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EFFECT OF FLUCONAZOLE ON FERTILITY OF COCKS

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ABSTRACT

This work was performed to investigate the possible adverse effect; if any; of fluconazole (2.5 mg/kg) on cock's (40 cocks) fertility after its administration in drinking water for 7 successive days. Cocks were classified into 2 equal groups (each of 20 cocks), the 1st group was served as control, while the 2nd group was administered therapeutic dose (2.5 mg/kg) of fluconazole. Samples (from 5 cocks each time) were collected after 1 day, 7 and 14 days post drug administration (for semen and serum samples) and at 28 days (for serum and histopathological examination of testis and epididymis). The obtained results revealed that, there was a significant decrease in serum testosterone level in medicated group with fluconazole at therapeutic dose. Semen picture revealed a significant decrease in sperm count and a non-significant change in abnormal sperm % and live/dead ratio. Histopathological finding of testes from treated group showed vacuolated spermatocytes with more widening diameter of seminiferous tubule, while the epididymis showed desquamation of epithelial lining inside lumen filled with mature sperm. Serum analysis for antioxidant markers showed a significant increase in total antioxidant capacity and serum malondialdehyde level.

INTRODUCTION

In many countries around the world, chicken meat consumption has been increased in the last few years. So people considered chicken's by-products the most suitable form of protein to satisfy their needs **(Roenigk, 1999)**.

Verma, et al., (2012) stated that any drop in fertility lead to great economic losses in animal production.

Yimer and Rosnina, (2014) mentioned that maintaining high fertility rate and good reproductive performance are the main goal for a successful animal production. The influencing factors on male fertility can be listed in the following " certain drugs, nutritional status, infectious diseases, hormonal disorders, environmental condition, genetic abnormalities and semen quality ".

Favareto, et al., (2011) recorded that exposure of male to certain drugs and chemicals increase the incidence of sexual dysfunction, due to a resultant damage in the level of spermatogonia cells which the reservoir of male genome or in the level of spermatozoid undergoing maturation.

Fluconazole is a triazole antifungal agent. It is highly water-soluble drug, absorbed rapidly and distributed well in all body fluids. It is excreted mainly through the kidney as unchanged and active drug **(Zervos and Meunier, 1993)**. It is used to treat yeast infections in the brain,vaginal area, mouth, and esophagus **(Molgaard, et al., 2016)**. Fluconazole has showed functional alterations in male fertility that could be reversed back to the normal condition again **(El-Medany and Hagar, 2002)**.

The aim of this work was designed to explain the possible effect of fluconazole on cock's fertility by using therapeutic dose on:

1- Male fertility which represented by:

a. Measuring of testosterone level in serum.
b. Sperm picture (sperm cell count, percent of live and dead sperms and assessment of total sperm abnormalities).
c. Histopathological studies on testes and epididymis.

2-Oxiditive stress and antioxidant markers:

a. Total antioxidant capacity.
b. Serum malondialdehyde 'Lipid peroxide'.

MATERIAL AND METHODS

Material

A) Drug

Fluconazole

Fluconazole **(flucoral)^{R}** was obtained from SEDICO company (6 October City, Egypt). Each capsule contains 150 mg fluconazole.

Dose: The recommended dose is 2.5 mg kg daily **(Reynes, et al; 1992)**.

B) Experimental animals:

Fourty (40) adult cocks were purchased from local poultry farm in El-Sinbillawein city. They were accommodated for two weeks before the experiment in the laboratory at Animal Health Research Institute, Mansoura. Cocks were kept under good hygienic condition. They were fed on finisher ration including 14% protein. The water was given ad-libitum. The bedding was wood shaving. The cocks were divided into two groups each of 20 cock.

Methods

Bird grouping:

This study was conducted on 40 clinically healthy mature cocks (1500 gm) which divided into 2 groups (20 cock each) as the following:

1st group: cocks were served as non-treated group (control).

2nd group: cocks were given the therapeutic dose of fluconazole (2.5 mg/kg b.wt /day) orally in drinking water for 7 successive days.

Serum sampling:

Blood sample was collected from 5 cocks of each group by slaughtering with sharp knife at 1day, 7, 14 and 28 day post drug administration. Samples were collected then they were put in the centrifuge at 3000 /15 rpm to get a clear serum. The obtained sera were kept in deep freezer at – 20^{o} C until assayed for serum total testosterone and antioxidant markers **(Stoffregen, et al; 1997)**.

