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EFFECT OF ESTRADIOL AND TAMOXIFEN ON SERUM AND SEMEN ESTRADIOL AND TESTOSTERONE CONCENTRATION, EPIDIDMYAL HISTOLOGY AND SEMEN QUALITY IN BALADY BUCKS

(With 5 Tables and One Figure)

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تأثير حقن الاستراديول والتاموكسفين على مستوى كل من الاستروجين والتستستيرون فى كل من مصل الدم والسائل المنوى والتركيب النسيجى للبربخ وجودة السائل المنوى فى ذكور الماعز البلدية

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هدفت الدراسة الحالية الى تحديد اثر حقن كل من الاستراديول والتاموكسفين (احد مضادات الاستروجين النوعية) على مستوى هرمونى الاستراديول والتستستيرون فى الدم وبلازما السائل المنوى والتركيب النسيجى للبربخ وكذلك جودة السائل المنوى فى ذكور الماعز البلدى كنموذج لما يمكن أن تسببه شبيهات ومضادات الاستروجين. أظهرت نتائج الدراسة الحالية أن هناك زيادة معنوية فى تركيز هرمون الاستروجين فى كل من الدم وبلازما السائل المنوى للحيوانات المعاملة بكل من الاستراديول والتاموكسفين. بالنسبة لهرمون التستستيرون فقد بينت النتائج أنه بينما كانت هناك زيادة معنوية فى تركيزه فى بلازما السائل المنوى فى كل من الحيوانات المعاملة بالاستراديول والتاموكسفين وكذلك فى مصل الحيوانات المعاملة بالاستراديول لم يكن هناك تغيير معنوى فى تركيز االتستسترون فى مصل الحيوانات المعاملة بالتاموكسفين بين الفحص الهستولوجى لانسجة البرنخ فى الحيوانات المعاملة بالاستراديول أن هناك زيادة فى إرتفاع وعدد الخلايا المبطنه للبربخ وكذلك فى طول وعدد أهدابها مقارنة بالمجموعة الضابطه. على النقيض من ذلك فقد كان هناك انخفاض فى ارتفاع الخلايا المبطنه للبرنخ وكذلك عدد وطول اهدابها فى الحيوانات المعاملة بالتاموكسفين. بالنسبة لخصائص السائل المنوى فقد كانت اشد تاثرا فى الحيوانات المعاملة بالاستراديول حيث كان

هـناك نقصا معنويا فى حجم القذفة والحركة الجماعية والفردية للحيوانات المنوية وكذلك فى تركـيز الحيوانات المنوية كما كان هناك زيادة معنوية فى نسب الحيوانات المنوية المشوهه والميته والغير ناضجة فى كل من فترتى العلاج وما بعد العلاج. لم تتـأثر خصائص السائل المـنوى كثـيرا بالتاموكسفين وانحصر التأثير فى زيادة معنوية فى نسبة الحيوانات الميته ونقص معنوى فى حجم القذفة. ويستنتج من هذه الدراسة ان الاضطراب فى تركيز هرمونى الاستروجين أو التستيرون أو فى نسبة أى منهما إلى الاخر فى مصل الدم وبلازما السائل المـنوى أى كـان سببه قد يؤدى الى خلل فى التركيب الهستولوجى للبربخ مما يترتب عليه انخفاض فى جودة السائل المنوى لذكور الماعز.

SUMMARY

The aim of the present study (Model study) was to identify the effects of Estradiol and Tamoxifen (Selective Estrogen Receptor Modulators, SERMS) on the serum and semen steroid hormones profile, integrity of the epididymal structure and semen quality in normal Balady bucks that might be mimicked or antagonized by environmental SERMS with estrogenic and/or anti-estrogenic properties. There was a significant ($P < 0.01$) increase in the serum and seminal plasma estrogen level in both Estradiol and Tamoxifen treated bucks. Regarding the testosterone concentration, it was noted that while there was a significant increase in the testosterone concentration in seminal plasma of both Estradiol and Tamoxifen treated groups as well as in the serum of Estradiol treated group, there was non-significant variation in serum testosterone concentration of Tamoxifen treated group. Histological examination of epididymal tissues from Estradiol treated bucks revealed an increase in the height of epithelial cells and length of their cilia. Also, there was an increase in the number of the nuclei indicating active division of the cells with consequent increase in the number of cilia. Conversely; in Tamoxifen treated group there was marked reduction in the number and length of the cilia and height of the cells. The semen quality was adversely affected in the Estradiol treated group as indicated by significant ($P < 0.01$) decrease in ejaculate volume, sperm motility and concentration as well as significant ($P < 0.01$) increase in the percentages of sperm abnormalities, dead sperms and sperms with protoplasmic droplets during treatment and post-treatment periods. However, the effect of Tamoxifen on the semen quality was comparatively mild and restricted to significant increase in the dead sperm percentage and decrease in the ejaculate volume. It could be concluded that whatever the cause of disturbed estrogen/testosterone ratio in both serum and seminal

plasma, it would disturb the integrity of epididymal structure with consequent reduction in the semen quality of bucks.

