

## **Study The Effect of Ketoconazole and Vitamin E on Some Arabi Ram Sperm Characteristics**

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**Received: -Feb., 25, 2022; Accepted: March. 22, 2022 ; Available Online March 31, 2022**

### **Abstract**

Experiment aims to investigate the effect of ketoconazole on certain sperm characteristic of Arabi rams .fifteen adult rams were randomly assigned in 3 groups, 5 animals in each group which treated daily as the following : control group, ket group received orally (25 mg/kg body weight of ketoconazole and ket-E group received (25 mg/kg body weight of ketoconazole+50 mg vit E / head). The study lasted 28 days. Collection of semen was done each week by artificial vagina. Sperm traits were estimated in addition to assessment the level of testosterone and some anti\_oxidative enzyme and oxidative marker (MDA ). It was concluded that orally administered Ketoconazole has a negative impact on fertility of Arabi rams and vit E at 50 mg/head can prevent adverse effect of ketoconazole on sperm characteristic of Arabi rams.

**Keywords:** ketoconazole, vit E , Arabi rams

### **Introduction**

Reproduction is considered an important part for production process, therefore any reducing in fertility of animal can cause

economic loss in animal production (1). Fertility is described as the ability of animals to create pregnancy and is kept by insemination of good-management female using good- value

semen that is placed in the female reproductive tract at the suitable time. Males should have a superior reproductive performance and a good genetic profile as the males fertility and genetic efficiency appears more vital than those of the females (2).

The exposure of male to some chemicals and drugs may disturb their sexual function. Some drugs can hurt spermatogonia cells that denote the male genomes, and so disturb the spermatozoa before maturation (3). The fungal diseases are rendered a serious risk for human and animal health. Fungal infections may be cause of hypersensitivity due to fungal protein, toxicity due to fungal mycotoxin. Various fungal infections are happen due to opportunist pathogen that may be endogenous(e.g,Candida infections) or are came from the environments (e,g' *Cryptococcus*, *Aspergillus* infection) (4).

Ketoconazole is one of imidazole family which has a broad spectrum antifungal activity. It comes first amongst the azole antifungal derivatives. It has established to be the most effective and most widely used antifungal azole derivatives to date [5].

As well, ketoconazole is generally used for dealing with progressive prostate cancer. However, ketoconazole has adverse effects on the male genital system in both human and animal which researcher reported that the uses

of ketoconazole cause a decrease in the weight of male genital organ (6). In additionally found that reduce epididymal sperm count and serum testosterone level (7) as well as have serious adverse events effect on sperm parameters (8) subsequently on fertility capacity. Furthermore, affected a testicular structure which has a toxicity effect on the testicular tissues (9). Recent studies suggested that ketoconazole causes an increase in the level of reactive oxygen species (10 and 11).

Vit. E is an additive which is termed as anti\_sterility meanwhile it is essential for regular function of female genital system. (12), and (13), showed that vit E is a chief chain break antioxidant in spermatozoal membrane because it can instantly scavenge free radicals such as hydrogen peroxide ( $H_2O_2$ ), superoxide an ion( $O_2^-$ ), and hydroxyl radical (OH). Because of lipid solubility of vit. E ,it is consider the major protection lines from peroxidation of the poly-unsaturated fatty acids of phospholipid in spermatozoal membranes (14). Thus, the present study aimed to indicate and evaluate the adverse effect of ketoconazole on some semen characteristics and the protective effect of vit E on reducing these toxicity on sperm parameters of Arabi rams.

## **Materials and Methods**

### **Animal and semen collection**

The study were carried out in an animal field in Shatra , (Thi-qar)from 20 March 2021 to 17 April 2021. 15 Arabi rams ( 3.5-4.5 years old with  $51 \pm 4$  kg B.W) they placed in pen with semi slatted flooring and feeding a diet established on (15) , the rate of forage to concentrate was 60: 40 *ad libitum* . Rams were randomly divided into 3 groups(5 ram per group) . daily treatments were done orally as follow: control group, ket group (25 mg/kg body weight of ketoconazole and ket-E group (50 mg vit E / head +25 mg/kg body weight of ketoconazole). After adaptation on new ration, 4 ejaculate (each week) from each ram were collected by using artificial vagina as labeled by (16).