1) Determination of serum total testesterone:

The level of total testosterone in serum were measured by Enzyme Linked Immunosorbant Assay (ELISA) method according to **Tietz, (1995)**.

2) Semen analysis:

1- Spermatozoa count: The count was done as the method reported by **Khaki, et al., (2008)**.

2- Spermatozoa live/dead ratio: The test was done by the method described by world health organization as mentioned by **Barth and Oko, (1994)**.

3-Sperm morphology%: The abnormal sperm morphology % were counted according to **Evans and Maxwell, (1987)**.

3) Oxiditive stress and antioxidant markers:

a)Determination of serum malondialdehyde (lipid peroxide): according to the method applied by **Ohkawa, et al., (1979).**

b)Determination of serum total antioxidant capacity: The serum levels of total antioxidant capacity (TAC) were assayed by spectrophotometer according to the methods adopted by **Koracevic, et al., (2001)**.

4) Histopathological technique:

Sampling:

Specimens of testes and epididymis were taken after 28 day from the end of treatment and they were fixed in 20% neutral buffered. Finally, the samples were blocked in hard paraffin, cut into sections of 5 micron thickness, stained by Haematoxyline and Eosin stain **(Bancroft and Stevens, 1996)**.

Statistical Analysis:

After collection of all data, it has been analyzed statistically by computerized SPSS program (Version 17) using two way ANOVA compare different groups at different times. The significant difference between groups and times were determined by using Dunnet test. The results considered significant when ≤ 0.05 according to **Howell (1995)**.

Table (1): The effect of fluconazole (2.5 mg/kg) on total serum testosterone hormone (ng/ml) of clinically health adult cocks. (M± S.E) (n=5)

Sampling time / Group	Total serum testosterone (ng/ml)				
	1day	**7day**	**14day**	**28day**	**P value**
G1 (Control) Non treated	4.36 ± 0.18^{A}	4.73 ± 0.37^{A}	4.53 ± 0.17^{A}	5.16 ± 0.08^{A}	**0.15 (NS)**
G2 fluconazole (2.5mg/kg)	2.5 ± 0.28^{aB}	2.6 ± 0.11^{aC}	2.8 ± 0.05^{abB}	4 ± 0.11^{cC}	**0.001**
P value	**0.001**	**0.001**	**0.002**	**0.001**	

The different capital letters in the same column means that there were significant changes at P <0.05.

The different small letters in the same raw means that there were significant changes at P <0.05.

Table (2): The effect of fluconazole (2.5 mg/kg) on Total Antioxidiant Capacity TAC (mmol/l) of clinically health adult cocks. (M± S.E) (n=5)

Sampling time / Group	Total Antioxidiant Capacity TAC (mmol/l)				
	1day	**7day**	**14day**	**28day**	**P value**
G1 (Control) Non treated	0.32 ± 0.06^{aA}	0.89 ± 0.05^{bA}	0.36 ± 0.08^{aA}	0.40 ± 0.05^{aA}	**0.001**
G2 fluconazole (2.5mg/kg)	0.81 ± 0.06^{abBC}	0.82 ± 0.06^{abA}	0.99 ± 0.05^{aB}	0.21 ± 0.05^{cA}	**0.01**
P value	**0.001**	**0.001**	**0.001**	**0.001**	

The different capital letters in the same column means that there were significant changes at P <0.05.

The different small letters in the same raw means that there were significant changes at P <0.05.

Table (3): The effect of fluconazole (at 2.5 mg/kg) on Malondialdehyde MDA (nmol/l) of clinically health adult cocks. (M± S.E) (n=5)

Sampling time / Group	Malondialdehyde MDA (nmol/l)				
	1day	**7day**	**14day**	**28day**	**P value**
G1 (Control) Non treated	0.41 ± 0.06^{aA}	0.39 ± 0.05^{aA}	0.90 ± 0.05^{bA}	0.72 ± 0.06^{bA}	**0.001**
G2 fluconazole (2.5mg/kg)	0.61 ± 0.04^{B}	0.52 ± 0.06^{AB}	0.94 ± 0.07^{B}	0.52 ± 0.06^{A}	**0.05 (NS)**
P value	**0.003**	**0.001**	**0.001**	**0.001**	

The different capital letters in the same column means that there were significant changes at P <0.05.

The different small letters in the same raw means that there were significant changes at P <0.05.

