

The effect of unilateral vasectomy on testosterone and testicular parameters in the adult male African giant rat (*Cricetomys gambianus*)

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Abstract

Background: The effects of vasectomy on spermatogenesis and reproductive parameters are recognized to be specie-dependent with marked differences in levels of perturbations observed.

Objectives: To assess the impact of unilateral vasectomy on testosterone level and other testicular parameters in the male African giant rat (AGR) (*Cricetomys gambianus*).

Methods: Sixteen adult male AGRs weighing 500-1300 g were recruited for the experiment. Animals were randomly divided into three experimental groups (1-3) and one control (sham operated) group with four rats per group. Experimental vasectomy was done by carefully ligating the vas deferens of the right testis of all the experimental groups (1, 2, and 3) and animals were allowed either 8, 6 and 2 weeks respectively before sacrifice. Sham-operated animals served as the control. Blood samples were collected and assayed for testosterone while testicular tissue was further processed for seminal fluid and histo-pathological analyses.

Results: Spermatogenic parameters indicate a pattern of decline in sperm count and motility between the experimental groups and the control and azoospermia in the eight-week group. Histological alterations were marked by atrophy of seminiferous tubules which was proportional to the duration of vasectomy. Serum testosterone levels were significantly reduced at eight weeks. There was no statistically significant difference between sperm counts of right and left testes except for group 3. Results suggest that unilateral vasectomy of the AGR may have negative impact on the contralateral testis in the male African giant rat.

Conclusion: These preliminary results reveal that unilateral vasectomy in the AGR may result in perturbations of the histo-architecture of the testes with possible decline in function.

Key words: African giant rat, vasectomy, testis.

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Introduction

Vasectomy which is the surgical ligation of the vas deferens has been in use as a popular method of contraception^{1,2} for men. However, despite the controversies surrounding the applicability of vasectomy as a male contraceptive method³, it has continued to attract continued interest from researchers and urologists. The contraceptive effectiveness of this technique has been established by investigators but they continue to generate sharp divisions as to the potential deleterious consequences thereafter⁴.

The vas deferens, a tubular structure that carries sperm from the cauda epididymis to the ejaculatory duct, consists of three muscular layers and a mucosal inner layer⁵. This important structure has been a subject for manipulations specifically in respect to male contraception. It appears that injury to the vas deferens will impact negatively on fertility parameters in the male¹ as this is supported by experiments showing increased seminiferous tubular diameters, obstruction of spermatogenesis and sperm maturation process⁶. Although evidence from studies in animals and humans suggest that vasectomy does not affect reproductive hormones⁷, reports of spermatogenic damage indicated by sloughing of seminiferous epithelium⁸ have been observed. In the light of divergent views/reports by authors, our current knowledge of the alterations following vasectomy remains but incomplete and requires further investigation. Against this standpoint,

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factors like species/strain of animal, differences in experimental designs and parameters evaluated, technique of vasectomy applied and time interval after vasectomy may have contributed to the different views expressed by authors.

Since the rat is an animal model frequently used for the study of vasectomy⁴ and they continue to exist variability in data regarding outcome of this procedure on various parameters, the current pilot study was undertaken to observe how the African giant rat (AGR) (*Cricetomys gambianus*) responds to vasectomy procedure. The AGR is known to be wild and nocturnal species which is gradually becoming domesticated for various purposes and the anatomy is attracting intense interest⁹; hence knowledge of how it responds in this experimental study would stimulate a more detailed investigation that would add to the comparative data with other species/strain.

There is paucity of data reporting effects of unilateral obstruction on contralateral testicular function in the AGR. This is despite works carried out on other animals like rabbit¹⁰ and monkeys¹¹ amongst others. Antypas et al.¹ showed that a bilateral deficiency in both Leydig and Sertoli cell secretory functions occurs in unilaterally vasectomized animals with an overall result of bilaterally impaired spermatogenesis and inhibited sperm maturation process. Previous studies by Saifzadeh and Derakhshanfar¹² and Kong et al.¹³ utilized 12 native Sprague-Dawley rats and 19 Japanese rabbits in their investigations on unilateral vasectomy. Our present study focuses on the effects of unilateral vasectomy on testicular histology, testosterone and seminal parameters in the AGR.

