The influence of pubertal hemi-castration on the reproductive hormone profile, sperm reserves and histoarchitecture of the testis of male albino rats

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Abstract

This study evaluated the effects of hemi-castration on the reproductive hormone profile, sperm reserves and testis histoarchitecture of pubertal male albino rats. Seventy two male albino rats of seven weeks of age were used for the study. They were randomly assigned to three groups: Sham operated control (SOC), Left hemi-castrated (LHC) and Right hemi-castrated (RHC) groups. The surgical procedures were performed under general anaesthesia. Blood samples were collected from three rats in each group on Days 1, 3, 7, 14, 28 and 63 days post-surgery for hormonal assay, and afterwards the rats were euthanized and the testis and epididymis were collected. Determination of serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone, and evaluation of cauda-epididymal sperm reserves and testes histoarchitecture were done following standard methods. Testis and epididymis weight percent of body weight (WPOBW) were calculated. The LHC rats had significantly ($p < 0.05$) higher mean LH concentrations when compared to SOC rats on day 7 and significantly lower ($p < 0.05$) mean FSH levels compared to SOC rats on day 1. The mean testosterone concentrations and caudal epididymal sperm reserves did not significantly vary ($p > 0.05$) among the three groups. The seminiferous tubular diameter and epithelial heights of RHC rats was significantly higher ($p < 0.05$) than that of the SOC rats on day 63 only. There were no obvious alterations in the histoarchitecture of the testes in all groups. The RHC rats had significantly higher ($p < 0.05$) testicular WPOBW than the SOC rats on days 7 and 14. There were no significant variations ($p > 0.05$) in the epididymal WPOBW among the groups all through the study period. It was concluded that no significant laterality to hormonal compensation or augmentation of extra-gonadal sperm reserves occurred in the pubertal hemi-castrated rats. Testosterone compensation in the hemi-castrated rats in the absence of FSH or LH elevation substantiated the theory that enhanced testicular LH-sensitivity, mediated by the secretion of more potent isoforms of FSH, underpins testosterone augmentation in hemi-castrates.

Keywords: Hemi-castration; Reproductive hormone profile; Sperm reserves; Pubertal rats; Testis, Epididymis.

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Introduction

Interventions that disrupt gonadal homeostasis can be utilised to examine how various factors control testicular activity. The excision of one testis (hemi-castration) has been found to result in diverse changes in the remaining gonad, including a compensatory upsurge in testicular growth, spermatogenesis and androgenesis (Brown *et al.*, 1994). Although numerous studies have been conducted in several species, the exact mechanisms responsible for these compensatory changes are still not fully understood. Apparently, the withdrawal of testicular determinants subsequent to a hemi-castration procedure interferes with and transforms the hypothalamic-pituitary-testicular axis, resulting in hormone-stimulated increases in both tubular and inter-tubular components of the remaining testis (Brown *et al.*, 1994).

The most consistently reported endocrine response to hemi-castration in pre-pubertal rats is increased serum follicle-stimulating hormone (FSH) concentrations (Frankel and Wright, 1982). Also, FSH levels were found to be elevated for a two to three week period in association with compensatory hypertrophy of the testis in boars hemi-castrated at ten days of age (Leidl *et al.*, 1980). It has been posited that following hemi-castration, intensified FSH production may be responsible for mediating testicular hypertrophy responses (Cunningham *et al.*, 1978). It is conventionally agreed that FSH has at least a qualified contribution to compensatory hypertrophy through its actions on seminiferous tubule components; however, there is much contestation about whether its influence is mediated through cellular hyperplasia, hypertrophy or both (Brown, 1994).

Contrary to what has been elucidated in the pre-pubertal rat, hypertrophy has not yet been observed in the pubertal rat, and testosterone production was observed to return to normal some hours following hemi-castration (Frankel and Mock, 1982; Frankel and Wright, 1982; Frankel *et al.*, 1984). Studies have been unable to establish noticeable changes in luteinizing hormone (LH) pulsatility or gonadotropin hormone-releasing hormone (GnRH)-stimulated

LH secretion in hemi-castrated rats (Frankel and Mock, 1982).

The capacity of animals to reproduce efficiently is a critical component of animal husbandry and agriculture at large. However, infertility is a problem to some degree in all animal production systems and reproductive failure is one of the most significant factors that limit productivity in animal production systems, and results in loss of profit worth millions of money annually.

In a context where hunger and food insecurity affects a large percentage of the world population, and millions of humans experience acute food scarcity on a daily basis, studies of this nature could ultimately provide information critical for developing more efficient methods of treating animal infertility and/or increasing the sperm producing capabilities of genetically and economically valuable males. Thus, continued investigations into the nature of and the mechanisms controlling the hemi-castration response are warranted. The aim of this study therefore was to evaluate the influence of pubertal hemi-castration on the reproductive hormone profile, sperm reserves and histoarchitecture of the testis of pubertal male albino rats.

