): one week treated group (T1) group, bent tail (black arrow) and Headless tail (red arrow). (d-f): two weeks treated group (T2), heads without tails (green arrow), head without hook (blue arrow), Pairing phenomenon (orange arrow) and bent tail (yellow arrow). (Scale bar 2 μm, x:400).

Table 3
Showing the effect of Filgrastim on the reproductive hormones profile.

Groups Parameters	Control	T1	T2
LH (mIU/ml)	4.57 ± 0.116	10.57 ± 0.42[a,b]	19.5 ± 1.0[a,b]
FSH (mIU/ml)	5.07 ± 0.08	8.37 ± 0.26[a,b]	12.85 ± 0.47[a,b]
T (ng/ml)	4.20 ± 0.12	2.10 ± 0.07[a,b]	1.20 ± 0.04[a,b]
Semen Quality test (RRT)	1.85 ± 0.12	1.58 ± 0.11[a,b]	1.26 ± 0.09[a,b]

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Tukey's test, the $p < 0.05$ statistically significant different; (a) significant compared to control, (b) significant compared between treated groups.

Table 4
Showing the effect of Filgrastim on the Redox state.

Groups Parameters	Control	T1	T2
GSH (mg/g. tissue)	63.88 ± 7.02	37.38 ± 6.02[a,b]	7.44 ± 1.29[a,b]
CAT (u/g. tissue)	2.22 ± 0.11	1.36 ± 0.11[a,b]	0.716 ± 0.14[a,b]
SOD (u/g. tissue)	867.5 ± 21.7	915.5 ± 13.8[b]	646.9 ± 74.7[a,b]
MDA (nmol/g. tissue)	31.7 ± 5.1	41.9 ± 5.0[a,b]	47.26 ± 1.1[a,b]

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Tukey's test, the $p < 0.05$ statistically significant different; (a) significant compared to control, (b) significant compared between treated groups.

group, where all pathological alterations of the pervious group were pronounced in addition to obvious deformed, disorganized, atrophied tubules, severe loss of normal histoarchitecure. Complete loss of interstitial tissues, sperm deficiency, dilated intratubular blood vessels, notable apoptotic changes with marked depletion in germ cells were seen with detachment of basement membrane and intracellular vacuoles between germ cells (Fig. 2e, i).

3.6. Immunohistochemical

Immunoreactivity of caspase-3 in the testicular tissue was slightly observed in the Control Group (Fig. 3a, b). In contrast, moderate immunohistochemical caspase-3 expression was apparent in the one week treated group (T1) (Fig. 3c, d), and moderate to severe immunoexpression of caspase −3 was observed in the two weeks treated group (T2) (Fig. 3e, f).

The mean area % and mean optical density of caspase-3 immunoexpression were raised significantly response to the Filgrastim administration in comparison with the Control Group likewise, both treated groups displayed a significant change in mean area % and MOD when compared with each other (Table 5).

4. Discussion

The current study was intended to determine the effect of Fligrastim on reproductive parameters of male Wistar rats. The sperm morphology in both treated groups, compared to the Con-

