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Original Article *Pharmacology*

The effects of oral ketoconazole and griseofulvin on the fertility of male rabbits

Amal Mohamad[1*], Aza Hassan[1], Magdy Amer[2], El-Said Abdel-Aziz[3]

[1]Animal Health Research Institute, Mansoura, Egypt
[2]Department of Pharmacology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, P.O 35516, Egypt
[3]Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Egypt

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Address correspondence to Amal Mohamed; Tel: +201208652860; E-mail: amal2325764@yahoo.com

ABSTRACT

Objectives: To investigate possible side effects of ketoconazole and griseofulvin on fertility of clinically healthy male rabbits.

Design: Randomized controlled experimental study.

Animals: Thirty mature male rabbits.

Procedures: Rabbits were randomly allocated into three equal sized groups (10 animals each). The first group served as a control group (received no treatment), the second group received griseofulvin (25 mg/kg B.W) orally for 30 days, and the third group received ketoconazole (50 mg/kg B.W) orally for 30 days. Semen samples were collected after 1 day, 1 week and 2 weeks post-administration to determine sperm motility, % of live and dead sperms, total sperm abnormalities and sperm cell concentration. Two weeks post-administration, testes and epididymides were removed for histopathological examination.

Results: Both drugs produced a significant reduction in the serum testosterone level, sperm cell concentration, percent of live sperms and percent of sperm motility.

Conclusion and clinical relevance: Ketoconazole and griseofulvin have a negative impact on fertility of male rabbits, and the effect is more pronounced with ketoconazole.

Keywords: Ketoconazole, Griseofulvin, Fertility, Rabbit

1. INTRODUCTION

Rabbit is an excellent laboratory animal that offers many advantages in the field of reproduction [1]. Rabbit farming is a promising and developing industry that can have a positive impact on national economy in Egypt. Considerable efforts have been done in epidemiological studies to highlight the nature of rabbit diseases and the control measures, and to immediately plan for national surveys of the current rabbit problems [2].

Reproduction is considered an important part for production process, therefore any drop in animal fertility can lead to economic losses in animal production [3]. Fertility is defined as the ability of a cyclic animal to establish pregnancy and is maintained by breeding good–managed female using high-quality semen that is introduced into the female genital tract at the appropriate time. Each male should have a good reproductive performance and a superior genetic profile as the male fertility and genetic value appears more important than those of the female [4].

The exposure of males to some drugs and chemicals may affect their sexual functions. Some drugs can damage spermatogonia cells that represent the male genome, and so disturb the spermatozoid under maturation [5]. The fungal diseases are considered a serious hazard for human and animal health. Fungal infections can cause allergy due to fungal proteins, toxicity due to mycotoxins found in fungi. Many fungal infections are caused by opportunistic pathogens that may be endogenous (e.g. Candida infections) or are acquired from the environment (e.g. *Cryptococcus*, *Aspergillus* infections) [6].

Griseofulvin is one of the most selective antifungal inhibitory agents. It has an effect against many fungi. Griseofulvin acts by interfering with the intracellular microtubule production and thus inhibits fungal mitosis. The selective toxicity of griseofulvin for fungi and its spectrum of action is restricted to the dermatophyte fungi mainly (causes of ringworm and athlete's foot)[6].

Ketoconazole is a member of imidazole family which has a broad-spectrum antifungal activity. It is considered the gold standard among the azole derivative antifungal drugs. It has proven to be the most successful and most widely used antifungal azole-derivative to date [7].

The aim of this study was to investigate the effect of ketoconazole and griseofulvin on the reproductive performance of male rabbits through the assessment of serum testosterone level and semen quality as well as the histopathological effect on testes and epididymis of rabbits.

2. MATERIALS AND METHODS

2.1. Animals

This study was performed on 30 clinically healthy New Zealand rabbits with an average age of 5-6 months and average weight of 3.5 ± 0.5 kg. Animals were apparently healthy with normal external genitalia. The animals were randomly divided into three equal groups, each of 10 rabbits. Group (1) was kept as a control and received no treatment. Group (2) was given griseofulvin as a solution orally (Griseofulvin, Glaxo Laboratories, white to creamy- or yellowish-white, crystalline powder) [8] at a dose of 25 mg/kg once daily for 30 days [9]. Group (3) was given Ketoconazole as a solution (El Nile pharmaceutical Company, Cairo, Egypt) to rabbits orally at a dose of 50 mg/kg/day for 30 days [10]. The experimental protocol of this work was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University.