Keywords: *Estradiol, tamoxieen, serum, semen, testosterone, bucks*

INTRODUCTION

Estrogen and functional alpha estrogen receptors (ERα) are required for normal fertility in males of all mammalian species (Olivera *et al.*, 2001). Goyal *et al.* (1997) and Zhou *et al.* (2001) reported that estrogen may be important for maintenance of structural and functional integrity of specific segments of the male reproductive tract. Estrogen is present in high concentration in the rete testis and seminal fluid of several species and targets estrogen receptors along the male reproductive tract (Hess *et al.*, 2001). Estrogen is formed from the conversion of androgens to estrogen under the effect of aromatase enzyme produced by both germ cells and spermatozoa (Kwon *et al.*, 1995 and Janulis *et al.*, 1998).

Estrogen receptors (ER) mainly alpha type (ERα) are present in high concentration in the testis and non-ciliated cells of efferent ductules of goats (Goyal *et al.*, 1997). Also, ERα were detected in the epididmyis of rat (Pelletier *et al.*, 2000), Mouse (Igushi *et al.*, 1991) and human (Ergun *et al.*, 1997). Estrogen stimulates reabsorption of testicular fluid from the efferent ductules under normal physiological conditions (Clulow *et al.*, 1998), but treatment with steroid hormone creates complications owing to interference with feedback regulation of gonadotrophin release (Hess *et al.*, 2001). Estrogen treatment produces harmful effects on the epididmyis and reduces the fertilizing ability of epidimyal sperms (Lubicz-Nawrocki, 1974), alters the function of the seminal vesicle and the endocrine system and reduces the epididymal sperm reserve (Gray *et al.*, 1989).

SERMs are diverse group of compounds that bind with specific high affinity to estrogen receptors (ER) and can act as either an ER agonist or antagonist depending on the tissue (Thieband and Secrest, 2001). They added that clinically available SERMs include clomphence, Tamoxifen and Toremifene which are Triphenylethylenes and Raloxifene (benzothiophene). Hansen *et al.* (1997) found that Tamoxifen stimulated the greatest stimulation of fluid reabsorption in the efferent ductules of treated rats. Olivera *et al.* (2001) attributed the infertility

produced by chemical blockage of ER in rats to functional and structural alteration in the function of the male genital tract mainly in the efferent ductules and epididymis rather than in the spermatogenesis or direct effect on the testis. The aim of the present work (a model study) was to identify the important effects of estrogen (Estradiol) and Tamoxifen (SERMs) on the serum and semen steroid hormone profile, integrity of the epididmyis structure and semen quality of normal Balady bucks that might be mimicked or antagonized by environmental (SERMs) with estrogenic or anti-estrogenic properties.

MATERIALS and METHODS

Animals management and treatments:

Nine Balady bucks (12 to 15 months in age and 22 to 28 kg in weight) were purchased from local markets in Kafr El-Sheikh. They were kept under the natural conditions of temperature and day-light. They were fed on commercial caked diet and berseem and allowed free access to tap water during the whole experimental period extending from March to June. After an accommodation period of 3 weeks, the bucks were allocated into 3 groups (3 bucks each):

1. Estradiol treated group:

Each buck in this group was injected with slowly released Estradiol at a dose of 1 mg/10 kgm B.W. weekly for 3 weeks. Estradiol was supplied by Schering Plough Company in a commercial preparation called Premogen^{R} Depot. It is available in ampoules of 1 ml capacity that contains 10 mg of Estradiol. The effect of this treatment may represent a model for the probable effects of environmental estrogen on the male fertility.

2. Tamoxifen treated group:

Each buck in this group was injected by Tamoxifen at a dose of 4 mg/1 kg B.W. daily for 21 days. Tamoxifen was supplied by Amrya company for pharmaceutical industries in the form of Tablets, each contains 40mg Tamoxifen. The determined dose for the 3 bucks was dissolved in 3 ml of absolute ethyl alcohol and then completed to 9 ml by normal saline. Each buck was injected by 1 ml i.m and 2 ml s/c.

3. Control group:

The bucks in this group were left untreated control. obtained seminal plasma were stored at -20°C till both estrogen and testosterone hormones were assayed.

