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COMPARATIVE STUDY OF THE MORPHOLOGY OF THE TESTES AND EXCURRENT DUCTS SYSTEM IN THE UNILATERAL CRYPTORCHID WEST AFRICAN DWARF GOATS

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SUMMARY

Laboratory investigations were carried out to study the comparative morphology of testes and the excurrent ducts system in the unilateral crytorchid West African dwarf goats. Two groups of 10 bucks were used: Prepubertal (2-4 months old; pubertal groups (5-8 months old).The result showed that the retained testis had a progressive significant reduction (P<0.05) in the seminiferous tubular diameters from the prepubertal to the pubertal goats and a reduction in the number of spermatogenic cells and increasing number of degenerating sertoli cells. Epididymis of the intra-abdominal testis showed significant increase (P<0.05) in the lumen diameter. The epithelia were flattened, occasionally cuboidal and were devoid of characteristic stereocilia. The vas deferens had poorly developed longitudinal folds and smooth muscles. It was however concluded that the alterations observed in the morphology of the intra-abdominal testes and the excurrent system compared to the contralateral scrotal testis were progressive and age dependent.

KEY WORDS: Unilateral cryptorchidism, Testes, Excurrent ducts, Goat, Epididymis

INTRODUCTION

Crytorchidism is the absence of testicles in the scrotum due to retention in the abdominal or inguinal region. It can either be unilateral or bilateral (Agrawal and Mitra, 1999 and Hadziselimovic and Huff, 2002). Unilateral cryptorchidism is a common condition among West African Dwarf (WAD) goats of south eastern Nigeria, with the right testis being consistently retained and abdominal in position (Ezeasor, 1985). Cryptorchidism is not only associated with morphologic changes in the gonads but also the excurrent duct system, that may lead to additional fertility problems (Depalma *et al.*, 1988; D'Agostino *et al.*, 1994; and Nistal and Paniagua, 1996). Several investigators have described the degenerative changes in the undescended testes in mammalian testes in both naturally and experimentally induced cryptorchidism (Ezeasor, 1985; Ezeasor and Singh, 1987; Takayuki and Sofikitis, 1999; Zakaria *et al.*, 2000; Izzet *et al.*, 2001 and Zaidi *et al.*, 2005). The effects of cryptorchidism on the epididymis and vas deferens morphology in unilateral cryptorchid West African Dwarf (WAD) goats have not been investigated. The present study therefore investigated the morphological changes associated with the testes and spermatic ducts system in the prepubertal and pubertal naturally unilateral cryptorchid West African Dwarf goats.

MATERIALS AND METHODS

Ten West African Dwarf bucks were used in each group for this study. The animals were purchased from a local market in Nsukka, Enugu State. All the animals used were inspected for health status before purchase and slaughter. The ages of the animal ranged from 2 8 months and were grouped into two; prepubertal (2 4 months) and pubertal (5 8 months). They were euthanized with 50g/kg Magnesium sulphate by intravenous injection through the jugular venipuncture. The retained and descended testes as well as vas deferens and epididymis were collected by blunt dissection.

Sections of the testes, epididymis and vas deferens were made and fixed in Bouin's fluid. The tissues were dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax. About 6 μm thickness of the tissue sections were cut using standard rotary microtome (Shandon 0325) and were stained using Haematoxylin and Eosin (H and E) techniques and mounted on a glass slide.

Histomorphometry and Histomorphology
The following parameters were measured using calibrated eye-piece micrometer (Graticules Ltd, Toubridge Kent): The epithelial height and lumina diameter of the epididymis and vas deferens and the seminiferous tubular diameters. Ten measurements were made per sections for each of the parameters, and the mean recorded for each animal. Selected sections were photographed with Leica (Galen III) photomicroscope with Moticam 1000 digital camera attached to a laptop computer.

Statistical analysis
All data obtained were expressed as means with the standard deviation. The data were subjected to compared sample students t-test with computer based SPSS, at $P<0.05$ being considered statistically significant.