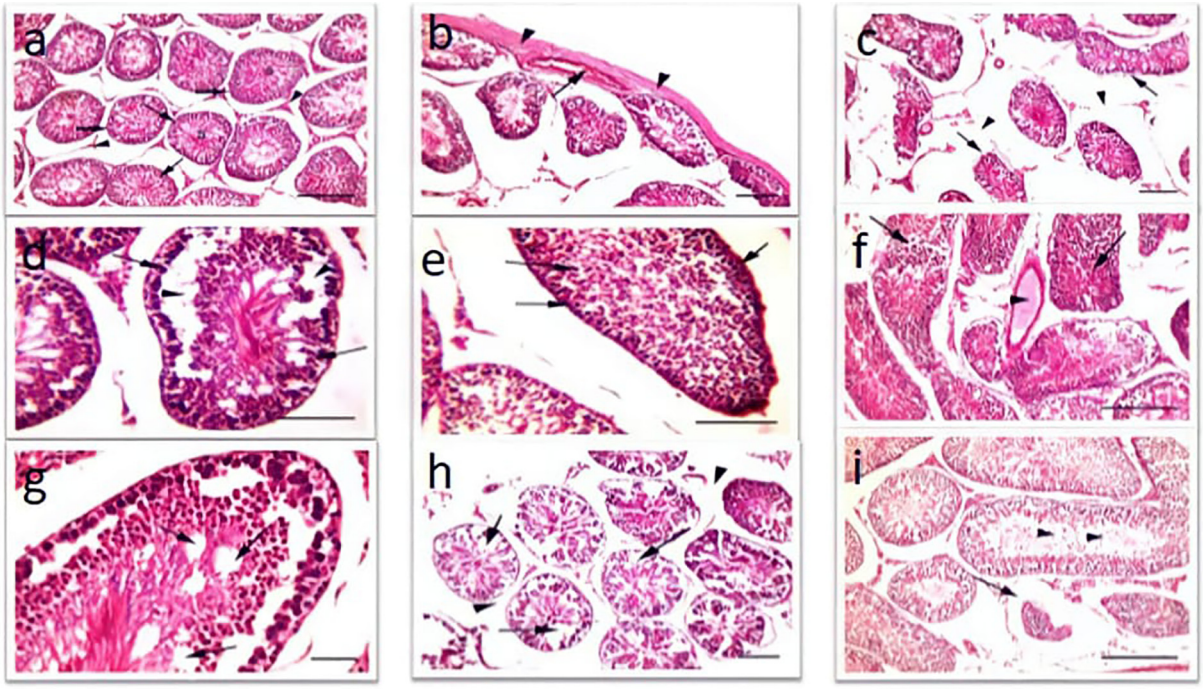

Fig. 2. Photomicrographs of testicular tissues H&E. (a) from control group. a: showing seminiferous tubules (thick arrow), interstitial tissues (arrow head), spermatozoa (S), germinal cells (thin arrow). (b-d) from one week treated group (T1) (b): showing congested dilated blood subscapular vessel (arrow), fibrotic capsule (arrow head). (c): lack of interstitial tissues (arrow head), irregularity and detachment of basement membrane (arrow). (d): showing moderate apoptotic changes in different types of germ cells (arrow), depletion of germ cells (arrow head). (e–i) from two weeks treated group (T2). (e): showing congested dilated intratubular blood vessel (arrow head), disorganized, deformed tubules (arrow). (f): showing apoptotic changes in germinal cells (arrow). (g): intracellular vacuoles between germinal cells (arrow). (h): showing loss of interstitial tissues (arrow head), loss of germ cells (arrow). (i): showing sperm deficiency (arrow head), atrophied tubules (arrow). (Scale bar a, c, e, g 10 μm, x: 100) (Scale bar b, d, f, h 5 μm, x:200).

trol Group, were significantly influenced. Several authors have recorded relapsing sperm morphology with elevated leukocyte concentration (Eggert-Kruse et al., 1992; Yanushpolsky et al. 1996). However, Fedder et al (1993) stated that the increasing number of leukocytes had no significant relation with sperm morphology abnormality. Smith et al (1990) listed sperm destruction related to sperm fragments within digestive vacuoles of phagocytic cells. Studies have proposed that there is no relation between the raise of anomalies in spermatozoa morphology and the increment in leukocytes (Keissling et al. 1995).

The current work has shown a significant reduction in the resazurin reduction test (RRT) ratio. El-Battawy and El-Nattat (2013) showed that the highest RRT ratio was manifested with sperm motility, count, morphology and viability. The resazurin reduction test supports the identification of metabolically efficient spermatozoa (Zalata et al. 1998). It has been used in semen evaluations in humans (Mahmoud et al. 1994; Reddy and Bordekar 1999; Zalata et al. 1998), bulls (Dart et al. 1994), rams (Wang et al. 1998), stallions (Carter et al. 1998), and boars (Zrimsek et al. 2004).

Mahmoud et al (1994) found significant correlations between RRT results and sperm cell concentrations, such as concentrations of motile spermatozoa. In the study performed by Reddy and Bordekar, it was found that the RRT ratio of human semen was significantly correlated with the percentage of motile sperm cells (Reddy and Bordekar 1999). The previous articles concluded that the evaluation of RRT results may provide more accurate information on the quality of semen (Strzeżek et al. 2013). In the present study, the reduction in weight of both left and right epididymis was noticed with the two weeks Filgrastim treatment which was explained by decrease in sperm reserve.

There are links between ROS and sperm motility reduction, may be due to a sequence of proceedings that cause quick reduction of intracellular ATP and eventually lead to axonemal injure and sperm immobilization (de Lamirande and Gagnon, 1995; Bansal and Bilaspuri, 2010; Agarwal et al., 2014). Our investigation also revealed that exposure to Filgrastim decreased testosterone level, indicating interference with steroidogenesis.

