

## The influence of natural unilateral cryptorchidism on sperm reserves and haematology of West African Dwarf (WAD) goats (*Capraaegagushircus*).

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**Abstract:** The prevalence of unilateral cryptorchidism and the influence of this reproductive disorder on sperm reserves and the haematology of West African Dwarf (WAD) bucks were studied in Enugu North Agricultural Zone of Enugu state, Nigeria. All the hemicryptorchid had the right testicle retained in the abdominal cavity. In situ morphometric measurements revealed scrotal circumference that was higher ( $P < 0.05$ ) in normal bucks than in the hemicryptorchids. The *ex situ* scrotal testicular weight and volume of the hemicryptorchids were greater ( $P < 0.001$ ) than that of the contralateral intra-abdominal testis but compared well with the corresponding values from the left testis of the normal bucks. In both groups, there was positive correlation between gonadal/extragenital weights and gonadal/extragenital sperm reserves, with the combined testicular sperm reserves of bucks with fully descended testis being higher ( $P < 0.05$ ) than the combined values from the hemicryptorchids. Percentage sperm motility and sperm viability were lower ( $P < 0.05$ ) for the hemicryptorchids ( $48.64 \pm 4.38$ ;  $63.22 \pm 6.36$  respectively) than for normal bucks ( $87.25 \pm 5.45$ ;  $83.29 \pm 2.25$  respectively). The haematology of the two groups were similar. The study showed no benefit in the preferential use of hemicryptorchid bucks for breeding by some livestock farmers.

**Keywords:** goats, haematology, hemicryptorchids, spermatozoa, testis.

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### I. Introduction

In most mammals, the testes migrate from the posterior pole of the kidneys, pass through the abdominal wall to eventually become lodged in the scrotum [1,2]. In ruminants, the testes are already located in the scrotum at birth, whereas in dogs, cats, pigs and horses, descent is completed after birth [3]. The hormone, Insulin-like peptide 3 (Insl 3), produced by the foetal Leydig cells is reported to play a key role in testicular descent by acting on the gubernaculum through its protein coupled receptor, relaxin-family peptide receptor 2 (RXFP2) [4,5]. In addition, epidermal growth factor (EGF), anti-Mullerian hormone (AMH) and an androgen independent gubernaculum growth factor (descendin) have all been shown to be involved in the process of testicular migration from their original embryonic location in the abdominal cavity [6,7,8].

Cryptorchidism refers to a situation where one (unilateral) or both (bilateral) testes are retained along their migration path and hence fail to descend into the scrotum. This reproductive disorder is reported to be rare in ruminants in general [9] but with high incidence in the West African Dwarf (WAD) goats [10,11]. In Enugu North Agricultural Zone (ENAZ) of Enugu state, Nigeria, the hemicryptorchid WAD bucks are preferred for breeding due to the locally held belief that they have better sex drive and high reproductive turnover in terms of number of kids sired per buck compared with normal bucks with fully descended testes. The present study therefore sets out to examine the breeding/production potential of hemicryptorchid WAD bucks vis-a-vis normal bucks with fully descended testes in this agricultural zone. The study has the objective of establishing the magnitude or prevalence of unilateral cryptorchidism in WAD bucks from selected Local Government Areas of this agricultural zone and determining if there is any relationship between the testicular morphometry and sperm reserves of the animals. The research will provide proof(s) as to whether there are benefits to be derived from keeping hemicryptorchid bucks for breeding as presently being practiced in Enugu North Agricultural Zone of Enugu State, Nigeria.

### II. Materials And Methods

#### 2.1 Study Area

The study was carried out in ENAZ, Enugu State, Nigeria. This agricultural zone is made up of six Local Government Areas (LGA) and lies within latitude  $6^{\circ}45''$  and  $7^{\circ}N$  and longitude  $7^{\circ}12.5''$  [12]. The ecology is derived savanna, with annual rainfall of 1500 - 1800 mm and ambient temperature range of  $21^{\circ}$  -  $30^{\circ}C$ . Four of the 6 LGA were randomly selected for the purpose of this study, with the selected town in each of the study areas indicated in parentheses: Nsukka (Opi), Udenu (Orba), Igboeze North (Enugu Ezike), and Igbo Eze South

(Iheaka).