RESULTS

Histomorphometry
The histomorphometry showed a significant reduction ($P<0.05$) in the mean seminiferous tubular diameters of the retained intra-abdominal testis (Table 1). Significant reduction at ($P<0.05$) of the mean epithelial height of epididymis (head) of the retained intra-abdominal testis were recorded in the prepubertal and pubertal goats (Table II).The pubertal goats showed significant increase ($P<0.05$) in the mean luminal diameter of the body of the epididymis of descended testis compared to retained (Table III). Table IV showed a significant difference ($P<0.01$) of the mean values of the epithelial height and luminal diameter of the tail of epididymis in descended and retained testis of the prepubertal and pubertal goats. In Table V, There was a reduction in the mean epithelial height and luminal diameter of vas deferens of retained testis in the prepubertal and pubertal goats ($P>0.05$).

TABLE I: Seminiferous tubular diameter of the descended and retained testes in the prepubertal and pubertal goats

Seminiferous tubular diameter pubertal goats (Mean SD)		
	Descended [Tubular diameter]	Retained [Tubular diameter]
Group A [Prepubertal]	153.59 41.25 [a]	64.82 22.22 [a]
Group A [Pubertal]	**159.72 39.99	**96.73 14.48 [b]

Mean values with same superscript across each row are significantly different at P < 0.01
Mean values with same superscript across each column are significantly different at P < 0.05
**** are significantly different at P < 0.05.**

TABLE II: Epithelial height and luminal diameter of the descended and retained head of the epididymis of the prepubertal and pubertal goats

Epithelial height and luminal diameter of the descended and retained head of the epididymis of the prepubertal and pubertal goats				
	Descended		Retained	
	Epithelial Height (μm	Lumen Diameter (μm	Epithelial Height (μm	Lumen Diameter (μm
Group A [Prepubertal]	24.34 4.47 [a]	69.19 48.22	14.53 1.19 [a]	56.89 2.60
Group B [Pubertal]	27.64 8.59 [b]	53.47 2.89	14.99 2.89 [b]	112.53 56.8

Mean values with same superscript across each column are significantly different at P < 0.05

TABLE III: Epithelial height and luminal diameter of the descended and retained body of the epididymis of the prepubertal and pubertal goats (Mean)

	Descended		Retained	
	Epithelial Height	Lumen	Epithelial Height	Lumen
Group A [Prepubertal]	35.87 21.89	85.77 20.27[a]	16.25 6.66	25.12 13.28[a]
Group B [Pubertal]	43.10 4.94**	42.23 17.15[c]	16.69 6.39**	126.32 42.53[c]

Mean values with same superscripts across each row are significantly different at [P < 0.05]
Mean values with same superscript across each column are significantly different at [P < 0.01]

TABLE IV: Epithelial height and luminal diameter of the descended and retained tail of the epididymis of the prepubertal and pubertal goats

Epithelial Height and Luminal diameter (µm) of the descended and retained tail of the epididymis of the prepubertal and pubertal goats (Mean ± SD)				
	Descended		Retained	
	Epithelial Height (µm)	Lumen Diameter (µm)	Epithelial Height (µm)	Lumen Diameter (µm)
Group A [Prepubertal]	36.70 ± 7.30	223.36±34.52[a]	35.09 ± 7.62	75.07±27.48[a]
Group B [Pubertal]	47.47 ± 8.89	212.04±60.87[b]	37.39 ± 9.02	85.99 ±16.14[b]

Mean values with same superscripts across each row are significantly different at [P < 0.01]

TABLE V: Epithelial height and luminal diameter of the descended and retained vas deferens of the prepubertal and pubertal goats

Epithelial Height and Luminal diameter (µm) of the descended and retained vas deferens of the prepubertal and pubertal goats (Mean ± SD)				
	Descended		Retained	
	Epithelial Height (µm)	Lumen Diameter (µm)	Epithelial Height (µm)	Lumen Diameter (µm)
Group A [Prepubertal]	54.28 ± 7.35[a]	161.52±5.14b[b]	40.59±10.03[a]	132.11±42.44[b]
Group B [Pubertal]	42.06±17.20[a]	190.72±35.12[b]	44.20 ± 8.26[a]	141.10±41.68[b]

Across each row and within each column, mean values with same superscripts are not significantly different at [P < 0.05]

