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Influence of natural unilateral cryptorchidism on endocrine profile and testicular histomorphology of West African Dwarf goats (Capra aegagushircus)

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Abstract:*The study was designed to examine the endocrine profile and testicular histology of natural hemicryptorchid West African Dwarf (WAD) bucks with the view of establishing their breeding potential. The results of the study showed serum FSH, LH, and testosterone concentrations that did not differ significantly between the hemicryptorchids and the normal bucks with fully descended testes. Histologically, there were evidences of sparsely populated tubular Sertoli cells with basal cytoplasm that were filled with osmiophilic (lipid) droplets in the scrotal testis of the hemicryptorchids. The intraabdominal testis of the hemicryptorchids also contained seminiferous tubules that were hypoplastic,much smaller in diameter and non-canalized, with germinal epithelia having cells at various stages of degeneration. The intertubular connective tissues were comparatively wider than that of the scrotal testis, with pockets of adipose cells. It is concluded that unilateral cryptorchidism adversely affects the scrotal and abdominally retained testes with likely consequenceson the breeding potential of affected animals.*

Keywords*: goats, hemicryptorchids, histology, Sertoli, testosterone*

I. Introduction

Cryptorchidism is one of the common congenital defects seen in man and animals. The word 'cryptorchidism' literally means hidden or obscure testis and generally refers to an undescended or maldescended testis. This reproductive disorder can be unilateral or bilateral where one or both testicles are retained at a point along their migration path to the scrotum, with unilateral cryptorchidism being more commonly reported than bilateral [1].

In most animal species, the right testis is more frequently retained than the left [2,3,4] most probably due to its embryonic location, being more cranially located with longer migration path than the left counterpart. However, some studies have shown that the left and right retention are nearly equal in occurrence [5]. The location of the undescended testis also differs among the species. For cats, dogs and horses, 50, 92 and 47 – 60 % of retained testes were in the abdominal cavity [6] whereas in the West African Dwarf (WAD) goats, the undescended testis was constantly found in the abdominal cavity [7] although subcutaneous location has been reported in 20 % of Nigerian Sahel bucks [8]. For humans, abdominal retention is considered unusual and most retained testes are subcutaneous in the groin, just outside the external inguinal ring, or near the neck of the scrotum [9,10].

Undescended testes are rare in ruminants in general [11]. For example, an overall prevalence of 0.6 % and 3.3 % were reported among Nigerian Sahel breed of goats [8] and goats slaughtered in South India [12] respectively. Goats in Iran [13] and Algeria [14] had prevalence of 2.9 % and 2.1 % respectively, while in newborn lambs in West Australia, a prevalence of 1.5 % was reported [15]. However, high prevalence of this urogenital disorder has been reported in WAD goats [7,16] in South Eastern Nigeria, ina locality where unilateral cryptorchid bucks are preferentially used for breeding among livestock farmers. This is based on the assumption that hemicryptorchids have better sex drive, higher conception rate and reproductive turnover than animals with fully descended testis. It is known that sex drive in the male is linked to plasma testosterone concentration, which in turn has important bearing on the process of spermcell formation, maturation and function. The present study therefore sets out to examine the endocrine profile and testicular histology of hemicryptorchid WAD bucks vis-à-vis normal animals with fully descended testis in Enugu North Agricultural zone of Enugu State in South Eastern Nigeria with the view of establishing the breeding potential of this animals and the veracity of the claimed enhanced sexual virility of hemicryptorchidWAD bucks in this area.

II. Materials and methods

Twenty sexually mature and clinicallyhealthy WAD bucks (10 normal, non-cryptorchidand 10 hemicryptorchids), weighing between 10 and 13 kg and 1-1.5 years of age were used for the study. They were purchased from local markets in Enugu North Agricultural Zone of Enugu State, South East Nigeria. The

animals were kept in the Small Ruminants section of the Veterinary Teaching Farm, University of Nigeria, Nsukka, Nigeria. Fresh herbage was provided for the animals twice daily, supplemented with commercial concentrate ration containing 15 % crude protein, 10 % crude fibre, and 2550 Kcal/Kg metabolizable energy. Water was provided *ad libitum*. No artificial lighting was supplied. The animals were allowed three weeks to acclimatize before the commencement of the experiments.