Materials and Methods

Experimental design: Seventy two male albino rats, of five weeks of age, were procured for this study. They were obtained from the albino rat breeders in the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. These animals were housed in fly-proof aluminium cages kept in a well-aerated building and fed commercially-prepared feed (Vital® Grower, Grand Cereals Ltd., Jos, Nigeria) and fresh drinking water *ad libitum*. Routine hygiene was maintained in the rat cages. The rats were handled in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria Nsukka, from which an approval was obtained for the use of the rats for the study (Approval Reference Number: FVM-UNN-IACUC-2021-0467).

The rats were identified with stripe marks on their tails that was made using a permanent marker and randomly assigned to three groups (n = 24), as follows: Group 1: Sham-operated Control (SOC; both testes intact), Group 2: Left-Hemicastrated (LHC) (right testis intact); and Group 3: Right-Hemicastrated (RHC) (left testis intact).

The five week-old rats were acclimatized for 2 weeks prior to the commencement of the experiment. The rats were seven weeks old when the study proper commenced and thus hemi-castration (LHC and RHC) and sham operation (SOC) procedures were carried out at seven weeks of age. After surgery, the rats were kept in their cages for 9 weeks and blood samples were collected from three randomly selected rats on days 1, 3, 7, 14, 28 and 63 post-surgery for hormonal assay. After blood sample collection from each of the rats, they were humanely euthanized and the testis and epididymis were collected, weighed and subjected to further laboratory analysis. The study lasted for a period of eleven weeks (the two weeks of acclimatization inclusive).

Surgical procedures: Hemi-castration of the rats in groups 2 and 3 was performed under general anaesthesia, using xylazine and ketamine. A mid-scrotal incision was done to expose the testis, and all testicular and epididymal vessels were ligated. The testis and associated epididymis were then excised. The incision was thereafter closed with chromic catgut. The rats were then kept warm until they regained consciousness. For the sham operated control group, following general anaesthesia using xylazine and ketamine, a mid-scrotal incision was made, the testis was palpated and the incision sutured without removal of the testis. The rats were then kept warm until they regained consciousness. Rats in all the groups were subsequently kept under the same experimental conditions all through the duration of the study. The purpose of this procedure was to avoid errors due to homeostatic responses resulting from the operational procedures alone.

Sample collection: Prior to sample collection, three rats were randomly selected from each group and weighed using a weighing balance (WANT Digital Electronic Weighing Balance, China; WT60001KF). Blood samples for laboratory assays were collected on days 1, 3, 7, 14, 28 and 63 post-surgery, from the retrobulbar plexus in the medial canthus of the eye, using microcapillary tubes, after mild anaesthesia with diethyl ether. The blood samples were collected in labelled plain sample bottles without anti-coagulant and allowed to stand for two hours to clot. Later, the clotted blood samples were centrifuged (at 2,000 revolutions per minute for 20 minutes) and then the resultant sera were collected in labelled plain sample bottles and stored at -20°C for hormonal (follicle-stimulating hormone, luteinising hormone and testosterone) assay. The three rats in each group were later humanely sacrificed. The testes and epididymis of each rat was carefully dissected out and weighed. The testicular and epididymal weight percentage of the body weights of the rats was calculated using the formula: Organ weight (g) × 100 / Body weight (g). The testes were fixed in 10% neutral buffered formalin for histological studies, while the dissected epididymides were put in Bijou bottles containing phosphate buffered saline, pH 6.8 and used for determination of cauda-epididymal sperm reserve.

Determination of the Cauda-epididymal sperm reserve: The cauda-epididymal sperm reserves were determined following the haemocytometric procedure (Almquist and Amann, 1961) as follows: The cauda-epididymis was first dissected from the caput and corpus together with other associated tissues. Thereafter, the epididymal tissues were macerated in a ceramic mortar with a ceramic pestle. The macerated tissues were homogenized with 10 ml of normal saline. The mixture was then filtered through wire gauze mesh to separate particulates from fluid. With a sterile syringe, 1 ml of the filtrate was aspirated and made up to 10 ml in a test tube by adding 9 ml of WBC diluting fluid. A drop of the 10 ml solution was used to load the Neubauer chamber of the hemocytometer for counting of the sperm cells. The sperm cells in the four large

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corner squares were counted and the total multiplied by a factor of 250,000 to give the number of sperm cells per ml.

Blood Sample (Serum) Analyses: Serum FSH, LH, and Testosterone levels were quantified using commercially available test kits (Accubind ELISA kits, obtained from Monobind Inc., USA). The hormonal assay followed standard procedures as described by Uotila *et al.* (1981) and Tietz (1995).