Sperm functions as capacitation needing little amount of reactive oxygen species (ROS) formation. However, the imbalance between ROS and antioxidant defense system led to higher production of ROS in the male reproductive system that caused sperm dysfunction and affected the sperms number and motility. Thus, the seminal ROS level can be used as an indicator for male infertility (Agarwal et al., 2006; Muratoğlu et al., 2018). In the current study, suppression in the testosterone concentrations was observed in the Filgrastim-treated rats in comparison with the Control Group and this disagrees with Aponte et al. (2019) that reported that the level of testosterone level was unaltered in Guinea pig and increased in ram lambs after administration with G-CSF. FSH and LH level in the two treated groups were significantly higher than in the Control Group. A study by Aponte et al. (2019) reported the elevation of LH level and unchangeable of FSH level in guinea pig while both FSH and LH are increment in ram lambs.

The abnormal oxidative stress manifested in our study subjects may be due to the fact that Filgrastim induces a transient inflammatory status described by circulating activated polymorphonuclear neutrophil granulocyte (PMN), which releases ROS consistent with (Cella et al., 2006). It is apparent that oxidative stress is a primary cause in the pathogenesis of chronic inflammation (Zuo et al., 2019). Concerning the nucleophilic and reducing properties of glutathione, it has a main role in the defense mechanisms of cellular antioxidant and acts as a substrate for glutathione peroxidase (Guarnieri et al., 1980). The reduction in GSH increases ROS activity in various tissues (Jackson and Bushell, 1999). In the