Table (4): The effect of fluconazole (2.5 mg/kg) on sperm count (10^6/ml), live/dead ratio (Live sperm %) and morphology% (abnormal sperm) of clinically health adult cocks during 14th days post treatment. (M± S.E) (n=5)

Groups	Sperm count (10^6/ml)				Live/dead ratio (Live sperm %)				Morphology% (abnormal sperm)			
	1day	7day	14day	P.value	1day	7day	14day	P.value	1day	7day	14day	P.value
G1 (Control) Non treated	4.53 ± 0.15[a]	4.60 ± 0.15[a]	4.53 ± 0.15[a]	0.95 (NS)	92.66 ± 9.19	94 ± 9.19	90.66 ± 9.19	0.10 (NS)	3.06 ± 0.48[a]	3 ± 0.48[a]	4 ± 0.48	0.31 (NS)
G4 Fluconazole (2.5mg/kg)	3.36 ± 0.15[b]	3.43 ± 0.15[b]	3.50 ± 0.15[c]	0.63 (NS)	89.66 ± 9.19	62.66 ± 9.19	91 ± 9.19	0.41 (NS)	5 ± 0.48[b]	5.66 ± 0.48[b]	5 ± 0.48	0.59 (NS)
P. value	0.006	0.008	0.001		0.4 (NS)	0.3 (NS)	0.79 (NS)		0.009	0.02	0.42 (NS)	

The different capital letters in the same column means that there were significant changes at P <0.05.
The different small letters in the same raw means that there were significant changes at P <0.05.

Semen examination

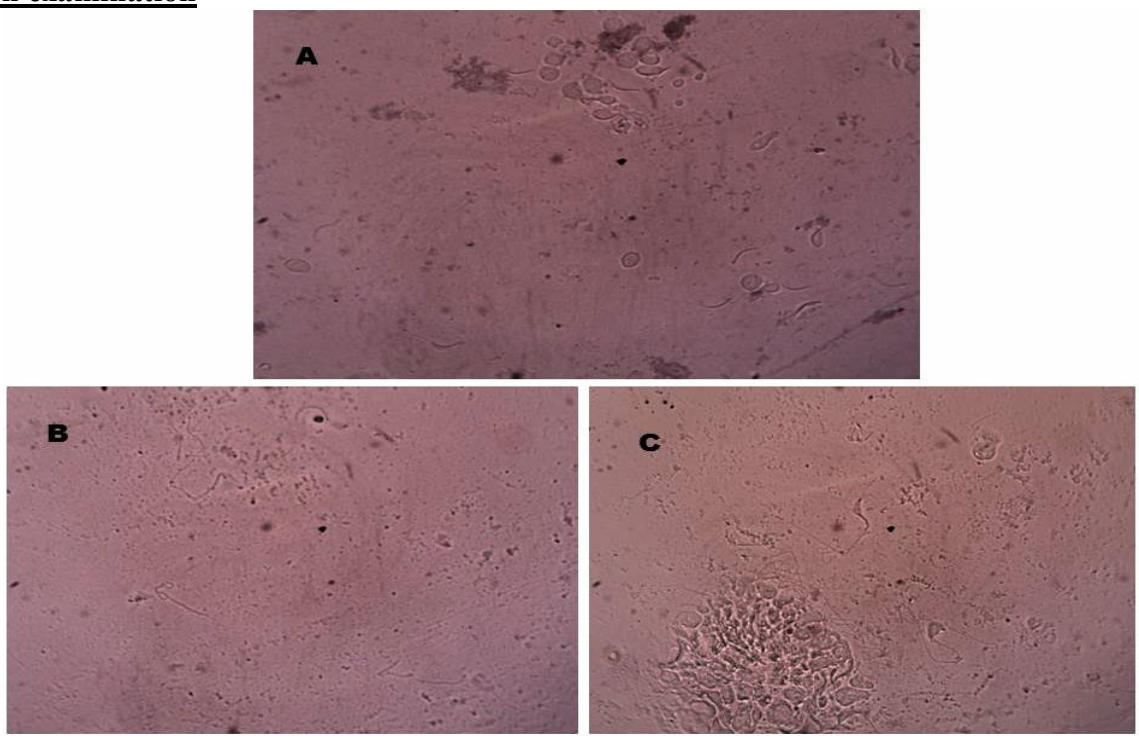

Figure (1): Showing the effect of fluconazole (at 2.5 mg/kg) on morphology of sperms (abnormal sperm):
(A) Normal sperm morphology (G1).
(B) Coiled tail and curved tail sperms (G2).
(C) Curved tail sperms (G2).