The FSH test kit was based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The test was performed as follows: Fifty microlitre of standard, serum specimen and control fluids were dispensed into appropriate wells in the microtitre plate. Enzyme Conjugate Reagent (100 μl) was then dispensed into each well and thoroughly mixed for 30 seconds to ensure complete mixing. The mixture was then incubated at room temperature for 60 minutes after which the contents of the microtitre plate were decanted and the plate blotted dry with absorbent paper. Wash buffer solution (350 μl) was pipetted into the microtitre wells and blotted again. The wash procedure was repeated two additional times. Substrate solution (100 μl) was added to all wells and incubated at room temperature for fifteen minutes. Stop solution (50 μl) was added to each well and gently mixed for 20 seconds. The absorbance in each well was read at 450 nm in a microtitre plate reader (MR-9602A Microplate Reader, Bio Trust Diagnostics Inc., CA, USA.) within thirty minutes of adding the Stop solution. A standard curve was generated by plotting the mean absorbance of each standard against its concentration, with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis. FSH concentrations were calculated using the corresponding values obtained from the standard curve.

The LH test kit was based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The determination procedure was as follows: The microtitre plate wells for each serum reference, control and test serum specimen to be assayed in duplicate were formatted. Fifty microlitre of the appropriate serum reference, control or serum specimen was pipetted into the assigned wells using a micropipette. LH-Enzyme Reagent (100 μl) was then added to all wells after which the microtitre plate was swirled gently for 30 seconds to mix. It was then covered. The mixture in the plate wells was incubated for 60 minutes at room temperature. The contents of the microtitre plate were discarded by decantation and the plate dried with absorbent paper. The wells were washed three times using 350 μl of wash buffer solution. Working Substrate solution (100 μl) was then added to all the wells and incubated at room temperature for fifteen minutes. Stop solution (50 μl) was added to each well and gently mixed for 20 seconds. The absorbance in each well was read at 450 nm in a microtitre plate reader (MR-9602A Microplate Reader, Bio Trust Diagnostics Inc., CA, USA.) within thirty minutes of adding the stop solution. A standard curve was generated by plotting the mean absorbance of each standard against its concentration, with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis. LH concentrations were determined using the corresponding values obtained from the standard curve.

Determination of serum levels of testosterone was based on the competitive binding between testosterone in the test specimen and testosterone-horse radish peroxidase conjugate for a constant amount of rabbit anti-testosterone in the incubation. The procedures were carried out as follows: The microtitre plate wells for each serum reference, control and test serum specimen to be assayed in duplicate were formatted. Ten microlitre of the appropriate serum reference, control or serum specimen was pipetted into the assigned wells using a micropipette. Testosterone Enzyme reagent (50 μl) was added to all wells and the micro-titre plate swirled gently for 20 – 30 seconds to mix properly. Testosterone Biotin Reagent (50 μl) was added to all the wells and the micro-titre plate gently swirled again for 20 – 30 seconds to mix. The mixture was covered and incubated for 60 minutes at room temperature after which the

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contents of the microtitre plate were discarded by decantation. Wash buffer solution (350 µl) was pipetted into the micro-titre plate and blotted again. The wash procedure was repeated for two more times. Working Substrate solution (100 µl) was added to all wells and incubated at room temperature for fifteen (15) minutes. Stop solution (50 µl) was added to each well and gently mixed for 20 seconds. The absorbance of each well was read at 450 nm in a microtitre plate reader (MR-9602A Microplate Reader, Bio Trust Diagnostics Inc., CA, USA.) within thirty (30) minutes of adding the stop solution. A standard curve was generated by plotting the mean absorbance of each standard against its concentration, with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis. Testosterone concentrations were determined using the corresponding values obtained from the standard curve.

Histological preparations: The fixed testicular specimens were dehydrated in graded levels of ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. Five micrometre thick sections were cut, mounted on glass slides, and stained with haematoxylin and eosin for light microscopy (Bancroft and Steven, 1990).

Data analyses: Quantitative data obtained from the study were subjected to one way analysis of variance, and variant means were separated post-hoc using the least significant difference method. Significance was accepted at probability less than 0.05 (Chatfield, 1983). The software used for the analysis was SPSS version 23 for Apple MacOS (IBM Corp, New York, USA). The results were presented as bar charts with standard error bars.

