

Influence of Unilateral Cryptorchidism on Libido, Haematology and Serum Reproductive Hormones, Total Protein, Lipid Profile and Oxidative Stress in West African Dwarf Goats

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Abstract

The failure of testicular descent (cryptorchidism) is known to cause abnormal testicular development and function. Unilateral cryptorchidism is prevalent in West African Dwarf (WAD) goats particularly in some areas where affected bucks are presumed to have better libido and reproductive efficiency by farmers. Androgens produced by the testes can influence libido and other hormonal and metabolic processes in the body. The study investigated the influence of natural unilateral cryptorchidism on serum reproductive hormones, total protein, lipid profile, oxidative stress, and haematology and libido in WAD bucks. Ten WAD bucks (aged 12 - 14 months) comprising 5 bucks with normal descended testes (N group) and 5 unilaterally-cryptorchid bucks (UC group) were studied. Blood and sera were collected and evaluated for haematology, serum total proteins and lipids (cholesterol, triglycerides and lipoproteins) concentrations, serum oxidative stress (catalase and lipid peroxidation activity), and serum levels of reproductive hormones (testosterone, FSH and LH). Libido testing of bucks was also performed by randomly isolating and confining each individual male with a doe on standing heat and recording the number of mountings made by the male within a period of 5 minutes as the libido score. The evaluated haematological indices and serum levels of proteins, lipids, oxidative stress indicators and reproductive hormones were not significantly different between the N and UC groups ($p > 0.05$). Although a higher mean libido score was recorded in N bucks compared to the UC group, this difference was not significant. Whereas no negative effects were observed in the evaluated physiologic indices in UC bucks, these findings do not support the presumption by farmers that UC bucks

have better libido than bucks with fully descended testes. Therefore, these animals should not be selected for breeding to avoid increasing the prevalence of unilateral cryptorchidism due to its genetic attribute.

Keywords

Cryptorchidism, Libido, Reproductive Hormones, Oxidative Stress, Goat

1. Introduction

Cryptorchidism is an abnormality of the male reproductive system resulting from the failure of one testis (unilateral) or both testes (bilateral) to descend from the fetal position into the scrotal sac, before or shortly after birth [1]. Unilateral cryptorchidism is common in small ruminants and typically affects the right testis compared to the left [2] [3]. Unilateral cryptorchidism has been reported in different breeds of goat worldwide [2] [4] [5] [6] [7]. In Nigeria, a high prevalence (up to 63%) of unilateral cryptorchidism has been observed in West African Dwarf (WAD) goats in regions where bucks with this condition are selectively bred by goat farmers who consider them to have better libido (sexual drive) and reproductive efficiency than bucks with fully descended testis [8].

The testis is an important organ involved in steroidogenesis (testosterone production) and spermatogenesis (sperm production), two functions that are critical for normal male reproduction. Failure of descent of the testis alters the normal scrotal environment required for optimal testicular development and function. Thus, Cryptorchidism has been associated with abnormal testicular structure and functions [9]. Some studies have reported sperm abnormalities in bucks [7] [8] [10] and other male species [11] [12] [13] with unilateral cryptorchidism.

Testosterone is secreted by the interstitial (Leydig) cells of the testis under the stimulation of luteinizing hormone (LH). An impairment in the synthesis or functions of testosterone leads to infertility due to abnormal sperm production [14] [15] and poor libido [16] [17]. The gonadotrophins, follicle stimulating hormone (FSH) and LH also have crucial roles in spermatogenesis [18]. The influence of UC on circulating testosterone and gonadotrophins is highly variable depending on several factors including the species affected, the duration of cryptorchidism and whether cryptorchidism is natural or experimental [19] [20] [21]. Previous reports on the effect of UC on circulating levels of reproductive hormones in WAD bucks are conflicting [22] [23] [24]. There is also no available information on the effect of UC on libido in WAD bucks. Testosterone is a steroid known to regulate protein, lipid and glucose metabolism [25], and oxidative stress [26]. UC have been linked with increased oxidative stress in some studies [27] [28]. However, it is unknown if UC can influence these physiologic processes in affected WAD bucks. Therefore, the aim of this study was to inves-

investigate the influence of natural UC on libido, haematology and serum reproductive hormones, total protein, lipid profile and oxidative stress in WAD bucks.