Hormones assay:

Blood samples were collected by Jugular vein puncture once weekly (3 weeks pre-, 3 weeks during and 7 weeks after treatments). The samples were centrifugated at 3000 rpm for 10 minutes. After completion of all evaluation processes, the remainder of the ejaculates were centrifugated at 3000 rpm for 15 minutes to obtain seminal plasma. The obtained sera and seminal plasma were stored at -20°C till both estrogen and testosterone hormones were assayed. *Testosterone*(ng/ml) concentration of both serum and seminal plasma was measured by RIA according to Adam *et al.* (1994) using active kits RIA DSL 4000 supplied by Diagnostic System Laboratories Inc. Corporate Head quarters 445 Medical Centre Blvd. Webster, Texas 77598-4217 USA. *Estrogen* concentration (pg/ml) of both serum and seminal plasma was measured by Enzyme-linked immuno-assay kits (ELISA).

Semen evaluation:

By means of Electro-ejaculator; semen was collected from each buck of the 3 experimental groups at the rate of 2 ejaculates per week for 15 weeks (3 pre-, 3 during and 9 post-treatments). Each ejaculate was directly transferred to water bath at 35°C while various evaluation examinations were made. The volume of the ejaculate was recorded directly after collection by the graduated collecting tubes. Mass motility (0-5), individual motility percentage and sperm abnormalities were determine according to Salisbury *et al.* (1978). Sperm cells concentration and total sperm count per ejaculate were determined using Neubauer heamocytometer. The percentage of alive sperms was determined in Eosin-Nigrosin stained films according to Swanson and Bearden (1951).

Histological examination:

At the end of the experiment, both control and treated bucks were castrated to obtain epididymal tissues. Tissue specimens from the corpus were fixed in Boune's solution and transverse sections were prepared and stained with heamtoxylin-eosin to study the effect of Estradiol and Tamoxifen on epididymal histology.

Statistical analyses:

The means $\pm$ SD were calculated for the concentrations of testosterone and estrogen in both serum and seminal plasma and as well as for some semen parameters in the 3 experimental groups pre, during and post-treatment periods. The obtained means were compared using ANOVA.

RESULTS

Effect of Estradiol and Tamoxifen injection on the serum estrogen concentration in treated Balady bucks:

The overall means of the serum estrogen concentration showed highly significant ($P < 0.01$) increase in Tamoxifen treated group during treatment period as well as in Estradiol treated group during post-treatment period and significant ($P < 0.05$) increase in Estradiol treated group during treatment period as well as in Tamoxifen treated group during post-treatment period compared with control group (Table 1).

The weekly serum estrogen concentration showed significant ($P < 0.05$) increase during the 3 weeks of treatment as well as during 1st 5th and 6th post-treatment weeks; highly significant ($P < 0.01$) increase during 2nd and 4th post-treatment weeks and non-significant variation during 3rd and 7th post-treatment weeks in Estradiol treated group compared with control group (Table, 1).

In Tamoxifen treated group, the serum estrogen concentration showed highly significant ($P < 0.01$) increase during treatment periods, significant ($P < 0.05$) increase during 1st, 2nd and 4th; non-significant variation during 3rd and 7th and significant ($P< 0.05$) decrease during 5th and 6th post-treatment weeks compared with control group (Table, 1).

Effect of Estradiol and Tamoxifen injection on the serum concentration of estrogen hormone in seminal plasma of treated Balady bucks:

The overall means of seminal plasma estrogen concentration showed significant ($P < 0.05$) increase in both Estradiol and Tamoxifen treated groups during treatment periods as well as in Estradiol treated group during post-treatment period and highly significant ($P < 0.01$) increase in Tamoxifen treated group during post-treatment period compared with the control group (Table, 2).

With the exception of the 1st and 2nd weeks of the treatment period and 1st and 2nd post-treatment weeks where there was significant ($P < 0.05$) increase in the concentration of seminal plasma estrogen there was non-significant variation in the remainder weeks (Table 2).

In Tamoxifen treated group, the seminal plasma estrogen concentration showed significant ($P < 0.05$) increase in the 3 weeks of treatment, 1st, 3rd, 4th, 5th and 7th post-treatment weeks, highly significant ($P < 0.01$) increase during 2nd post-treatment week and non-significant

variation during 6th post-treatment week compared with control group (Table, 2).

Effect of Estradiol and Tamoxifen injection on the serum testosterone concentration in Balady bucks:

The overall mean of serum testosterone concentration showed significant ($P < 0.05$) increase in Estradiol treated group and non-significant variation in Tamoxifen treated group compared with the control group (Table, 3). The serum testosterone concentration showed significant ($P < 0.05$) increase during all of the 3 treatment weeks as well as during the first 3 post-treatment weeks but showed non-significant variations during 4th, 5th, 6th and 7th post-treatment weeks in Estradiol treated group compared with control one.