Results

Rats in the LHC group had a significantly ($p < 0.05$) higher mean serum LH level when compared to rats in the SOC group on day 7 post surgery (Figure 1). There were no significant ($p > 0.05$) variations in serum LH levels of rats in all the three groups (LHC, RHC and SOC) on days 1, 3, 14, 28 and 63 post-surgery (Figure 1). The LHC rat group had significantly ($p < 0.05$) lower mean

serum FSH levels when compared to rats in the SOC group on day 1 post-surgery, but there were no significant variations in the serum FSH levels of all the rat groups on days 3, 7, 14, 28 and 63 post-surgery (Figure 2). There were no significant variations ($p > 0.05$) in the mean testosterone levels of all the rats groups (LHC, RHC and SOC) all through the post-surgery experimental period (Figure 3).
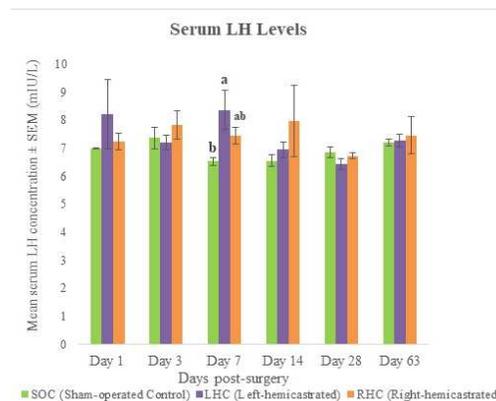

Figure 1: Serum levels of luteinizing hormone in hemi-castrated male albino rats, compared to sham-operated control. [***** columns with different superscript letters are significantly ($p < 0.05$) different; SEM: standard error of mean.]
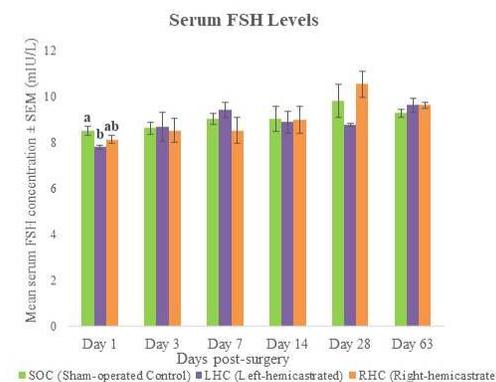

Figure 2: Serum levels of follicle stimulating hormone in hemi-castrated male albino rats, compared to sham-operated control. [*****columns with different superscript letters are significantly ($p < 0.05$) different.]

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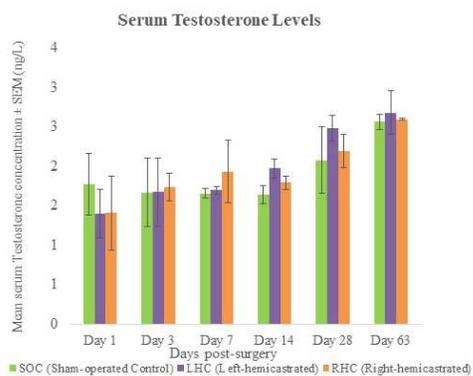

Figure 3: Serum levels of testosterone in hemi-castrated male albino rats, compared to sham-operated control.

There were no significant variations (p > 0.05) among the rats groups in their mean cauda-epididymal sperm reserve all through the post-surgery experimental period (Figure 4). The seminiferous tubular diameter of rats in the RHC group were significantly (p < 0.05) wider when compared to those of rats in the SOC group on day 63 post-surgery (Figure 5). There were no significant (p > 0.05) variations in the mean seminiferous tubular diameter on other days (days 1, 3, 7, 14 and 28) post-surgery (Figure 5). The seminiferous tubular epithelial heights of rats in the RHC group were significantly (p < 0.05) higher when compared to those of rats in the SOC group on day 63 post-surgery (Figure 6). There were however no significant variations (p > 0.05) in the mean seminiferous tubular epithelial heights of all the rat groups on all other days (days 1, 3, 7, 14 and 28) post-surgery (Figure 6).
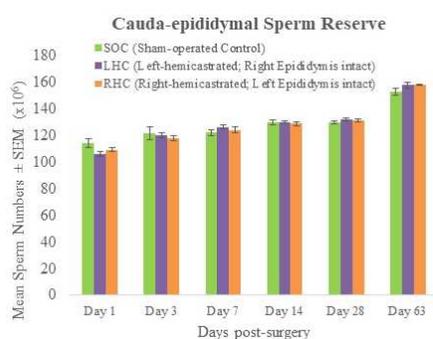

Figure 4: Cauda-epididymal sperm reserve of pubertal hemi-castrated male albino rats, compared to sham-operated control.
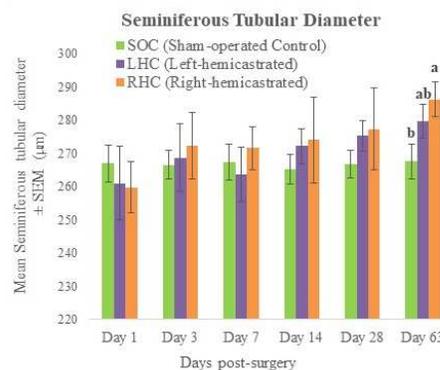

Figure 5: Seminiferous tubular diameter of pubertal hemi-castrated male albino rats, compared to sham-operated control. [* columns with different superscript letters are significantly (p < 0.05) different.]