2.2. Collection of Samples

Semen and blood samples were collected from each animal at 31, 38 and 45 days of initial administration. Serum was used for assaying the testosterone level. The semen was collected by means of an artificial vagina filled with a warm liquid (about 45 °C). A doe was fitted with this device and presented to the buck. Some authors reported that previous stimulation of the buck increases sperm concentration, and that leaving a doe on the top of the buck's cage for several minutes may help in stimulating bucks. The collected semen samples were used for the determination of sperm characterizations including the following:

2.2.1 Serum testosterone level

Serum testosterone levels were measured according to the method described by Castro et al. [13].

2.2.2. Sperm motility

The semen was initially diluted with warm sodium-citrate solution 2.9 % and the sperm motility was assessed according to the method described previously [11].

2.2.3 Percentage of live and dead sperms

The percentages of dead sperms were measured according to Parker and McDaniel [11].

2.2.4. Percentage of total sperm abnormalities

The percentages of morphologically abnormal sperms were measured according to the method described previously [11].

2.2.5. Sperm cell concentration

Sperm cell concentration was determined using hemocytometer and the number of sperm/mL was calculated according to the method described by Habeeb et al. [12].

2.3. Histopathological analysis

Two weeks from the administration of the last dose of the drugs, tissue samples (testes and epididymis) were removed and immersed immediately in 10 % buffered formalin for histopathological examination [14].

2.4. Statistical analysis

Data were statistically analyzed using Statistical Package of Social Sciences (SPSS), Version 17 (Inc., Chicago, IL, USA) computer software. Data were expressed as mean ± SE. The effects of treatment on each variable measured were analyzed using general linear model repeated measures ANOVA [15]. When there is a significant result, one-way ANOVA with post hoc Duncan multiple comparison tests were used to detect the specific variations and the level of significance was set at $P<0.05$ [16].

3. RESULTS

3.1. Serum testosterone level

The effect of oral administration of griseofulvin and ketoconazole 30 consecutive days on the serum level of testosterone hormone in rabbits is presented in figure (1). Results revealed a significant a decline in total serum testosterone hormone level in treated groups along the whole experimental period ($P< 0.05$) in comparison to the control group (Figure 1). Both griseofulvin and ketoconazole-treated groups induced a significant decrease ($P< 0.05$) in total serum testosterone level at 31 days (0.89 ± 0.24 and 0.80 ± 0.24 respectively) post-dosing. The level of serum testosterone then increased in 38 day and in 45 day post-administrations compared to that of 31 day in all groups however the increase was not significant (Figure 1).

3.2. Semen picture

3.2.1. Semen volume

The effect oral administration of griseofulvin and ketoconazole on semen volume in rabbits is presented in figure (2). There was a significant decline ($P< 0.05$) in semen volume in treated groups along the whole experimental period in comparison to the control group (Figure 2). Both griseofulvin and ketoconazole-treated groups showed a significant decrease ($P< 0.05$) in semen volume at 31 day (0.75 ± 0.14 and 0.3 ± 0.1 respectively), 38 day (1.2 ± 0.14 and respectively) and at 45 day (1.2 ± 0.2 and 1.3 ± 0.13 respectively) post-dosing. The semen volume then increased in 38 day and in 45 day post-administrations in all groups compared to that of 31 day however the increase was not significant (Figure 2).

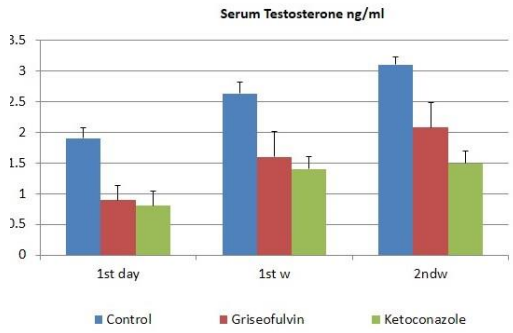

Figure 1. Effect of oral administration of griseofulvin and ketoconazole on serum testosterone levels in rabbits.

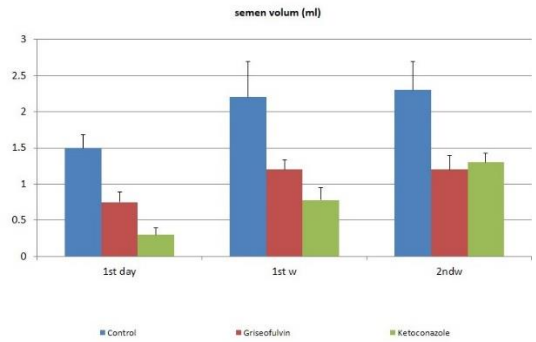

Figure 2. Effect of oral administration of griseofulvin and ketoconazole on semen volume in rabbits.