Histopathological findings:

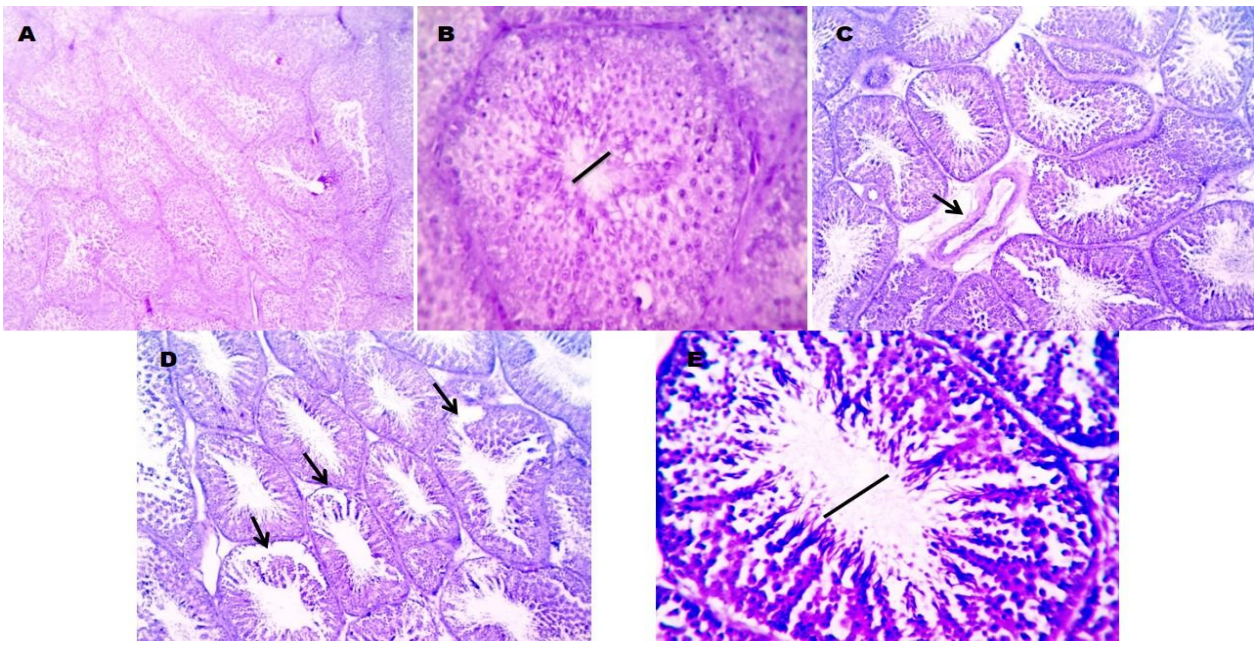

Figure (2) (A-E): (A) Testes, Gp 1control shows normal seminiferous tubules, **(B)**, Gp1control shows normal seminiferous tubule lined with spermatogonia then several layers of spermatocytes . Spermatids are attached to head of Sertoli cells. **(C),** Gp 2 shows mild perivascular edema (arrow),**(D)**, Gp 2 shows multifocal loss of spermatocytes with more widening diameter of seminiferous tubules (arrows). **(E)**, Gp 2 high power of **(D)**, shows dissoluted spermatid with widening diameter of seminiferous tubule (black line). H&E, X: A,C& D:100-B&E :200.

