Assessment of the Reproductive Potentials of Intact and Unilateral Cryptorchid Bucks of West African Dwarf (WAD) Goats in Makurdi

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Abstract
The assessment of the serum testosterone concentration, testicular morphometry and semen evaluation of unilateral cryptorchid West African Dwarf bucks was studied with the view of establishing their reproductive potential. A total of 20 West African Dwarf bucks comprising of 10 unilateral cryptorchid and 10 intact West African Dwarf bucks were used for the study. All the unilateral cryptorchids had their right testicles retained abdominally. The result of the study showed serum testosterone concentration that did not differ significantly ($P>0.05$) between the unilateral cryptorchids and the intact West African Dwarf bucks with fully descended testes. The testicular-epididymal morphometry revealed contralateral testicular measurements of the unilateral cryptorchids that did not differ significantly ($P>0.05$) with the two descended testes of the intact WAD bucks. Percentage sperm viability was significantly lower ($P<0.0001$) for the unilateral cryptorchids (68.78 ± 1.46) than for intact bucks (83.7 ± 2.055). Percentage sperm abnormality for the unilateral cryptorchids (31.22 ± 1.46) was also significantly higher ($P<0.0001$) compared to the value for the intact WAD bucks (16.3 ± 2.055). In both groups, the testicular-epididymal parameters were significantly correlated with each other.
Based on the findings of this study, it can be concluded that unilateral cryptorchidism has adverse effect on the semen quality of the affected animals and as a result their reproductive potential is compromised.

Keywords*: goats; unilateral cryptorchids; spermatozoa, testis; epididymides; serum testosterone*

Introduction

Cryptorchidism is a common birth defect regarding male genitalia. The condition is heritable and occurs where there is inbreeding over several generations, but some cases may be caused by fetal exposure to estrogen, androgen inhibitors and certain endocrine disruptors (Thonneau *et al*., 2003, Amann and Veeramachaneni, 2007). It may be unilateral or bilateral, but unilateral cryptorchidism occurs more frequently than bilateral cryptorchidism; and unilateral cryptorchidism is more common with the right than the left testis (Slatter, 1985, Foster,

2007). The position of the undescended testis may be subcutaneous, inguinal or abdominal (Amann and Veeramachaneni, 2007). Unilateral cryptorchids may be fertile if the descended contralateral testis is functional (Kaki and Sofikitis, 1999). Fertility of unilateral cryptorchid WAD goats was presumed because they sired does and boosted the population of cryptorchids among the population (Emehelu *et al*., 2005).

Unilateral cryptorchids are expected to be fertile as earlier reported (Zaidi *et al*., 2005), but some level of sub-fertility; a delay in conception would be expected when they had been associated with decreased levels of serum testosterone (Hall and Gomes, 1975). This study is based on the assumption that Unilateral Cryptorchid West African Dwarf bucks have better sex drive, higher conception rate and reproductive turnover in terms of number of kids sired per buck compared with Intact WAD bucks with fully descended testes. This present study will compare the sperm characteristics and serum testosterone levels of Intact and Unilateral Cryptorchid WAD bucks so as to proffer solution to the preference of Unilateral Cryptorchid WAD bucks to intact bucks by some livestock farmers.

Materials and Methods

This study was carried out at a laboratory in the Veterinary Teaching Hospital, Annex, of the University of Agriculture Makurdi, Benue State, Nigeria.

Experimental animal

A total of twenty (20) sexually mature and healthy West African Dwarf bucks of 1 year (12 months) to 2 years (24 months) of age were used for this study. The goats were purchased from markets within Makurdi and its environments and comprised ten (10) unilateral cryptorchid and ten (10) intact WAD bucks.

Sample Collection and Experimental Design

Fresh testes with their epididymides contained in scrotal sacs were collected from mature West African Dwarf bucks slaughtered at a metropolitan abattoir in Makurdi, Benue State, Nigeria. Samples were individually packaged in polythene bags and placed in ice packs for transportation to laboratory.

Blood collection and determination of serum testosterone level

Blood samples were collected from via the jugular vein using a 5 ml syringe and transferred into plain sample tubes. The blood samples were allowed to clot at room temperature and the serum separated by centrifuging. The serum samples were stored at -20℃ for hormonal assay. Testosterone levels were determined with the use of Testosterone Enzyme Immunoassay test kit, Catalog Number: 10007 (Chen *et al*., 1991, Bricaire *et al*., 1991 & Tietz, 1995).