Histomorphology

In the seminiferous tubules of the descended testis of the 2-3 months old prepubertal goats, sertoli cells were seen lining the seminiferous tubules as simple columnar epithelium and gonocytes were seen sparsely distributed among the sertoli cells. Leydig cells were seen in the interstitium (Plate 1).In contrast, the tubules of the retained counterpart were non-canalized, but some sertoli cells were seen present. There were no sign of spermatogenesis, except few gonocytes seen sparsely distributed among the sertoli cells (Plate 2).In 6 months pubertal goats, the spermatogenic cells were much more apparent, with the spermatogonia, spermatocytes spermatids and spermatozoa differentiating progressively and with few degenerating sertoli cells (Plate 3). The contralateral retained testis showed a lot of degenerating sertoli cells and presence of few spermatogonia which were almost basally located in the epithelium of the tubules. The lumen of the tubules was filled with debris from the degenerating sertoli cells (Plate 4).

A cross section of the epididymis in 3-4 months old prepubertal goats in the descended testis showed clear pseudostratified columnar epithelium with the stereocilia obviously distributed on the epithelium (Plate 5). In the contralateral intra-abdominal epididymis, the tubular lumen was dilated and was lined by flattened and occasionally columnar epitheliums which were devoid of characteristic stereocilia. Fibrovascular tissues were in between the tubules (Plate 6). In the 7 months old pubertal goats, the descended epididymis showed marked feature of maturation. The columnar epithelia were very tall and well ciliated and contained clumps of spermatozoa (Plate 7).In contrast, the degenerative changes that occurred in the retained epididymis were marked compared to the pubertal goats. The tubules were highly distorted and were lined by cuboidal epithelium. The lumen was also dilated and were devoid of spermatozoa (Plate 8).The descended vas deferens showed normal morphology in the prepubertal goats, i.e. the longitudinal folds were developed, the muscle layers were distinct (inner longitudinal layer, intermediate circular layer and outer longitudinal layer), tubular epithelium were ciliated (Plate 9).In the intra-abdominal vas deferens, there were marked degeneration in the tunica muscularis which showed no distinct layering of the smooth muscle. The longitudinal folds were poorly developed and tubular lumen was devoid of cilia. The pubertal goats showed more serious degeneration in the muscle layers of the retained testis (Plate 10.).

PLATES

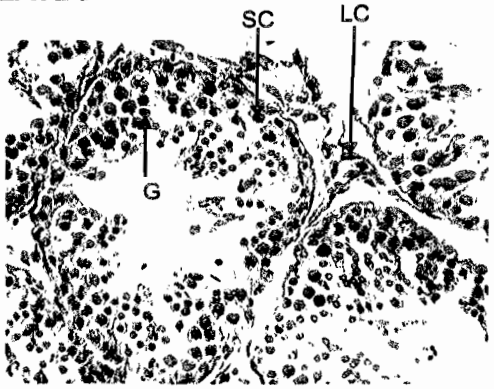

PLATE I: Section of seminiferous tubules in the descended testis of prepubertal goats (3-4 months old). Observe Sertoli Cells (SC) lining the Seminiferous tubules as simple columnar epithelium and Gonocytes (G) distributed amongst the Sertoli Cells (SC), Leydig Cells (LC) x 400, H&E

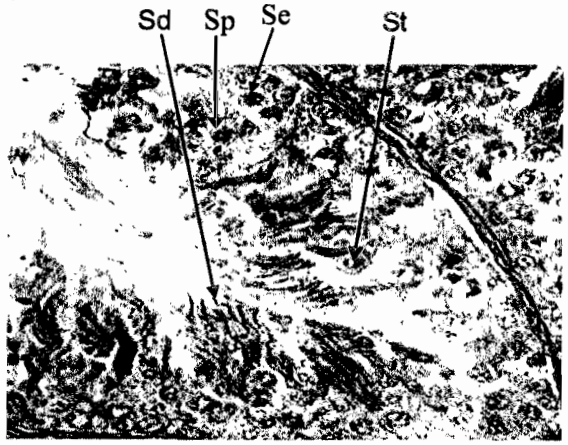

PLATE 3: Seminiferous tubles inn the scrotal testis of pubertal goats (7 months old). Note the spermatogenic cells in active differentiation from Spermtogonia (Se), Spermatocytes (Sp) , Sertoli cells(St) and Spermatozoa (Sd). x 600, H &E

