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# Sexual behavior and fertility of male rats following subacute hemi-orchidectomy

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## Abstract

This study was conducted to investigate the sub-acute response of testicular size, body weight, fertility and mating indices in hemi-orchiectomy induced suppression of testosterone levels (<0.5 ng/mL) among adult male Wistar rats (Four months old). Libido, fertility and extended implication of the procedure on $F_1$ generation were also observed. After exposure to relevant experimental procedure, each of the male rats was housed with a proven female breeder to assess mating and fertility indices. Obtained data was statistically analyzed by analysis of variance. Hemi-orchidectomy resulted in compensatory increase in testosterone secretion due to contralateral testicular hypertrophy, mating and fertility indices of hemi-orchidectomized and fully orchidectomized rats were 80%:60% and 20%:0% respectively. The male progeny belonging to $F_1$ generation showed no sign of infertility. The findings suggest that hemi-orchidectomy induces sudden compensatory testicular hypertrophy, immediate Loss of fertility after full orchidectomy, diminished libido rather than complete loss of libido occurs. In conclusion, number of offspring delivery is independent of testosterone level, however testosterone level and its interference with the complex modifications in the hypothalamo-hypophyseal secretions are involved in the central control of male sexual activity.

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## Introduction

The clinical picture of male hypogonadism depends on its etiology (Soliman *et al.,* 2001). About 30% of men between the ages of 40 and 79 are hypogonadal (Rhoden and Morgentaler, 2004), they present with symptoms such as erectile dysfunction, depression, anemia, reduced muscle mass and bone density, diminished libido and sense of vitality (Allan and McLachlan, 2004). Decline in testosterone (T) production can be achieved through castration, this may either be surgically conducted by orchidectomy or chemically induced by the use of drugs such as anti-androgens, stimulants and down regulators of gonadotropin releasing hormone (GnRH) receptors in the adenohypophysis (GnRH agonists) for example, triptorelin, buserelin, goserelin, and leuprorelin (Okada, 1997; Pucarelli *et al.*, 2003; Fontana *et al.,* 2003). In addition to prostate cancer therapy, orchiectomy is also applied in the treatment of testicular cancer, cryptorchidism, in sex reassignment surgeries and also as punishment for sex offense (Norman-Eady, 2006; Bilefsky, 2009).

Hemi-orchidectomy has been proved as an effective treatment for ipsilateral scrotal or testicular diseases (Ivany *et al*. 2002), however, compensatory testicular hypertrophy was reportedly observed in prepubertal unilateral orchidectomy (McCoard, 2001). Testicular size has direct correlation with sperm production capacity (Lustra *et al*., 2002), nonetheless, the sperm viability in relation to quantification has not been properly addressed. Definitive studies on the sub-acute impact of unilateral orchidectomy on sexual function and some physical parameters are yet to be reported.

The aim of the present study was to assess the libido and fertility status of hemi-orchidectomized Wister rats as compared to their bilaterally orchidectomized and intact counterparts. In addition, its effects on testicular size and body weight were evaluated alongside the extended effect of the procedure on fertility and libido of their male $F_1$ progeny.

## Materials and methods

*Animals and grouping*

This work was performed in accordance with the recommendations of International Standards for the Protection of Animals (Rollin, 2006). Twenty five healthy adult male Wistar rats, *Rattus norvegicus,* 3-4 months old, (200–250g) and Twenty five proven female breeders of same strain, 2-3 months old (150-180g) were used for this study. Animals were obtained from the animal facility of the Department of Human Physiology, Bayero University Kano, Nigeria. Twenty male alongside twenty female rats (in separate cages) were allowed to acclimatize in animal studies laboratory for a week before commencement of the study. The male rats were randomly divided into five groups (n=5) each as following;

Group I were bilaterally orchidectomized rats, Group II unilaterally orchidectomized rats respectively, group III were sham operated while group IV were not surgically manipulated (laboratory control). In order to eliminate the effect of compounders such as laboratory stress, change of environment and impact of isolation on rats sexual behavior, a passive group was introduced (n=5), animals belonging to this group were allowed to remain in their accustomed environment (animal facility) without isolation and were not operated. The animals were placed in appropriate cages (38 cm x 46 cm x 24 cm), maintained at room temperature conditions (30 – 33$^{o}$C) with natural light, obeying the circadian cycle and were fed with standard diet and water given *ad libitum*. Female rats were not included in the grouping, they were only introduced at the stage of fertility studies (days four through eight post-orchidectomy and at day 25 through day 42 post-orchidectomy).