2. Materials and Methods

2.1. Animals

Ten adult WAD bucks consisting of 5 bucks with normal descended scrotal testes (normal or N group) and 5 bucks with one scrotal testis and one cryptorchid testis (unilaterally-cryptorchid or UC group) were obtained from the local livestock market and used for the study. Unilateral cryptorchidism was confirmed in retrospect at the time of slaughter. All the animals were aged between 12 - 14 months using dental estimation based on the presence of permanent first incisors. Appropriate animal welfare was observed by following the regulations specified by the National Research Council's Guide for the Care and Use of Laboratory Animals [29]. The research protocol was approved by the Experimental Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (UNFVM/2017/11-178314). The animals weighed between 6 - 9 kg with mean body weights of 7.3 kg and 7.1 kg for the N and UC groups, respectively. Two adult WAD doe goats aged between 12 - 14 months were also used in the study to serve as oestrous does. Animals were housed in well-ventilated pens while water and feed were provided at all times. They were acclimatized for a month and routinely screened for gastrointestinal and blood parasites. General physical, musculoskeletal and reproductive tract examinations were carried out on all animals to confirm normal health status before the onset of sample collection.

2.2. Haematological, Biochemical and Hormonal Assays

Blood and sera were collected twice (replicate) from each buck with an interval of 7 days from the jugular vein. All samples were collected at the same time in the morning to avoid diurnal variations. Blood samples collected in potassium (K_3)-EDTA anticoagulant was used for haematology. Serum samples were collected from the supernatant following centrifugation of coagulated blood at 2000 \times g and 25°C for 10 min, and then stored at -20°C until assay. The sera were evaluated for serum total protein, lipid profile, oxidative stress (catalase activity and lipid peroxidation level) and hormonal levels. Serum analyses of all samples were performed in the same assay to avoid inter-assay variation.

Haematological parameters were evaluated using the standard laboratory methods as described previously [30]. Packed cell volume (PCV) was measured with a microhaematocrit centrifuge and reader (Hawksley, England). The concentration of haemoglobin (Hb) was measured using the cyanmethaemoglobin method. The concentrations of white blood cells (WBC) and red blood cells (RBC) were evaluated microscopically using the Neubauer haemocytometer (Gallenkamp, England). Serum total protein concentration was measured using a clinical refractometer (Atago; Bellview, WA, USA). Serum lipid profiling was

performed by measuring the serum concentrations of total cholesterol, triglycerides, low density lipoproteins (LDL) and high density lipoproteins (HDL) using commercial kits (Dialab; Wiener Neudorf, Austria) and a spectrophotometer (Chem-5V3; Erba, Mannheim, Germany).

Serum catalase activity was determined according to the method described previously by Sinha [31] and modified by Hadwan [32]. The test is based on the production of chromic acetate when dichromate in acetic acid is reacted with hydrogen peroxide (H_2O_2). The level of chromic acetate is then determined colorimetrically at 570 nm. Samples were run alongside a control, standard and blank tests. Briefly, 100 μ l of serum was added to 1 ml of H_2O_2 -sodium, potassium phosphate buffer (50 mM, pH 7.4). This was incubated at 37°C for 3 min and then mixed with 2000 μ l Dichromate/acetic acid reagent. The tubes were heated at 100°C for 10 min, cooled and then centrifuged at 2500 \times g for 5 min to remove precipitated proteins. The changes in absorbance were recorded at 570 nm against the reagent blank using a spectrophotometer (Jenway 6305; Jenway, Essex, UK). Catalase activity was expressed in kU.

Lipid peroxidation level in the serum was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS) based on the method of Stocks and Dormandy [33] modified by Sicinska *et al.* [34]. Briefly, 1 ml of serum was mixed with 20% trichloroacetic acid (1:1) and incubated at room temperature. Samples were centrifuged at 1000 \times g for 10 min. Then 1.0 ml of 1% Thiobarbituric acid was added to the supernatant and samples were placed in boiling water bath (100°C) for 15 min. The contents were cooled on ice and centrifuged for 10 min at 2500 \times g. The absorbance of the supernatant was read at 532 nm against a reagent blank using a spectrophotometer. Lipid peroxidation (TBARS) was expressed in absorbance units (AU)/ml of serum.

Serum concentrations of the reproductive hormones testosterone, FSH and LH were measured by using enzyme immunoassay (EIA) test kits (Monobind; Lake Forest, CA, USA) and a microplate reader (StatFax 4200; Awareness Tech, Palm City, FL, USA). The testosterone EIA is based on the principle of competitive binding between testosterone in the test specimen and testosterone-enzyme conjugate for a constant amount of rabbit anti-testosterone antibody while the FSH and LH EIA assay system utilizes a mouse monoclonal anti- α -FSH (LH) antibody for solid phase (microtitre wells) immobilization and another mouse monoclonal anti- β -FSH (LH) antibody in the antibody-enzyme conjugate [35]. The EIA test systems had lower limits of detection of 0.0576 ng/ml, 0.134 mIU/ml and 0.054 mIU/ml for testosterone, FSH and LH respectively.