2.1 Hormonal assays

At the end of the acclimatization period, each animal was restrained routinely and about 5 ml of blood collected by venipuncture via the external jugular vein. The blood was transferred into commercial sample bottle, allowed to stand for 1 hour, and centrifuged at 10,000 rates per minute. The resultant supernatant serum was decanted into fresh sample bottle and stored at -21°C. The hormonalassayswere carried outwithin 48 hours of collection of the blood sample.

For follicle stimulating hormone (FSH) and luteinizing hormone (LH) assays, the standard procedure of microplateimmunoenzymometric assay(type 3) [17] was used; whereas the serum testosterone concentration was evaluated by a competitive microplate enzyme immunoassay (type 7) [18]. The within and between assay coefficient of variation for FSH and LH were 2.9 %, 3.6 % and 4.8 %, 6.0 % respectively, with a cross reactivity of less than 0.0001 for both hormones. For testosterone, the within and between assay coefficient of variations were 5.2 % and 5.9 % respectively, with a cross reactivity with selected steroids of less than 0.0001.

2.2 Testicular histomorphology

Soon after the collection of the blood samples, the animals were euthanized by an overdose of pentobarbital sodium. The intraabdominal and scrotal testes were fixed by vascular perfusion through the testicular artery by a method described by Ezeasor [7]. Briefly, the abdominal aorta was exposed after laparotomy, cleared of extraneous tissues to unravel the origins of its branches. Thereafter, it was ligated below the origin of the left renal artery and again at the bifurcation where it gave origin to the external iliac arteries.

The posterior mesenteric artery and the dorsal lumbar arteries were also ligated. This procedure afforded access to the testicular arteries and their perfusion through the segment of the aorta from which they took their origin. Perfusion was by gravity from a reservoir containing mammalian Ringer's solution and 0.4 % procaine hydrochloride, suspended from a height of approximately 90 cm. The caudal vena cava was incised to allow drainage of the perfusate. As soon as the testes blanched the rinsing was stopped and perfusion resumed with the fixative (cold 4 % glutaraldehyde in 0.1 M cacodylate buffer; pH 7.3) for 30 minutes.The testicular tissues were diced into 1 mm cubes, fixed for an additional two hours by immersion in fixative of the same composition. They were rinsed in 0.1 M cacodylate buffer and postfixed in 1 % osmium tetroxide in the same buffer. This was followed by dehydration in graded concentrations of acetone and embedded in Araldite.

Sections 1 µm thick were cut with glass knives for light microscopy, stained with 1 % toluidine blue in 1 % borax.

III. Analysis of data

GraphPad software (USA) was used for the statistical analysis of data obtained. Results are presented as the Means ± standard error of the means. Differences between the means of the groups were compared using Students' *t*-test. P values less than 0.05 were considered statistically significant.

IV. Results

4.1 Hormonal assays

The results of the FSH, LH, and testosterone assays showed values that did not differ significantly between the normal bucks and the hemicryptorchids (Fig.1; $P>0.05$). The following values, in parentheses, were recorded for the normal bucks (FSH (mIU/ml), 0.55±0.15; LH (mIU/ml), 0.98±0.39; testosterone (ng/ml), 2.80±0.99) as against the following values (FSH, 0.35±0.13; LH, 1.38±0.13, and testosterone, 2.26±0.39) for the hemicryptorchids. Inaddition, sex drive manifested by anogenital sniffing and bouts of mounting of other animals in the herd were also adjudged to be similar in both groups.

4.2 Testicular histomorphologic studies

4.2.1 Non-cryptorchid, normal bucks

Both the right and left testes of the normal, non-cryptorchidgoats showed seminiferous tubules with normal spermatogenic cells and interstices (Fig 2 a and b) The tubules were of variable sizes with a mean diameter of 177.20 ± 33.0 µm and 179.80 ± 30.5 µm respectively. The tubular germinal cells at various stages of development were abundant in most sections of the tubules. The spermatocytes and the spermatids were well differentiated and were interspersed by Sustentacular (Sertoli) cells that exhibited normal appearance. Within the wider intertubular connective tissue were the Leydig cells.