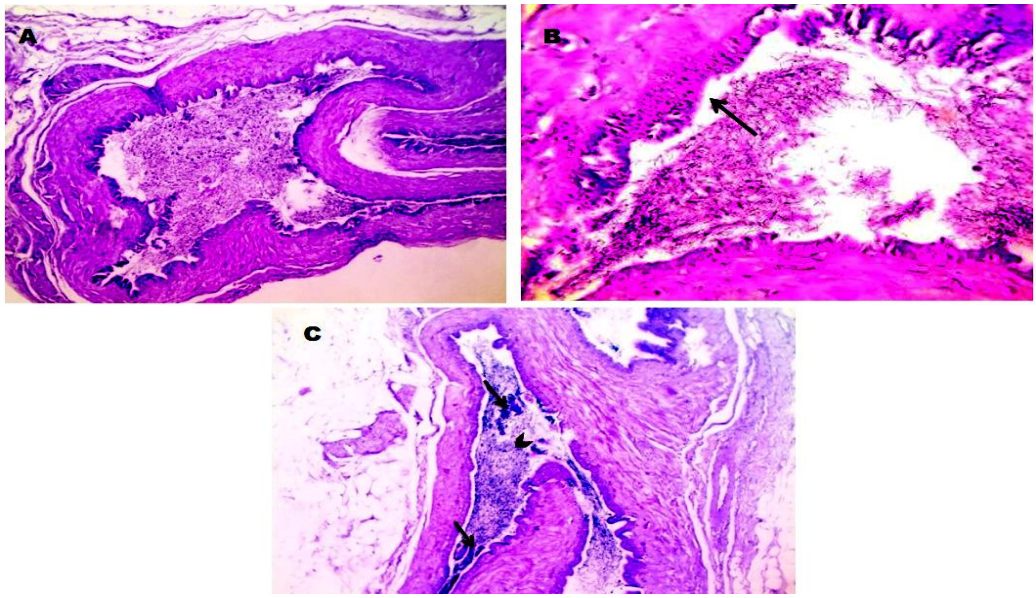

Figure (3) (A-C): (A) Couda of epididymis, Gp1control shows normal histological picture, **(B)**, Gp1control high power of Fig A to show normal epididymal lining (arrow), **(C)**, Gp2 shows desquamation of epithelial lining (arrows) inside lumen filled with mature sperm (arrowhead), H&E, X: A&C:100-B:200.

RESULTS AND DISCUSSION

1) Serum testosterone:

The obtained data showed a significant decrease in serum testosterone level in medicated group. That was supported by many auhers, **Clissold, (1997**) said that in mammalians tissues, fluconazole has inhibited lanosterol conversion to cholesterol as a result of many enzymatic reactions inhibition. Therefore gonadal and adrenal steroidogenesis have been blocked .The suitable explanations for the reduction in serum testosterone level was regarded to the decline in cholesterol availability and inhibition of testosterone synthesis from both Leydig's cells and adrenal gland as mentioned by **De Coster, et al.;(1998)**. In addition , **Gal, et al., (1998)** reported that fluconazole has androgen suppressive properities. It has ability to inhibit CP450 mixed function oxidase enzymes. So it can inhibit steroidogenesis by inhibiting C17-20 layase enzyme, which involved in steroid hydroxylation " such as progestins turned into androgens". **Meredith, et al.; (1998)** found that fluconazole has enzyme inhibiting not activating propriety by finding those enzymes in bound with microsomes of rat and mouse liver. As a result it can cause decline in serum testosterone level. **Grosso, et al.; (1998)** stated that fluconazole can reduce testosterone level by facilitating its rapid metabolism after globulin separation. Similar findings have showed a decline in testosterone level by **Irsy and Koranyi, (1999)**.

In addition, high-dose of fluconazole might cause adrenal insufficiency, decreased cortisol level as recorded by **Albert, et al., (2001)**. And this supported by the results previously obtained by **El-Medany and Hagar, (2002)** who stated that oral administration of fluconazole (50 mg/kg) to sexually mature male rabbits induced a significant decrease in serum testosterone and significant increase in serum prolactin, LH and FSH. Moreover, **Santhana Krishnan and Cobbs (2006)** said that antifungal azoles inhibit the production of adrenal steroids and can cause acute adrenal insufficiency in man. In addition, ketoconazole administered at relatively high dosages, has inhibited adrenocortical steroidogenesis by blocking steroidogenic enzymes **(Ohlsson et al. 2010)**. Finally there was a significant decrease in testosterone level in fluconazole treatment d cells of the two primary cultures of normal adrenal glands as mentioned by **van der Pas et al., (2012)**.

2)The effect of fluconazole (2.5 mg/kg) on sperm count (10^6/ml), live/dead ratio (Live sperm %) and morphology% (abnormal sperm):

The obtained data showed that there were a significant decrease in the sperm count in fluconazole medicated group and a non-significant change in abnormal sperm % and live/dead ratio. This findings could be collectively explained in the light that fluconazole might have reduce the sensitivity of steroids receptors to androgens as declared by **Baulieu, (1996)**. There was a concomitant decrease in testosterone as stated by **Pont, et al.; (1998)**. Also our findings were in accordance to **El-Medany and Hagar (2002)** who stated that oral dosing of fluconazole to mature male rabbits revealed a significant decrease in testosterone level, semen volume, sperm count and percentage of viable sperms.