Methods

Animals

Sixteen adult male African giant rats weighing between 500 and 1300grams were procured from Awolowo market in Mushin, Lagos and allowed to acclimatize in the Animal house of the Department

of Anatomy, College of Medicine, University of Lagos for a period of two weeks. Confirmation of species was sought from the Zoology Department of the University of Lagos. They were maintained in well aerated iron cages at room temperature (27-30°C) and were fed omnivorous diet composed of sweet potatoes, corn, beans, Pawpaw and other roots daily similar to what is fed at research centers^{14,15}. Tap water was provided *ad libitum* during the period of experimentation. Animals were divided into 4 groups of 4 rats each and treated as in table I below:

Surgical maneuver was carried out in a sterile environment within the laboratory. Animals were anaesthetized for surgery by chloroform inhalation in a desiccating jar and maintained by intramuscular ketamine (Laboratory Pharmaceutical, India). The right scrotum was surgically opened and the vas deferens located and clamped using mosquito artery forceps 2 cm apart before ligation. The proximal end was tied using absorbable sutures and the testis returned into the scrotum. The scrotum was closed in two layers; 4-zero absorbable sutures were used for the musculature and 4-zero absorbable sutures for the skin. Sham operations were performed in

the same manner except that the vas deferens was neither ligated nor divided. The Departmental Postgraduate Committee approved this study which was also approved by the Postgraduate Education Committee of the College of Medicine of the University of Lagos before experimentation was started. All procedures were in conformity with the International Guidelines for handling of Animal for experimentation.

The rats were sacrificed at the end of 2, 6 and 8 weeks duration of the experiment respectively. Due to the wildness of the AGRs, each of the rats was lowered with its cage into a desiccator containing chloroform and subsequently injected with i.m. ketamine. The testes were dissected out, weighed and fixed in Bouin's fluid for further histological processing. Testicular tissue was sectioned at 5 µm thickness and stained with haematoxyline & eosin

Table 1: Experimental grouping of animals

Groups	Treatment	Duration
1	Unilateral vasectomy on right vas deferens	8 weeks
2	Unilateral vasectomy on right vas deferens	6 weeks
3	Unilateral vasectomy on right vas deferens	2 weeks
4	Sham-operated (control)	8 weeks

(H & E) before being viewed with an Olympus® binocular light microscope at magnifications X10 and X40.

Seminal fluid analysis included sperm motility, count and morphology. The caudal epididymis was excised and minced using a sterile scalpel blade before being placed in 0.5 ml physiological saline (0.85% NaCl) to allow the spermatozoa swim out freely. After two minutes of incubation, the solution was pipetted onto clean glass slides and cover-slipped for light microscopy at X40 magnification. Percentage of sperm motility was assessed using graded semi-quantitative scale of 0 to 5 and the spermatozoa were evaluated for the rate of forward movement and graded accordingly, WHO classification method i.e., 0 = No movement, 1 = Sluggish or tail movement alone, 2 = Intermittent sluggish movement, 3 – 4 = Fair & Good movement and 5 = Maximum movement in forward direction¹⁶. Motility was defined as a sperm that showed any movement in the flagellum during a 30-seconds observation period and was expressed as a percentage of motile sperm in total sperm¹⁷.

Sperm count was done using the improved counting chamber (Haemocytometer) and counting was done as described in Freund and Carol¹⁸. The spermatozoa count was obtained by counting the number of sperm cells in the four WBC chambers of the Neubauer's slide.

Sperm morphology: The fixed sperm were smeared on a glass slide and stained with phosphate buffered saline solution of Giemsa (Merck, Germany). Morphological alterations in spermatozoa head and flagellum were observed under 40X magnification as in Suresh et al.¹⁶.

Testosterone assay

Skin over the neck region was excised open immediately after anaesthesia and blood drawn from the common carotid artery using a 5 ml syringe and needle. Blood sample was centrifuged at 3000 rpm for 10 minutes using a bench centrifuge and the serum collected stored at 4°C before assay was carried out. The enzyme-linked immunoabsorbent assay (ELISA) technique (TECO Diagnostics, Anaheim, CA. Biotech Laboratory Ltd, UK) was used to assay for testosterone.