### **Ketoconazole administration**

Ketoconazole tablets 200mg/tables were bought from a local drug store. The doses were calculated according to the practice guide of dose conversion by (17).

### **Semen Processing**

Collection of semen was done with an artificial vagina every week , placed in ranked test tube, located in a thermoflask at  $37C^{\circ}$ , and conveyed to the lab for valuation of semen volume , PH , sperm count , motility (mass and individual ), live sperm and deformities.

### **Estimation of serum testosterone**

The blood collection and separation of serum was done weekly and the level of testosterone was done according to the way of (18)

### **Acrosomal integrity of sperm**

The integrity of the membrane of acrosomal was determined by using eosin nigrosine stain; 20 microliters of eosin nigrosine blended with 20 microliters of semen and then was smeared on a microscopic slide for evaluation under a phase-contrast microscope (19).

### **Estimation of testicular Malondialdehyde (MDA Concentration):**

MDA was estimated by the way of (20).Reaction of (M D A) with thio barbituric acid in an acid medium give a stained thio\_barbituric acid complex assessed by spectro photometer , at 520 to 535 nm in compare to blank and the amount of MDA were identified as n moles MDA / mL semen.

### **Measurement the level of Super oxide Dismutase (SOD) :**

Testicular (SOD) was evaluated according to (21). The optical absorbances were assessed at wave lengths(560 n m) against blank reagent . SOD equal to appearing absorbances of SOD / mL semen.

### **Estimation of Glutathione peroxidase GPx:**

Level of GPx in semen was estimated with spectrophotometer using Ransel reagent from

Randox-Laboratory Ltd . (Crumlen ,UK ). GPx activity was indicated as unit(U) / mL semen.

**Estimation of activity of catalase(CAT):**

This way designated by (22) which used for the assessment of activity of CAT in the semen sample.

**Statistical analysis:**

SPSS-Version 19 was used for Data analyzing. One way (ANOVA ) and least significant difference(LSD ) were done to evaluate significant differences amongst means  $P \leq 0.05$ .

**Table(1) composition of feed materials and additives of diet**

<b>Diet</b>	<b>control</b>	<b>ket</b>	<b>Ket-E</b>
Soybean	15	15	15
Barley	31	31	31
Wheat flour	11	11	11
Bran	24	24	24
Ground corn cob	14	14	14
Mineral and vitamin	4	4	4
Salts	1	1	1
Overall	100	100	100
<b>Additive</b>			
Ketoconazole mg /kg B.W		25	25
Vit E mg / head			50

**based on value for ruminant (15)**

**Results**

The result in Table 2 proved that the sperm concentration of the ketoconazole group decreased significantly ( $P < 0.05$ ) lower than that of control and ket-E groups ( $2.4 \pm 0.33$  vs.  $3.8 \pm 0.20$  and  $3.6 \pm 0.24$  respectively). As well as

there are different values of sperm motility in mass and individual motility among the groups. The data demonstrated that administration of ketoconazole at (25 mg/kg BW for 28 days) exhibit a decrease significantly ( $P < 0.05$ ) for ket group comparative with control and ket-E

groups in all sperm motility, both mass motility ( $80 \pm 4.4$  vs.  $94 \pm 4.5$  and  $95 \pm 6.4$  respectively) and individual motility ( $80 \pm 2.68$  vs.  $90 \pm 3.20$  and  $88 \pm 3.16$  respectively) and viability (live sperm) ( $88 \pm 3.14$  vs.  $95 \pm 4.46$  and  $95 \pm 5.68$  respectively).. , and significant increase ( $P < 0.05$ ) of abnormalities of ket control and ket-E groups ( $91.5 \pm 0.2$  vs.  $99.5 \pm 0.3$  and  $99.8 \pm 0.2$  respectively) .

group comparative with control groups and ket- E group ( $9.8 \pm 1.76$  vs.  $6.5 \pm 0.62$  and  $6.8 \pm 1.26$  respectively). it has been found out significant decrease at ( $P < 0.05$ ) in the integrity of acrosomal membrane of the induced group ket group comparative with