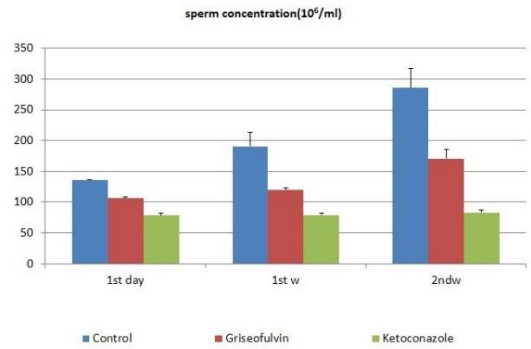

Figure 3. Effect of oral administration of griseofulvin and ketoconazole on sperm motility in rabbits.

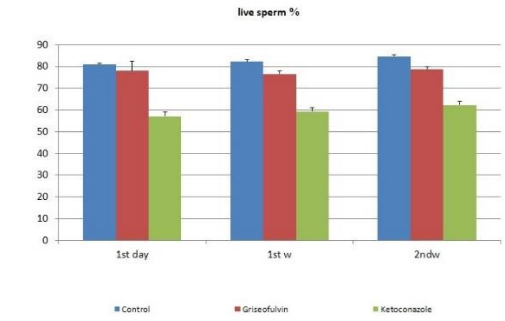

Figure 4. Effect of oral administration of griseofulvin and ketoconazole on total sperm abnormalities in rabbits.

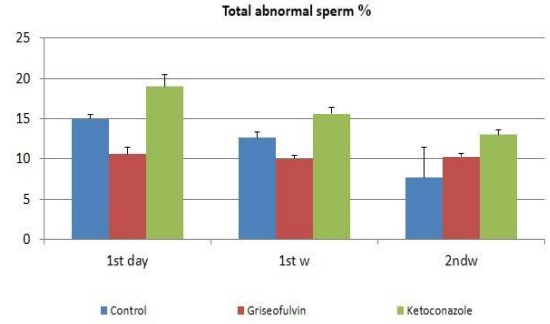

Figure 5. Effect of oral administration of griseofulvin and ketoconazole on sperm cell concentration in rabbits.

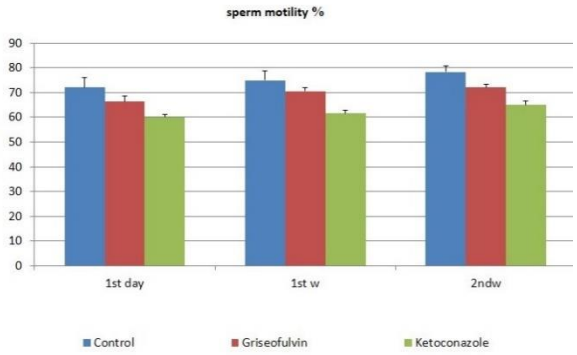

Figure 6. Effect of oral administration of griseofulvin and ketoconazole on live sperms in rabbits.

3.2.2. Sperm motility

The effect of oral administration of griseofulvin and ketoconazole on sperm motility in rabbits is presented in figure (3). The therapeutic dose of griseofulvin did not alter the percent of sperm motility at 31day, 38 day and 45 day post stop-dosing compared to the control group (66.3 ± 2.3, 70.6 ± 1.4 and 72 ± 1.5 respectively). On the other hand, ketoconazole produced a significant decrease in the percent of sperm motility ($P< 0.05$) at 31 day, 38 day and at 45 day post-dosing compared with control group (60 ± 1.1, 61.6 ± 1.2 and 65 ± 1.5 respectively) (Figure 3).

3.2.3. Percent of total sperm abnormalities

The effect of oral administration of griseofulvin and ketoconazole on total sperm abnormalities in rabbits is presented in figure (4). Results showed a significant reduction in the percent of total sperm abnormalities ($P< 0.05$) in griseofulvin-treated group in 31 day post-dosing compared to the control group (10.6 ±0.8 and 15± 0.5 respectively), while after 38 day and at 45 day post-dosing (10 ± 0.5 and 10.3 ± 0.33 respectively) this effect disappeared compared to the control group (12.6 ± 0.8 and 7.7 ± 3 .8, respectively). Meanwhile, the administration of ketoconazole produced a significant increase in the percentage of total sperm abnormalities ($P< 0.05$) in 31 day, 38 day and 45 day post-dosing (19 ± 1.5, 15.6 ± 0.8 and 13 ± 0.57 respectively), compared to control group (15 ± 0.5, 12.6 ± 0.8 and 7.7± 3.8, respectively) (Figure 4).

