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Quantitative and cytological studies of interstitial (Leydig) cells in the scrotal and retained testes of unilateral cryptorchid West African Dwarf goats

Godwin Chidozie Okpe*, Daniel Nwagbo Ezeasor

Department of Veterinary Anatomy, University of Nigeria, Nsukka, Nigeria

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ABSTRACT

Cryptorchidism is a common condition in West African Dwarf goats in Eastern Nigeria. The cytology and morphometry of interstitial cells were investigated using light and electron microscopy. Twenty adult male goats were used for the study. Ten of the goats were natural unilateral cryptorchids, while the remaining ten had fully descended testes and served as control. Significant hyperplasia of the interstitial cells were observed in scrotal and retained testes of the unilateral cryptorchid bucks compared to the control (normal) bucks. The number of interstitial cells per cross section of the testes was also significantly higher ($P < 0.05$) in the contralateral scrotal testes of the unilateral cryptorchids (757.88 ± 29.92) than in the testes of the normal bucks (677.30 ± 25.92). In the contralateral scrotal testes of the unilateral cryptorchids, the interstitial cells had abundant cytoplasmic organelles, particularly mitochondria, Golgi apparatus and smooth endoplasmic reticulum, while the retained testes showed evidence of degeneration characterized by irregular nuclear profile, accumulation of nuclear heterochromatin, abundant lipid droplets, cytoplasmic vacuolations and focal intercellular dilations of interstitial cells. These results suggest that the intra-abdominal testes were significantly degenerated and the impairment was probably from exposure of the testes to higher abdominal temperature. However, the interstitial cells of the contralateral scrotal testes of the unilateral cryptorchid bucks showed normal histology and compensatory hyperplasia.

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1. Introduction

West African Dwarf (WAD) goat is a short-legged goat, indigenous to the humid rain forest ecological zone of West Africa. It is the predominant breed of goat in Southern part of Nigeria (Oyeyemi et al., 2011). These goats are largely unimproved genetically but show a certain degree of tolerance or resistance to trypanosomiasis (Chiejina and Behnke, 2011). Among the WAD goats of Eastern Nigeria, unilateral cryptorchidism has been described as a common condition (Emehelu et al., 2005). The difference in prevalence of this condition between places may be attributed to some genetic, endocrine and environmental factors (Boisen et al., 2004; Preikša et al., 2005). The impact of cryptorchidism on testicular histology and spermatogenesis has been extensively reported (Forest, 2006; Thorup et al., 2010).

It is well established that testosterone is produced by interstitial cells (Shima et al., 2013a,b) and is essential for maintaining and stimulating normal spermatogenesis (Baker and O'Shaughnessy, 2001; Ramaswamy and Weinbauer, 2015). Bergh and Damber (1978) in a morphometric and functional investigation of the interstitial cells of experimental cryptorchidism in rats observed that interstitial cell function in the cryptorchid testis was maintained at 30 days but highly impaired at 100 days of age. In contrast to the above findings, Ezeasor (1985) observed impaired interstitial cell morphology characterized by extensive degeneration of the cell in cryptorchid testes. Interstitial cell hypertrophy and hyperplasia in scrotal testes were also reported in cryptorchid cases in other animal species (Carucci et al., 2003; Zaidi et al., 2005). These later authors inferred that the hypertrophy resulted from doubling of cell volume and nuclear volume. Clegg (1965) interpreted interstitial cell hyperplasia and hypertrophy as reflecting compensatory changes in the contralateral scrotal testis of unilateral cryptorchids. Ironically, other investigators noted atrophy of interstitial cells with various degrees of regressive changes in cell organelles (Chung and Brock, 2011). The conflicting reports on the

* Corresponding author.
E-mail addresses: godwin.okpe@unn.edu.ng, chiokpe@yahoo.com (G.C. Okpe), daniel.ezeasor@unn.edu.ng (D.N. Ezeasor).

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effect of cryptorchidism on the interstitial cell need further investigations to elucidate if there are hyperplasia and/or hypertrophy of the interstitial cells in unilateral cryptorchid cases. Therefore, the present study was aimed to examine the cytology and quantitative histology of interstitial cells in the scrotal and retained testes of the unilateral cryptorchid goat in Nigerian environment.

2. Material and methods

2.1. Compliance with ethical standard

All institutional and National guidelines for the care and use of animals for experiment were followed. Permission was obtained from the University of Nigeria Ethics committee on the use and care of animals for this experiment. The animals used for this study were later used for Gross Anatomy practical for our students.

Twenty adult male West African Dwarf (WAD) goats weighing between 6 and 10 kg were used for the study. The ages of the bucks were determined by dentition method as described by Wilson and Durkin (1984). The bucks were divided into two groups, Group A (10 bucks with fully descended testes) and Group B (10 bucks with unilaterally descended testes). The animals were dewormed using ivermectin (Ivomec®), and vaccinated against pest de petit ruminants (PPR) using tissue culture vaccine obtained from Nigerian Veterinary Research Institute, Vom. They were fed with giant star grass, elephant grass and spent maize grain. Water was provided *ad libitum*. The animals were allowed to acclimatize for a period of two weeks before the onset of the experiment.