**Table (2) *In vivo* impact of ketoconazole and Vit. E on Some Spermatozoal characteristics of Arabi Ram (means $\pm$ SE).**

parameter	control	ket	Ket-E	LSD
<b>Volume ml</b>	$1.35 \pm 0.24$	$1.14 \pm 0.32$	$1.30 \pm 0.28$	NS
<b>PH</b>	$6.8 \pm 1.2$	$7.0 \pm 1.3$	$7.0 \pm 0.9$	NS
<b>Concentration mil/ml</b>	$3.8 \pm 0.20$	$2.4 \pm 0.33$	$3.6 \pm 0.24$	1.2
	a	b	a	
<b>Mass motility%</b>	$94 \pm 4.5$	$80 \pm 4.4$	$95 \pm 6.4$	14.0
	a	b	a	
<b>Individual motility%</b>	$90 \pm 3.20$	$80 \pm 2.68$	$88 \pm 3.16$	8.0
	a	b	a	
<b>Viability%</b>	$95 \pm 4.46$	$88 \pm 3.14$	$95 \pm 5.68$	7.0
	a	b	a	
<b>Abnormality%</b>	$6.5 \pm 0.62$	$9.8 \pm 1.76$	$6.8 \pm 1.26$	3.3
	b	a	b	
<b>Acrosomal integrity</b>	$99.5 \pm 0.3$	$91.5 \pm 0.2$	$99.8 \pm 0.2$	8.0
	a	b	a	

Value with small letters within a row differ significantly ( $p < 0.05$ )

Results in Table (3) revealed that the level of serum sex hormone (testosterone) dramatically dropped ( $P < 0.05$ ) in KET group compared with the control and ket-E groups ( $12.0 \pm 1.16$  vs.  $16.2 \pm 1.13$  and  $16.0 \pm 2.22$  respectively).

In KET\_treated rams, testicular MDA level were significantly ( $P < 0.05$ ) increased, compared with the control and ket-E groups ( $7.22 \pm 0.45$  vs.  $5.17 \pm 0.78$  and  $4.02 \pm 0.32$  respectively).

compared with the control and ket-E groups, KET\_treated group presented a significant reduction ( $P < 0.05$ ) in SOD activity ( $14.65 \pm 1.66$  vs.  $18.15 \pm 2.62$  and  $18.92 \pm 1.16$  respectively) and elevation ( $P < 0.05$ ) in GPx ( $3.97 \pm 0.88$  vs.  $1.97 \pm 0.97$  and  $1.76 \pm 0.62$  respectively) and CAT activity ( $5.54 \pm 0.75$  vs.  $3.11 \pm 0.40$  and

$3.62 \pm 1.42$  respectively) . The level of these oxidative stress markers didn't differ significantly between control and ket-E groups.

**Table(3) *In vitro* impact of ketoconazole and Vit. E on Some Sperm characteristics of Arabi Ram (means  $\pm$  SE).**

parameter	control	ket	Ket-E	LSD
Testosterone ng/mL	$16.2 \pm 1.13$ a	$12.0 \pm 1.16$ b	$16.0 \pm 2.22$ a	4.0
MDA(nomL/mL)	$5.17 \pm 0.78$ b	$7.22 \pm 0.45$ a	$4.02 \pm 0.32$ b	2.05
GPx (U /mL)	$1.97 \pm 0.97$ a	$3.96 \pm 0.88$ a	$1.76 \pm 0.62$ b	1.99
SOD (U /mL)	$18.15 \pm 2.62$ a	$14.65 \pm 1.66$ b	$18.92 \pm 1.16$ a	3.50
CAT(U/mL)	$3.11 \pm 0.40$ b	$5.54 \pm 0.75$ a	$3.62 \pm 1.42$ b	1.92

Value with small letters within a row differ significantly ( $p < 0.05$ )

## Discussion

Ketoconazole is commonly used for fungal diseases treatment in domestic animals. It is a synthetic , broad spectrum imidazole that exert their effect by a way that cause increase permeability of membrane, prevents exploit of precursor of DNA and RNA and production of oxidative and peroxidative enzyme (23). In the current study, demonstrated a slight reduction in the volume of semen, and obvious drop in sperm count , percent of live sperms and

motility , increase in the morphologically abnormal sperms and deterioration of acrosome integrity of rams treated with ketoconazole at all-time points. All these changes can be instructed to the oxidative effect of ketoconazole on the testis, which that proved by a previous study (24) and (25). ketoconazole is possible to reactive oxygen species production and the oxidative damages of lipid membranes then lead to severe testicular

toxicity (26). As well as found that ketoconazole effect on the male genital system in both human and animal (9).

