

Animal Reproduction Research Institute

## UNTOWARD EFFECTS OF OXYTETRACYCLINE ON REPRODUCTIVE CAPACITY OF BARKI RAMS (With One Table and 2 Figures)

By

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الآثار الجانبية لعقار الاوكسى تتراسيكلين على الكفاءة التناسلية  
للكباش البرقى

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استخدم في هذه الدراسة خمس كباش جيدة يبلغ عمرها حوالي اربعة اعوام حقنت بعقار الاوكسى تتراسيكلين (تراميسين طويل المفعول) في العضل بجرعة تبلغ ٢٠٠مجم/١٠كجم من وزن الحيوان ولقد تم تجميع عينات سائل منوى محكمة من كل حيوان مرة واحدة اسبوعيا لمدة ثلاث اسابيع على التوالي قبل الحقن، على انه بعد الحقن قد تم تجميع عينات السائل المنوى في اليوم الثانى والثالث والسابع ثم بعد ذلك مرة واحدة اسبوعيا لمدة خمسة اسابيع. وبعد التجميع مباشرة تم تقييم الصفات البيولوجية (حجم العينة سم<sup>٣</sup>، تركيز الحيامين فى سم<sup>٣</sup>، ونسبة الحركة الامامية) والصفات الشكلية للحيامين (نسبة الحى والاشكال الشاذة للذيل وكذا نسبة التشوهات الشكلية للجسم الطرفى %) وكذا الصفات الطبيعية للعينة (تركيز ايون الهيدروجين والضغط الاسموزى) هذا بالإضافة إلى انه قد تم قياس بعض الدوال التناسلية لكل حيوان على حدة وهى (محيط غشاء الصفن سم وزمن الوثب بالثانية) وقد تم ايضا قياس درجة حرارة كل حيوان على مدى تجربته واخيرا اخضعت كل عينات السائل المنوى كلها للاختبار البكتريولوجى حيث تم عد جميع المستعمرات الناتجة واخضاعها للتصنيف الكيماوى الحيوى. ولقد اظهرت النتائج بالنسبة للصفات البيولوجية بان هناك انحطاط معنوى لحجم العينة ونسبة الحركة الامامية، على انه لم يظهر اى تأثير معنوى على تركيز الحيامين) اما بالنسبة للصفات الشكلية فلقد كان هناك انخفاض معنوى فى النسبة المؤية للاشكال الشاذة للذيل للحيامين فى اليوم السابع ما عدا نسبة الاشكال الشاذة للجسيم الطرفى ونسبة الحيامن الحية وايضا محيط غشاء الصفن وزمن الوثب حيث انها لم تتأثر خلال التجربة ولقد اثبتت النتائج بأن حقن التراميسين طويل المفعول قد زاد من تركيز ايون الهيدروجين فى العينات فى الاسبوع الرابع من الحقن حيث جعلها تقرب من الحد القلوى وبعد ذلك انخفضت فى الاسبوع الخامس من التجربة ولقد انخفض ايضا الضغط الاسموزى للسائل المنوى انخفاض غير معنوي فى اليوم السابع من الحقن. هذا ولم يتأثر زمن

الوثب ولا محيط غشاء الصفن بعد حقن التراميسن طويل المفعول . ولقد كانت درجة حرارة الحيوانات ثابتة على طول التجربة ولم تتأثر خلال التجربة ومن الملاحظ بأنه كان هناك إنخفاض حاد في عدد المستعمرات البكتيرية المعزولة بعد حقن التراميسن طويل المفعول وايضا كان التأثير الميثيط للتراميسين على العصبيات القولونية والمكورات العنقودية بنفس الدرجة . خلاصة القول بأنه يمكن استعمال التيراميسين طويل المفعول في علاج الكباش البرقي بدون أى أخطاء يجب تجنب إنهاك الكباش خلال موسم التزاوج .