2.3. Libido Testing

After the period of blood collection, libido testing of the bucks was performed as previously described for ruminants [36]. Oestrus (sexual receptivity) was synchronised in the two doe goats using double intramuscular injection of dinoprost tromethamine (Lutalyse; Zoetis, NJ, USA) at 5 mg/kg given ten days apart,

followed by close observation for the typical signs of oestrus for 1 - 4 days post-treatment. During oestrus, all the bucks were randomly assessed for libido by isolating and confining each individual male with a doe on standing heat and recording the number of mountings made by the male within a period of 5 minutes. A mounting was defined as penile erection and each successful placement of both male forelimbs on the rump (croup) of the doe. Each male was assessed twice with each of the oestrous females on standing heat and the average number of mountings was defined as the libido score, and was determined per male.

2.4. Data Analysis

Data were analysed statistically using GraphPad Prism version 6.01 (GraphPad Software Inc.). The mean of the replicate values for each animal were compared between the N and UC groups using the *t*-test. Values represent mean \pm standard error (Mean \pm SE) and were considered significant when $p < 0.05$.

3. Results

The results following the evaluation of serum reproductive hormones, total protein, lipid profile, oxidative stress, haematology and libido in N and UC WAD bucks are presented in **Table 1**.

Table 1. Comparison of serum levels of reproductive hormones, proteins, lipids, oxidative stress, and haematology and libido in normal and unilaterally-cryptorchid (UC) WAD goats.

Parameter	Normal (N)	Cryptorchid (UC)
<i>Serum hormonal assay</i>		
Testosterone (ng/ml)	3.87 \pm 0.59	2.94 \pm 0.38
Follicle-stimulating hormone (FSH; mIU/ml)	0.86 \pm 0.05	1.03 \pm 0.10
Luteinising hormone (LH; mIU/ml)	1.96 \pm 0.13	1.88 \pm 0.04
<i>Serum protein and lipid profile</i>		
Total protein concentration (g/dl)	6.02 \pm 0.11	6.08 \pm 0.04
Total cholesterol (mg/dl)	63.70 \pm 2.6	65.68 \pm 4.0
Triglycerides (mg/dl)	25.82 \pm 1.7	22.98 \pm 3.2
Low density lipoproteins (LDL; mg/dl)	25.61 \pm 3.0	29.85 \pm 2.9
High density lipoproteins (HDL; mg/dl)	38.09 \pm 3.0	35.84 \pm 3.5
<i>Serum oxidative stress</i>		
Catalase activity (kU)	1.36 \pm 0.17	1.44 \pm 0.30
Lipid peroxidation level (AU/ml)	0.73 \pm 0.11	0.88 \pm 0.16
<i>Haematology</i>		
Packed cell volume (PCV) (%)	32.8 \pm 1.3	32.2 \pm 1.5
Haemoglobin (Hb) concentration (g/dl)	10.76 \pm 0.4	10.78 \pm 0.5
White blood cells (WBC; $\times 10^3/\mu\text{l}$)	13.89 \pm 0.55	14.19 \pm 0.23
Red blood cells (RBC; $\times 10^6/\mu\text{l}$)	8.51 \pm 1.18	8.72 \pm 1.07
<i>Libido testing</i>		
Libido score (mountings/5 min)	16.7 \pm 2.6	11.5 \pm 2.3

Values represent mean \pm SE (n = 5). No significant differences ($p > 0.05$).

Evaluation of the serum concentrations of reproductive hormones showed that the mean serum testosterone concentration appeared to be higher in N goats compared to the UC goats, although this difference was not significant ($p > 0.05$). Similarly, serum concentrations of the gonadotrophins FSH and LH were not significantly different between both groups. Serum biochemical parameters were also compared between both groups of bucks. Serum total protein concentration ranged from 5.7 - 6.3 g/dl (N group) and 6.0 - 6.2 g/dl (UC group) but their means did not differ between both groups. Lipid profiling showed that the serum concentrations of the measured lipids (total cholesterol, triglycerides, LDL and HDL) did not differ between both groups. The results of the serum oxidative stress evaluation showed that both serum catalase activity and lipid peroxidation levels were not significantly different between both groups. Following haematological evaluation, the mean values for PCV, Hb, and WBC and RBC concentrations for the N and UC groups were not significantly different.

The results of libido testing revealed that all the bucks from both groups exhibited sexual response, penile erection and mounting behaviour when confined with the does on standing heat. The number of mountings ranged from 9.0 - 25.5 for the N group and 6.5 - 17.5 for the UC group. Although the mean number of mountings (libido score) appeared to be higher in N bucks (16.7 ± 2.6) compared to the UC group (11.5 ± 2.3), this difference was not significant; $p > 0.05$.