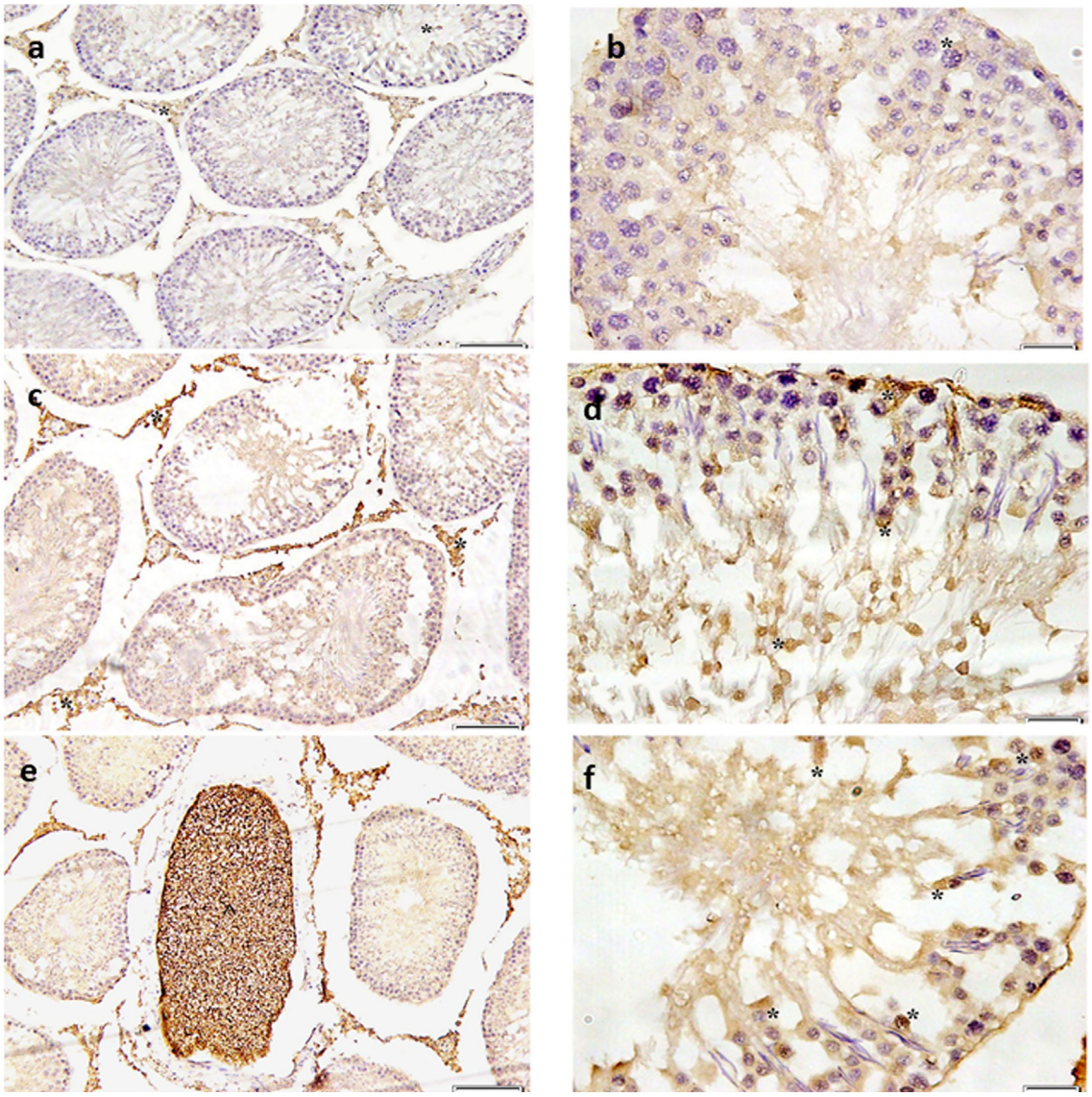

Fig. 3. Photomicrographs of testicular tissues caspase-3 immunostaining. (a, b) from control group. (a): showing weak caspase 3 + ve IE (*) among some interstitial cells and few germ cells. (b): showing weak caspase 3 + ve IE (*) in few 1ry spermatocytes. (c, d) from one week treated group (T1). (c): showing mild to moderate caspase 3 + ve (*) among interstitial tissue. (d): showing moderate caspase 3 + ve (*) in spermatogenic cells. (e,f): from two week treated group (T 2).(e): showing sever caspase 3 + ve (*) among whole an atypical tubule (^). (f): showing moderate to severe caspase in 3 + ve (*) (IE) multiple in spermatogenic cells. (Scale bar a, c, e 10 μm, x: 100) (Scale bar b, d, f, 2 μm, x:400).

Table 5
Showing mean area % and Mean optical density (MOD) of caspase3 immunoexpression.

Groups Parameters	Control	T1	T2
Mean area %	1.9 ± 0.078	7.52 ± 0.1212^{a,b}	13.78 ± 0.122^{a,b}
MOD	0.19 ± 0.013	0.48 ± 0.0125^{a,b}	0.81 ± 0.013^{a,b}

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Tukey's test, the $p < 0.05$ statistically significant different; (a) significant compared to control, (b) significant compared between treated groups.

treated samples under investigation, it showed a significant decrease in intracellular GSH concentration that ticked the occurrence of this reactive species. SOD is a metallic protein located inside the cell that converts superoxide anion into H_2O_2 and O_2, and therefore removing free radicals. Thus, the elevation in the output of superoxide radical stimulates SOD (Röth et al., 1998). Subsequently, we considered the significant change in SOD activity in both treated groups due to the greater amount of O_2-radical arising from either stimulated PMN or due to H_2O_2 and OH reaction, that also outcomes in superoxide radicals (Halliwell and Gutteridge, 1990).

In our study, there is significant increment in MDA in the two treated groups compared with the Control Group. Sperm plasma membrane is rich in polyunsaturated fatty acid PUFA and plays a great role in membrane performance and constitution. PUFA is also one of the prime targets in the lipid peroxidation process (Lenzi et al., 1996).

Our biochemical results revealed that Filgrastim elevated the MDA level, pursued by a rise in oxidative stress and lower levels of CAT were observed in the both treated groups in comparison with the Control Group. On the other hand, Filgrastim injection caused a significant change in SOD activity in the treated groups when compared with the Control Group. Drug stimulates lipid peroxidation by protein glycation and spontaneous glucose oxidation

causing free radical formation. The production of free radicals led to increment of antioxidant enzymes concentration such as SOD and CAT (Davì et al., 2005). Hence, the deterioration of testicular tissue results mainly from either the failure in the equilibrium of pro-oxidant and antioxidant or the exaggerated level of oxidative stress due to the free radical formation. It has been proposed that the estimation of MDA levels can be used to determine the testicular tissue injure degree (Özkan et al., 2004).

Meanwhile, histological analysis of testes from the rats treated with Filgrastim show effects on seminiferous tubules including irregularity in basement membrane, apoptotic changes in different types of germinal cells and loss of their normal histoarchitecure in addition to lack of interstitial tissues. The change in shapes of the tubular affects the smoothness of spermatogenesis in the two treatment groups as previously reported by (Mutalip et al., 2013).

In warm blooded creatures, the fundamental entity of the testis is seminiferous tubules. The sperm differentiation processes are controlled by hormonal incitement and dynamic associations between the Sertoli cells and the germ cells of the seminiferous epithelium. Sertoli cells emit hormonal and nourishing elements that enhance the proliferation and differentiation of the germ cells. The intricate organization and cell communications that happen in the testis give a wide range of focuses by which toxicants can intrude on spermatogenesis (Orazizadeh et al., 2010).