With the exception of 1st post-treatment week where there was significant ($P < 0.05$) decrease and 7th post-treatment week where there was significant ($P < 0.05$) increase in the serum testosterone concentration, there was non-significant variation in all of the treatment and post-treatment weeks of serum testosterone concentration in Tamoxifen treated group compared with the control group.

Effect of Estradiol and Tamoxifen injection on the Tstosterone concentration in the seminal plasma of Balady buck:

The overall means of seminal plasma testosterone concentration showed highly significant ($P < 0.01$) increase in Estradiol treated group and significant ($P < 0.05$) increase in Tamoxifen treated group compared with control group during treatment and post-treatment periods (Table, 4).

In Estradiol treated group, the weekly seminal plasma testosterone concentration showed significant increase at $P < 0.01$ during all of the 3 weeks of treatment as well as 1st and 2nd post-treatment weeks and at $P < 0.05$ during 3rd and 4th post-treatment weeks but afterwards it showed non-significant variation during 5th, 6th and 7th post-treatment weeks compared with control group (Table, 4).

In Tamoxifen, treated group, the weekly seminal plasma testosterone concentration showed significant ($P < 0.05$) increase during all of the treatment and post-treatment periods with the exception of the 5th post-treatment week where there was non-significant variation compared with the control group (Table, 4).

Histological findings of the epididmyis:

Compared with the control bucks; Estradiol injection resulted in an increase in the height of the epithelial cells, an increase in the number of the nuclei indicating division of cells with subsequent increase in the number of cilia protruding in the lumen of the epididmyis and their length as well (Fig. 1b). The nuclei of the cells were distributed at more than one level in contrast to the control where the nuclei wee arranged at the mid level of cells.

In the Tamoxifen treated bucks, there was a decrease in the height of the epithelial cells and reduction in both number and length of the cilia protruding into the lumen as well as the nuclei became elongated in shape compared with both control and Estradiol treated bucks (Fig. 1c).

Effect of Estradiol and Tamoxifen injection on the semen characteristics of normal Balady bucks:

1. Effect of Estradiol injection on the semen characteristics:

Estradiol injection resulted in significant ($P < 0.01$) increase in ejaculate volume and percentages of dead sperms, sperm abnormalities and protoplasmic droplets and decrease in the percentage of both mass and individual motility as well as in the sperm cells concentration in treated group compared with non-treated control group during-and post-injection periods (Table, 5).

2. Effect of Tamoxifen injection on the semen characteristics:

There were non-significant variations in all of the studied semen, characteristics with the exception of sperm cell concentration where there was a significant ($P < 0.01$) increase compared with the control group during injection periods (Table, 5). During the post-injection period, there were significant ($P < 0.01$) decrease in the ejaculate volume and percentage of abnormal sperms; increase in the percentage of dead sperms and non-significant variation in the sperm cell concentration and the percentage of both individual motility and protoplasmic droplet compared with control group.

DISCUSSION

The caprine model may be useful for studies designed to determine mechanisms through which androgen and estrogen regulate development and function of the testes and exocurrent ducts (Goyal *et al.*, 1997). Screening the results of the current study revealed that the

testosterone concentrations were lower while the estradiol concentrations were higher in the seminal plasma than in the serum of non-treated control bucks, a finding which coincided with those of Luboshitzky *et al.* (2002) in normal men. They added that blood and seminal plasma hormone levels are not correlated and the higher seminal plasma estradiol levels compared with blood levels suggest local production of estradiol in normal men. They also added that the balance between estrogen and androgen in the seminal plasma is important for normal fertility. Gray *et al.* (1989) stated that chemicals having hormonal activity such as testosterone cyrproterone acetate, tamoxifen, estradiol and diethylstilbestrol disturb the synchrony of the endocrine events in male.

The significant increase in the serum and seminal plasma estrogen concentrations in Estradiol treated bucks may be considered as logical result for the injection of slowly released Estradiol preparation. Wolf *et al.* (1992) recorded linear significant increase in the serum estradiol level in male bovines as the number of estradiol implants was increased.

The increased both serum and seminal plasma estrogen concentration in Tamoxifen treated bucks compared with the control ones may be attributed to the competition of Tamoxifen (estrogen agonist) with estrogen for its receptors displacing it from its receptors in certain tissues leading to an increase in both serum and seminal plasma levels. However, this explanation may be supported by Clulow *et al.* (1998) who recorded comparable results with the anti-androgen flutamide that caused an increase in the systemic androgen concentration in rats. Also, Hampl *et al.* (1988) found that Tamoxifen treatment in men resulted in significant increase in the serum estradiol level and added that adrenal steroidogensis was positively influenced by this antiestrogen.