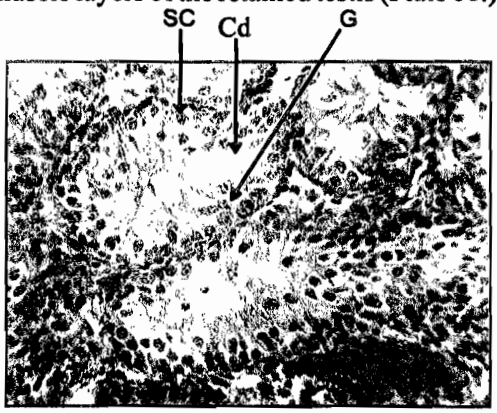

PLATE 2: Seminiferous tubles of the retained testis of prepubertal goats. Shows non canalized lumen and few Sertoli Cell (SC),Cytoplasmic debris (Cd), Gonocytes (G) x 400, H&E

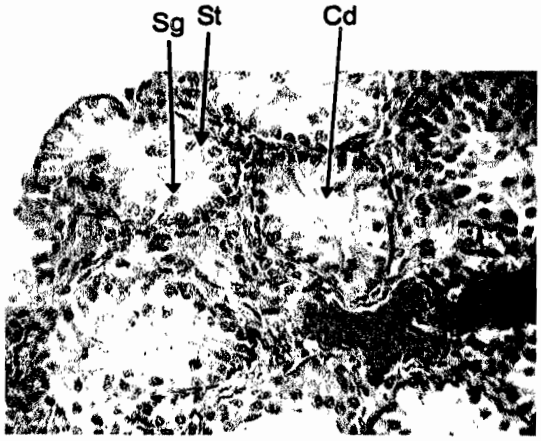

PLATE 4: Seminiferous tubules of retained testis of the pubertal goats. Observe degenerating Sertoli cells(St), spermatogonia (Sg) and Cytoplasmic debris (Cd) in tubular lumen with few Leydig cells and interstitial tissue present. x 400 , H&E

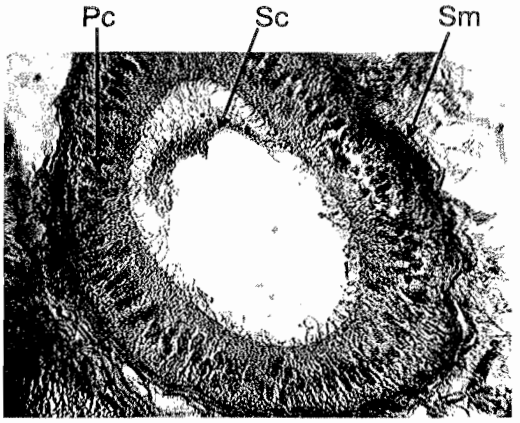

PLATE 5: Section of the epididymis of the prepubertal goats (3-4 months old). Note Stereocilia (Sc) on the Pseudostratified columnar epithelium cells (Pc), Smooth muscle fibers (Sm). x 400. H &E

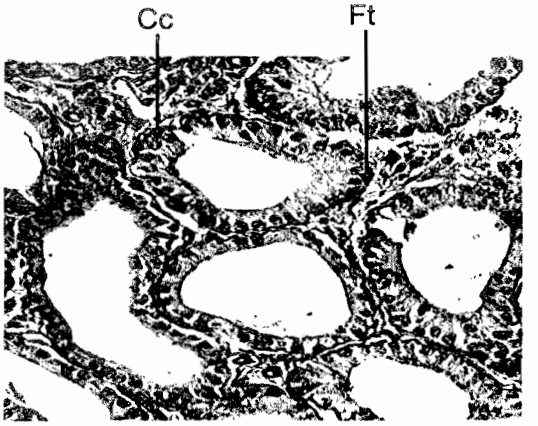

PLATE 6: Epididymis of the retained testis of the prepubertal goats. The dilation of the tubular lumen and loss of stereociliation with apparent, Columnar cells (Cc), Fibrovascular tissue (Ft) x 100. H&E