The current study has shown histological abnormalities in the treated rats' testicular tissue as sloughing and shortening of the seminiferous epithelium that led to reducing the number of spermatogenic cells. It has been known that the interstitial tissue of Leydig cells is responsible for testosterone producing and it is a needful requirement to keep good and successful spermatogenesis (Zirkin et al., 1989; Ramaswamy and Weinbauer, 2014). The interstitial damage in the testes of the treated rats might have caused a decrease in testosterone level and finally suppressed the spermatogenesis process (Sunday et al., 2019).

Moreover, the apoptosis inducement in different tissues depends on the caspase-cascade stimulation causing cell death (Saulsbury et al., 2009). Referring to Dadhich et al., cell death and proliferation have a vital role in maintaining the testicular cell counts depending on a hormonally mediated process that regulates the generation of Sertoli and germ cells balance. Apoptosis has two important roles in the normal spermatogenesis, which are lowering the number of germ cells supported by Sertoli cells and sweeping up sperms abnormality and injured cells that are omitted (Angelopoulou et al., 2007; Dadhich et al., 2010; Anbarkeh et al., 2019).

The immunohistochemical results showed an increase in positive caspase-3 immunoexpression in different germ cells in the treated groups. The previous studies have reported the necessity of apoptosis process for normal spermatogenesis in mammals and sustain cellular homeostasis. This physiological process protects the equilibrium between Sertoli cells and germ cells. The members of the caspase family play a significant role in the regulation of the apoptosis in the seminiferous tubules (Saygin et al., 2015). The previous studies have reported that the testicular epithelium germ cells are killed by apoptosis. The percentage of apoptosis in testicular tissue increased in the infertile males and this emphasized the relation between infertility and increased apoptosis (Said et al., 2004).

Caspases are actively involved in cleaving the proteins at aspartic acid and it is known that they play a part in cleaving the neighboring amino acids (Sakai and Suqasawa, 2014). Indeed, once caspases are activated, there seems to be an immutable commitment to cell death (Mc Ilwain et al., 2013). Therefore, here in the present study we aimed to investigate the expression of caspase-3, following exposure to Filgrastim.

The apoptosis process might be initiated as a result of increased levels of ROS as fundamental trigger at the beginning of the cell death pathways, and antioxidants scavenge molecules to neutralize ROS subsequently diminished the apoptotic events (Zeidan-Chulia et al., 2014; Shooreia et al., 2019). The increment in the testicular tissue apoptosis is correlated with the reduction in the testosterone level (Shetty et al., 1996; Nandi et al., 1999), proposing that testosterone functions as a germ cell survival factor.

5. Conclusion

Based on the above results, we suggest that Filgrastim induces a disorder in sperm morphology associated with an increase in the oxidative stress. Filgrastim affects the germinal epithelium that influences the spermatogenesis and enhances caspace-3 expression.

Authors' contributions

S.S. has collected the required data, performed analyses, interpreted the data and wrote the manuscript. N.B. S.S. has participated in collecting the data and performing analyses. A.R. and H.A. have helped with the study design, evaluation and the interpretation of data and the final approval of the manuscript. All authors have read and approved the final manuscript. All data were generated in-house and no paper mill was used.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank everyone who has given us assistance, support (including assistance with writing and editing) or special equipment and instruments.