3.2.4. Sperm cell concentration

The effect of oral administration of griseofulvin and ketoconazole on sperm cell concentration in rabbits is presented in figure (5). Griseofulvin caused a significant decrease in sperm cell concentration ($P< 0.05$) at 31 day, 38 day and 45 day post-dosing (106 ± 3.0, 120 ± 2.8 and 170.6 ± 15 respectively). Similarly, ketoconazole induced a significant reduction ($P< 0.05$) in sperm cell concentration in 31 day, 38 day and 45 day post-dosing (78 ± 3.6, 79 ± 3.5 and 83.3 ± 4.1 respectively) post-administration compared to the control group (136.3 ± 0.6, 190.9 ± 22.9 and 285.6 ± 31 respectively) (Figure 5).

3.2.5. Percentage of live sperms

The effect of oral administration of griseofulvin and ketoconazole for 30 consecutive days on percentage of live sperms in rabbits is presented in figure (6). Results revealed a non-significant reduction in the percentage of live sperms in griseofulvin-treated groups at 31day post-administration, however the reduction was significant ($P< 0.05$) in the 38 day and at 45 day (76.6 ± 1.2 and 78.6 ± 1.4 respectively) post-dosing compared to the control group (82.3 ± 0.8 and 84.6 ± 0.8 respectively). The therapeutic dose of ketoconazole evoked a significant reduction ($P< 0.05$) in the percent of live sperms at 31 day, 38 day and at 45 day post-administration (57 ± 2.08, 59.3 ± 1.7 and 62.3 ± 1.7 respectively) compared to the control group (81 ± 0.5, 82.3 ± 0.8 and 84.6 ± 0.8 respectively) (Figure 6).

3.3 Histopathological findings

3.3.1. Testes lesions

Normal histology of seminiferous tubules in non-treated control group associated with all the successive stages of spermatogenesis, these active mature tubules were separated from each other by a thin layer of interstitial connective tissue containing endocrine cells and Leydig cells (Figure 7).

The testes of griseofulvin-treated animals showed mild changes characterized by vacuolar degeneration in spermatocytes and spermatocytes giant cells to moderate tubular degeneration (Figure 7). The testes of ketoconazole-treated animals showed marked interstitial fibrosis and edema with severe tubular degeneration and desquamation of germ cells in lumen, arrested spermatogenesis characterized by wide lumen of tubules, few germ cells and spermatocytes lining tubules with absence of sperms, or characterized by wide lumen of tubules lined by few germ cells with absence of spermatocytes and sperms in addition to separation of germ cell layer from basement membrane (Figure 7).

3.3.2. Epididymis lesions

Normal histology of ducts with normal sperms in non-treated control group is presented in Figure 8. The epididymis of griseofulvin-treated animals showed few sperms with large amount of exfoliated germ cells from ducts (Figure 8). The epididymis of ketoconazole-treated animals showed severe changes characterized by hyalinization of sperms in lumen of ducts, absence of sperms, and blebbing of epithelial lining ducts and vacuolization of epithelial lining ducts (Figure8).