4.2.2 Hemicryptorchids

The histologic features of the scrotal testis of the hemicryptorchids were similar to that of the normal bucks. Although the mean tubular diameter was slightly wider (181.30 ± 24.60) than that of the normal bucks, the difference was not significant (P>0.05).The Sertoli cells were sparsely populated (Fig 2a) with some having basal cytoplasm that were filled with osmiophilic (lipid) droplets (Fig 3 a and b). The interstices also contained Leydig cells that were uniformly distributed. In contrast, sections of the retained, intraabdominal testis of the hemicryptorchids showedhypoplastic, non-canalisedseminiferous tubules (Fig 4a). The mean tubular diameter was significantly smaller (99.24 ± 14.53 µm; P<0.05) compared with its scrotal counterpart (181.30± 24.60).

The tubular germinal epithelium contained cells at various stages of degeneration, with vacuolation of some of the Sertoli cells. The intertubular connective tissue was comparatively wider and contained adipose cells, which were not discernible in the scrotal testis. The tubular wall was thicker than normal and appeared compromised. There were also aggregates (clumps) of dark staining interstitial cells of Leydig in the interstices (Fig 4b).

V. Discussion

The results of this study showed no significant difference in the endocrine profile of the hemicryptorchids and normal bucks. The serum level of FSH, LH, and testosterone for both groups fell within the normal range reported for this [19, 20] and other breeds of goat [21, 22, 23]. It could therefore be reasonably inferred that the hypothalamic-pituitary-gonadal axis,with the corresponding hormonal output were not significantly compromised by abdominal retention of the testis. This is of interest because it is known that the expression of male sexual behaviours is controlled essentially by plasma levels of testosterone [24, 25] mediated via androgen receptors in the brain [26], especially in the medial preoptic area [27]. That the serum testosterone concentration did not differ significantly between the two groups therefore forecloses the claimed higher sexual virility of hemicryptorchid bucks by livestock farmers in the study area since expectedly, a higher concentration of serum testosterone would be required to support the claimed higher expression of maleness in this group.

The structural defects that accompany abdominal retention of the testis in goats have been noted. Very remarkable is the significant reduction in weight [8, 13] and the attendant decrease in the diameter of the hypoplastic, non-canalisedseminiferous tubules as well as the vacuolation of some of the tubular Sertoli cells reported in the present study.We attribute this tubular degeneration to the thickening of the basement membrane, which hindered metabolic interchange between the seminiferoustubular epithelium and the interstitium. Basal lamina formation and indeed extracellular matrix development takes place only when there is functional synergy between the peritubularmyoid and tubular Sertoli cells [28]. The myoid cells, in addition,influence Sertoli cell number, function, and ability to support germ cell development and by extension regulate the physical state of the sperm cells as well as total sperm output [29, 30]. They also influence Leydig cell development and steroidogenesis. Thus, the degeneration of the tubular wall contributed significantly to the hypogonadism reported in this study. Such structural aberrations will not be able to adequately support germ cells development.This may be the reason why this urogenital disorder is usually associated with reduced fertility [31]. Furthermore, the appearance of adipose cells in the interstices of the retained testisdoes suggest some degree of dysfunction in the Leydig cells. This may explain the comparatively lower (eventhough not statistically significant) basal level of testosterone in the hemicryptorchid bucks due perhaps to thermal effect on the abdominal testis. Adipose cells are known to be storage depots of precursors required for androgen synthesis and their accumulation in the interstices in this instance could have arisen from non-utilization by the Leydig cells. This argument is further strengthened by the reversible increase in cholesterol ester and triglyceride levels reported in normal mice testes subjected to brief scrotal hyperthermia [32].

It would also appear, from the present study, that the abdominal retention of one testicle, to some extent, impactednegatively on the functionality of the tubular Sertoli cells of the scrotal testis of the hemicryptorchids as evidenced by the appearance of lipid droplets in their cytoplasm. We consider this development a possible defect in lipid metabolism by the Sertoli cells in this group.Previous studies in rat cryptorchid models have similarly demonstrated a detrimental effect of unilateral cryptorchidism on the contralateral scrotal testicular function especially with regard to sperm cell concentration and fertilizing capacity [33]. This was attributed to an increase in scrotal testicular temperature as a result of an increase in testicular blood flow, which was thought to hinder Leydig and Sertoli cells secretory functions [34].Similar lesions have also been described in the pathology of the testis involving germ cell degeneration [35, 36].