References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
Advani, S.H., Achreckar, S., Thomas, D., Krishnankutty, B., 2010. Granulocyte colony-stimulating factor (filgrastim) in chemotherapy-induced febrile neutropenia. Indian J. Med. Paediatr. Oncol. 30 (3), 125–128.
Agarwal, A., Prabakaran, S., Allamaneni, S.S., 2006. Relationship between oxidative stress, varicocele and infertility: ameta-analysis. Reprod. Biomed. 12, 630–633.
Agarwal, A., Virk, G., Chloe Ong, C., du Plessis, S., 2014. Effect of oxidative stress on male reproduction. World J. Mens. Health 32 (1), 1–17.
Angelopoulou, R., Plastira, K., Msaouel, P., 2007. Spermatozoal sensitive biomarkers to defective protaminosis and fragmented DNA. Reprod. Biol. Endocrinol. 5, 36.
Anbarkeh, F.R., Nikravesh, M.R., Jalali, M., Sadeghnia, H.R., Sargazi, Z., 2019. The effect of diazinon on cell proliferation and apoptosis in testicular tissue of rats and the protective effect of vitamin E. Int. J. Fertil. Steril. 13 (2), 154–160.
Aponte, P.M., Gutierrez-Reinoso, M.A., Sanchez-Cepeda, E.G., Garcia-Herreros, M., 2019. Differential role of r-met-hu G-CSF on male reproductive function and development in prepubertal domestic mammals. PLoS ONE 26.
Bansal, A.K., Bilaspuri, G.S. 2010. Impacts of oxidative stress and antioxidants on semen functions. Vet. Med. Int. 686137.
Benbella, A., Aboulmakarim, S., Hardizi, H., Zaidouni, A., Bezad, R., 2018. Infertility in the Moroccan population: an etiological study in the reproductive health center in Rabat. Pan. Afr. Med. J. 30, 204.
Beutler, E., Duron, O., Kelly, M.B., 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882.
Carter, R.A., Ericsson, S.A., Corn, C.D., Weyerts, P.R., Dart, M.G., Escue, S.G., Mesta, J., 1998. Assessing the fertility potential of equine semen samples using the reduction dyes methylene green and resazurin. Arch. Androl. 40, 59–66.
Cella, G., Marchetti, M., Vignoli, A., Randi, M.L., Saggiorato, G., Pasetto, L., Pagnan, A., Barbui, T., Falanga, A., 2006. Blood oxidative status and selectins plasma levels in healthy donors receiving granulocyte-colony stimulating factor. Nature Publishing Group All rights reserved 20, 1430–1434.