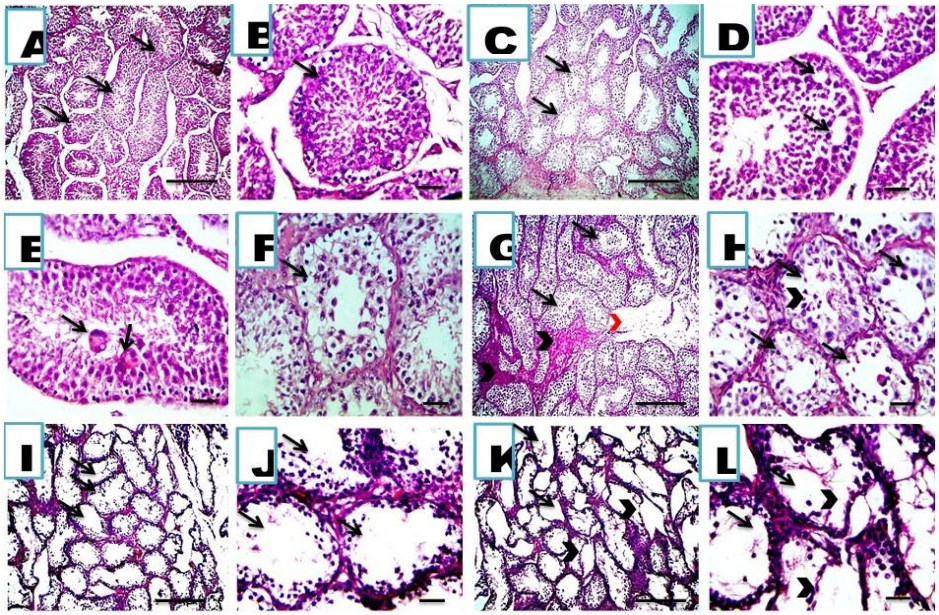

Figure 7. Testes sections showing normal histology of seminiferous tubules (black arrows) in control group (A & B), from mild changes characterized by vacuolar degeneration in spermatocytes (black arrows) (C & D) and spermatocytes' giant cells (black arrows) (E) to moderate tubular degeneration (black arrows) (F) in ketoconazole-treated group, marked interstitial fibrosis (black arrowhead) and edema (red arrow) (G) with severe tubular degeneration (black arrows) and desquamation of germ cells in lumen (black arrowheads) (H), arrested spermatogenesis characterized by wide lumen of tubules, few germ cells and spermatocytes lining tubules with absence of sperms (black arrows) (I & J) or characterized by wide lumen of tubules lined by few germ cells with absence of spermatocytes and sperms (black arrows) in addition to separation of germ cell layer from basement membrane (black arrow) (K & L) in griseofulvin-treated group. H & E, X: 100 bar 100 (A, C, G, I, K) and X: 400 bar 50 (B, D, E, F, H, J, L).