2.2. Preparation of samples for light microscopy

Five bucks from each group were euthanized with an overdose of pentobarbitone sodium (Sagatal®). Testes samples collected from each buck were processed for light microscopy as described below.

Fixation by perfusion was done using a mixture of equal volumes of 10% neutral buffered formalin and Bouin's fluid as the fixing agent according to the methods of Ezeasor (1985). With a sharp blade, the testes were dissected out and weighed. The testes were then cut into thin slices of about 3–4 mm thickness. These slices were processed for light microscopy using the paraffin embedding technique. The sections were stained with haematoxylin and eosin (H&E) and toluidine blue stains, and then examined using the Leica binocular light microscope. Photomicrographs were captured using a Moticam Image plus 5.0 digital camera (Motic China Group Ltd.) attached to the Leica binocular microscope.

2.3. Preparation of samples for electron microscopy

Five bucks from each group were also euthanized using an overdose of pentobarbitone sodium (Sagatal®) as described above. Testes samples were processed for semi and ultrathin sections as described below;

Modified Karnovsky fixative composed of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 was used as primary fixative. The fixing agent was administered by perfusion as described by Ezeasor (1985). Very thin testis samples from each buck were post fixed in OsO_4 in Millonig's buffer, dehydrated in increasing concentrations of ethanol, cleared in propylene oxide and embedded in epoxy resin. Semi thin sections, 1 μm thick sections were cut using an ultra microtome, stained with toluidine blue and examined using a Leica microscope.

Ultrathin 50–90 nm thick sections were obtained using ultra microtome. They were stained with Reynold's lead citrate and saturated aqueous uranyl acetate. The sections were examined using a Philips CM 10 Transmission Electron Microscope. Micrographs were produced using an Olympus Mega View III digital camera (Olympus Corporation Japan) attached to the Transmission Electron Microscope.

2.4. Cell counts and dimensions

The numbers of interstitial cells per testis and per gram of testis were determined using the formula described by Castro et al. (2002). The dimensions (cell diameter and nuclear diameter) of the interstitial cells were determined using a standardized eyepiece micrometer.

2.5. Statistical analysis

Quantitative data were subjected to one way analyses of variance (ANOVA) for factorial design. Differences between means in groups were verified by Duncan's test, using Statistica 9.0 software (soft incorp.) Differences among means were considered statistically significant at $P < 0.05$.

3. Results

3.1. Number and dimensions of the interstitial cells

The quantitative histological parameters of interstitial cells are presented in Table 1. The number of interstitial cells per cross section of testis was found to be higher ($P < 0.05$) in the contralateral scrotal testes compared to the retained testes and also the normal testes, respectively. The number of interstitial cells per cross section of the retained testis was also higher ($P < 0.05$) than those of the normal testes.

From the findings, the total numbers of interstitial cells per testis and per gram of testis were significantly higher ($P < 0.05$) in the retained and contralateral scrotal testes of the unilateral cryptorchid bucks compared to the testes of the normal bucks ($P < 0.05$). However, both the interstitial cell numbers per testis and per gram of testis were observed to be similar ($P > 0.05$) in the retained and contralateral scrotal testes of the cryptorchid bucks.

The nuclear and the interstitial cell diameters were found to be similar ($P > 0.05$) in the scrotal testes of the unilateral cryptorchid bucks when compared with the testes of the normal bucks.

Some of the interstitial cells were arranged in clusters of 3–4 cells per cluster. The mean number of clusters per cross section of the testis was significantly higher ($P < 0.05$) in the contralateral scrotal testes compared to the retained testes and also the normal testes. However, there was no significant difference ($P > 0.05$) between the mean numbers of clusters in the retained testes of unilateral cryptorchid bucks and the testes of normal bucks. The mean numbers of interstitial cells per cluster were similar ($P > 0.05$) in all the groups.

3.2. Light and electron microscopy

In light microscopy, interstitial cells of the normal and the unilateral cryptorchid bucks were found in the intertubular tissues in close association with blood vessels. The cells were arranged singly but may appear in clusters of 3–4 cells (Figs. 1–3). The interstitial cells were irregular in shape, their nuclei were oval or elongated within which are aggregations of heterochromatin and single nucleolus. The cytoplasm contained numerous granules. Differences were not observed in the light microscopy of the interstitial cells of the testes of normal bucks and the contralateral scrotal testes of the unilateral cryptorchid bucks. Some Leydig cells in the retained testes were degenerated (Fig. 3).

In electron microscopy, the testes of the normal (control) and the contralateral scrotal testes of the unilateral cryptorchid bucks showed that adjacent interstitial cells were joined by junctional

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