Cooper, K.L., Madan, J., Whyte, S., Stevenson, M.D., Akehurst, R.L., 2011. Granulocyte colony-stimulating factors for febrile neutropenia prophylaxis following chemotherapy: systematic review and meta-analysis. BMC Cancer 11, 404.
Crobu, D., Spinetti, G., Schrepfer, R., Tonon, G., Jotti, G.S., Onali, P., Di Stefano, A., 2014. Preclinical and clinical phase I studies of a new recombinant Filgrastim (BK0023) in comparison with Neupogen®. BMC Pharmacol. Toxicol. 15 (1), 7.
Dadhich, R.K., Real, F.M., Zurita, F., Barrionuevo, F.J., Burgos, M., Jime¬nez, R. 2010. Role of apoptosis and cell proliferation in the testicular dynamics of seasonal breeding mammals: a study in the Iberian mole, Talpaoccidentalis. Biol. Reprod. 83, 83–91.
Dart, M.G., Mesta, J., Cremshaw, C., Ericsson, S.A., 1994. Modified resazurin test for determining the fertility potential of bovine spermatozoa. Arch. Androl. 33, 71–75.
Davì, G., Falco, A., Patrono, C., 2005. Lipid peroxidation in diabetes mellitus. Antioxid. Redox. Signal 7, 256–268.
de Lamirande, E., Gagnon, C., 1995. Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects. Hum. Reprod. 10, 15–21.
D'Souza, A., Jaiyesimi, I., Trainor, L., Venuturumili, P., 2008. Granulocyte colony–stimulating factor administration: adverse events. Transfus. Med. Rev. 22 (4), 280–290.
Eggert-Kruse, W., Bellmann, A., Rohr, G., Tilgen, W., Runnebaum, B., 1992. Differentiation of round cells in semen by means of monoclonal antibodies and relationship with male fertility. Fertil. Steril. 58, 1046–1055.
El-Battawy, K.A., El-Nattat, W.S., 2013. Evaluation of rabbit semen quality using resazurin reduction test. Global Veterinaria 11, 767–770.
Fedder, J., Askjaer, A.A., Hjort, T., 1993. Non-spermatozoa cells in semen: relationship to other semen parameters and fertility status of the couple. Arch. Androl. 31, 95–103.
Filler, R. 1993 Methods for evaluation of rats epididymal sperm morphology. In: R.E. Chapin JHH (ed.), Male reproductive toxicology, Academic Press, California, pp. 334–343.
Guarnieri, C., Flaming, F., Caldarera, C.M., 1980. Role of oxygen in the cellular damage induced by re-oxygenation of hypoxic heart. J. Mol. Cell. Cardiol. 12, 797–808.
Hafeez, M., Ahmed, A., Usmanghani, K., Mohiuddin, E., Asif, H.M., Akram, M., Reham, R., 2011. Clinical evaluation of herbal medicine for oligospermia. Pakistan J. Nutr. 10, 238–240.
Halliwell, B., Gutteridge, J.M.C., 1990. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. 186, 1–85.
Jackson, M.J., Bushell, A. 1999. Antioxidants. In: Grace, P.A., Mathie, R.T. (eds.), Ischaemia Reperfusion Injury, Blackwell Science Oxford, pp. 322–327.
Keissling, A.A., Lamparelli, N., Yin, H.Z., Seibel, M.M., Eyre, R.C., 1995. Semen leucocytes: friends or foes?. Fertil. Steril. 60, 196–198.
Kiernan, J.A., Horobin, R.W., 2009. A special issue devoted to hematoxylin, hematein, and hemalum. Biotech. Histochem. 85 (1), 5–6.
Lenzi, A., Picardo, M., Gandini, L., Dondero, F., 1996. Lipids of the sperm plasma membrane: From polyunsaturated fatty acids considered as markers of sperm function to possible scavenger therapy. Hum. Reprod. Update 2 (3), 246–256.
Liongue, C., Wright, C., Russell, A.P., Ward, A.C., 2009. Granulocyte colony-stimulating factor receptor: stimulating granulopoiesis and much more. Int. J. Biochem. Cell Biol. 41 (12), 2372–2375.
Mahmoud, A.M., Comhaire, F.H., Vermeulen, L., Andreou, E., 1994. Comparison of the resazurin test, adenosine triphosphate in semen and various sperm parameters. Hum. Reprod. 9, 1688–1693.
Mc Ilwain, D.R., Berger, T., Mak, T.W., 2013. Caspase functions in cell death and disease. Cold Spring Harb. Perspect. Biol. 5, a008656.
Muratoğlu, S., Dizakar, O.S.A., Aktan, A.K., Ömeroğlu, S., Akbulut, K.G., 2018. The protective role of melatonin and curcumin in the testis of young and aged rats. Andrologia 51 (3), 1–10.
Mutalip, S.S.M., Singh, G.K.S., Shah, A.M., Mohamad, M., Mani, V., Hussin, S.N., 2013. Histological changes in testes of rats treated with testosterone, nandrolone, and stanozolol. Iran J. Reprod. Med. 11 (8), 653–658.
Nair, A.B., Jacob, S., 2016. A simple practice guide for dose conversion between animals and human. J. Basic Clin. Pharm. 7 (2), 27–31.
Najafi, G., Farokhi, F., Jalali, S.A., Akbarizadeh, Z., 2016. Protection against cyclosporine-induced reprotoxicity by Satureja khuzestanica essential oil in male rats. Int. J. Fertil. Steril. 9 (4), 548–557.
Nandi, S., Banerjee, P.P., Zirkin, B.R., 1999. Germ cell apoptosis in the testes of sprague dawley rats following testosterone withdrawal by ethane 1,2-dimethanesulfonate administration: relationship to fas?. Biol. Reprod. 61, 70–75.
Orazizadeh, M., Khorsandi, L.S., Hashemitabar, M., 2010. Toxic effects of dexamethasone on mouse testicular germ cells. Andrologia 42 (4), 247–253.
