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## FERTILITY IN RAMS TREATED WITH CLOMIPHENE CITRATE (With 1 Table and 3 Figures)

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### الخصوبة فى الكباش المعالجة بسترآت الكلوميفين

وائل محمد بهجت

تم دراسة تأثير سترات الكلوميفين الجرعة بالفم ، على الخصوبة فى الكباش. تم تجميع عينات السائل المنوى وأيضاً عينات الدم من خمس من الكباش الرحمانى البالغة والخصبة ، وذلك بواقع مرتين فى الأسبوع لمدة ٣ أسابيع. وقد تم معالجة كل كبش ب ١٠٠ مجم سترات الكلوميفين لمدة ٥ أيام. ثم تم تجميع عينات السائل المنوى وأيضاً عينات الدم مرة أخرى من الكباش المعالجة بسترآت الكلوميفين. وقد تم تقييم صفات السائل المنوى للكباش (الحركة الفردية للحيوانات المنوية ، النسبة المئوية للحيوانات المنوية الحية ، تركيز الحيوانات المنوية) قبل وبعد التجريب بسترآت الكلوميفين. كما تم قياس تركيز هرمون التستستيرون فى عينات الدم قبل وبعد التجريب بسترآت الكلوميفين. كان هناك انخفاض فى القيم الخاصة بصفات السائل المنوى للكباش المعالجة بسترآت الكلوميفين. كذلك كان هناك انخفاض ملحوظ فى تركيز هرمون التستستيرون (٥٠% تقريباً) فى الكباش المعالجة بسترآت الكلوميفين. ويمكن الاستنتاج أن لسترآت الكلوميفين تأثير ضار على صفات السائل المنوى وعلى تركيز هرمون التستستيرون عند تجرعها بالفم للكباش البالغة والخصبة.

### SUMMARY

The effect of orally administered clomiphene citrate on the fertility of rams was studied. Semen and blood samples were collected from a five mature and fertile Rahmani rams (control) twice per week for 3 weeks. Each ram was treated with 100mg-clomiphene citrate for 5 days. Semen and blood samples were collected again from the clomiphene citrate treated rams (CC treated) for the same period. Semen parameters (sperm motility, live sperm, and sperm concentration) were assessed in the two groups. Blood samples were subjected to radioimmunoassay for

assessment of testosterone concentration in the control and CC treated rams. A reduction in the values of semen parameters was observed in the CC treated rams. Testosterone concentration was significantly decreased (approximately 50% from control rams) by clomiphene citrate treatment. It could be concluded that clomiphene citrate has a deteriorative effect on semen quality and testosterone concentration when it is orally administered to mature and fertile rams.

*Key words: Fertility in rams treated with clomiphene citrate*

## INTRODUCTION

Clomiphene citrate is a drug having augmenting influences on fertility. In the female, clomiphene affects fertility in a positive way by tricking the body into thinking that less estrogen is present than there really is, by blocking estrogen's attachment to its receptor, and the body responds by releasing greater amounts of FSH that stimulates ovogenesis (Irmssb, 1997). In the male rats, it is effective in increasing the male's serum level of LH and FSH, which stimulate testosterone and sperm production by the testes (Fertilitext, 1997). In infertile male rats, clomiphene citrate treatment together with testosterone administration has maintained their fertility (Rej *et al.*, 1988). On the other hand, Sokol *et al.* (1988) have shown that clomiphene citrate is not a useful drug in the treatment of male infertility. Clomiphene citrate treatment in intact male rats was found to inhibit their reproduction (Brown and Chakraborty, 1988). No adequate or well-controlled studies demonstrate the effectiveness of clomiphene citrate on male fertility and whether has a specific androgenic or antiandrogenic effects (Mosby, 1997). The aim of this study is to determine whether clomiphene citrate can improve fertility of rams, measured by semen evaluation and serum testosterone concentration, or not.

## MATERIALS and METHODS

Five Rahmani rams from Alexandria veterinary faculty farm were assigned for this study. Semen samples were collected from each ram (control) twice per week for 3 weeks. Blood

samples were collected from each ram following semen collection. A period of rest (10 days) was given to the rams. Then each ram was given clomiphene citrate (CC) orally (Clomiphene, ADCO, Egypt) at a daily dose of 100mg for 5 days. Semen and blood samples were collected again from the CC treated rams.

Semen samples were collected by the electroejaculator. The percent of motile sperm, percent of live sperm, and sperm cells concentration (using haemocytometer method), were subjected for evaluation.

Blood samples were collected by jugular venipuncture. Serum samples were obtained by centrifugation (3000rpm/min for 15 minutes) for assessment of testosterone concentration by radioimmunoassay using a testosterone kits (Diagnostic Systems Laboratories, Texas, USA).

T-test (Norusis, 1986) was used for comparing the obtained data of semen analysis and serum testosterone concentration, before and after CC treatment.

## RESULTS

The values of studied semen parameters (percentage of motile sperm, percentage of live sperm, and sperm cells concentration) before CC treatment were found within the normal range reported on Rahmani ram's semen characteristics (Noseir, 1993). After CC treatment, these values showed a significant decrease ( $P \leq 0.01$ ), in the percentage of sperm motility and in the percentage of live sperm (Table 1 & Fig. 1). Moreover, there was a noticeable significant decrease ( $P \leq 0.01$ ) in sperm cells concentration after CC treatment (573.75 Vs 2212.5 million/ml in control rams, Fig. 2).

The mean serum testosterone concentration in the studied rams before CC treatment was  $4.82 \pm 0.89$  ng/ml. A significant decrease ( $P \leq 0.01$ ) in testosterone concentration ( $2.75 \pm 0.53$  ng/ml) was noticed after CC treatment. This serum testosterone drop due to CC treatment could be estimated to approach 50% below control value (Fig. 3).