## SUMMARY

Five mature rams of four years old were injected intramuscularly (I/M) once as 200 mg/ 10kg B.W. Terramycin long Acting (TLA) for each. Semen samples were collected from each ram 3 weeks before injection as a control samples, at 2<sup>nd</sup> , 3<sup>rd</sup> and 7<sup>th</sup> day after injection then weekly for up to 5 weeks. Immediately after collection semen samples were evaluated for semen characteristics. Also some reproductive performance and the body temperature of the male had been measured all over the experiment. Also, each semen sample was subjected to bacteriological examination where colonies were counted and subjected to biochemical identification. The obtained results revealed that significant reduction in semen volume and percent of individual motility, whereas there was no detectable significant effect was shown in sperm concentration. A significant reduction in the percent of coiled tail was proved at the 7<sup>th</sup> day post treatment, where as TLA injection did not evoke any effect on percent of live spermatozoa, acrosomal integrity, the reaction time and the scrotal circumference throughout the experiment. On the other hand, TLA was highly significantly increase the pH of the ram semen toward alkalinity (7.5±0.0) at the 4<sup>th</sup> week post treatment. While the osmotic pressure of the semen was decreased non significantly at 7<sup>th</sup> day. The body temperature was proved to be stable throughout the period of TLA treatment. A sharp reduction in the total bacterial count was obtained in the 3<sup>rd</sup> day after TLA injection, also the bactericidal action of TLA on the isolated E.coli and Staphylococci was nearly the same. In conclusion, Oxytetracycline long acting can be safely used in treatment of Barki rams, but during breeding season precaution should be taken to avoid over use.

*Key Words: Untoward, Oxytetracycline, Reproductive, Barki Rams*

## INTRODUCTION

Oxytetracyclines were proved to be relatively safe drugs for cattle, sheep, horses, dog and swine (Booth and Mc Donald, 1988 and Prescott and Baggot, 1988). However, adverse effect may be attributed to their severely irritant nature which causes tissue damage at injection site (Nouws, 1984). Disturbance of intestinal flora due to their excretion through the bile into the gastro-intestinal tract (Woods *et al.*, 1973 and Bennett *et al.*, 1983). They are also implicated in nephrotoxicosis (Lairmore *et al.* 1984). Oxytetracycline contaminated with 4-epianhydro-Tetracycline and other degradation products were incriminated as the agents that caused edema, renal necrosis and hepatic degeneration in dehydrated calves (Teuscher, *et al.*, 1982), yellow or brown discoloration of bones and teeth had been observed when Tetracycline administered to puppies or pregnant bitches (Owen, 1963). Further more I/V injection in cattle was followed by acute collapse probably due to calcium binding and cardiovascular depression (Gyrd-Hansen *et al.*, 1981). Finally administration of oxytetracycline to pigs reduce immuneresponse (Zhakov *et al.*, 1978).

However, little reports have been conducted on the possible adverse effects of oxytetracycline on the capacity of the male animals, Timmermans (1974) proved that oxytetracycline was deleterious to spermatogenesis in the rat. Also, Abbitt *et al.* (1984) and Barth and Wood (1998) concluded that oxytetracycline injection to beef bulls did not affect spermatogenesis, seminal pH, ejaculated volume, percentage of motile spermatozoa, rate of sperm motility and sperm concentration. However, they proved that there were a treatment by day effect on sperm concentration. In addition, Fattouh *et al.*, (1991) proved that oxytetracycline injection in therapeutic dose bears no harmful effect on the buck semen. So, the purpose of the present study is to through a light on the effect of long acting oxytetracycline on the reproductive capacity of Barki rams.

## MATERIAL and METHODS

### 1- Animals:-

Five mature apparently healthy male rams of four years old were used. They were raised on the experimental farm of the Animal Reproduction Research Institute (ARRI), Giza, Egypt.

## **2- Oxytetracycline:**

Terramycin Long acting (TLA Pfizer Egypt Animal Health Division) was injected to rams intramuscularly (I/M) once as 200 mg / 10 kg B.W for each.

## **3- Semen Samples:**

Semen samples were collected from each ram using artificial vagina that was set up to proper condition where 1<sup>st</sup> and 2<sup>nd</sup> ejaculates were collected and pooled together. Control semen samples were collected from the same rams weekly for 3 successive weeks before injection of TLA for the study purpose, semen samples were collected at the 2<sup>nd</sup>, the 3<sup>rd</sup> and the 7<sup>th</sup> day after injection, Then weekly for up to 5 weeks.

