FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 50, 3: 131—133, 2006

# THE REPRODUCTIVE IMPLICATIONS OF CLOMIPHENE CITRATE ON SPERM CELLS DURING THE EPIDIDYMAL TRANSIT OF SPERMATOZOA IN MALE WISTAR RATS

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## ABSTRACT

**The reproductive implications of Clomiphene citrate (CC) on male Wistar rats revealed the reactions of the rats to CC at different dosages (varied). Results revealed detrimental effects at a higher dosage. In the *caput* of the epididymidis, the mean percentage motility in group B Wistar rats (20.0 ± 8.66) and group C Wistar rats (23.3 ± 3.33) was significantly lower (P < 0.05) than group A Wistar rats (42.0 ± 2.00). The drug did not influence the percentage livability of the spermatozoa in the *caput* epididymidis. In the *corpus* epididymis the mean percentage motility for groups A — C were 56.00 ± 2.45, 25.56 ± 7.50, 13.00 ± 5.77, respectively. Group A value was significantly higher than those of groups B and C. The percentage livability was higher in-group A than B and C. The concentration of the spermatozoa was significantly higher in group A (78.25 ± 6.97. 106) than groups B (53.75 ± 1.89. 106), though this is not significantly different (P < 0.05). These findings were discussed in relation to the significance of the drug as a fertility enhancer in infertile animals. The reproductive implications of the drug were studied in the epididymis of Wistar rats with no adverse effects when used at the recommended dose. The drug can therefore be used to boost the reproductive potential of infertile male animals.**

**Key words: Clomiphene; epididymis; fertility; rat; semen**

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## INTRODUCTION

Clomiphene citrate (CC) is non-steroidal drug; it has a molecular formula of $C_{26}$ $H_{28}$ CINO. $C_6$ $H_8$ $O_7$. 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 gonadotrophin (1, 2). Some scientists have reported that a significant improvement following CC treatment in terms of semen volume, density, motility and sperm concentration (3, 4). Clomiphene citrate (Clomid®) is used to trigger ovulation in women with anovulatory circles in those having secondary amenorrhoea with normal levels of follicles stimulating hormones (FSH), luitenizing hormone (LH) and prolactin (1). Women with low estradiol levels respond poorly to clomiphene citrate. When it is used as recommended, it is a safe, reliable, inexpensive therapeutic modality (5).

Epididymal transit is the movement of the sperm cells from *caput* (head) through *corpus* (body) to tail of the epididymis. Sperm cells stored in the *caudal* epididymis at the end of spermatogenesis can be influenced by age, nutrition, drug, temperature and successive ejaculations (6, 7). The normal sperm cells of Wistar rats consist of a hook shaped head (question mark shape), a thin neck, midpiece and a long tail. It is only in rats and mice that the heads of spermatozoa terminate in a distinct "hook" shape (8).

The aim of this study was to determine if clomiphene would enhance fertilization potential of the spermatozoa in terms of the motility, livability and integrity of the sperm cells. This is important in animal breeding and artificial insemination programmes.

## MATERIALS AND METHODS

Sixty Wistar rats aged between 12 and 14 weeks and with a body weight of 150—180 grams were used in this study. They were divided into three groups A, B, C, of twenty rats each and were kept in a cage of about 18 by 25 inches in the Experimental Animal Unit (EAU) of the Faculty of Veterinary Medicine University of Ibadan, Nigeria. All the rats were fed on commercial rat pellets and 50 mg of Clomid® tablets were administered to group B. Each table was dissolved in 1.0 ml of distilled water and deposited into the stomach of each rat using the cannular for five consecutive days. Group C rats were treated with 0.6 mg of Clomiphene citrate that was dissolved in 1.0 ml distilled water as in earlier groups. Group A rats which is the control received 1.0 ml of distilled water. The rats from all the groups were then left for two weeks before semen samples were collected for analysis.

Spermatozoa were obtained and examined from the *caput*, *corpus* and *caudal* epididymidis. The methods of collection from these locations were generally similar to that of Akusu *et al.* (9).

The spermatozoa recovered were used to study motility, live/dead or livability (using eosin-nigrosin stains). The improved Neubaur haemacytometer method was used to determine sperm cell concentration as described by Zemjanis (10).

## DATA ANALYSIS

Data obtained were subjected to Student *t*-test and chi-square test for the establishment of significance (11).

## RESULTS

Results of the signs, symptoms and observable activities of the rats in groups B and C given 50 mg and 0.6 mg clomiphene citrate (CC) respectively are presented in Table 1.

Motility in the control experiments (Group A) and percentage livability were increasing as the sperm cells migrate along the *caput* through *corpus* to *caudal* epididymis. While the same increase goes for the groups B and C given 50 mg and 0.6 mg per body weight decrease ($P<0.05$) in motility when group A and the groups B and C values were compared.

Table 2 indicated that the treated group B had motility of $20.00\pm8.66\%$ in the *caput* $25.56\pm7.50\%$ in the *corpus* and $42.50\pm2.50\%$ in the *caudal* these values are significantly lower ($P<0.05$) than the control group. The values of the concentration in group B treated with 50 mg CC is significantly lower ($P>0.05$) than that of group A treated with 1.0 ml of distilled water (Table 2). The concentration of group A control to the experiment is $78.25\pm6.97.10^6$ spermatozoa.ml$^{-1}$ of the 0.6 mg. In the *caudal* (tail) epididymis the motility of group A $76.00\pm2.4.40\%$ is significantly higher ($P<0.05$) than group C ($46.67\pm3.33\%$). The percentage of the spermatozoa that were alive in the *caudal* epididymis group A was $88.00\pm3.39\%$. This is significantly higher ($P<0.05$) than $73.33\pm6.67\%$ of *caudal* epididymis of group C. The percentage livability in *corpus* and *caput epididymidis* was higher in group A than in groups B and C.