Figure 6: Seminiferous tubular epithelial heights of pubertal hemi-castrated male albino rats, compared to sham-operated control. [* columns with different superscript letters are significantly (p < 0.05) different.
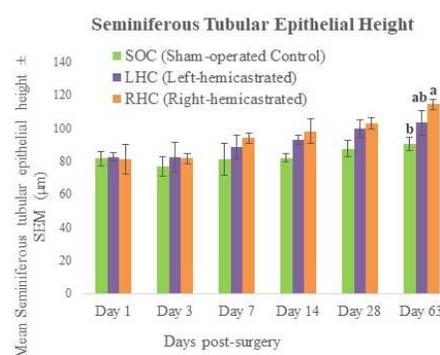
Histological evaluation of the testis of the rat groups showed no obvious alterations in the histoarchitecture of the sections of the testes of all the rat groups (SOC, LHC and RHC) all through the post-surgery experimental period (days 1, 3, 7, 14, 28 and 63) [Figure 7].

Rats in the RHC group had a significantly (p < 0.05) higher testicular weight percent of body weight on day 7 post-surgery when compared to the SOC and LHC groups , while on day 14 post-surgery, the testicular weight percent of body

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weight of the LHC group was only significantly (p < 0.05) higher than that of the SOC group (Figure 7). There were however no significant variations (p > 0.05) in the testicular weight percent of body weight on days 1, 3, 28 and 63 post-surgery (Figure 8). There were no significant variations (p > 0.05) among the rat groups in their epididymal weight percent of body weight all through the post-surgery (days 1, 3, 7, 14, 28 and 63) experimental period (Figure 9).
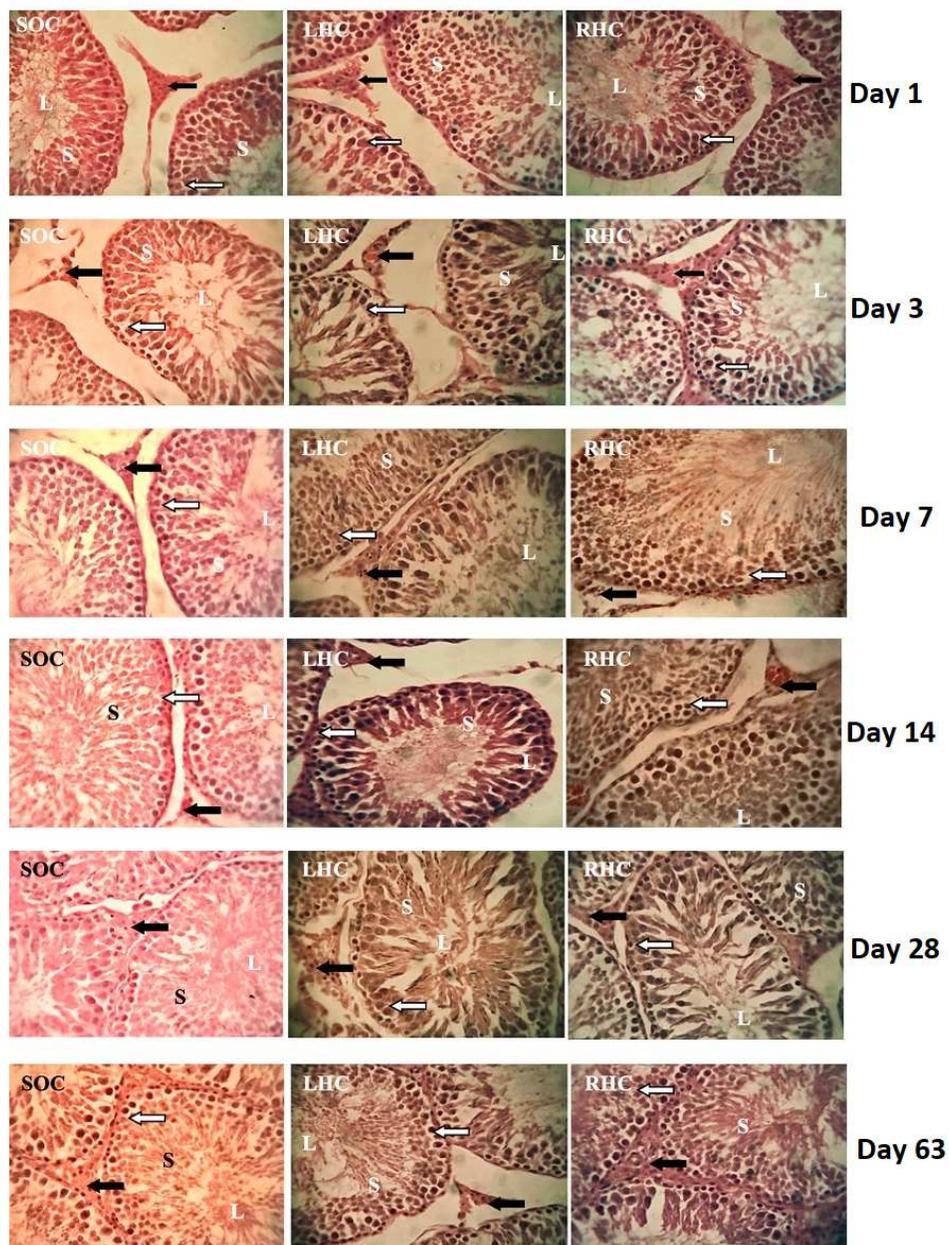