Immediately after collection semen samples were evaluated (Salisbury *et al.*, 1978) for semen volume (ml), sperm concentration ( $\times 10^6$  sperm/ml), percentage of individual motility, percentage of live spermatozoa (Compbell *et al.*, 1956), coiled tail, acrosomal integrity (Bloom 1983), pH of the semen and the osmotic pressure. The osmotic pressure (M/osmol) was determined in seminal plasma (Salisbury *et al.*, 1978) using MOsmette TM Precision System Inc (USA). Also some reproductive performance of the male had been measured as scrotal circumference (mm) and the reaction time (sec). At last the body temperature was taken all over the experiment (°C).

## **4- Bacteriological examination:-**

Bacteriological examination was carried out according to Quinn *et al.*, (1994). Briefly, semen samples were collected in sterile bottles and were quantitatively inoculated into Nutrient agar, Blood agar, MacConkey and standard plate count agar. After incubation at 37°C for 18-24 hours colonies were counted and subjected for further biochemical identification following the method of Cruickshank, *et al.*, (1975)

## **5- Statistical analysis:-**

All data were statistically analyzed using Costat Computer Program, Version 3.03 Copyright (1986) cottort software.

## **RESULTS**

The effect of long acting oxytetracycline (TLA) injection on the ram semen characteristics were listed in table (1) where the semen volume

was reduced significantly after TLA injection ( $F=4.0$ ,  $p<0.05$ ). It was proved that the reduction in semen volume was reached to its maximum level at 7<sup>th</sup> day post treatment ( $1.7 \pm 0.2$  ml), then it started to rise at the 2<sup>nd</sup> week ( $2.5 \pm 0.2$  ml) until it reached near to the normal level at 5<sup>th</sup> week ( $2.3 \pm 0.3$  ml). Also the percentage of individual motility was suppressed all over the post treatment period ( $F=2.6$ ,  $p<0.05$ ) where the minimum figure was obtained at the 7<sup>th</sup> day ( $73.0 \pm 4.4\%$ ). On the other hand no detectable significant effect of TLA on sperm concentration was observed. A non significant reduction in the percentage of live spermatozoa was observed at the 7<sup>th</sup> day after treatment ( $72.4 \pm 3.1\%$ ). At the same time, The percentage of coiled tail was increased highly significantly ( $F = 32.1$ ,  $P<0.001$ ) at the 2<sup>nd</sup> and the 3<sup>rd</sup> day after injection ( $13.0 \pm 0.3$  and  $13.8 \pm 0.2$  respectively), then decreased at the 7<sup>th</sup> day ( $2.6 \pm 0.5\%$ ) till the last week of the experiment. While the I/M injection of (TLA) did not evoke any effect on the acrosomal integrity throughout the experiment.

Analysis of variance showed a highly significant increase ( $F = 25.8$ ,  $P<0.001$ ) in the level of semen pH toward alkalinity ( $7.5 \pm 0.0$ ) at the 4<sup>th</sup> week post treatment, then reduced significantly to ( $7.3 \pm 0.1$ ) at the 5<sup>th</sup> week post treatment. While the osmotic pressure of the seminal plasma did not affected significantly throughout the experiment, however, it was observed to be reduced apparently at the 7<sup>th</sup> day post treatment ( $273.2 \pm 15.6$  m/osm). The effect of oxytetracycline treatment on the reaction time of rams had been gradually increased non significantly after injection till the 4<sup>th</sup> week post treatment where it decreased and became proportional to the time before treatment ( $74.8 \pm 7.6$  vs.  $93.6 \pm 10.1$  sec). Also, the scrotal circumference did not affect significantly by TLA injection. Data gathered in Table (1) indicated that there was no significant effect of TLA on the body temperature.

The total bacterial count in semen of treated rams was remained progressive till 48 hours after injection ( $55.0 \pm 0.6 \times 10^3$ ) then sharply decreased in the 3<sup>rd</sup> day after injection (Fig. 1). Figure (2) showed that the action of TLA on Isolated E.coli and staphylococci organisms was nearly the same.

## DISCUSSION

Adverse effect of antibiotic agents on spermatogenesis or sperm function have been demonstrated in animals and man (Dukes and Elis,

1982 and Schelegel *et al.*, 1991). In conjunction with this statement the present results reflect significant ( $p < 0.05$ ) reduction in semen volume and percentages of individual motility recorded after oxytetracycline injection, which are in agreement with the results obtained by Biswas (1980) who found a significant decrease in sperm motility and live sperm count after oxytetracycline injection (20 mg/kg/day) for 6 days in ram. Furthermore Abbitt *et al.*, (1984) found that oxytetracycline injection in beef bulls reduced significantly ( $p < 0.01$ ) semen volume and sperm motility in the 3<sup>rd</sup> day after injection.