Regarding the effect of Estradiol injection on the testosterone concentration in both serum and seminal plasma, the results of the present study revealed that there was a significant increase in the serum and seminal plasma testosterone concentration during treatment as well as during first 3 and 4 post-treatment weeks respectively. The elevated serum testosterone concentration may be explained in the light of the findings of Dechaud *et al.* (1999) who found that xenoestrogens displace endogenous testosterone and estradiol from human plasma sex hormone binding globulins (hsHBG) binding sites resulting in a dose dependent

increase in the concentration of hsHBG unbound testosterone and/or estradiol and eventually disrupt the estrogen to androgen balance. Moreover, since the major portion of circulating estradiol in males arises from peripheral conversion of androgens (Cupps, 1991) in non-glandular tissues such as adipose tissues, muscles and brain (McDonald *et al.*, 1971), the increased serum estradiol level recorded in the present study may interfere with such conversion process by means of negative feedback effect leading to elevated testosterone level. Similarly the increased seminal plasma estrogen concentration as has been recorded in the present study may reduce the conversion of seminal plasma androgen into estrogen by means of negative feedback effect since the conversion of seminal plasma androgen into estrogen under the effect of aromatase enzyme produced by sperms remains the primary source of estrogen in the male reproductive tract (Hess *et al.*, 2001). Carreaus *et al.* (1999) reported that the aromatase enzyme produced by both germ and sperm cells represents 62% of the total testicular aromatase activity.

The non-significant variations in the serum testosterone concentration in the Tamoxifen treated bucks compared with control ones may come in accordance with the findings of Gill-Sharma *et al.* (2003) who found that testosterone concentration remained unchanged throughout the 90 days of Tamoxifen treatment in monkeys. The significant increase in the seminal plasma testosterone concentration in Tamoxifen treated bucks compared with non-treated ones as well as with the serum testosterone concentration of Tamoxifen treated and non-treated bucks suggest local intra-testicular testosterone production induced by Tamoxifen. Carppo *et al.* (2003) attributed the increase in the seminal plasma testosterone concentration in Tamoxifen treated human male to the concurrent increase in the seminal plasma hCG concentration that thought to have a paracrine effect on the intratesticular regulation of testosterone secretion. Shore *et al.* (2003) detected a rise in the testosterone concentration of seminal plasma in ram one day after i.m injection of 500 i.u. of hCG.

Regarding the histological finding observed in the Estradiol treated bucks, the increase in the number and height of the epithelial cells lining the epididmyis and their microvilli as well may be attributed to the mitogenic effect of estradiol. Cooke *et al.* (2001) stated that estradiol stimulates epithelium proliferation in the male and female reproductive tract and attributed such activity to the paracrine effect of estradiol on its stromal hormone receptors. Szego *et al.* (1988) showed

that the endometrial epithelium cells in female quickly responded to estrogen treatment by increasing number and height of the microvilli. The histological finding shown in Tamoxifen treated bucks in the current study may be comparable with the finding of Olivera *et al.* (2001) who noted a decrease in both the height of the epithelium and microvilli of the efferent ductules of ICI (antiestrogen) treated mice.

The adverse effects of Estradiol injection on the semen characteristics of normal bucks during treatment and post-treatment periods are believed to be due to the disturbances in the function of the epididmyis rather than the testes. Gray *et al.* (1989) found that administration of estrogen alters the function of endocrine system, seminal vesicle and epididmyis while the testicular measures are relatively unchanged. This belief may be supported firstly by the earlier appearance of the adverse effects in the semen characteristics during treatment i.e. before elapse of complete spermatogenic cycle and secondly by the marked histological changes in the epithelial lining of the epididmyis that were certainly accompanied by functional disturbances (fig.1b). Sakai *et al.* (1998) found that structural changes in the microvilli of the epididymal epithelium can alter fluid reabsorption. Hess *et al.* (1997) found that estrogen regulates the reabsorption of luminal fluid in the head of the epididmyis and a disturbance of such function causes sperms to enter the epididmyis diluted rather than concentrated with subsequent increase in the ejaculate volume and decrease in the sperm cell concentration as has been recorded in the present study. Cooper (1998) found that dysfunction of epididymal cells lead to abnormal concentration of ions and accumulation of cytoplasmic droplet material which eventually disturb motility and live sperm percentage. Also Eddy *et al.* (1996) speculate that sperm abnormalities shown in the cauda epididmyis may be attributed to the dysfunction of the narrow, apical and clear cell. The comparatively higher percentage of protoplasmic droplets may be explained in the light of results of Hess *et al.* (2001) who concluded that estrogen treatment in rats decrease the sperm transit time through the epididmyis and eventually the passage of immature sperms.