Figure 7: Photomicrograph of sections of the testes of pubertal hemi-castrated male albino rats, compared to sham-operated control, days 1, 3, 7, 14, 28 and 63 post-surgical castration; H & E × 400. [SOC – Sham operated control; LHC – Left hemi-castrated; RHC – Right hemi-castrated; S - Seminiferous tubular epithelium; L - Lumen of seminiferous tubular epithelium containing spermatids; White arrows - Active spermatocytes; Black arrows - Interstitium with Leydig cells].
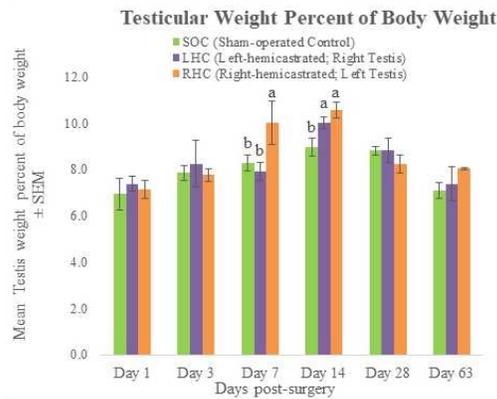
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Figure 8: Testicular weight percent of body weight of pubertal hemi-castrated male albino rats, compared to sham-operated control. [* columns with different superscript letters are significantly (p < 0.05) different.]
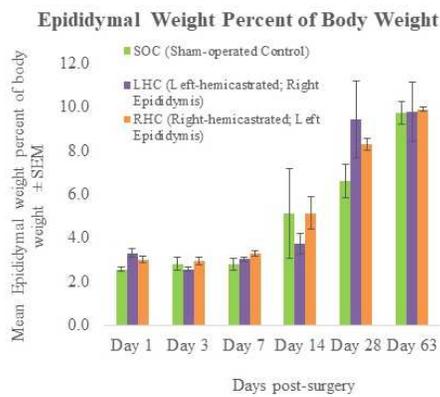

Figure 9: Epididymal weight percent of body weight of pubertal hemi-castrated male albino rats, compared to sham-operated control.

Discussion

Hemi-castration is a procedure that remodels the homeostasis of the entire gonadal axis. In several studies, removal of one testis resulted in various alterations in the remaining gonad, including a compensatory testicular hypertrophy in adult (Tabassi *et al*., 2009) and pre-pubertal animals (Minton and Wetteman, 1988). These were thought to be primarily due to modifications in seminiferous tubule diameter (Putra and Blackshaw, 1985) and, perhaps, a minor interstitial cell hyperplasia (Agee *et al*., 1988) and enhanced spermatogenic activity in the remaining testis characterized by augmented germ cell numbers and Sertoli cell occupancy

(Putra and Blackshaw, 1982). Some researchers noted a rapid, significant upsurge in serum testosterone levels of hemi-castrated rats (Frankel and Mock, 1982) and boars (Walton *et al*., 1978), transient serum FSH elevation (Brown and Chakraborty, 1991) while LH secretion was reported to remain unaffected (Rivier *et al*., 1989; Brown and Chakraborty, 1991).

In the present study, rats in the LHC and RHC groups did not record significant (p > 0.05) differences in serum LH levels when compared to rats in the SOC group on all but day 7 of the experiment, when LHC rats recorded a significantly higher mean serum LH level than SOC rats. The general trend of these findings corroborates studies which found serum LH secretion to be unaltered by hemi-castration (Orth, 1984; Rivier *et al*., 1989; Brown and Chakraborty, 1991; Brown *et al*., 1994).

Testicular steroidal hormones have been found to downregulate GnRH pulses but substantial direct action on the pituitary to affect LH secretion has not been demonstrated (Tilbrook and Clarke, 2001). This could possibly explain why the decline in testosterone associated with unilateral gonadectomy, however transient it may be, given the rapid compensation that is observed to occur in hemi-castrates, failed to cause any effect on LH secretion. Other researchers have speculated that amplified gonadal sensitivity to LH may be responsible for the upsurges in testosterone secretion secondary to hemi-castration which have been demonstrated in pre-pubertal and adult animals (Selin and Moger, 1979; Brown *et al*., 1994). Given, however, that attempts to demonstrate an increase in expression of testicular LH receptors have not yielded results (Brown *et al*., 1994), there is growing evidence that FSH possibly augments Leydig cell function (Kerr and Sharpe, 1985; Selin and Moger, 1977).