*Orchidectomy*

All surgical procedures were carried out as previously described (Sulaiman, *et al.,* 2014). The procedure was carried out in adherence to the technical rules of asepsis under general anesthesia. A premedication of atropine (Atropine, Laborate Pharmaceuticals, Panipat, India. 132103-NF, A4-1953) was administered (0.02 mg/kg, *i.p*). Five minutes

afterward, a combination of Ketamine hydrochloride (25 mg/kg, Ketajet ®, Sterfil Laboratories, India) and diazepam (2 mg/kg, Valium, Roche LTD, Basel, Switzerland)were co-administered intraperitoneally. The rats were immobilized in supine position, fur in the scrotal area was depilated, skin was exposed and disinfected, testes were externalized by opening the tunica vaginalis through 1cm median anterior scrotal incision. The ductus deferens, main arteries and veins were isolated and ligated before severing and subsequent testicular removal below the point of ligation. This was performed on both testes for members of group I, only the left testicle was removed from members of Group II, while members of Group III were sham operated (no testis was removed). The incision was closed by suturing the scrotal skin then swabbed with 10% povidone iodine solution. The rats were kept under warmth till they regain consciousness. They were then housed in separate cages, and had free access to food and water *ad libitum*.

*Hormonal assays*

After the follow-up period of 42 days, the rats were anaesthetized and 5 ml of blood was collected in plain containers via retro-orbital plexus technique under aseptic condition as previously described (Sulaiman *et al.*, 2014). Isolated sera of all the animals were assayed for the testosterone, LH and FSH concentrations using testosterone enzyme immunoassay test kit (TEIA Test Kit, Catalog No: TEST-96), LH and FSH Microplate Immunoenzymometric Assay (Accubind Elisa microwells, Monobind Inc. Lake Forest, CA 92630, USA, Product code: 625-300) respectively according to manufacturer's instruction. The principle of the test was based on competitive binding between testosterone in the test specimen and testosterone-conjugate for a constant amount of rabbit anti-testosterone. The lower detection limit for testosterone assay was 0.08 ng/mL and the intra- and interassay coefficients of variation were 10% and 9%, respectively.

*Weight assessment*

A weighing scale (American weigh triple beam scale, model: TB-2610. Readable load of 610 g with readability and sensitivity of 0.1g) was used to measure the precastration (on the day at which orchidectomy was performed 2 hrs prior to commencement of orchidectomy) and postcastration (on day 42 post-orchidectomy period, 2 hrs after withdrawal of feeds and prior to collection of blood sample) body weight of each male rat. Weight gain was calculated by subtracting initial (pre-castration) weight from final (postcastration) weight, the percentage weight gain was calculated as previously described (Oloyo *et al,* 2011).

Weight Gain (WG) = Final Postcastration Weight (PoW) –Initial Precastration Weight (PrW).

$$\text{Percentage Weight Gain} = \frac{\text{Weight Gain (g)}}{\text{Final Postcastration Weight (g)}} \times 100$$

Measurements of testicular weights was group dependent:

Group I: Both testes were orchiectomized and weighed immediately after orchidectomy was performed (i.e on day 1 post-orchidectomy).

Group II: The left testis was orchiectomized and weighed on the day 1 while the right testis was removed and weighed on the last day of the study (Day 42).

Group III, IV and V: Both testes were orchiectomized and weighed on the last day of the study (Day 42).

*Fertility and mating indices*

Four days after castration, each male rat of the $F_0$ generation was individually housed with a proven female breeder for four more days (day 4 through day 8). A rat was designated to be a proven female breeder if it had given birth to at least 2 offspring. The female rats were assessed for mating every morning and evening throughout the four days of exposure. Mating was judged by the presence of sperm in the vaginal smear of female rats and this was considered as day zero of pregnancy. Haemocytometeric method was used to identify presence of sperm cell with the

aid of improved Neubauer's counting chamber. At day 25, non-pregnant female rats were reintroduced to respective male counterparts and allowed to remain together until day 42 postcastration period (day 25 through day 42), this served as a sterility confirmatory period for the earlier exposed male subjects. The female rats were then examined for pregnancy. Pregnant females were separated from males and housed in separate cages. The number and sex of offspring (males and females) was recorded. Fertility index for male rats was calculated using the method applied by D'Souza and colleagues, (2004) while the same idea was used to calculate the mating indices for each group.

The Fertility Indices of all groups were calculated using the following formula:

$$\text{Fertility Index (\%)} = \frac{\text{Number of Males Siring Offspring}}{\text{Total Number of Males in the Group}} \times 100$$

Mating index of all groups was calculated using the formula below:

$$\text{Mating Index (\%)} = \frac{\text{Number of males that mated}}{\text{Total Number of Males in the Group}} \times 100$$

Fertility and mating indices of the $F_1$ generation were similarly determined. After weaning, 5 offspring males (seven weeks old) were randomly selected from each generation Groups (II-V). Each male was housed with a proven female breeder and assessed for fertility and libido. These four groups were designated as Groups VI-IX. Pregnant females were separated from males and housed in separate cages. After birth, the numbers of offspring (males and females) was also recorded.