Statistical analysis

Data obtained were analyzed using the Student's t-test to compare variables and ANOVA with Turkey's post hoc test to compare between the groups and

results expressed as mean + SD. P<0.05 was considered significant.

Results

Mortality

Only one rat died in group 1 after 6 weeks of treatment. Autopsy findings showed that death was due to trauma and respiratory pathologies.

Histopathology of testicular tissues: Testicular cross sections of left testis in control and all treated groups showed normal spermatogenic epithelium with 6 layered thickness of spermatogenic cells with small luminal diameter filled with immotile spermatozoa. There was severe seminiferous tubular atrophy with dense aggregation of inflammatory cells within the interstitium in the right testis of group 1 animals. Testicular sections in group 2 were essentially normal with slight reduction of spermatogenic layers to 4 in the right testis. In group 3, most of the right testis were atrophied with widely spaced tubules, few luminal spermatozoa and reduced spermatogenic layers. Figures 1 & 2 represent low (x100) and high (x400) power magnifications for same slide. All sections were viewed by a Histopathologist and representative slide presented here for each group.

Morphology: Spermatozoa recovered from control group and left testes of other experimental groups were normal in shape. They were no spermatozoa seen in group 1 (azoospermia) while those seen in group 3 had rounded head with short tail.

Sperm count

There was significant decrease in sperm count of right and left testes in groups 1-3 when compared with respective control. Though the sperm count in left testes were higher than the right testes, only group 3 values were significant (p<0.05) (table 2).

Spermatozoal motility: Significant declines in motility (more than 73% decrease) (p<0.05) were seen in groups 1 and 2 while group 3 was not significant (table 2).

Testicular weight (TW)

The right testes of groups 1, 2 and 3 showed significant decline in TW when compared with those of control group of same side. However, there was a significant increase (p<0.05) in TW between right and left testes of group 3 (table 2).

Testosterone assay: Though they were no significant difference in TT levels between other groups, the values for group 1 was significantly lower than control (table 2).

Table 2: Testicular weight, seminal fluid analysis and testosterone assay for experiment groups

Group	Mean BW (g)	TW (g)BW (kg)		Sperm count ($\times 10^6$ /ml)		Motility (%)	TT (ng/ml)
		Right testis	Left testis	Right testis	Left testis		
1	850 \pm 338	1.57 \pm 1.11 ^a	2.26 \pm 1.6	0.25 \pm 0.18 ^a	0.35 \pm 0.25 ^a	22.50 \pm 15.9 ^a	0.65 \pm 0.46 ^a
2	850 \pm 273	2.53 \pm 0.34 ^a	2.68 \pm 0.34	0.15 \pm 0.74 ^a	0.18 \pm 1.13 ^a	19.00 \pm 9.00 ^a	1.33 \pm 0.14
3	795 \pm 226	3.31 \pm 0.31 ^a	6.03 \pm 1.44 ^a	7.08 \pm 7.93 ^a	14.21 \pm 7.93 ^a	38.0 \pm 20.61	1.40 \pm 0.20
4	847 \pm 204	5.51 \pm 0.40	5.35 \pm 0.24	71.31 \pm 12.09	50.00 \pm 1.56	83.50 \pm 5.45	1.33 \pm 0.14

Key: ^a* p<0.05; TT is testosterone; BW represent mean is body weight; TW is testicular weight