The non-significant variations in all of the semen parameters except sperm cell concentration in Tamoxifen treated bucks during treatment period may come in accordance with Gill-Sharma *et al.* (2003) who observed that oral administration of Tamoxifen has no effect on semen parameters vz. Volume, count, morphology and motility in human

and non-human primates. However, during the post-treatment period, the significant decrease in the ejaculate volume may be explained in the light of the results of Hess and Nakai (2000) who found that Tamoxifen showed the greatest stimulation of fluid reabsorption with subsequent decrease in the ejaculate volume. It could be concluded that whatever, the cause of disturbed estrogen/testosterone ratio in both serum and seminal plasma, it would disturb the integrity of epididymal structure with consequent reduction in the semen quality of bucks.

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Table 1 : Serum estrogen concentration (pg/ml) pre-, during and post-treatment with Estradiol and Tamoxifen in balady bucks.

Period	Pre-treatment				During treatment				Post-treatment						
Group	1st wk	2nd wk	3rd wk	Overall mean	1st wk	2nd wk	3rd wk	Overall mean	1st wk	2nd wk	3rd wk	4th wk	5th wk	6th wk	7th wk	Overall mean
Control (n = 3)	277.33 ± 28.56 c	274.00 ± 31.09 c	274.33 ± 38.68 c	275.22 ± 19.41 c	277.00** ± 24.64 c	280.67** ± 31.26 c	269.00** ± 25.06 c	275.56** ± 24.05 c	280.67** ± 39.27 b	289.33** ± 35.28 c	269.33** ± 17.04 b	283.33** ± 30.55 b	286.00** ± 41.68 b	283.67** ± 26.10 b	282.33ns ± 50.36 c	281.38** ± 18.51 c
Estradiol treated group (n = 3)	273.00 ± 17.52 c	273.33 ± 10.53 c	275.33 ± 59.18 c	273.89 ± 20.03 c	489.67** ± 28.55 b	591.00** ± 22.91 b	685.00** ± 33.79 b	588.22** ± 25.72 b	686.00** ± 354.25 a	393.33** ± 19.21 a	726.33** ± 123.44 a	671.00** ± 33.51 a	518.00** ± 24.06 a	402.00** ± 24.10 a	315.00ns ± 22.67 c	586.52** ± 30.73 a
Tamoxifen treated group (n = 3)	273.67 ± 24.42 c	281.33 ± 33.58 c	276.33 ± 46.93 c	277.11 ± 22.91 c	892.33** ± 87.69 a	851.33** ± 61.46 a	875.00** ± 66.86 a	872.89** ± 53.02 a	849.67** ± 55.99 a	606.00** ± 28.16 b	378.00** ± 32.71 a	391.00** ± 38.51 b	204.00** ± 7.81 c	189.33** ± 16.21 c	264.33ns ± 18.45 c	499.53** ± 41.00 b

Mean carrying different letters with in the same column are significantly different
** = P < 0.01

Table 2: Seminal plasma estrogen concentrations (pg/ml) pre-, during and post- treatments with Estradiol and Tamoxifen in balady bucks.

Period / Group	Pre-treatment				During treatment				Post-treatment							
	1st wk	2nd wk	3rd wk	Overall mean	1st wk	2nd wk	3rd wk	Overall mean	1st wk	2nd wk	3rd wk	4th wk	5th wk	6th wk	7th wk	Overall mean
Control group (n = 3)	487.00 ± 27.84b	493.67 ± 22.55 b	478.00 ± 48.04 b	486.22 ± 32.66 b	482.33** ± 36.56 b	482.13** ± 6.09 b	476.67** ± 60.19 b	480.44** ± 25.62b	473.33** ± 56.86 b	483.33** ± 30.33 c	494.67** ± 110.39 b	490.00 ± 20.00 b	492.33** ± 28.57 b	487.60** ± 166.32 ab	686.67* ± 80.21 b	486.76** ± 43.91 c
Estradiol treated group (n = 3)	474.00 ± 26.23 b	482.33 ± 33.56 b	490.67 ± 114.07 b	482.33 ± 42.19 b	1499.0** ± 166.59 a	1508.70** ± 98.78 a	1499.7** ± 210.83 a	1502.3** ± 65.65 a	1130.0** ± 66.78 a	919.33** ± 46.00 b	700.67** ± 62.07 ab	514.00 ± 20.66 b	445.67** ± 47.65 b	356.33** ± 17.62 b	481.67* ± 11.50 b	649.67** ± 15.80 b
Tamoxifen treated group (n = 3)	483.67 ± 33.17 b	475.00 ± 82.61 b	482.00 ± 88.61 b	480.22 ± 36.84 b	1562.70** ± 126.62 a	1538.70** ± 161.51 a	1553.7** ± 244.30 a	1539.3** ± 68.81 a	1274.3** ± 33.71 a	1133.30** ± 75.06 a	912.00** ± 94.60 a	841.67 ± 87.51 a	770.00** ± 30.00 a	671.67** ± 92.78 a	653.67* ± 79.60 b	893.81** ± 39.29 a