## **2.2 Experimental animals for *in vivo* studies**

Twenty sexually mature and healthy WAD bucks, weighing between 10 - 12 kg and between 1 - 1.5 years of age were used for the study. The animals were sourced from the local markets in the study area and comprised 10 normal bucks and 10 unilateral cryptorchids. They were kept in the Small Ruminants Research Unit of the Veterinary Teaching Farm, University of Nigeria, Nsukka, Nigeria. Fresh herbage was provided for the animals daily, supplemented with commercial concentrate ration containing 15% crude protein, 10% crude fibre and 2550 kcal/kg metabolisable energy. Water was given *ad libitum*. No artificial lighting was provided. The animals were allowed three weeks to acclimatise before the commencement of the experiments.

## **2.3 *In situ* morphometry**

### **Scrotal circumference**

This was measured in the standing buck using a measuring tape, with the circle of the tape placed at the widest part of the scrotum (mid circumference) and at right angle to the long axis of the testis and parallel with the ground [13].

### **2.4 Scrotal skin thickness**

This was measured by first displacing the testis upwards and then measuring the thickness of the scrotum using a vernier callipers. Corrected values for the *ex situ* testicular mid-circumference were obtained by deducting values obtained from the scrotal skin thickness.

### **2.5 Testicular length and diameter**

The testicular length was measured with the tape from the distal to the proximal pole of the testis, avoiding as much as possible the epididymis. The testicular diameter was taken at the same point as the scrotal circumference.

## **2.6 *Ex situ* gross morphometry**

The animals were humanely sacrificed and the hair over the scrotum shaved with a scalpel blade. Thereafter, a longitudinal incision was made on the skin parallel to the median raphe of the scrotum but about 3 cm away from it. The underlying muscles were similarly incised and the testis exteriorised. The spermatic cord was securely tied with chromic catgut and the testis excised about 0.5 cm away from the ligature. The testis was placed in a petri dish containing normal saline for morphometric studies. The testicular weight was measured with a sensitive weighing balance (Mettler PM 600, Switzerland) after removing remnants of the spermatic cord and the tunica vaginalis. The longitudinal length and the mid-circumference were measured with the aid of a vernier calliper. The caudaepididymis was neatly excised from the rest of the epididymis and weighed in a similar manner as the testis. Individual testis volume was determined by water displacement using 1 litre measuring cylinder.

For the intraabdominal testis of the hemicryptorchids, after exteriorising the descended testis, the associated spermatic cord (together with the ductus deferens) was traced through the inguinal canal and the posterolateral wall of the abdomen to the posterior aspects of the bladder and the ampulla. Thereafter, the intestines were displaced, to expose the abdominally located testis, which was excised, along with the ductus deferens and the ampulla. The abdominal testis was then subjected to the same measurements as the scrotal testis of the normal and hemicryptorchid bucks.

All the procedures described were performed in accordance with the ethical standards prescribed by the Research and Ethics Committee of the University of Nigeria, Nsukka, Nigeria.

## **2.7 Determination of gonadal and extra gonadal sperm reserves**

This was determined according to the method of Amann and Almquist [14]. Briefly, a known weight was removed from the middle portion of each testis and transferred into a beaker containing 20 ml of semen diluting fluid and ground in a laboratory mortar. The content of the mortar was transferred into a 50 ml beaker. The mortar was rinsed with 20 ml of the semen diluting fluid and the washings added to the homogenate. After mixing gently, 5 ml of the homogenate was measured out and transferred into a test tube and one drop of eosin stain added. The sperm cells were counted by placing one drop of the stained homogenate on either ends of the improved Neubauerhaemocytometer. This was covered with cover slip and the sperm cells counted using x 40 magnification and x 10 wide angle eyepiece of the light microscope. For caudaepididymal sperm reserve, the caudal was carefully excised from the rest of the epididymis and weighed in a sensitive balance. Thereafter, the same procedure as described for the testis was adopted for the assessment of the sperm reserve in this segment of the epididymis.