Testicular-epididymal morphometry

The testes of the intact bucks and the contralateral scrotal testes of the unilateral cryptorchid bucks (with their epididymides) were obtained after the bucks have been slaughtered. These testes and epididymides were collected and transported in ice packs to the laboratory. At the laboratory, the scrotal sacs, the tunica vaginalis, the vas deferens and the remnants of the spermatic cords were removed to obtain the testes and the epididymides. Then the epididymides were neatly excised from the testes and both were weighed. Using a pair of surgical scissors, the epididymides were separated into caput, corpus and caudal segments. The following parameters were taken; Testicular length, mid-Testicular circumference, Testicular weight, Epididymal length, Epididymal weight and weight of various epididymal sections, with the aid of a meter rule and a sensitive electric weighing balance. The positions

of the undescended testes were also determined and recorded.

Determination of sperm motility, sperm viability and sperm abnormality

Sperm was collected from the caudal epididymides by incision method. Several incisions were made on the distal end of the cauda to expose the sperm cells to the outer environment. The sperm cells were rinsed with 3ml of sodium citrate in a petri-dish and placed in a water bath at 37^{o}C for 30 mins to allow the sperm cells to swim out into the medium.

Sperm motility was evaluated by placing a drop of the suspended sperm on a warm slide with a cover slip. The preparation was examined under ×40 magnification of a microscope for percentage of motile spermatozoa.

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 (1958). A smear of the suspended semen was made on a clean microscope slide after mixing with a drop of eosin-nigrosin stain and was air-dried. The air-dried smear was then examined under oil immersion objective (×100 magnification) of the microscope. The number of unstained (viable) cells and morphologically abnormal cells were then counted and expressed in percentage. About 200 sperm cells were evaluated in each replicate.

Determination of extra gonadal sperm cell concentration

The caudaepididymal sperm cell concentration was determined by the dilution of 20µl of suspended semen with 380µl of formal saline (1:20 dilution factor) to kill the live sperm cells and facilitate count. Sperm cell concentration in the caudaepididymal tissues were then counted by placing a drop of the diluted semen on the Neubauerhaemocytometer and it was viewed under the microscope at ×40 magnification. Sperm heads were counted on both chambers from 5 central squares containing 16 smaller squares and their average was calculated. Sperm cell concentration of the sample was obtained by the formula;

Concentration/ml = (Dilution factor) (Count in 5 squares) (0.05×10^{6})

The sperm concentration was expressed in terms of sperm $\times 10^{6}$ /ml

Statistical analysis

Results were presented as means and standard error of the means (SEM). Differences between the groups were compared using unpaired t-test with Graphpad Prism statistical software version 7.03 for windows. For analysis, $P<0.05$ was considered statistically significant.

Results

Hormonal assay

The value of the serum testosterone level in the intact bucks was 4.39± 0.59 ng/ml while the value obtained in the unilateral cryptorchids was 3.36 ± 0.26 ng/ml. The result of the serum testosterone assay in this study showed no significant difference ($P>0.05$) between the intact and the unilateral cryptorchid West African Dwarf bucks.

Testicular-epididymal morphometry

The weights of the left and right scrotal testes of the intact WAD bucks were 26.87±1.36g and 26.46±1.64g respectively, while that of the left scrotal testis of the unilateral cryptorchids was 25.67±3.18g (Table 1). There was no significant difference ($P>0.05$) between them. The epididymal weights, epididymal lengths, testicular lengths and mid-testicular circumference for left and right testes of the intact bucks and the left contralateral testes of the unilateral cryptorchids also showed no significant differences ($P>0.05$) between the values from both groups.

Caudaepididymal Sperm cell concentration, Sperm motility, Sperm viability and Sperm abnormality

The result for the sperm cell evaluation of the intact and unilateral cryptorchid WAD bucks is presented in Table 2.The values of the caudaepididymal sperm cell concentration of the intact and unilateral cryptorchid WAD bucks were 19.82±49.69× 10^7/ml and 11.01 ± 17.92×10^7 /ml respectively. The value of the sperm cell concentration for the intact bucks was higher but did not differ statistically (P>0.05) from the sperm cell concentration value obtained from the unilateral cryptorchids. The sperm motility value for the intact and unilateral cryptorchid WAD bucks were 76.0 ± 3.23% and 75.56 ± 3.58% respectively. There was no significant difference (P>0.05) between the values. However, the values of the sperm viability and sperm abnormality for both groups were highly significant (P<0.0001). The sperm viability values for the intact and unilateral cryptorchid WAD bucks were 83.7± 2.06% and 68.78± 1.46% respectively. The values of the sperm abnormality for the intact and unilateral cryptorchid WAD bucks were 16.3 ± 2.06% and 31.22 ± 1.46% respectively.

Discussion

The expression of male sexual behavior is controlled essentially by plasma levels of testosterone (Wu and Gore, 2010) mediated through androgen receptors in the brain (Wersinger *et al*., 1997), especially in the medial preoptic area (Simerly *et al*., 1990). In this study, the serum testosterone level in both groups fell within the range reported by Daramola *et al.* (2006a) and Daramola *et al*. (2006b) for intact WAD bucks and Uchendu and Ezeasor (2015) for both groups. The serum testosterone level in the unilateral cryptorchid bucks was comparatively lower but not statistically significant; therefore, abdominal retention of the testis did not compromise the hypothalamic-pituitary-gonadal axis and its hormonal output significantly. This does not support the claim by some livestock farmers that unilateral cryptorchid bucks have higher sexual virility because higher expression of male sexual behavior would require higher serum testosterone.