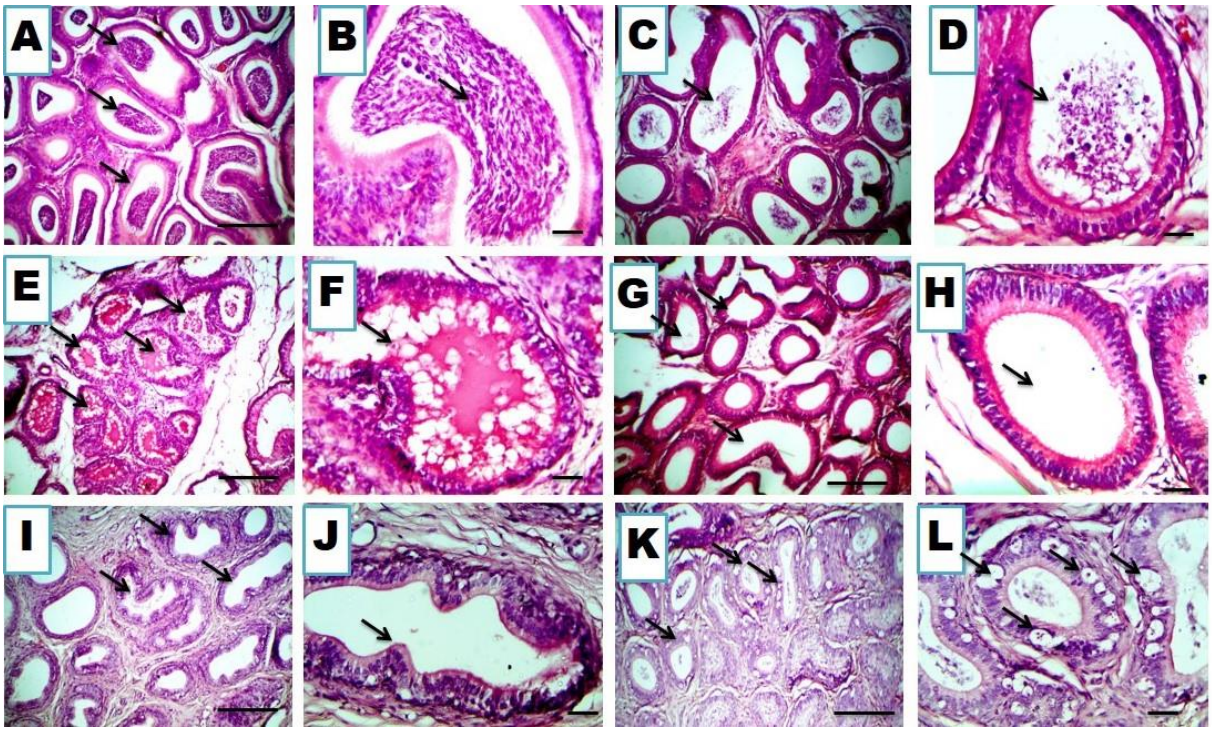

Figure 8. Epididymis shows normal histology of ducts with great quantity of sperms (black arrows) in control group (A & B), few sperms with large amount of exfoliated germ cells (black arrows) (C & D) from ducts in ketoconazole treated group, severe changes in griseofulvin-treated group characterized by hyalinization of sperms in lumen of ducts (black arrows) (E & F), absence of sperms (black arrows) (G & H), blabbing of epithelial lining ducts (black arrowheads) (I & J) and vacuolization of epithelial lining ducts (black arrow) (K & L). H & E, X: 100 bar 100 (A, C, E, G, I, K) and X: 400 bar 50 (B, D, F, H, J, L).

4. DISCUSSION

Infertility is considered as a real challenge to animal production. Several factors can affect the production and quality of sperms including drugs, toxins and environmental factors [17]. Antifungal agents are widely used in veterinary practice. They are playing an important role in the control and treatment of fungal diseases in different animals [18].

In the current study, oral administration of griseofulvin and ketoconazole reduced serum testosterone levels in rabbits at all tested time points. The histopathological changes induced by the drugs support the biochemical finding. Similar finding has reported that administration of griseofulvin evokes necrosis in the seminal vesicle and minimized the output of the sperm in rats [21]. In another study, it was shown that griseofulvin induced oligospermia in sheep [22] and elicited adverse effects on sperms of mice [23]. In the same line, griseofulvin has been found to affect the germ cells of male mice and to reduce the serum level of testosterone [28]. Similarly, ketoconazole has been shown to block testosterone biosynthesis in rat Leydig cells [24], and to induce a transient decrease in the level of serum testosterone in human [25]. Administration of ketoconazole has also been reported to induce a significant reduction in the level of serum testosterone in male rats [19, 26, 27]. In addition, a testicular damage and reduced testosterone level in serum of ketoconazole-treated males has also been reported [29].

The results of the present study also demonstrated a marked decrease in semen volume, sperms concentration, percent of live sperms and percent of sperm motility and an increase in the morphologically abnormal sperms of rabbits treated with griseofulvin and ketoconazole at all-time points. Administration of griseofulvin has been reported to increase the abnormalities of sperm in mice [9, 32] and rabbit [33]. On the other hand, fluconazole has been shown to decrease sperm cells motility and progressivity [34]. High doses of griseofulvin have been shown to cause a deterioration of frozen semen quality [22]. It has been reported that ketoconazole had a spermicidal action if mixed with ejaculated sperm (in vitro) obtained from dogs, monkeys and humans [30]. Furthermore, Drobnis and Nangia [31] stated that oral administration of ketoconazole could induce inhibition in cauda epididymal sperm motility post-dosing and arrest in the epididymal spermatozoa motility.

In the current study, griseofulvin elicited mild changes in the testes and epididymis particularly at the 45 day of supplementation.These results agree with those obtained previously. It has been shown that the use of griseofulvin in rats induced necrosis and damage in the seminal epithelium reducing total sperm output [21]. Similarly, griseofulvin has been reported to have a genotoxic effect on both male and female germ cells [28]. In addition, Amin et al [26] demonstrated that ketoconazole induced severe testicular histopathological lesions such as degeneration of the seminiferous tubules and depletion of germ cells. On other

hand, Iamsaard et al. [35] stated that the seminiferous tubules of ketoconazole-treated mice showed sloughing of germ cells and early cell degeneration.

Conclusion

Oral daily administration of griseofulvin and ketoconazole for 30 consecutive days has a negative impact on male rabbit fertility.

Conflict of interest statement

The authors declare that there is no conflict of interest in the current research work.

Research Ethics Committee Permission

The current research work was conducted according to standards of Research Ethics committee, Faculty of Veterinary Medicine, Mansoura University.

Authors´ contribution

A. M. and M. A. conducted the experiment, the analytical procedures, and wrote the manuscript, A. M. designed the experiment, and revised the manuscript, M. A. revised the manuscript.

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