## 2.8 Determination of sperm motility and sperm vitality (viability)

These were assessed according to the World Health Organization's (WHO) guidelines[15]. For sperm motility assessment, one end of the cauda was punctured with a 21 G needle and a drop of semen from the opening was placed on a clean and pre warmed glass slide and covered with a cover slip. The preparation was examined under x 10 magnification of the light microscope for mass motility and x 40 magnification for percentage of motile spermatozoa. The assessment was subjective.

The percentage of viable sperm cells from the two groups was evaluated by a modification of the eosin red exclusion test of Hanks and Wallace[16]. Briefly, a smear of the semen was made on a clean microscope slide after mixing with a drop of eosin-nigrosin stain. The air-dried smear was then examined under x 40 magnification of the light microscope. The number of stained (dead) and unstained (viable) cells were then counted and expressed in percentage. About 200 sperm cells were evaluated in each replicate.

## 2.9 Haematology

The Packed Cell Volume (PCV) and the Haemoglobin concentration (Hb) were determined by the microhaematocrit method of Coles [17] and cyanomethaemoglobin method of Kachmar[18] respectively. For the Red Blood Cells (RBC) and White Blood Cell (WBC) counts, the improved Neubauerhaemocytometer was used with formolcitrate diluting fluid for RBC and Rees-Teller diluting fluid for WBC. The differential leucocyte counts were obtained by examining the thick blood smears stained with Leishman stain and observing under the high power (x 100) objective of the light microscope. The erythrocytic indices were deduced, using routine standard procedures.

## 2.10 Analysis of data.

Data generated from the study were analysed using GraphpadInstat<sup>®</sup> (USA) statistical software. Results are presented as the means  $\pm$  standard error of the means (SEM). Differences between the means of the groups were compared using Student's t-test and one way Analysis of Variance (ANOVA) as appropriate.

# III. Results

## 3.1 Prevalence of unilateral cryptorchidism

Farmers in the study area kept a good mix of normal and unilateral cryptorchid bucks. The highest number of hemicryptorchids (relative to normal bucks with fully descended testes) was found in Opi (62.5 %) in Nsukka LGA, with the least (32.5%) from Orba in Udenu LGA (Table 1). All the normal bucks examined had non-split scrotum while all the hemicryptorchids, on palpation had only the left testicle in the scrotal sac. Many farmers also kept hemicryptorchid bucks for breeding. About 80 % of the farmers believed that unilateral cryptorchids are sexually more virile, aggressive and achieve higher conception rate and annual reproductive turnover than animals with two testicles. About 10 % were of the opinion that the reproductive capacities of both groups are similar, while the remaining 10 % would prefer bucks with two testicles for breeding.

## 3.2 *In situ* morphometric measurement

The results of the live body weight, mean scrotal length, and scrotal skin thickness did not show any significant difference ( $P > 0.05$ ) between the normal and hemicryptorchid bucks (Table 2). However, the scrotal circumference differed ( $P < 0.05$ ) between the groups and was higher in the normal than in the hemicryptorchids.

## 3.3 *Ex situ* morphometric studies

*Ex situ* morphometric measurements showed no significant difference between the left and right testicular weights of the normal bucks. Similarly, no difference was found between the left testis weight of the normal bucks and the corresponding scrotal testis weight of the hemicryptorchids. The scrotal testicular lengths, volume, and diameter were also similar between the groups. In contrast, the abdominally retained testis of the hemicryptorchid was soft to touch and much smaller in size compared with the scrotal testis of the hemicryptorchids and the normal bucks (Fig 1). The mean weight of the abdominal testis was approximately one seventh (1/7) of the weight of the scrotal testis. The difference was very significant ( $P < 0.001$ ). Values for peripheral longitudinal length and mid-circumference of the scrotal testis were about twice the value recorded for the abdominal testis (Table 3). In addition, the combined testes and cauda epididymides weights of the normal bucks was greater ( $P < 0.05$ ) than the combined testes and epididymides weight of the hemicryptorchids.