**Table 1. Reactions of the Wistar rats after the treatment with Clomiphene citrate**

| Parameter studied | Group A | Group B | Group C |
|---|---|---|---|
| Activity | Normal | Decreased | Normal |
| Ataxia | Absent | Present | Absent |
| Coma | Absent | Absent | Absent |
| Cyanosis | Absent | Absent | Absent |
| Defaecation | Normal | Increased | Normal |
| Forces consistency | Normal | Soft | Normal |
| Faecal colour | Normal | Light brown | Dark |
| Irritability | Absent | Decreased | Absent |
| Respiration | Normal | Dyspnea | Normal |
| Mortality | Absent | High | Absent |

**Table 2. Spermiogram of rats treated with Clomiphene citrate and distilled water control**

| Parameters | Control/Group A | 50 mg CC/Group B | 0.6 mg CC/Group C |
|---|---|---|---|
| **A.** Head of epididymis (*caput*) | | | |
| Motility (%) | $42.0\pm2.00^a$ | $20.00\pm8.66^b$ | $23.33\pm3.33^b$ |
| Percentage live | $58.0\pm2.00$ | $52.62\pm7.50$ | $53.33\pm6.67$ |
| **B.** Body of epididymis (*corpus*) | | | |
| Motility (%) | $56.00\pm2.45^a$ | $25.56\pm7.50^b$ | $13.00\pm5.77^b$ |
| Percentage live | $66.00\pm2.48^a$ | $42.50\pm2.50^b$ | $56.67\pm3.33$ |
| **C.** Tail of epididymis (*caudal*) | | | |
| Motility (%) | $76.00\pm2.40^a$ | $42.50\pm2.50$ | $46.67\pm3.38^b$ |
| Percentage live | $88.00\pm3.39^a$ | $47.50\pm4.79^b$ | $73.33\pm6.67^c$ |
| Concentration.$10^6$ | $78.25\pm6.97$ | $53.75\pm1.89$ | $62.67\pm6.17$ |

CC — Clomiphene citrate

$^{a, b, c}$ — numbers differently superscripted in horizontal column are significantly different ($P < 0.05$)

## DISCUSSION

The decrease in the activity with some obviously abnormal symptoms and death are due to the over dosage of the rats, the signs decreased with no death when the dosage was reduced. The spermiogram in the epididymal transit revealed that more matured sperm cells exhibit highest percentage of motility increase as the maturation increase in the epididymis. This is similar with the findings of Oyeyemi *et al.* (6).

The percentage livability (percentage live) decreased significantly when compared with the value at the *caudal* epididymis 88.00 ± 3.39 % in control group A to 47.50 ± 4.79 % in group B when 50 mg CC was used. The findings when 0.6 mg CC was used differed with a the percentage livability value of 73.33 ± 6.67 %. This was higher than group B but lower than that of group A. This observation was similar to the report of Noseir-Wael (1) that the percentage livability of fertile animals is not superior when Clomiphene citrate was used.

In the concentration (sperm count) of the sperm cells in control group A the value $78.25 \pm 6.97.10^6$ spermatozoa.ml$^{-1}$ was significantly higher ($P < 0.05$) than group B value of $53.75 \pm 1.89.10^6$ spermatozoa.ml$^{-1}$. While in group C the value of concentration is $62.67 \pm 6.17.10^6$ spermatozoa.ml$^{-1}$. This indicates that Clomiphene citrate reduced sperm count in a fertile male animal.

It is an established fact that matured sperm cells are in the *caudal* (tail) epididymis (6, 10). The maturation of spermatozoa usually starts in the head (*caput*) epididymis. The maturation continues at the *corpus* (body) before it gets to the *caudal*.

Clomiphene citrate in this study has shown potential to increase male fertility but the recommended dosage must be used. The semen collections form the *caudal* epididymis or from the rats must be after four weeks of treatment when spermatogenic cycle will have been completed. After this a sperm cell will migrate through the *caput* and *corpus* to *caudal* epididymis.

This study concludes that it is important that rats either for natural breeding and or artificial insemination programme should be adequately fed for maximum performance. Clomiphene citrate can be used to boost the percentage livability and concentration of sperm cells of an infertile male rat.

These two parameters are necessary for male reproductive potential on which others' motility, volume and mass activity depend (12, 13). Also the number of spermatozoa in the ejaculate is of paramount importance in fertilization (14).

The adverse effect of CC treatment on the fertile rats in the present study may be due to CC influencing effect on testosterone and or due to its antiestrogenic properties, while simultaneous use of CC in fertile male rats could have a controversial effect. Therefore Clomiphene citrate treatment that could improve citrate treatment that could improve fertility in infertile male rats (15) infertile ram(1) could also induce a deteriorative effect on sperm cells and their characteristics on sperm cells and their characteristics when used in fertile Wistar rats

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*Received March 9, 2005*