The serum FSH levels in the hemi-castrated groups in this present study, showing no significant differences when compared to control, did not align with findings from earlier researchers who conducted studies using pre-pubertal and adult rats. Hemi-castration experiments using pre-pubertal and adult

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subjects reported a transient elevation of FSH immediately after removal of the testis (Brown and Chakraborty, 1991; Minton and Wetteman, 1988). The precise physiological means through which FSH upsurge following hemi-castration is accomplished remains unclear. However, the foremost hypothesis, particularly for the pre-pubertal animal, may attribute this phenomenon to a reduction in testicular inhibin feedback inhibition following removal of the testis (de Jong and Robertson, 1985). This was supported by the establishment of an inverse correlation between inhibin concentrations and serum FSH in pre-pubertal rats (Rivier *et al.*, 1989; Brown and Chakraborty, 1991). However, this inverse relationship could not be discerned in adult rats (Rivier *et al.*, 1989; Brown and Chakraborty, 1991), suggesting that inhibin feedback regulation of FSH secretion may have a significant age component. The findings of this present study in pubertal rats, showing no serum FSH elevation following hemi-castration, seems to suggest that testosterone compensation following hemi-castration is not necessarily dependent on the upsurge of FSH as observed in other studies. It is pertinent, however, to note that studies which established a transient elevation of FSH secretion following hemi-castration could not explain the upsurge of FSH within the context of the adult rat's fully developed steroid negative feedback system.

The results of serum testosterone assay in this study, which showed no significant difference in serum testosterone levels elevation when compared to control, indicates a clear and rapid compensatory upsurge in testosterone secretion in the hemi-castrated groups to bring them to par with the intact sham-operated control. This is in consonance with reports of studies conducted in several species that established a swift hormonal response to removal of a testis (Frankel and Mock, 1982: Walton *et al.*, 1978). As previously discussed, the precise physiological mechanisms responsible for this compensatory response are still somewhat controversial. The most widely accepted submission is that FSH augments testicular responsiveness to LH (Selin and Moger, 1979). However, a detailed

physiological explanation of how exactly this happens is yet to be agreed upon. One theory is that gonadectomy, unilateral or bilateral, interrupts a neural connection between the testis and central nervous system, instigating alterations in the hypothalamo-pituitary axis. One study of hemi-castrated rats was able to establish an elevation of GnRH in the arcuate nucleus (ipsilateral to the excised gonad), associated with a concomitant upsurge in FSH secretion, but not LH (Mizunuma *et al.*, 1983).

Another possibility that could explain the testosterone compensation, even in the absence of FSH elevation as observed in this study, is an increase in the potency or biological activity of secreted FSH. Variations in gonadotrophin efficacy have been linked to the capacity of the pituitary gland to produce various forms that differ in molecular weight, isoelectric profile, receptor-binding affinity and half-life in circulation (Chappel and Ramaley, 1985; Dahl *et al.*, 1988). Chappel and Ramaley (1985) established a pubertal correlation to the variations in the isoforms of FSH. Apparently, as progression is made through puberty in readiness for sexual maturity, both the absolute amounts and, crucially, the isoelectric focusing profile of FSH were observed to vary. It is possible, that hemi-castration triggers secretion of isoforms of FSH which could produce more potent augmentation of the LH-sensitivity of interstitial tissues of the remaining gonad resulting in the rapid compensation observed in this study, even in the absence of elevation of serum FSH levels beyond levels observed in the sham-operated control group. The dynamics underlying alterations in the biopotency of FSH (or the relative concentrations of its isoforms) following hemi-castration are ambiguous, but implicate multi-faceted interfaces within the hypothalamo-pituitary-testicular axis.

In this study, there were no discernible differences in the testosterone hormone response between left and right hemicastrates. Brown *et al.* (1994) suggested that there were disparities in the observed hemi-castration response dependent on which of the left or right

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testes were removed. They noted a more robust response associated with removal of the left gonad compared to excision of the right testis, although they were unable to provide explanations for this submission. The disparity mirrors similar observations in studies of ovarian compensatory hypertrophy in hemi-ovariectomized female rats, where there is a significantly higher number of ovulated ova upon excision of the left ovary than the right (Chavez *et al.*, 1987).

Analysis of extragonadal sperm reserve revealed no statistical significant differences between the hemi-castrated and sham-operated groups in this study. Studies on sperm production in hemi-castrated animals have reported conflicting results: one study noted significant elevations in total sperm production and rete testis flow (Mirando *et al.*, 1989); another recorded higher spermatozoa numbers in the caput of the epididymis (Santolaya and Burgos, 2009); others observed no differences between hemi-castrates and intact subjects (Vreeburg *et al.*, 1974; Thompson and Berndtson, 1993). These findings appear to have a strong age dependence, with test subjects hemi-castrated before puberty showing greater tendency for compensatory changes while adult test subjects rarely showcasing notable alterations in testicular morphology, although there may be increased spermatogenic efficiency (Hochereau-de Reviers *et al.*, 1976).