Means carrying different letters within the same column are significantly different:
* = P < 0.05
** = P < 0.01

Table 3: Serum testosterone concentrations (ng/ml) pre-, during and post-treatments with Estradiol and Tamoxifen in balady bucks.

Period / Group	Pre-treatment				During treatment				Post-treatment							
	1st wk	2nd wk	3rd wk	Overall mean	1st wk	2nd wk	3rd wk	Overall mean	1st wk	2nd wk	3rd wk	4th wk	5th wk	6th wk	7th wk	Overall mean
Control	1.87 ± 0.40 b	1.90 ± 0.10b	1.97 ± 0.38 b	1.9 ± 0.07b	1.87** ± 0.57 b	1.93** ± 0.06 b	1.93** ± 0.47 b	1.91** ± 0.32 b	1.97** ± 0.21 b	1.83** ± 0.38 b	2.00** ± 0.17 b	1.90 ± 0.44 b	1.90 ± 0.53 b	1.87** ± 0.58 ab	1.63** ± 0.15 b	1.87** ± 0.14 b
Estradiol	1.90 ± 0.33 b	1.87 ± 0.40 b	1.93 ± 0.32 b	1.90 ± 0.19b	7.13** ± 0.31 a	5.57** ± 0.37 a	6.03** ± 0.58 a	6.44** ± 0.27 a	5.37** ± 0.37 a	4.33** ± 0.12 a	3.67** ± 0.15 a	2.17 ± 0.12 b	1.47 ± 0.21 b	1.30** ± 0.10 b	1.80** ± 0.20 b	2.87** ± 0.01 a
Tamoxifen	1.87 ± 0.55 b	1.97 ± 0.21 b	1.93 ± 0.25 b	1.92 ± 0.16 b	1.23** ± 0.49 b	1.27** ± 0.21 b	1.13** ± 0.21 b	1.21** ± 0.22 b	1.33** ± 0.22 a	1.33** ± 0.23 b	1.93** ± 0.25 b	2.13 ± 0.25b	2.17 ± 0.21 b	2.67** ± 0.06a	2.33** ± 0.06a	1.93** ± 0.10 b

Means carrying different letters within the same column are significantly different
* P < 0.05
** = P < 0.01

Table 4: Seminal plasma testosterone concentrations (ng/ml) pre-, during and post- treatments with Estradiol and Tamoxifen in balady bucks.

Period	Pre-treatment				During treatment				Post-treatment					
Group	1st wk	2nd wk	3rd wk	Overall mean	1st wk	2nd wk	3rd wk	Overall mean	1st wk	2nd wk	3rd wk	4th wk	5th wk	6th wk	Overall mean
Control group (n =3)	0.34 ± 0.01 c	0.33 ± 0.09 c	0.35 ± 0.29 c	0.34 ± 0.04 c	0.34** ± 0.16 c	0.33** ± 0.08 c	0.33** ± 0.11 c	0.33** ± 0.11 c	0.33** ± 0.10 c	0.38** ± 0.06 b	0.38** ± 0.06 b	0.36** ± 0.07 b	0.34 ± 0.09 b	0.33* ± 0.07 b	0.36** ± 0.04 b	0.34** ± 0.01 c
Estradiol treated Group (n = 3)	0.36 ± 0.07 c	0.36 ± 0.11 c	0.34 ± 0.07 c	0.35 ± 0.06 c	3.17** ± 0.26 a	2.29** ± 0.37 a	2.44** ± 0.27 a	2.36** ± 0.13 a	1.82** ± 0.11 b	1.35** ± 0.09 b	0.94** ± 0.12 c	0.62** ± 0.10 a	0.78 ± 0.05 b	0.42* ± 0.01 ab	0.31** ± 0.04 b	0.83** ± 0.05 a
Tamoxifen treated group (n = 3)	0.36 ± 0.07 c	0.32 ± 0.05 c	0.32 ± 0.07 c	0.33 ± 0.06 c	1.56** ± 0.09 b	1.52** ± 0.19 a	1.32** ± 0.10 b	1.46** ± 0.14 b	1.05** ± 0.07 b	0.86** ± 0.08 b	0.76** ± 0.22 a	0.62** ± 0.06 a	0.55 ± 0.11 b	0.52* ± 0.09 a	0.93** ± 0.09 a	0.69** ± 0.01 b

Means carrying different letters within the same column are significantly different at:
* - $P < 0.05$
** - $P < 0.01$

Table 5: Effect of Estradiol and Tamoxifen injection on the semen characteristics of Balady bucks before, during and after treatments.