Özkan, K., Boran, C., Kilinc, M., Garipardic, M., Kurutaş, M., 2004. The effect of zinc aspartate pretreatment on ischemia-reperfusion injury and early changes of blood and tissue antioxidant enzyme activities after unilateral testicular torsion-detorsion. J. Pediatr. Surg. 39, 91–95.
Ramaswamy, S., Weinbauer, G.F., 2014. Endocrine control of spermatogenesis: Role of FSH and LH/ testosterone. Spermatogenesis 4 (2), e996025.
Reddy, K.V., Bordekar, A.D., 1999. Spectrophotometric analysis of resazurin reduction test and semen quality in men. Indian J. Exp. Biol. 37, 782–786.
Röth, E., Nemes, J., Kapronczay, P., Varga, G., Jaberansari, M.T., Borsiczky, B., 1998. Free radical reactions and the endogenous antioxidant system in essential hypertension. Eur. J. Int. Med. 9, 263–270.
Said, T.M., Paasch, U., Glander, H.J., Agarwal, A., 2004. Role of caspases in male in fertility. Hum. Reprod. 10, 39–51.
Sakai, W., Suqasawa, K., 2014. FANCD is a target for caspase 3 during DNA damage-induced apoptosis. FEBS Lett. 588, 3778–3785.
Satoh, K., 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin. Chim. Acta 90 (1), 37–43.
Saulsbury, M.D., Heyliger, S.O., Wang, K., Johnson, D.J., 2009. Chlorpyrifos induces oxidative stress in oligodendrocyte progenitor cells. Toxicology 259, 1–9.
Saygin, M., Caliskan, S., Ozguner, M.F., Gumral, N., Comlekci, S., Karahan, N., 2015. Impact of L-carnitine and selenium treatment on testicular apoptosis in rats exposed to 2.45 GHz microwave energy. West Indian Med. J. 64 (2), 55.
Shetty, J., Marathe, G.K., Dighe, R.R., 1996. Specific immunoneutralization of FSH leads to apoptotic cell death of the pachytene spermatocytes and spermatogonial cells in the rat. Endocrinology 137, 2179–2182.
Shishehgar, S., Dolatian, M., Alavi Majd, H., Bakhtiary, M., 2014. Perceived pregnancy stress and quality of life amongst Iranian women. Global J. Health Sci. 6, 270–277.
Shooreia, H., Khakic, A., Afshin, A.K., Ali, A.H., Moghimian, M., Shokoohi, M., 2019. The ameliorative effect of carvacrol on oxidative stress and germ cell apoptosis in testicular tissue of adult diabetic rat. Biomed. Pharmacother. 111, 568–578.
Smith, D.C., Barratt, C.L.R., Williams, M.A., 1990. The characterization of non-sperm cells in the ejaculates of fertile men using transmission electron microscopy. Andrologia 21, 319–333.
Strzeżek, R., Filipowicz, K., Stańczak, M., Kordan, W., 2013. Spectrophotometric analysis of the resazurin reduction test as a tool for assessing canine semen quality. Bull. Vet. Inst. Pulawy 57, 281–285.
Sun, W., Su, Q., Cao, X., Shang, B., Chen, A., Yin, H., Liu, B., 2014. High expression of polo-like kinase 1 is associated with early development of hepatocellular carcinoma. Int. J. Genomics 312130.
Sunday, A.A., Victor, O.U., Babatunde, S.O., Julius, A.A., Ayooluwa, G.I., 2019. Nitrite-induced testicular toxicity in rats: therapeutic potential of walnut oil. JBRA Assisted Reprod. 23, 15–23.
Wang, S., Holyoak, G.R., Panter, K.E., Liu, Y., Evans, R.C., Bunch, T.D., 1998. Resazurin reduction assay for ram sperm metabolic activity measured by spectrophotometry. Proc. Soc. Exp. Biol. Med. 217, 197–202.
Yanushpolsky, E.H., Politch, J.A., Hill, J.A., Anderson, D.J., 1996. Is leucospermia clinically relevant?. Fertil. Steril. 66, 822–825.
Zalata, A.A., Lammertijn, N., Christoper, A., Comhaire, F.H., 1998. The correlates and alleged biochemical background of the resazurin reduction test in semen. Int. J. Androl. 21, 289–294.
Zeidan-Chulia, F., Gursoy, M., de Oliveira, B.-H.N., Gelain, D.P., Könönen, K., Gursoy, U.K., Moreira, F., Uittoc, V., 2014. Focused microarray analysis of apoptosis in periodontitis and its potential pharmacological targeting by carvacrol. Arch. Oral Biol. 59, 461–469.
Zhang, J., Chen, R., Yu, Z., Xue, L., 2017. Superoxide Dismutase (SOD) and Catalase (CAT) activity assay protocols for caenorhabditis elegans. Bio-protocol 7 (16), e2505.
Zirkin, B.R., Santulli, R., Awoniyi, C.A., Ewing, L.L., 1989. Maintenance of advanced spermatogenic cells in the adultrat testis: quantitative relationship to testosterone con-centration within the testis. Endocrinology 124 (6), 3043–3049.
Zrimsek, P., Kurc, J., Kosec, M., Mrkun, J., 2004. Spectrophotometric application of resazurin reduction assay to evaluate boar semen quality. Int. J. Androl. 27, 57–62.
Zuo, L., Prather, E.V., Stetskiv, M., Garrison, D.E., Meade, J.R., Peace, T.I., Zhou, T., 2019. Inflammaging and oxidative stress in human diseases: from molecular mechanisms to novel treatments. Int. J. Mol. Sci. 20, 4472.

Further reading

Fridovich, I., 1986. Biological effects of the superoxide radical. Arch. Biochem. Biophys., 247
Hess, R.A., Renato de Franca, L., 2008. Spermatogenesis and cycle of the seminiferous epithelium. Molecular mechanisms in spermatogenesis. Adv. Exp. Med. Biol. 636, 15.