VI. Conclusion

In conclusion, unilateral cryptorchidism is accompanied by structural defects of both the retained and scrotal testis of WAD bucks with no significant alteration in the hormonal output of the animals. Such defects will most likelyhave deleterious effects on the semen quality and output with serious negative implications on

the breeding potential of affected animals. There is therefore no advantage in the use of hemicryptorchid bucks for breeding.

Conflict of interest.
The authors of this work declare that they do not have any conflict of interest.

Ethical standards
The work reported herein was carried out in accordance with the guidelines of the Research and Ethics Committee of the University of Nigeria, Nsukka, Nigeria.

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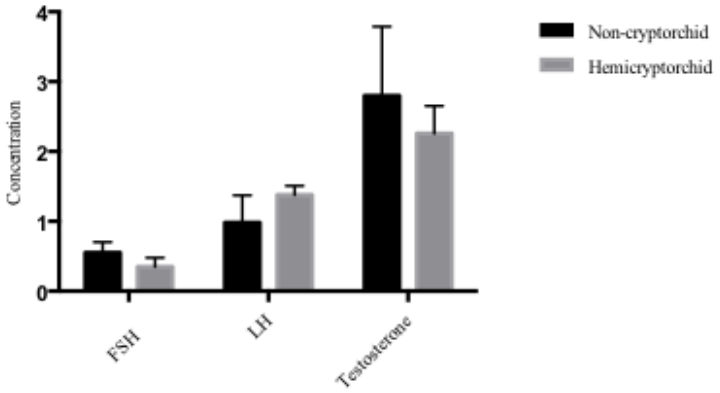
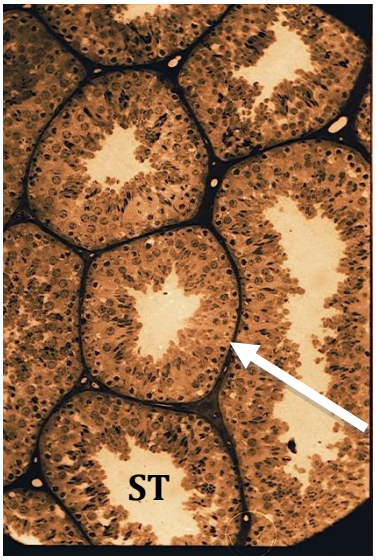
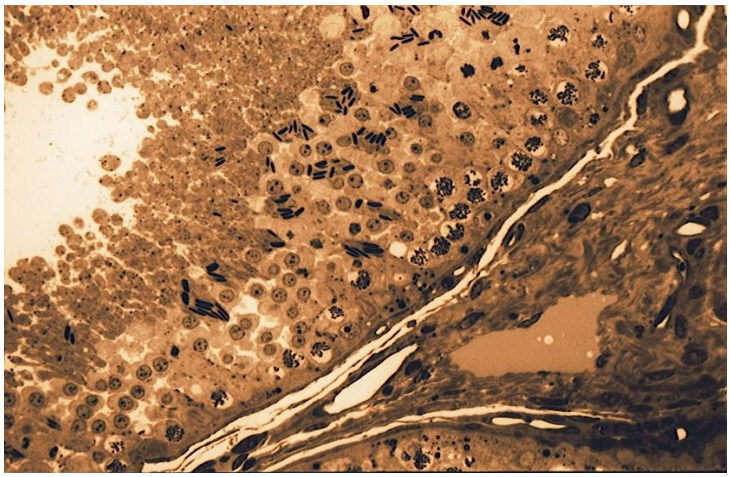


Fig 1 Serum concentration of FSH (mIU/ml), LH (mIU/ml) and testosterone (ng/ml) in non-cryptorchid and hemicryptorchid WAD bucks. Values are the mean ± SEM.

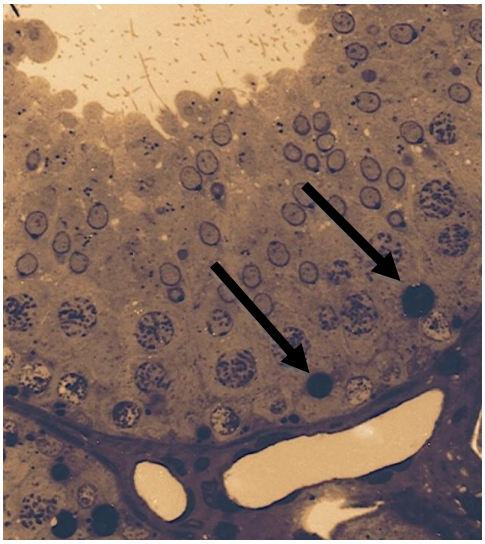


(a)