They interpreted the reduction of semen volume and motility in the 3<sup>rd</sup> day by proposing that lacking of penile protrusion during electroejaculation resulting in loosing of some semen volume and absence of some fluid component normally present in the preputal cavity also contamination of the collected portion.

It was previously known that oxytetracycline in therapeutic dose was attained in semen (Immelman and Dreyer, 1986 and Fattouh *et al.*, 1991). Also tetracycline have been shown to bind avidly to mammalian spermatozoa (Ericsson and Baker, 1967) and depress the survival rate of bull spermatozoa when it was added in media for thawing pelleted bull semen (Stoianov, 1987) due to its Ca-binding ability (Gyrd Hansen *et al.*, 1981) which was proved to be essential for sperm motility (Lapointe *et al.*, 1996). The spermatogenic cycle in ram is 49 days (Ortavant, 1958), so any alteration in the semen characteristics obtained during 40 days post oxytetracycline injection reflect changes in the physiology of the epididymis and accessory gland rather than in testis.

Although Biswas (1980) found that oxytetracycline injection reduced significantly the percentage of live spermatozoa, yet the present results did not found any significant changes in the percentage of live spermatozoa, which proved that TLA in therapeutic dose did not evoke any toxic effect on ram spermatozoa (Fattouh *et al.*, 1991).

It was clear from the present results (Table 1) that the sperm concentration was not affected significantly during post treatment periods. Kushniruk (1976) proved that the tetracycline hydrochloride either in therapeutic or toxic doses demonstrated only a negligible disruption of spermatogenesis after one month treatment period as evaluated histologically. It line with this finding Abbitt *et al.*, (1984) speculated that the number of germ cells per grams of beef bull were not influenced by oxytetracycline injection.

The highly significant ( $p < 0.01$ ) reduction in coiled tail percent reflect changes in the osmotic pressure and pH (Reid *et al.*, 1948 and Drevious and Eriksson, 1966) as it was proved by the obtained results. Also, the highly significant ( $p < 0.01$ ) changes in the pH of the seminal plasma were attributed to the reduction of the total bacterial count and in part to the presence of oxytetracycline in the semen (Immelman and Dreyer, 1986 and Fattouh *et al.*, 1991).

The depressing effect of oxytetracycline on reaction time, although non significant, it was proved to be week neuromuscular blocking agent in horse (Bowen and McMullan, 1975) and had a parasympathetic blocking effect in beef bulls (Abbitt *et al.*, 1984). The non significant effect of oxytetracycline on scrotal circumference in the present study was confirmed by Abbitt *et al.*, (1984). At last, it was clear from Table (1) that oxytetracycline injection did not alter the ram body temperature although Nouws (1984) proved that I/M injection of oxytetracycline causes marked irritation to the tissue.

From the bacteriological point of view, it was noted that there was a progressive increase of the total bacterial count in semen of treated rams within 48 hours after injection ( $55.0 \pm 0.6 \times 10^3$ ) and then a sharp decreased was detected in the 3<sup>rd</sup> day after treatment (Fig. 1). The progressive increase in total bacterial count within the 48 hours after treatment was due to day to day fluctuation which occur in the bacterial content of semen collected from apparently healthy animals (Foote and Salisbury, 1948 and Ghanem, 1980). A sharp decreased of total bacterial count in the 3<sup>rd</sup> day after injection is almost similar to that obtained in a previous study by Ericsson and Baker (1967) who concluded that tetracyclines are distributed to various body tissue including the prostatic fluid and semen. Furthermore Fattouh *et al.*, (1991) proved that oxytetracyclin was excreted in the goat semen with an average of  $0.28 \pm 0.01 \mu\text{g/ml}$ . After injection of double doses (18 mg/kg B.W) a significant increase in the excreted oxytetracycline in semen was obtained ( $0.33 \pm 0.02 \mu\text{g/ml}$ ). This may be due to a relatively high serum level for up to 3 days and moderate for up to 5 days resulting in the presence of the drug in equivalent concentration in the genital tract (Medina, 1988).