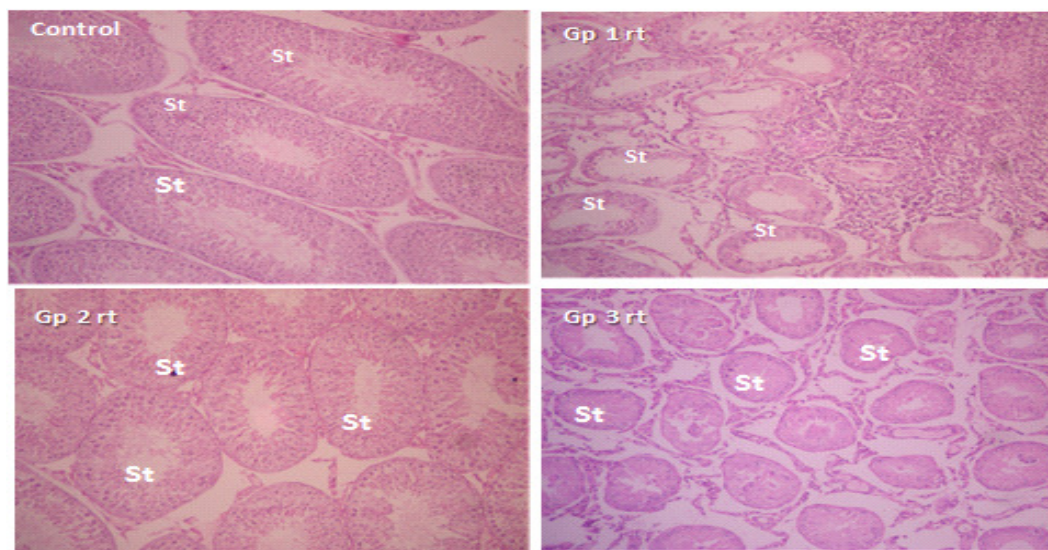


Figure 1: Testicular sections of right testis of control, right testis of groups 1, 2 and 3; H & E x 100. St represent seminiferous tubules, note the marked atrophy of St in groups 1 and 3 respectively.

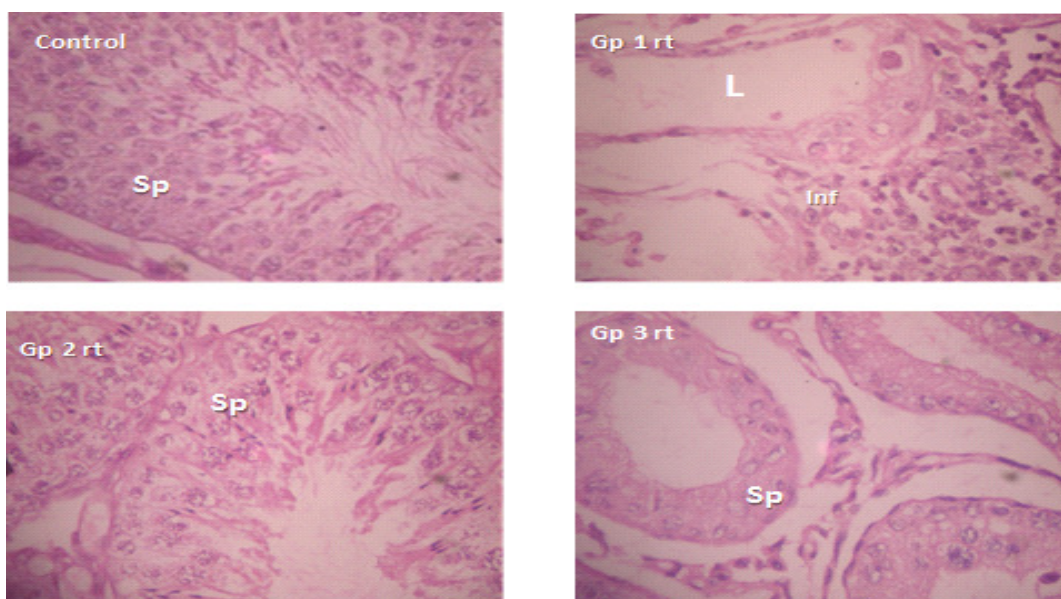


Figure 2: Testicular sections of right testis of control, right testis of groups 1, 2 and 3; H & E x 400. Sp represent spermatogenic cells in seminiferous tubules; L is lumen of seminiferous tubules; Inf is infiltrates in the interstitium; note extensive degeneration of spermatogenic cells in groups 1 and 3 respectively

Discussion

While an intact vas deferens is a necessary anatomical structure for the conveyance of spermatozoa needed for procreation, vasectomy outcomes have been associated with various pathologies of the prostate gland although this remains a debated subject¹⁹. In humans, damage of the vas deferens during childhood often occurs as a complication of inguinal surgical operations such as inguinal herniorrhaphy²⁰. The consequence of this has been noted and imbalance in sex hormones has been previously reported as a contributory factor responsible for pathological changes in the testis following vasectomy²¹. In our study, vasectomy in the AGR only resulted in a reduction in testosterone levels at 8 weeks indicating that this effect may likely be pronounced at longer duration.