In addition, a non-significant alteration in percentage of abnormal sperm forms was observed.

3) The role of fluconazole on oxiditive stress and antioxidant markers:

Free radicals and oxidants play a dual role as both toxic and beneficial compounds to the body. They are produced either from normal cell metabolism or from external sources "pollution, cigarette smoke, radiation, medication". With high levels of free radicals that could not be destroyed resulted in their accumulation in the body and generated a phenomenon called "oxidative stress". This process developed many chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases **(Pham-Huy, et al., 2008)**.

a) Total Antioxidant Capacity (TAC):

As showed in Table (2) there was a significant increase in total antioxidant capacity of fluconazole medicated group. This result was in accordance to **Mahl, et al.; (2015)** who stated that fluconazole increased generation of reactive oxygen species (ROS) and glutathione peroxidase (GPx), superoxide dismutase (SOD) in treated cells. **Zervos, et al., (1996)** said that the activity of fluconazole was attributed to the binding of the drug at cell surface receptors and up regulation of intracellular signaling pathways leading to enhanced oxygen free radical release and chemotaxis.

b) MDA 'lipid peroxide':

Lipid peroxidation occurred due to excessive damage in cell membrane and lipoproteins by hydroxyl radical and peroxynitrite. Malondialdehyde (MDA) and other compounds were resulted from this reaction, which have cytotoxic and mutagenic effect. Lipid peroxidation once started, rapidly affect high number of lipid molecules **(Frei, 1997)**.

The results recorded in Table (3) showed that fluconazole (at therapeutic dose) revealed a significant increase in serum Malondialdehyde level of treated cocks. This finding is in agreement with that obtained by **Schechter and Gladwin, (2003**) who reported that organ injury associated with oxidative stress has induced by fluconazole therapy. Also the previous results reported by **Ramadan, (2013)** showed that there was an elevation in the lipid peroxidation end product malondialdehyde (MDA) as well as nitric oxide (NO) in brain and heart tissues of pregnant rats.

4) Histopathological finding:

Testes lesions: testes of fluconazole (2.5 mg/kg) medicated group showed vacuolated spermatocytes with more widening diameter of seminiferous tubule. Mild perivascular edema and multifocal loss of spermatocytes inside the dilated lumen of seminiferous tubules. This may be attributed to differences in dose or duration of the drug administration.

Epididymal lesions: Figure (3) showed desquamation of epithelial lining inside lumen filled with mature sperm.

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الملخص العربي

تأثير الفلوكونازول علي خصوبه الديوك

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تم إجراء هذا العمل للتحقق من التأثير الضار المحتمل للجرعه العلاجيه لعقار الفلوكونازول (٢,٥ مجم / كجم) على خصوبة الديوك (عدد ٤٠ ديك) بعد إضافته في مياه الشرب لمدة ٧ أيام متتالية. قسمت الديوك إلى مجموعتين (بواقع ٢٠ ديك لكل مجموعة) ، واستخدمت المجموعه الاولي كمجموعه ضابطه بدون علاج ، بينما المجموعة الثانية أعطيت الجرعة العلاجية لعقار الفلوكونازول(٢,٥ مجم / كجم). تم تجميع العينات (بواقع ٥ ديوك في كل مرة) بعد يوم واحد و ٧ أيام و ١٤ يومًا من انتهاء اعطاء العلاج (لفحص عينات السائل المنوي ومصل الدم) وبعد ٢٨ يومًا (لفحص مصل الدم ونسيج الخصية والبربخ). فكشفت النتائج التي تم الحصول عليها ؛ أن هناك انخفاض كبير في مستوى هرمون التستوستيرون في مصل الدم للمجموعة المعالجه بالجرعه العلاجيه لعقارالفلوكونازول. وكشفت صورة السائل المنوي عن انخفاض معنوي في عدد الحيوانات المنوية وكذا تغير غير معنوي في نسبة الحيوانات المنوية المشوهه ونسبة الحيوانات المنوية الحيه. كما أظهر الفحص النسيجي المرضي للخصيتين والبربخ عن وجود تغيرات غير طبيعية. بالاضافه الي وجود زياده معنويه في مقدار القدرة المضادة للأكسدة ومستوى المالونديالدهيد بمصل الدم.