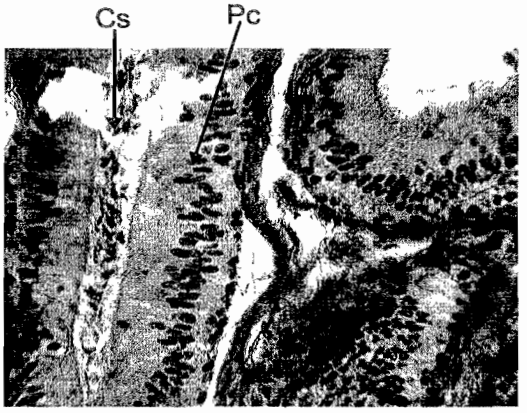

PLATE 7: Epididymis of scrotal testis of the pubertal goats (8 months old). Note the marked feature of maturation of the Pseudostratified columnar epithelium (Pc)and clumps of spermatozoa (Cs) x 400, H&E

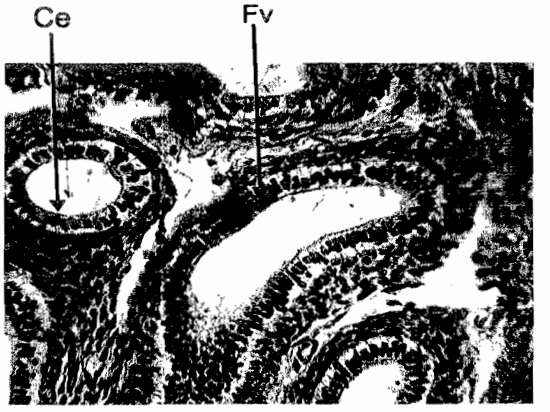

PLATE 8: Epididymis of retained testis of pubertal goats. Observe marked degeneration of the epithelial cell (Ce) and loss of cilia and absence of spermatozoa with degeneration of Fibrovascular tissue(Fv) x 400, H&E

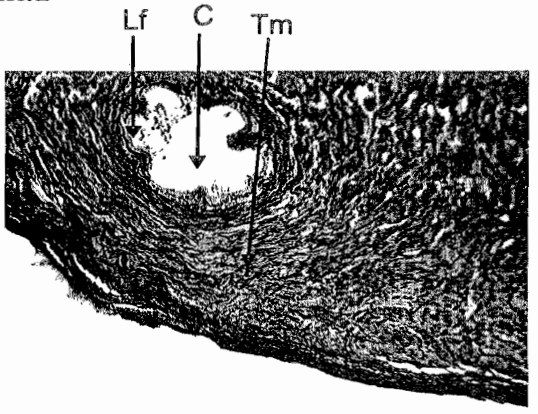

PLATE 9: Section of the vas deferens of the scrotal testis Note the developed Longitudinal fold (Lf), Stereocilition (C) and Smooth muscle layers (Tm) distinctively aligned. x 100. H&E

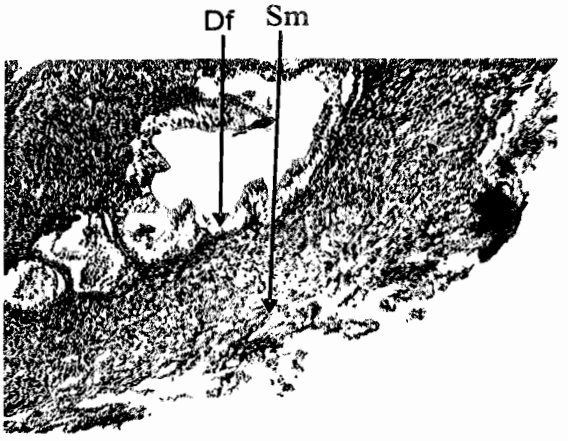

PLATE 10: Vas deference of the retained testis showing poorly developed Longitudinal folds (Df) and Smooth muscle (Sm).×100, H&E