The action of TLA on isolated E.coli and Staphylococci organisms was nearly the same (Fig. 2), however, the response of Staphylococcai organism was a markedly earlier than E.coli. Lew *et al.*, (1977) reported that this antibiotic posses a wide range activity against

gram positive and gram negative bacteria as well as those in sensitive to many chemotherapeutic agents. Moreover, Finland (1974) suggested that some forms of oxytetracycline appear to be active against *Staphylococcus aureus*.

In conclusion oxytetracycline long acting can be used safely in treatment Barki rams but during breeding season precautions should be taken to avoid over use.

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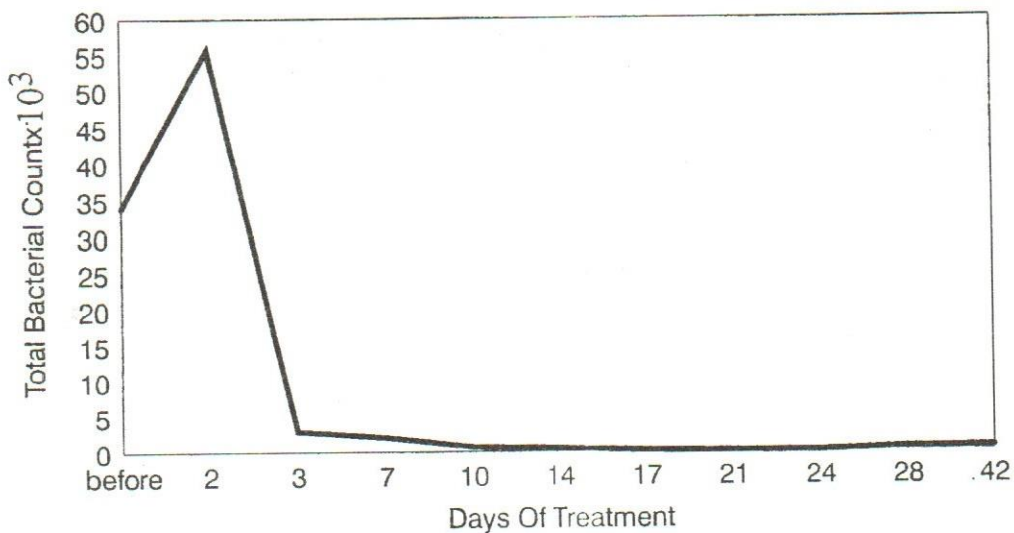
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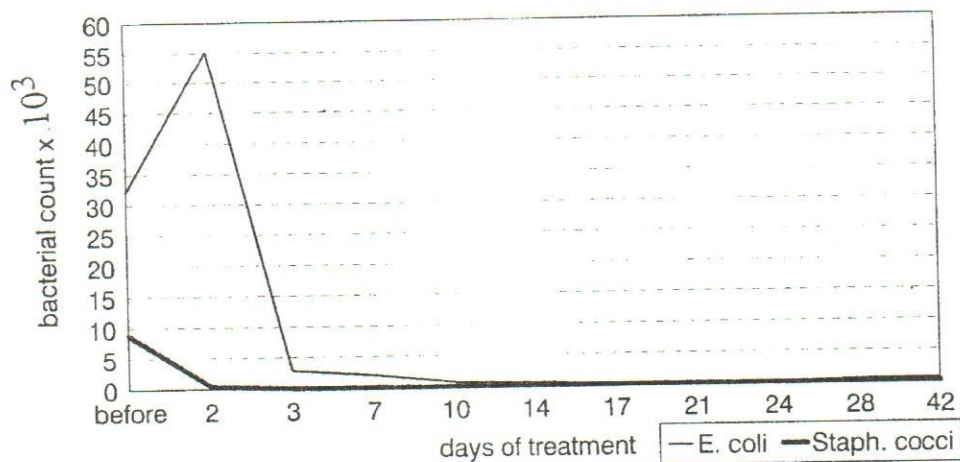
**Table 1: Effect of oxytetracycline long acting on the semen, reproductive characteristics and body temperature of Barki rams. (mean  $\pm$  S.E)**