Initial evaluation of the histoarchitecture of the testes in this study showed no obvious morphological changes at the level of the seminiferous tubules, however, upon more granular morphometric analyses, seminiferous tubular diameter and seminiferous tubular epithelial height changes observed in the right hemi-castrates on day 63 were interesting. These contrasted with reports by Mock and Frankel (1982) and Frankel *et al.* (1984) who demonstrated an asymmetric testicular response, being more consistent and vigorous when the left testis was removed compared to the right. They suggested that the testicular hemi-castration response, which they believe to be neurally controlled, shares the asymmetry

which was described in the neural regulation of hemi-ovariectomy.

Research work reported by Cunningham *et al.* (1978) in rats hemi-castrated at 5 days of age, proposed that the testicular hypertrophy they observed was as a result of enhanced Sertoli cell numbers due to substantial upsurges in testicular protein and DNA concentrations in Sertoli cell-enriched testes. However, working with cell-enriched hemi-castrates devoid of germ cells, they went further to suggest that the hemi-castration response may be more precisely explained by augmented Sertoli cell activity rather than multiplication of germinal constituents. In a study of rats hemi-castrated at 3-days, Orth (1984) attributed the hemi-castration response to Sertoli cell proliferation, having observed an escalation in the fraction of Sertoli cells in the residual testis labelled with [3H]. What remained unclear was whether this increased activity manifested as augmented Sertoli cell numbers (hyperplasia) and/or cell volume (hypertrophy). Another study, in which surgery was deferred until 10 days of age or later, found no proliferation of Sertoli cells using morphometric techniques (Putra and Blackshaw, 1982), rather, hypertrophy was linked to increased seminiferous tubule length and a greater cross-sectional area. In yet another study of 6-week-old lambs, no significant changes in Sertoli cell numbers were observed, rather, increased mean diameter of the seminiferous tubules and nuclear cross sectional area of Sertoli cells were primarily responsible for the hypertrophy (Hochereau-de Reviers *et al.*, 1980). The increases in seminiferous tubular epithelial height recorded in the present study lend more credence to increase in Sertoli cell size rather than hyperplasia as a vehicle for the hemi-castration response, specifically in older test subjects. Brown *et al.* (1994) noted that the differences in observations by different studies could be explained by a number of factors, viz; species differences; age at time of castration; time of evaluation relative to hemi-castration; and testing methodology.

Analysis of the weight percent of body weight of the testes and epididymides in the present study

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revealed only isolated disparities but no differences that could indicate a departure from control, especially on the later days of the experiment when there would have been enough time for morphological changes. Our findings were in contrast to those of Putra and Blackshaw (1982) who found compensatory testicular hypertrophy with increases in testis weight per 100 g of body weight and significant increases in the seminiferous tubule length of hemi-castrated rats, although they noted that the response was inversely correlated to sexual maturation. On the other hand, Thompson and Berndtson (1993) found no increase in testis weight months after hemi-castration in sexually mature male rabbits. It is clear that the age at time of hemi-castration (in the context of the sexual maturity of the animal) affects the scale and nature of the compensatory response, as Cunningham *et al.* (1978) found that hypertrophy of the residual testis was not observed in rats if hemi-orchidectomy was performed after 45 days of age.

Conclusion

The findings of this study on pubertal rats have bolstered observations that circulating luteinizing hormone concentration is essentially unaffected by disruptions at the level of the gonads, suggesting that luteinizing hormone may merely play a secondary role in the compensatory responses to hemi-orchidectomy. There were convincing indications in this study that FSH, even in the absence of elevated circulating levels, exerts an influence on testicular sensitivity to LH and produces swift upsurge of testosterone hormone levels in hemi-castrates within hours of operation. Given our findings in this study, we think that the secretion of more potent isoforms of FSH could be responsible for the augmented testosterone production in the hemicastrates. This study was unable to establish a consistent laterality to the hormonal compensation, however, a morphometric evaluation of the histoarchitecture yielded observations that suggested a slightly stronger response to

removal of the right testis which challenged the findings of other researchers who had indicated a more compelling reaction to excision of the left testis. Extragonadal sperm reserve assay in this study lends plausibility to suggestions that, beyond a certain age, hypertrophy of tubular constituents is not observed – and rather, compensatory responses are characterized by increases in Sertoli cell size without proliferation of germinal components. We posit, from our findings, that in cases of unilateral testicular injury or disease, a stud male can still be bred successfully.

Conflict of Interest

The authors do not have any conflict of interest to declare.

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