Severe atrophy of seminiferous tubules observed in the histological sections of testicular tissues of vasectomized AGRs after 8 weeks mirrors the concomitant decline in testicular weight which could also be a direct effect of reduction in levels of testosterone hormone. This is corroborated by our previous studies which showed that decline in testosterone levels adversely affected seminiferous tubular morphologic and reproductive capacities of the testes of Sprague-Dawley rats²² which ultimately would compromise spermatozoal production. The reduction in TW in our result is proportional to the duration of vasectomy with the 8 weeks duration having the maximum decline. Also, it has been shown that smaller testicles after vasectomy showed severe alterations in the seminiferous tubules compared to heavier testicles that presented with normal morphology. As a result, the degree of morphologic changes corresponds closely to the testicular weight²³. However, part of the noted limitation of this preliminary work is the absence of stereologic data on seminiferous tubular morphology to actually quantify these observations. But we can infer that the gross perturbations seen in the atrophy of the seminiferous tubules more likely would negatively impact on the integrity of the spermatogenesis process in the testes.

In our result, we observed atrophy in the right testicular tissue; the reason for this remains to be investigated but we suggest that early alterations after vasectomy displayed histological changes which consisted mainly of depletion of germ cells. Again the extent of the depletion varied greatly in different seminiferous tubules and a possible role for humoral antibody in this model have been advocated by

Flickinger et al.²⁴ since there is an association between testicular changes and serum antisperm antibodies.

The aggregation of inflammatory cells seen in testicular tissues of vasectomized AGRs is in tandem with the reports of Gupta et al.²⁵ and may likely further lead to fibrotic changes in the testicular epithelium due to inflammation. A sperm granuloma is a complex network of multiple minimal epithelialized channels that help to prevent high intraluminal pressure in the obstructed ducts. Obstructive vasectomy induces a rise in pressure affecting the epididymis and efferent ductules. These structures become markedly distended and then adapt to reabsorb large volumes of testicular fluids and sperm products. Animal Studies show that epididymal and testicular obstruction due to vasectomy will eventually lead to rupturing of epididymal tubules and formation of granulomas in these animals²⁰. However, data from our result shows that only testes of group 3 (left testicular weight) were significantly higher compared to the right testicular weight. But there was a significantly decreased TW of the ipsilateral testes in groups 1, 2 and 3 compared with the control which also mirrors similar pattern in hormonal aberrations. Antypas et al.¹ have shown that a bilateral deficiency in both Leydig and Sertoli cell secretory functions occurs in unilaterally vasectomized animals with an overall result of bilaterally impaired spermatogenesis and inhibited sperm maturation process.

Declines in sperm count and motility observed in this study as well as other morphological aberrations seen in sperm morphology post-vasectomy agrees with the works of Kim et al.²⁶ and Blanco-Rodriguez and Martinez-Garcia²⁷ that variations in circulating testosterone levels could adversely affect germ cell development. We reason that the defect in ipsilateral testicular function in the AGR testis with obstructed right vas deferens can be easily explained as it corroborates the work of Lamano-Carvalho et al.⁶. The high pressure within the epididymal and seminiferous tubules due to the obstruction leads to extension and dilation of the tubules and the exit of spermatozoa from epididymal and seminiferous tubules. This may result in interstitial fibrosis of the testes and secondary epididymal obstruction²⁰.

The reason why biochemical test results (testosterone assay) could not reach the level of statistical significance in other groups (except group 1) unlike histological observations remains to be understood. However, we aver that the longer duration of

animals in group 1 might be responsible for this observation but this certainly will be confirmed if a larger cohort of AGR is recruited for more elaborate study. But our preliminary conclusion from the results shown indicates that ipsilateral vasectomy does alter the contralateral testicular parameters in the AGR in the short duration. Further work is advocated on these observations in view of the increasing domestication of the African giant rat and a possible 'humanization' effects.

Conclusion

While studies to elucidate changes in the male reproductive organs following vasectomy procedures in the African giant rat is limited, we have highlighted in our study that unilateral vasectomy may disrupt testicular parameters that would eventually compromise the integrity of the seminiferous tubular function.

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