**Table 1:** Mean semen parameters and serum testosterone concentration in rams before and after CC treatment.

| Parameters                         | Before CC treatment (control) | After CC treatment (CC treated) |
|------------------------------------|-------------------------------|---------------------------------|
| Percentage of sperm motility       | 75.62±1.47 <sup>a</sup>       | 52.50±3.53 <sup>b</sup>         |
| Percentage of live sperm           | 74.37±1.47 <sup>a</sup>       | 46.62±3.61 <sup>b</sup>         |
| Sperm concentration (million/ml)   | 2212.50±387.96 <sup>a</sup>   | 573.75±53.05 <sup>b</sup>       |
| Testosterone concentration (ng/ml) | 4.82±0.89 <sup>a</sup>        | 2.75±0.53 <sup>b</sup>          |

Means ± St.E.

Means with different letters in same row are significantly different ( $P \leq 0.01$ ).

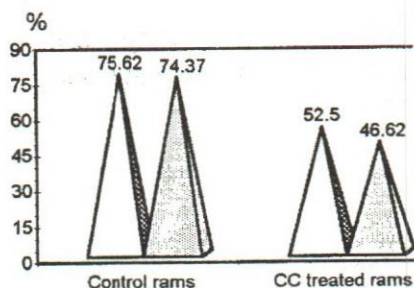


Fig. 1: Percentage of sperm motility and live sperm in control and CC treated rams

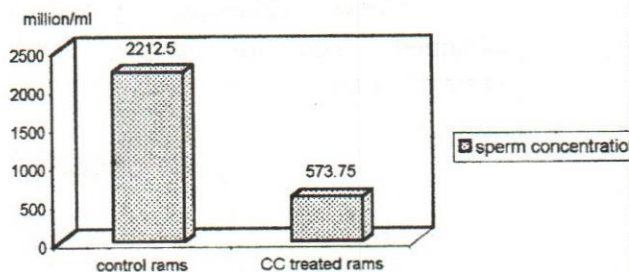


Fig. 2: Sperm cells concentration in control and CC treated rams

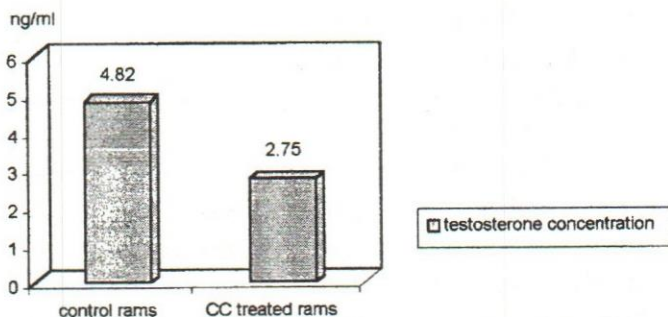


Fig. 3: Serum testosterone concentration in control and CC treated rams

## DISCUSSION

Clomiphene citrate is a non-steroidal drug, it has a molecular formula of  $C_{26}H_{28}ClNO.C_6H_8O_7$ . It is capable of interacting with estrogen receptor containing tissues. The first endocrine event in response to clomiphene treatment in male rats is an increase in the release of pituitary gonadotrophins (Mosby, 1997). Clomiphene citrate has been involved in many trials for the improvement of male fertility, but its beneficial effect is controversial (Shanis *et al.*, 1991). A significant improvement in semen volume, sperm density, and sperm motility was noticed in men treated with CC (Micic and Dotlic, 1985). Also a significant improvement in sperm counts occurred as a result of CC treatment in men (Soler Rosella *et al.*, 1980). On the contrary, Shanis *et al.* (1991) have shown that in men there was a significant deterioration in sperm function as measured by percentage of morphologically normal sperm. Weissenberg *et al.* (1992) have stated that in intact male rats LH and testosterone secretion were suppressed by clomiphene treatment. Hideki *et al.* (1990) have demonstrated that infertile males with low FSH and LH plasma levels tended to respond to CC treatment, while those with elevated levels of FSH and LH did not have any improvement in semen quality.

Testosterone may be the hormone that makes the male but it is estrogen, the so called female hormone, that gives sperm its reproductive punch, as estrogen regulates fluid reabsorption in the efferent ductules of the male, and with out reabsorption the sperm remains diluted and incapable of maturation in the epididymis, so any block in the estrogen receptor's function may result in infertility (Barlow, 1997). In this work, the studied semen parameters in the CC treated rams were low along with a significant decrease in testosterone concentration. There was also a positive correlation between testosterone concentration and studied semen characteristics. A similar postulate was reported by Doshi *et al.* (1994) who obtained a positive correlation between testosterone concentration, and total sperm count and sperm motility in buffalo-bulls treated with CC, indicating that low levels of testosterone was always associated with low values of semen characteristics. Brown and Chakraborty (1992) have suggested that clomiphene decreased the synthesis and/or release of gonadotrophins and also decreased serum LH and testosterone concentration in male rats.

The adverse effect of CC treatment on the fertility of rams, in the present study, may be attributed to its influencing effect on testosterone reduction (approximately 50%) and/or due to its antiestrogenic properties. It seems that good fertility response of clomiphene in mature males depends mainly on the presence of low fertility and/or deficiencies in serum gonadotrophins (Hideki *et al.*, 1990). While, simultaneous use of CC in normally fertile males could have a controversial effect. Therefore, CC treatment that could improve fertility in male infertile rats (Rej *et al.*, 1988) or infertile men (Micic and Dotlic, 1985), could also induce a deteriorative effects on semen characteristics when used in fertile rams. There is a still remains for investigation, a clomiphene good fertility response when administered to subfertile or infertile farm animals.

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