## 3.4 *In situ* versus *ex situ* morphometric measurements

For the normal bucks, results of the gross morphometry showed no difference ( $P > 0.05$ ) in the *in situ* and *ex situ* testicular diameter and testicular length. Mean values for *in situ* and *ex situ* left testicular diameter were  $2.67 \pm 0.15$  and  $2.64 \pm 0.14$  respectively, while the values for the *in situ* and *ex situ* right testicular diameter were  $2.62 \pm 0.13$  and  $2.58 \pm 0.11$  respectively. The mean values for the *in situ* and *ex situ* testicular

lengths were  $8.15 \pm 0.17$  and  $6.42 \pm 0.15$  respectively.

### **3.5 Gonadal and extra gonadal sperm reserves, Sperm motility and Viability**

The results of the gonadal and extra gonadal sperm reserves of the WAD bucks are presented in Table 4. The left and right testes of the normal bucks showed no difference ( $P>0.05$ ) in the mean sperm reserves. Sperm reserves in each of the testes of the normal bucks also compared well with that of the scrotal testis of the hemicryptorchids. However, the combined testicular sperm reserve in normal bucks was higher ( $P<0.05$ ) than the values obtained from the two testicles of the hemicryptorchids. Similarly, in the hemicryptorchids, the abdominal testis (and cauda) contained far less number of sperm cells ( $P<0.001$ ) than their scrotal counterparts. However, in both groups of animals, the mean caudaepididymal sperm reserves were higher ( $P<0.01$ ) than the mean sperm reserves in the testes. The total sperm reserves of the testes and cauda of the normal bucks were  $36.75 \pm 3.79 \times 10^7$  and  $97.37 \pm 3.26 \times 10^7$ /ml respectively while the hemicryptorchids had total sperm reserves of  $20.41 \pm 2.82 \times 10^7$ /ml and  $54.29 \pm 1.17 \times 10^7$ /ml in the testis and cauda respectively.

3.6 Both sperm motility and vitality were lower ( $P<0.05$ ) in the hemicryptorchids than in the normal bucks (Table 4). The predominant feature of the non-viable, stained cells of the hemicryptorchids was tailless heads and sperm cells with kinky tails.

### **3.7 Haematology**

All the haematological parameters evaluated viz PCV, Hb, RBC and erythrocytic indices, and WBC counts showed similar and comparable values between the normal and hemicryptorchid bucks (Table 5), with the exception of the mean neutrophil number which was higher ( $P<0.05$ ) in the hemicryptorchids than in their control (normal) counterpart. The mean values for the control and hemicryptorchids were  $31.25 \pm 2.90$  and  $45.50 \pm 4.97$  respectively.

## **IV. Discussion**

Earlier reports on the prevalence of unilateral cryptorchidism indicated that this reproductive disorder is rare in ruminants [9,19] but high in the Nigerian variant of the WAD goats [10,11]. This high incidence of unilateral cryptorchidism in this breed was attributed to the preferential use of unilateral cryptorchids for breeding due to their claimed higher reproductive turnover by local farmers. The results of the present study confirmed these reports, with the highest incidence of cryptorchidism recorded in Opi, an adjoining town to Nsukka metropolis. Most of the farmers interviewed posited that bucks with fully descended testes are frequently castrated to enhance their market value since they are adjudged to have better growth rate with fat and muscle deposition than castrated hemicryptorchid bucks. Since this reproductive disorder is sex linked and a heritable trait [20], the anomaly is passed on to future generations of the animal, resulting in widespread distribution of the gene in their flock and in high incidence of cryptorchidism in the area.