*Statistical analysis*

The data were expressed as mean ± standard error of mean (ẋ±SEM). One way analyses of variance (ANOVA) was used for comparison between groups. This was followed by Bonferonni's post hoc test for multiple comparisons. The analysis was conducted using SPSS software (version 20). The significance level was set as $P<0.05$.

**Results**

*Body and testicular weights*

Table I shows the body weight gain (g), percentage weight gain (%) and the mean right testicular weights in each group. In all groups, there was an increase in the body weight after six weeks of the study. However the percentage increase in weight in bilaterally orchidectomized group was significantly lower ($P < 0.05$) when compared with that of the control groups. The percentage weight gain of the sham operated group was insignificantly higher ($P > 0.05$) than that of the bilaterally and unilaterally orchidectomized groups, it was also recorded to be lower than that of the control groups (experimental and passive controls). The mean right testicular weight of the unilaterally orchidectomized group was significantly higher ($P < 0.05$) when compared to that of other groups.

**Table 1.** Body weights gain (g), percentage weight gain (%) and right testicular weights (g) (n=5) after 42 days.

| | Mean ± SEM | | | | |
|---|---|---|---|---|---|
| Variables | Group I | Group II | Group III | Group IV | Group V |
| WG (g) | 32.60±3.68* | 32.20±1.80* | 36.60±1.04 | 45.40±4.43 | 48.60±1.50 |
| WG (%) | 10.2±0.86* | 11.2±0.490 | 12.4±0.25 | 14.4±0.93 | 15.2±0.20 |
| RTW (g) | 2.20±0.20 | 3.40±0.245* | 2.20±0.20 | 2.60±0.25 | 2.20±0.20 |

*Significance at $P < 0.05$. WG: weight gain, RTW: right testicular weight, SEM; standard error of mean.

*Hormonal assays*

Table II shows the changes in serum concentrations of testosterone (T), LH and FSH. The statistical analysis of serum testosterone levels revealed a significant reduction of testosterone levels in orchidectomized animals ($P<0.05$) compared to non-orchidectomized ones. Significant increase ($P<0.05$) in serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) level was observed in the bilaterally orchidectomized group as compared to the

control groups, a marked increase in gonadotropins was also observed in unilateral groups but no significance (P>0.05) was recorded in the serum level of LH, nevertheless FSH was significantly increased as compared to the control.

*Fertility and mating indices*

Table III shows the number of offspring produced by rats in each group ($F_1$ and $F_0$ generations), along with their fertility and mating Indices. The animals in bilaterally orchidectomized group recoded zero fertility, while members of the other groups had significant fertility index.

**Table 2.** Serum concentrations some sex hormones in male Wistar rats (n=5) after 42 days.

| | Mean ± SEM | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| T (ng/ml) | 0.36±0.031* | 0.79±0.010* | 1.07±0.044 | 1.06±0.043 | 1.01±0.024 |
| LH (mIU/ml) | 10.00±1.673* | 9.60±0.748* | 5.00±0.447 | 6.80±1.393 | 4.60±0.927 |
| FSH(mIU/ml) | 9.60±2.462* | 9.40±0.748* | 4.80±0.663 | 6.00±1.049 | 3.80±0.663 |

*: Significance at P ≤ 0.05. T: testosterone, LH: luteinizing hormone, FSH: follicle stimulating hormone.

**Discussion**

In an effort to examine the effect of hemi-orchidectomy on libido, fertility, testosterone-gonadotropin proportions, body and testicular weight it was observed that both unilateral and bilateral orchidectomy resulted in significant (P < 0.05) reduction in weight gain and percentage weight gain as compared to non-operated intact rats in the laboratory and passive control groups. This can be explained by exposure of operated rats to physical and emotional stress. This contradicts the findings of Drori and Folman (1976), who stated that; castrated rats have higher rate of weight gain than intact rats due to higher feeding rate (eats 17% more food than intact) and that orchidectomized animals have 5% more body fat than intact ones. As previously reported, insignificant higher percentage weight gain was recorded among castrated rats as compared to intact ones after 30 days follow up period (Hassan, 2010). This contradiction as evident in lower weight gain could be explained by the lower feed consumption among the castrates and exposure to physical and emotional stress. This effect might have been influenced by decline in testosterone level, because as previously illustrated, testosterone and estradiol increases food intake (Mystkowski and Schwartz, 2000). It may also be suggested that the subject's state of mind may affects their appetite, as this can be explained by the effect of lowered testosterone level on mood among the castrated animals. The anabolic role of testosterone also plays an important role in modification of total body weight, muscle mass and even cell regeneration (Allan and McLachlan, 2004).