The result of the scrotal testicular and epididymal morphometry in this study showed no significant difference (P>0.05) between the intact bucks and the unilateral cryptorchids. The scrotal testes of the unilateral cryptorchids in this study did not undergo compensatory hypertrophy as reported by Inegedu *et al*. (2005) but is in agreement with the observation of Igbokwe *et al*. (2009) in Nigerian Sahel bucks and Uchendu *et al*. (2015) in West African Dwarf bucks. Although the retained testicle is hypoplastic, there may be no requirement for compensation by the descended scrotal testis, since retained testicle is known to maintain some level of functionality especially with regard to steroid production, with optimal plasma levels of testosterone in affected animals (Uchendu & Ezeasor, 2015). This may be the reason for the absence of compensatory hypertrophy in the gross morphometry of the scrotal testis of the unilateral cryptorchids in this study. Testicular morphometry is very important in establishing the breeding soundness of domestic animals and may provide reliable guide to sperm production capability of the testis (Oyeyemi, *et al*., 2002).

The values of the caudaepididymal sperm cell concentration for both groups in this study were much lower than the values reported for West African Dwarf bucks by Uchendu *et al*. (2015) but higher than the values reported for Sahel bucks by Igbokwe *et al.* (2013).The gap existing between the values may be as a result of differences in age, level of maturity and genetic makeup. The result of the sperm viability for both groups compared well with the values reported by Uchendu *et al*. (2015). The detrimental effect of unilateral cryptorchidism on testicular function observed in this study was a significant reduction in sperm cell viability and increase in sperm cell abnormality, together with poorly developed spermatogenic cells

seen in this group compared with intact bucks. The present study showed that the contralateral scrotal testis of the unilateral cryptorchid West African Dwarf bucks produced comparable spermatozoa with the intact bucks, but the sperm quality was reduced because of appearance of increased population of abnormal spermatozoa and this could reduce their productive efficiency. Normal semen morphology is believed to be one of the major elements that determine the semen ability to effect conception in the female, therefore, accurate determination of morphological abnormalities could assist in eliminating males with low fertility potential in breeding herds (Rodriguez-Martinez & Barth, 2007).

Conclusion

In conclusion, this study has demonstrated that the non-significant difference in the serum testosterone level from both groups indicates that the unilateral cryptorchids do not have higher virility as claimed by some livestock farmers. Also, the sperm viability of the contralateral testis of unilateral cryptorchids was lower than that of the intact bucks suggesting that the unilateral cryptorchid bucks might have reduced fertility. Therefore, unilateral cryptorchids are virile but their fertility is compromised.

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Table 1: Scrotal testicular and epididymal morphometry of intact and unilateral cryptorchid West African Dwarf bucks

	Intact bucks		Unilateral cryptorchid bucks
Parameters	**Left**	**Right**	**Left**
Testicular weight (g)	26.87 ± 1.36	26.46 ± 1.64	25.67 ± 3.18
Epididymal weight (g)	4.78 ± 0.21	4.56 ± 0.24	5.01 ± 0.33
Weight of epididymal segments			
Caput weight (g)	2.20 ± 0.12	2.11 ± 0.12	2.19 ± 0.17
Corpus weight (g)	0.44 ± 0.06	0.41 ± 0.04	0.58 ± 0.06
Caudal weight (g)	2.17 ± 0.10	2.09 ± 0.14	2.18 ± 0.15
Testicular length (cm)	5.19 ± 0.11	5.08 ± 0.13	4.78 ± 0.24
Testicular circumference (cm)	9.84 ± 0.20	9.60 ± 0.21	9.65 ± 0.45
Epididymal length (cm)	9.87 ± 0.20	9.84 ± 0.19	9.18 ± 0.38

Not significant (P>0.05)

Table 2: Caudaepididymal sperm concentration, sperm motility, sperm viability and sperm abnormality of intact and unilateral cryptorchid West African Dwarf bucks

Parameters	**Intact bucks**	**Unilateral cryptorchids**	**LOS**
Caudaepididymal sperm concentration ($\times 10^7$/ml)	19.82 ± 49.69	11.01 ± 17.92	ns
Sperm motility (%)	76.0 ± 3.23	75.56 ± 3.58	ns
Sperm viability (%)	83.7 ± 2.06[a]	68.78 ± 1.46[b]	****
Sperm abnormality (%)	16.3 ± 2.06[a]	31.22 ± 1.46[b]	****

Means with different superscripts in the same row are significantly different (P<0.0001)
LOS= level of significance