There's marked reduction in the level of testosterone in this study. Nevertheless, ketoconazole inhibit the role of the cytochromeP450 enzyme included in steroidogenesis and thus decreases the syntheses of testosterone (23). The KET\_ induced testicular damages in this experiment was accompany by raise in MDA (a marker of lipid peroxidation), which is the final product of lipid peroxidation . It is likely then that reactive oxygen species production and the oxidative damages of lipid membrane cause KET\_ induced testicular damage. GPx and SOD are main enzymes that sweep destructive ROS in male genital organ (27). In the current study, administration of KET has cause reduction of SOD level and the rising of GPx in testes. The subsequent overproductions of ROS may cause oxidative stress. Up regulation of CAT enzyme activity, which may be due to catalyzing the removal of hydrogen peroxide (28), which mean there's an adaptive reaction to the great generation of H<sub>2</sub>O<sub>2</sub> in the testicular tissues. Our result was in accordance to that obtained by (8 and 6).

To avoid the negative effect of drugs, the researcher suggested used the antioxidant substance and protective nutrient such as vit E

which can decrease the side effect of the different drugs such as chemotherapies (29).

Selective effect of Ketoconazole and vit E on the indication of antioxidant / pro-oxidant balance were detected in this investigation . Small amount of ROS is essential for sperms to reach fertilizing ability (30). Nevertheless, extreme ROS may be harmful, specially due to sperm cell don't possess DNA reparation mechanisms, in fact they have little cytoplasmic antioxidant enzyme (31), (32). Oral treatment of vit. E has important effect on the male genital system. The valuable effect was obvious by increasing in semen value, antioxidant condition and testosterones in mammal. Vit.E is a lipids soluble anti-oxidant that counterbalance free radicals and keep cellular membranes counter to ROS. It also prevent lipid peroxidation and so improve function of other antioxidant(33). It was concluded that ketoconazole has a negative impact on fertility of Arabi rams and while vit E at 50 mg/head can prevent these adverse effect of sperm characteristic of Arabi rams.

**Conclusion:** Orally administration of ketoconazole (25 mg/kg body weight) for twenty eight days induce negative impact on some sperm parameters and oxidative enzymes of Arabi rams. concurrent administration of vitamin E (50 mg/head) with ketoconazole can prevent these deterioration in sperm parameters of Arabi rams.

**Conflict of interest:** Author declare there is no conflict of interest.

### Acknowledgment

I extend my gratitude to the Deanship of the Technical Institute of Shatrah for providing me with material and moral support. I also extend my heartfelt thanks to those who helped me in completing the research, especially the members of the Animal Production Techniques Department and those working in the field, and I do not forget to support my family, for they have all my thanks and gratitude

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## دراسة تأثير الكيتوكونازول وفيتامين هـ في بعض صفات الحيامن للكباش العربية

على صفات الحيوانات المنوية في الكباش العربية

خيري الركابي

المعهد التقني الشطرة- ذي قار

**الخلاصة:** تهدف التجربة إلى معرفة تأثير مادة الكيتوكونازول على بعض خصائص الحيوانات المنوية في الكباش العربية . تم اختيار خمسة عشر كبشًا بالغًا بشكل عشوائي في 3 مجموعات ، 5 حيوانات في كل مجموعة. والتي تم علاجها يوميًا على النحو التالي: مجموعة السيطرة ، ومجموعة الكيتوكونازول (25 مجم / كجم من وزن الجسم من كيتوكونازول ومجموعة الكيتوكونازول+ فيتامين هـ (50 مجم فيتامين هـ / رأس + 25 مجم / كجم من وزن الجسم من كيتوكونازول). استمرت التجربة 28 يوم. تم جمع السائل المنوي كل أسبوع عن طريق المهبل الصناعي وتم تقدير صفات الحيوانات المنوية بالإضافة إلى تقييم مستوى هرمون التستوستيرون وبعض الإنزيمات المضادة للأكسدة وعلامة التأكسد (MDA) وقد استنتج أن تناول الكيتوكونازول عن طريق الفم له تأثير سلبي على خصوبة الكباش العربية ، في حين أن فيتامين E عند 50 مجم / رأس يمكن أن يمنع التأثير الضار للكيتوكونازول على معايير الحيوانات المنوية للكباش العربية.