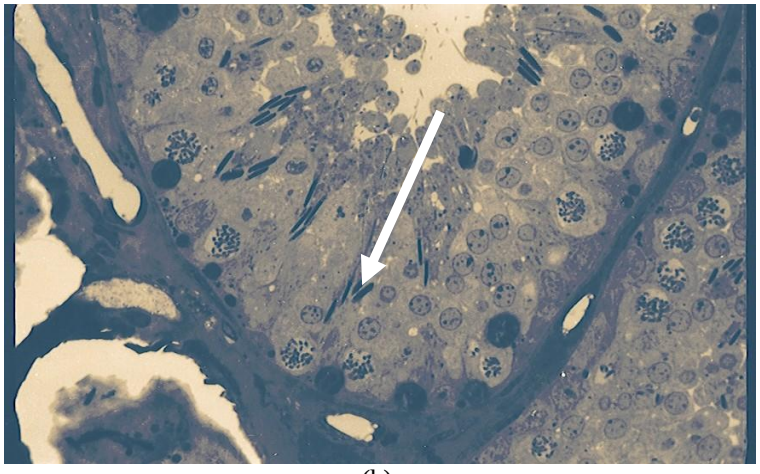


(b)

Fig 2 (a) section of the right testis of normal bucks with fully developed seminiferous tubules (ST), well delineatedintertubular connective tissue. X 300, and (b) section of the left testis of normal bucks showing seminiferous tubular lumen and differentiating spermatogenic cells with Leydig cells in the intertubular connective tissue. X 375.

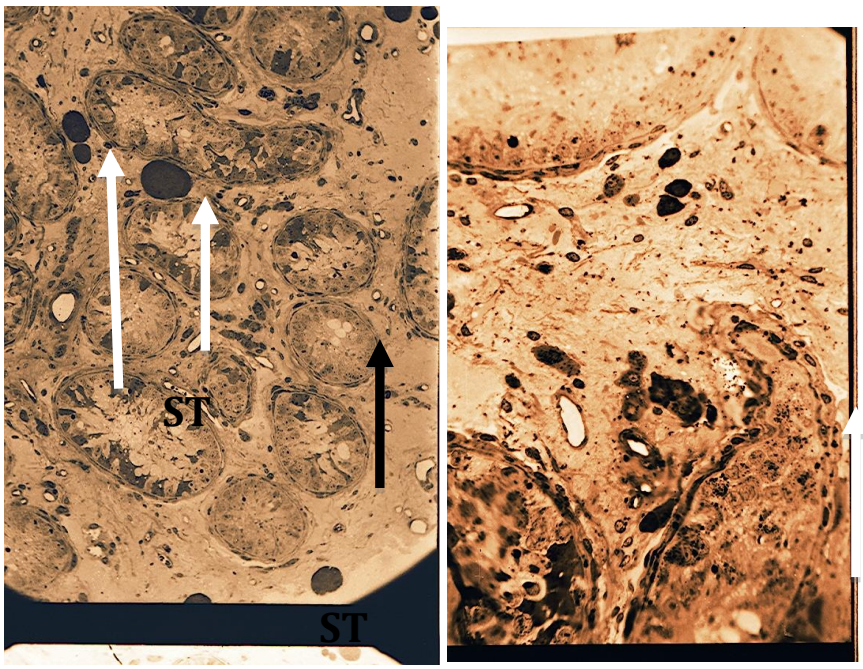


(a)



(b)

Fig 3 (a) section of scrotal testis of hemicryptorchid WAD bucks showing sparsely populated Sertoli and germ cells. Note lipid droplets in Sertoli cells (dark arrows), and (b) section of scrotal testis showing elongated spermatids (light arrow), and other germ cell associations. X 375



(a) (b)

Fig 4(a).Section of the abdominal testis of the hemicryptorchid showing hypoplastic and non-canalisedseminiferous tubules (ST), vacuolated Sertoli cell (dark arrow), and adipose cells (light arrows) in the interstices. X 300. Note the wide intertubular connective tissue, and (b) aggregates of dark staining Leydig cells (light arrow) in the testicular interstitium. X 375