DISCUSSION

It has been known for more than hundred years now that cryptochidism can lead to anatomical and physiological testicular impairment as was noted by (Bedford, 1991), all of the scrotal mammals tested to date has been rendered infertile by this condition. Laboratory mice for example, exhibited a complete inhibition of a 74% decrease in testicular weight only 4 weeks after being rendered cryptorchid (Mendis-Handagama, et al., 1990).From the result of the present study, it could be suggested that the degenerative changes associated with the testes and the excurrent duct system were progressive with age in the naturally unilateral cryptochid dwarf goats. Some authors indicate that testicular histology is more normal when the testes are situated at a lower position (Hadziselimovic et al., 1987 and Meyers-Wallan,1999), and the age related progressive changes in the undescended testis have also been widely believed to be secondary to the testicular exposure to an elevated extrascrotal temperature (Fonkalsrude , 1986 and Mieusset et al., 1993).

From the 3rd month of life onward, nuclear and cytoplasmic features, typical of normal mature Sertoli cells differentiated progressive in the scrotal testis of the contralateral intra-abdominal testis degenerated, perhaps because of the sensitivity of the cytoplasmic organelles to the unfavourable intra-abdominal environment (Ezeasor and Singh, 1987).The decrease in the number of spermatic cell in the retained testis as observed in the pubertal goats (6 months), may be secondary to the degeneration of the Sertoli cells, because each Sertoli cells supports a limited number of germ cells in a species-specific manner, (Russel and Peterson, 1984 and Franca and Russel, 1998). It is currently accepted that the number of Sertoli cells established during testis development determine the rate of spermatogenesis in sexually mature animals and that sertoli cells and peritubular myoid cells appeared to act in concert to synthesize various components of the extracellular matrix of the basal lamina (Hess et al., 1993 and Limanowski et al., 2001), and the splitting of the basal lamina of the seminiferous epithelium of the intra-abdominal testis, therefore, may be a reflection of the disruption of this process, (Ezeasor and Singh, 1987 and Zakaria et al., 2000). The degenerative changes observed in the spermatic ducts may also be secondary to androgen insensitivity by the ducts system or deficiency of 5á-reductase necessary for the conversion of testosterone into the more active dihydrotestesterone which maintains the integrity of the ducts system.

Marked effects of residence in the abdomen on epididymal function have been seen in several scrotal species and indeed it has been hypothesized that the need for cooler temperature in the epididymis rather then the testis have been the driving force behind the evolution of the scrotum (Bedford, 1991). This hypothesis is in the agreement with that of the present study, which showed that the intra-abdominal retention of the epididymis has a deleterious effect on its histomorphology and function. Abnormalities from simple epididymal elongation to more complex forms such as complete disruption between testes and epididymis have been found in cryptorchid human patients with an overall frequency of 36-90% (Koff and Scaletsky, 1990 and Mollaeian et al., 1994).These malformations may present problems for sperm maturation and transportation. The present study showed loss of stereocilia of the epithelium of the epididymis and vas deferens in the retained testes which may impede sperm transport. The tunica mucosa of the vas deferens showed poorly developed and less numerous longitudinal folds akin to disuse atrophic changes due to absence of spermatogenesis and hence absence of local factors.

The histomorphometry showed a significant reduction ($P < 0.05$) in the mean seminiferous tubular diameters of the retained intra-abdominal testis akin to atrophic changes due to degeneration of the germ cells in response to increased intra-abdominal temperature over time. Significant reductions at ($P < 0.05$) of the mean epithelial height of the epididymis of

the retained intra-abdominal testis were recorded in the prepubertal and pubertal goats. The pubertal goats showed a high significant increase ($P < 0.01$) in the luminal diameter of the epididymis, compared to prepubertal goats. This could be attributed to the degenerative changes that resulted to loss of cilia and dilation of the tubular lumen.

CONCLUSION

In conclusion, our work has to provide an insight into the morphologic features of the testes, epididymis and vas deferens and being the first work done to compare the alterations in the histology of the testes and the spermatic duct system in the prepubertal and pubertal naturally unilateral cryptochid dwarf goats and the degenerative changes which were age dependent.

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