**Table 3.** The number of offspring produced, fertility and mating indices in each group of $F_0$ and $F_1$ generations (n=5).

| | $F_0$ generation | | |
|---|---|---|---|
| Groups | Fertility Index % | Mating % | Number of offspring |
| I | 0 | 20 | 0 |
| II | 80 | 60 | 12 |
| III | 80 | 100 | 14 |
| IV | 100 | 100 | 15 |
| V | 100 | 100 | 14 |
| $F_1$ generation | | | |
| VI | 100 | 100 | 18 |
| VII | 80 | 100 | 17 |
| VIII | 100 | 80 | 9 |
| IX | 100 | 100 | 11 |

Weights of the offspring from $F_0$ and $F_1$ generations were recorded at the time of weaning (i.e., 30 days). Weight gain was used as an indicator of general health and development. At the time of weaning, there was no statistically significant difference in the weights of the litters between the Groups I to IX, indicating that parental hemi-orchidectomy status did not affect the development and growth of their respective offspring. This can be explained by the possible non-mutational or non-polymorphic effect of the surgical procedure as opposed to possible radiation or chemotherapy in prostate or testicular cancer.

Although, significant decrease in serum testosterone level was recorded in the hemi and fully orchidectomized groups, extra-testicular sources of testosterone production and secretion may explain the gradual rebound in its level as observed in the hemi-orchidectomized group. This also account for the minute amount of the hormone detected in the bilateral group. However, serum testosterone concentration among unilaterally castrated rats was significantly higher than that of the bilaterally orchidectomized groups. This is indicative of active compensatory role played by contralateral hypertrophied testis, an evidence of compensatory growth and development of the scrotal testis in hemi-castration.

Both Bilateral and unilateral treatments caused significant increase in serum FSH. Significant increase in LH was more conspicuous in the bilateral than the unilateral group. Accordingly, the presence of one hypertrophied testis in hemi-orchidectomy may suffice. Interestingly, this implies that a single normal testis has the capacity to produce sufficient seminiferous tubular agent, as well as testosterone for hypophyseal communication. The marginal difference between percentage increases in FSH (93.02%) and LH (75.5%) observed in hemi-orchidectomy may be due to the available serum testosterone level, which could exert less stimulatory effect on LH secretion (as compared to bilateral group), nevertheless the severing of the epididymis and removal of one testis is likely enough to decrease the concentration of inhibin to a sub-threshold level, thus not enough for inhibition of FSH secretion. The results reported here indicate that some factors other than peripheral serum testosterone, may be influential in the regulation of serum gonadotrophins. Particularly important was that; although bilateral orchidectomy caused a massive fall in serum testosterone level when compared to that of unilateral groups, the difference in rise of gonadotropin level was not proportionate with the level of decline in testosterone. Irrespective of the 20% mating recorded after bilaterally orchidectomy, fertility was completely lost. The mating indexes of the bilateral subjects indicates non-immediate loss of sexual drive, nevertheless, the mating did not yield offspring and no viable sperm was observed on the slide. This contradicts the findings of Pholpramool and Sornpaisarn (1980) who reported that fertility can be sustained for 2-8 days post-orchidectomy, however, it is possible that fertility was immediately lost due to severing of epididymis during the process of orchidectomy. Similarly, it was suggested that lack of fertility induced by treatment with the gonadotropin releasing hormone (GnRH) analog; orntide was due to testosterone suppression in male rats, an effect that was reversed after cessation of medication (D'Souza *et al.,* 2004). Meaning, even if the testicles were to be intact, the significant reduction in testosterone level of bilaterally orchidectomized rats could lead to complete loss of fertility. However, the unilaterally orchidectomized groups recorded 80% fertility and mating indices, this imply that; even though the group had decline in testosterone level, it was not significant enough to halt fertility and it did not reduce libido to any significant level. The compensatory role played by the contralateral testicle in enhancing increased testosterone production per testicle in the unilateral animals may had significant effect on sertoli cells. The number of offspring produced by a female rat may had no correlation with level of testosterone in male counterpart's serum. While as for the $F_1$ generation, it may be right to suggest that the physiological responses to hypothalomo-hypophyseal axis observed in $F_0$ was

transient rather than stable, as the $F_1$ generation shows no observable defect in sexuality.

**Conclusion**

Loss of fertility after bilateral orchidectomy was immediate, however, there was low mating index indicative of diminished libido not complete loss of libido. Numbers of offspring delivered were independent of testosterone level. Although fertility is spared and testosterone production was decreased among the hemi-orchidectomized animals, libido was not eliminated within the study period, thus this cannot be recommended as remedy for abnormal libido. Yet, it can be suggested that, the procedure may decrease libido among those with excessive and abnormal urge for mating as observed in sex offenders.

*Conflict of interest*

Authors declare no conflict of interest in conduct and preparation of this manuscript.

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