Measurements of scrotal circumference and testicular size have been used as veritable tools in establishing the breeding soundness of domestic animals and may provide useful and reliable guide to sperm production capability of the testis [21,22]. The results of the scrotal circumference and testicular measurements in normal bucks, in the present study, compared well with values reported by other investigators in this breed [22,23] but differed from values recorded for the hemicryptorchids. For example, even though the scrotal testis weight, diameter and volume of the hemicryptorchids were comparable with their corresponding counterpart in the normal bucks ( $P>0.05$ ), the combined values (left plus right) were greater ( $P<0.05$ ) in bucks with fully descended testes (Table 3). Apparently, the scrotal testis of the hemicryptorchids did not undergo compensatory hypertrophy, in agreement with the observation of Igboke et al. [24] in Nigerian Sahel goats but in contrast to the report of other investigators [25,26]. Compensatory hypertrophy of the remaining gonad has been widely reported among hemicastrates in goats [27] and other animal species [28,29,30] and has been associated with greater amounts of both interstitial and tubular tissue as well as increase in androgen production [31,32]. An improvement in the semen quality of goats following this procedure has also been observed [27]. However, unilateral cryptorchidism, as opposed to hemicastration, is a congenital natural phenomenon that exists in affected animals over time. The retained testicle, although anatomically retarded is known to maintain some level of functionality especially with regard to steroidogenesis, with optimal plasma levels of testosterone in affected animals [33]. There may therefore be no requirement for compensation by the descended scrotal testis. This may be the reason for the absence of compensatory hypertrophy in the gross morphometry of the scrotal testis of the hemicryptorchids in the present study.

In both the hemicryptorchids and normal bucks there was positive correlation between the testicular weights (Table 3) and testicular sperm reserves (Table 4). Even though the amount of sperm cells per gram of testicular tissue appeared similar between the two groups, the combined testicular sperm reserves of the normal bucks was higher ( $P<0.05$ ) than the reserves in the hemicryptorchids. The difference could be accounted for by

the significant reduction in the weight and size of the abdominal testis, which was approximately one seventh the weight of the scrotal testis. Such abdominal retention has been reported to give rise to degeneration of the tubular wall as well as its cellular constituents and supporting structures [34,35]. Testicular size has been shown to be positively correlated with testicular function [13,36]. Another detrimental effect of unilateral cryptorchidism on testicular function observed in the present study was a significant reduction in sperm cell motility and vitality, together with poorly developed spermatogenic cells seen in this group compared with bucks with fully descended testes. There were large numbers of vital but immotile cells in the hemicryptorchids, suggesting structural defects in the flagellum [37] with the potential of substantially reducing the fertilizing capacity of the sperm cells and the overall reproductive capabilities of the animal. Similar studies in canine cryptorchid models [38] and in the rat [39] in which there was impairment in sperm cell function of the scrotal testis was attributed to an increase in the temperature of the descended testis caused primarily by an increase in testicular blood flow [40]. Such an unfavourable testicular microenvironment will impair Leydig and Sertoli cells structural and secretory functions and by extension, the process of sperm cell maturation and function.

It was observed that the cauda epididymal sperm reserves were much higher ( $P < 0.01$ ) than the mean reserves in the testes in both groups (Table 4). This is not surprising because of the extensive fluid reabsorption that takes place between the rete testis and the cauda epididymis. It is estimated that 96 % of the fluid leaving the rete testis is reabsorbed by the time the cauda epididymis is reached thereby concentrating the sperm cells about 25 fold [41,42]. The driving force for fluid reabsorption in the cauda has been reported to be an active transepithelial transport of sodium ions from the epididymal cells to the lateral intercellular spaces, thus creating an osmotic gradient for passive chloride and water reabsorption [43,44]. Both potassium and hydrogen ions are in turn actively secreted in exchange for sodium reabsorption.

Although there was an increase ( $P < 0.05$ ) in the number of neutrophils in the hemicryptorchids relative to the normal bucks, the values for both groups fell within the normal range reported for this specie [45].

## V. Conclusion

This study has demonstrated that the abdominal retention of the testis has obvious deleterious effects on the semen quality and hence breeding soundness of hemicryptorchids compared with normal bucks with fully descended testis. The sexual behavior and phenotype of the animals may appear normal [33] but the reproductive potential is compromised. Since the defect is heritable, many well-informed farmers select strictly against it [46] with very low incidence of cryptorchidism reported in their flock. There is therefore no merit in using unilateral cryptorchids for breeding. An enlightenment campaign should be mounted to discourage livestock farmers in this agricultural zone from this unwholesome practice.

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Table 1 Prevalence of unilateral cryptorchidism in West African Dwarf (WAD) bucks in Enugu North Agricultural Zone (ENAZ), Nigeria

S/N	Town	Total number of bucks	Normal bucks	Hemicryptorchids	Prevalence (%)
1	Orba	166	112	54	32.5
2	Opi	96	36	60	62.5
	Enugu		62	38	38
3	-Ezike	100			
4	Iheaka	88	38	50	56.5
Total		450	248	202	44.9

Table 2 Body weight (g) and in situ scrotal measurements (cm) of normal and hemicryptorchid WAD bucks.