Treatment period / Semen characteristics	Pre-treatment period (3 weeks)			During treatment period (3 wks)			Post- treatment period (9 wks)		
	Control group	Estradiol group	Tamoxifen group	Control group	Estradiol group	Tamoxifen group	Control group	Estradiol group	Tamoxifen group
Volume (ml)	1.67± 0.15 b	1.16± 0.09 b	1.13± 0.09 b	1.18± 0.15 b	1.84± 0.19 a	1.33± 0.06 b	1.17± 0.05 b	1.50± 0.07 a	1.03± 0.05 c
Mass activity (score 1-5)	4.28± 0.10 b	4.44± 0.19 b	4.47± 0.32 b	4.50± 0.33 a	3.00± 0.33 b	4.50± 017 a	4.44± 0.10 a	3.91± 0.08 c	4.32 0.09 a
Individual motility (%)	85.56± 2.55 a	83.89± 3.47 a	82.22± 1.92 a	85.00± 01.67 a	48.89± 1.92 b	83.33± 1.67 a	84.26± 4.32	69.07± 2.74 b	81.30± 2.31 a
Sperm cell conc. (n x 10^9 m^{-1})	2.12± 0.26 b	2.12± 0.10 b	2.14± 0.22 b	2.13± 0.15 b	1.87± 0.06 a	2.55± 0.12 a	2.14± 0.21 a	1.66± 0.10 b	2.14± 0.05 a
Dead sperm percentage	4.11± 0.84 b	4.67± 0.58 b	4.78± 0.19 b	4.22± 0.69 b	12.67± 2.19 a	5.11± 0.83 b	4.30± 0.17 c	10.85± 0.68 a	5.48± 0.45 b
Abnormal sperm percentage	5.56± 0.69 b	5.44± 0.69 b	5.44± 0.51 b	5.89± 0.19 b	8.56± 1.07 a	4.67± 0.58 b	5.52± 0.46 b	10.37± 0.06 a	4.81± 0.39 c
Protoplasmic droplet percentage	1.55± 0.38 b	1.08± 0.84 b	1.78± 0.51 b	1.44± 0.19 b	4.78± 0.19 a	1.44± 0.38 b	2.07± 0.39 b	6.00± 0.29 a	2.51± 0.51 b

Values in the same row with different letters are significantly different at ($p<0.01$) within each period.

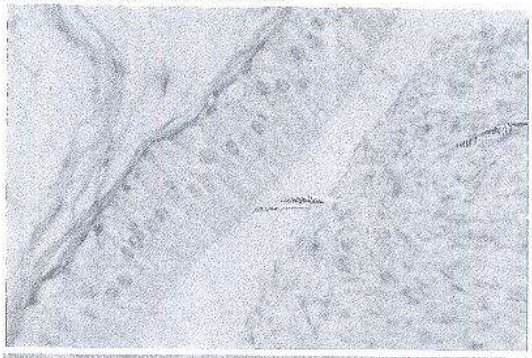

Fig.(1a): Epididymis(corpus) of control bucks (x 400) showing normal structure of the epithelial linning as well as normal distribution of the cilia.

Fig.(1b):Epididymis (corpus) of estradiol treated bucks (x 400) showing increased number and height of the epithelial cells linning of the epididmyis Also there is an increase in the number and length of the cilia.

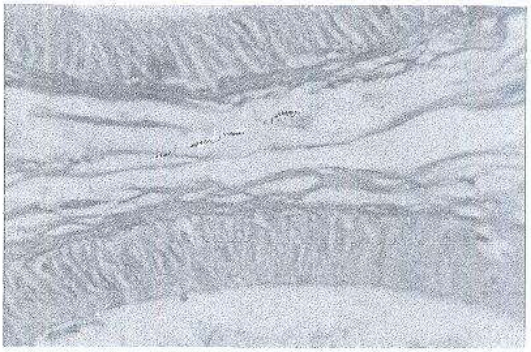

Fig.(1c): Epididymis(corpus) of Tamoxifen treated bucks (x 400) showing decrease in the height of epithelial cells as well as in the number and length of their cilia.