4. Discussion

The failure of descent of the testis is known to cause abnormal testicular development and function [9]. Androgens (testosterone) are steroid reproductive hormones produced by the testes that can influence libido [16] [17] and other hormonal and metabolic physiologic processes in the body [25] [26]. Whereas there is evidence of lowered semen and sperm quality in UC bucks [7] [8] [10], it is not clear if and to what extent UC can affect the circulating levels of reproductive hormones in WAD bucks. Therefore, this study investigated the influence of unilateral cryptorchidism on serum reproductive hormones, total protein, lipid profile, oxidative stress, haematology and libido in WAD bucks.

The effects of unilateral cryptorchidism on circulating levels of testosterone and gonadotrophins is highly variable, and is dependent on several factors including the animal species, location and duration of cryptorchidism and whether cryptorchidism is natural or experimental. Previous studies in WAD goats reported different observations. One study [23] reported higher serum levels of testosterone and LH but not FSH in UC bucks. In contrast, other studies did not find any effect on serum testosterone [24] and both testosterone and the gonadotrophins [22] which are in agreement with the findings in this study. These conflicting observations may be due to differences in the number of sampled animals or differences in the methods and times of sample collection relative to any sexual activation or breeding. However, our findings suggest that unilateral

cryptorchidism has no effect on the circulating levels of testosterone, FSH and LH in affected bucks.

The liver has important metabolic functions including the regulation of circulating levels of metabolites such as proteins, lipids and glucose. Testosterone is a steroid androgen known to exert direct and indirect effects on various aspects of protein, lipid and glucose metabolism that are mediated through the liver including the stimulation of protein anabolic effect and lipid oxidation [25]. Changes in serum testosterone levels have also been associated with altered serum lipid profile in human males [37] [38]. Serum levels of reproductive hormones and lipids have also been linked with altered seminal parameters in humans [39]. Unilateral cryptorchidism had no significant effect on the serum concentrations of total proteins and lipids (cholesterol, triglycerides and lipoproteins), and the values recorded in both groups were similar to the findings from previous studies on WAD goats [40] [41]. This observation may be related to the failure of unilateral cryptorchidism to alter serum levels of testosterone in affected bucks.

Oxidative stress is known to occur following an excessive production of reactive oxygen species (ROS) or an inadequate antioxidant activity, leading to increased levels of ROS and lipid peroxidation that cause damage to cells and cellular components [42]. Damage induced by oxidative stress has been linked with infertility in males [43]. Testosterone was reported to suppress lipid peroxidation (lowered MDA levels) in neutrophils suggesting antioxidant regulatory effects by plasma levels of testosterone [26]. Interestingly, unilateral cryptorchidism has been associated with increased oxidative stress (elevated MDA level) in UC male children [27] and male rats [28]. However, no differences were observed here in the measured serum oxidative stress indicators (catalase activity and MDA levels) between the normal and UC bucks. This is also consistent with the report that unilateral cryptorchidism did not affect these stress indicators in the semen of UC bucks [7].

Components of the blood are under the influence of different biochemical agents that are transported within the systemic circulation. Testosterone is known to have a stimulatory effect on erythropoiesis [44] which was observed via an increase in PCV and Hb concentration [45]. The haematologic values here were also within the range reported for WAD goats by other studies [22] [46]. In this study, UC did not alter the evaluated haematologic indices in affected bucks which is consistent with a previous finding [22]. This may also be related to the absence of any effects of unilateral cryptorchidism on circulating testosterone levels.

Libido is considered to be important in male farm animals and is routinely tested and estimated as libido scores by different methods including the use of oestrous females, and scoring the number and vigour of matings or mating attempts. Males with high libido scores are selected as part of breeding soundness examination for good reproductive performance and fertility [36] [47]. Poor li-

bido has been associated with low circulating testosterone levels in males [16] [17]. In this study, UC bucks recorded a lower mean number of mountings (libido score) than the non-cryptorchid bucks but this difference was not statistically significant. This is also consistent with the absence of any effects of unilateral cryptorchidism on systemic levels of testosterone, the steroid hormone associated with the stimulation of libido. These findings therefore provide evidence that unilateral cryptorchidism does not influence libido in affected bucks.

5. Conclusion

In summary, UC did not alter libido, some haematological indices (PCV, Hb, WBC and RBC concentration), serum total proteins and lipids (cholesterol, triglycerides and lipoproteins) concentrations, specific serum oxidative stress indicators (catalase and lipid peroxidation activity), and serum levels of the reproductive hormones (testosterone, FSH and LH) in WAD bucks. However, findings in this study may be limited with respect to observations in large populations of goat herds. Whereas no negative effects were observed with respect to the evaluated physiologic indices in the UC bucks, these findings do not support the presumption by farmers that UC bucks have better libido than bucks with fully descended testes. More importantly, bucks with unilateral cryptorchidism may have compromised reproductive efficiency due to abnormal sperm production [7] [24]. Therefore, these animals should not be selected for breeding to avoid increasing the prevalence of unilateral cryptorchidism due to its genetic attribute.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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