Animals	Body weight	Scrotal Circumference	Scrotal Length	Scrotal Skin thickness
Normal	10.20 ± 0.74	15.70 ± 0.43	8.15 ± 0.17	0.23 ± 0.02
Cryptorchids	11.20 ± 0.68	*11.65 ± 0.25	8.50 ± 0.21	0.28 ± 0.02

\*P<0.05 compared with normal bucks

Table 3 Ex situ testes weight (g), testes diameter (cm), testes volume (cm<sup>3</sup>), testes length (cm), and caudaepididymal weight (g) of normal and hemicryptorchid bucks.

Parameters	Normal bucks			Hemicryptorchids		
	Left	Right	Total	Left	Right	Total
Testes Weight	24.16± 2.61	23.68 ± 2.16	47.84±4.74	26.35±2.34	**3.57 ± 0.63	*29.92±2.59
Diameter	2.64± 0.15	2.58 ± 0.11	5.22 ± 0.26	2.76± 0.07	*1.25 ± 0.78	*4.01± 0.09
Volume	23.94± 1.94	24.38 ± 2.20	48.31±4.02	25.38± 2.31	**3.70 ± 1.15	*29.08±2.14
Length			6.06 ± 0.14	5.91± 0.08	*2.29 ± 0.04	
Caudaepididymides weight	1.69 ± 0.09	1.63 ± 0.08	3.32 ± 0.17	1.98 ± 0.10	0.55 ± 0.03	*2.53 ± 0.14

\*\*P<0.001 compared with normal bucks, and values from scrotal testis of hemicryptorchids

\*P<0.05 compared with combined values of normal bucks

Table 4 Gonadal and extragonadal sperm reserves, sperm motility and viability of normal and hemicryptorchid WAD bucks

Parameter	Normal bucks			Hemicryptorchids		
	Left	Right	Total	Left	Right	Total
Testes sperm Concentration (x10 <sup>7</sup> /ml)	20.25±2.69	17.13±2.01	36.75± 3.79	18.63±2.03	***1.78± 0.09	*20.41±2.82
Caudaepididymides sperm Concentration (x10 <sup>7</sup> /ml)	46.38±1.97	51.00±1.71	**97.37± 3.26	*50.88±3.61	***3.41±0.14	**54.29 ± 1.17
Sperm motility (%)	87.25 ± 5.45			*48.64 ± 4.38		
Sperm viability (%)	83.29 ± 2.25			*63.22 ± 6.36		

\*P<0.05 compared with values from normal bucks

\*\*P<0.01 compared with total sperm reserves in the testes

\*\*\*P<0.001 compared with contralateral scrotal testis and cauda of hemicryptorchids

Table 5 Haematology of normal and hemicryptorchid WAD bucks

Parameters	Normal bucks	Hemicryptorchids
PCV (%)	26.25 ± 0.77	28.00 ± 0.77
Hb (g/dL)	9.25 ± 0.55	10.45 ± 0.32
RBC (10 <sup>6</sup> /μl)	12.63 ± 1.28	12.99 ± 1.07
MCV (fL)	22.44 ± 2.54	22.56 ± 2.75
MCH (pg)	7.91 ± 1.03	7.96 ± 1.25
MCHC (g/dL)	35.16 ± 1.69	37.01 ± 1.07
WBC (x 10 <sup>3</sup> /μl)	12.05 ± 1.36	8.22 ± 2.32
Differential WBC (%)		
Neutrophils	31.25 ± 2.90	*45.50 ± 4.97
Lymphocytes	62.00 ± 3.01	49.10 ± 5.15
Monocytes	4.25 ± 0.96	4.00 ± 0.76
Eosinophils	3.71 ± 1.58	2.33 ± 0.76
Basophils	0.13 ± 0.01	0.00

\*P<0.05 compared with value from normal bucks



Figure 1. The left (L) and right (R) testes of hemicryptorchid West African Dwarf buck