Semen characters	Before treat.	Post treatment										Overall mean
		2nd day	3rd day	7th day	2nd W	3rd W	4th W	5th W				
Volume (ml)	2.9 a $\pm 0.1$	1.9 b $\pm 0.2$	1.8 b $\pm 0.1$	1.7 b $\pm 0.2$	2.5 b $\pm 0.2$	2.1 a b $\pm 0.1$	2.5 a b $\pm 0.2$	2.3 a b $\pm 0.3$	2.2 $\pm 0.1$			
Ind. Mot (%)	86.3 a $\pm 1.1$	78.0 b $\pm 3.7$	74.0 b $\pm 3.7$	73.0 b $\pm 4.4$	75.0 b $\pm 2.9$	73.5 b $\pm 1.9$	74.0 b $\pm 0.6$	74.5 b $\pm 0.9$	76.0 $\pm 1.1$			
Conc ( $\times 10^6$ /ml)	3281.3 $\pm 207.5$	2592.0 $\pm 282.8$	1174.9 $\pm 525.4$	3144.0 $\pm 652.6$	3546.0 $\pm 501.8$	2792 $\pm 378.9$	3124.0 $\pm 124.3$	2350.0 $\pm 196.8$	3027.0 $\pm 140.6$			
Live sperm (%)	88.2 $\pm 1.3$	81.2 $\pm 1.2$	81.1 $\pm 2.3$	72.4 $\pm 3.1$	83.2 $\pm 7.9$	84.9 $\pm 2.1$	85.0 $\pm 3.3$	89.9 $\pm 0.8$	83.1 $\pm 1.4$			
Coiled tail (%)	8.3 b $\pm 1.0$	13.0 a $\pm 0.3$	13.8 a $\pm 0.2$	2.6 c $\pm 0.5$	5.5 c $\pm 1.2$	5.4 c $\pm 1.2$	4.5 c $\pm 0.7$	5.0 c $\pm 0.2$	7.3 $\pm 0.7$			
Acrosomal defect (%)	3.2 $\pm 0.4$	3.4 $\pm 0.3$	3.2 $\pm 0.2$	2.2 $\pm 0.5$	2.4 $\pm 0.3$	2.4 $\pm 0.2$	2.2 $\pm 0.1$	3.1 $\pm 0.5$	2.8 $\pm 0.1$			
PH	7.0 c $\pm 0.0$	7.0 c $\pm 0.0$	7.0 c $\pm 0.0$	7.0 c $\pm 0.0$	6.9 c $\pm 0.1$	7.4 b $\pm 0.1$	7.5 a $\pm 0.0$	7.3 b $\pm 0.1$	7.1 $\pm 0.1$			
O.P (m/osm)	349.0 $\pm 7.1$	345.6 $\pm 26.3$	355.0 $\pm 22.1$	273.2 $\pm 15.6$	325.0 $\pm 28.4$	339.2 $\pm 14.8$	322.5 $\pm 18.6$	-	330.0 $\pm 7.7$			
Reaction time (sec)	93.6 $\pm 10.1$	106.6 $\pm 24.9$	114.6 $\pm 36.3$	109.1 $\pm 20.9$	122.2 $\pm 22.5$	114.1 $\pm 27.7$	100.8 $\pm 19.9$	74.8 $\pm 7.6$	104.5 $\pm 7.7$			
Scrotal Circum (mm)	32.0 $\pm 0.4$	32.0 $\pm 0.6$	31.4 $\pm 0.7$	31.6 $\pm 0.7$	30.9 $\pm 1.7$	32.2 $\pm 0.9$	31.1 $\pm 1.3$	31.3 $\pm 1.2$	31.6 $\pm 0.3$			
Temperature ( $^{\circ}$ C)	39.5 $\pm 0.1$	39.5 $\pm 0.1$	39.4 $\pm 0.0$	39.4 $\pm 0.1$	39.4 $\pm 0.1$	39.3 $\pm 0.0$	39.3 $\pm 0.0$	39.5 $\pm 0.1$	39.4 $\pm 0.1$			

Figures with different superscripts a,b,c,...are significantly at least  $p < 0.05$



**Fig.1: Effect of oxytetracycline long acting on total bacterial count in ram semen**



**Fig. 2: Effect of oxytetracycline long acting on E.coli & Staph.cocci in ram semen**