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https://dx.doi.org/10.4314/nvj.v44i3.2 **ORIGINAL ARTICLE**

Effects Of Exogenous GnRH Stimulation On Testicular Spermatogenesis And Characteristics Of The West African Dwarf Buck.

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SUMMARY

The testis in male domestic animals has two primary functions: spermatogenesis and steroidogenesis, coordinated by testicular cells under GnRH control. This study examined the features of the testes of the bucks of the West African dwarf breed to assess the effects of additional GnRH-stimulated levels of FSH, LH, T, and estradiol from 3 to 12 months of age. Twelve 3-month-old bucks were divided in a random manner into GnRH-treated (n = 6) and control (n = 6) groups. Monthly, bucks were weighed, their testicular diameter measured, and two blood samples taken (n = 12) at a 1-hour interval to establish peripheral concentrations. The GnRH-treated group received 0.5µg/kg of GnRH intramuscularly the next day, while the control group received saline subcutaneously. Blood samples were taken 1 and 2 hours later to establish GnRH-stimulated levels. At 12 months, bucks were castrated, and testes were weighed and prepared for histological analysis. Testes diameters showed no significant difference ($p<0.05$): 24.52±0.43 mm (GnRH-treated group) and 25.92±0.45 mm (control group). However, yearling testicular mass differed significantly ($p<0.05$): 41.50±5.80g (GnRH-treated group) and 38.80±7.58g (control group). The number and make-up of seminiferous tubular cells (cells of Sertoli, germ cells in the form of spermatogonia, developing into spermatocytes, further maturing into spermatids, and ultimately forming spermatozoa) indicated increased spermatogenesis in the GnRH-treated group. It is concluded that GnRH super-stimulation increased spermatozoa production in the West African dwarf buck.

Key words: WAD buck, peripheral, GnRH, super-stimulation, seminiferous tubular cells.

INTRODUCTION

Goats contribute 12% of the milk, 17% of the meat, and 30% of the ruminant animals in Africa (Wilson, 1991). The goat known as the West African dwarf (WAD) goat is the most widespread and valuable native breed of goat in the 18 nations of West and Central Africa (ILCA, 1987). In Nigeria, the population of West African goats numbers about 11 million (Chiejina and Behnke, 2011), and at least 90% of this livestock is thought to be kept by small-holder rural goat owners who regard the goats as a valuable asset (Jabbar, 1998). These goats not only ensure that regular homeowners have access to food in the form of meat and milk, but they also offer gifts (skin leather) and can be sold when necessary to become a vital source of cash (Peacock, 2005). However, as economically important as these small ruminants are, their reproductive capacity has yet to be fully exploited during breeding. The testis of the buck is charged with two primary functions: steroidogenesis and spermatogenesis (Potter and DeFalco, 2017; Griswold, 2018; Houda *et al*., 2021). Steroidogenesis is the primary biological function of Leydig cells and is essential for the formation of secondary sex as well as the start and maintenance of spermatogenesis (Smith and Walker, 2014; Zirkin and Papadopoulos, 2018). As part of the spermatogenesis process, Sertoli cells play multiple important roles, including but not limited to creating an optimum environment for spermatogonia growth and nourishment (Mita *et al.*, 1982; Meroni *et al.*, 2019) and producing testicular fluid and testosterone-binding proteins, thereby increasing the concentration of intracellular testosterone (Wang and Han, 2019). For the testis to accomplish its functions, Leydig cells as well as Sertoli cells must be activated, respectively, by LH and FSH, generated by GnRH binding to pituitary gonadotropin receptor membranes (Dufau and Catt, 1978; Plant, 2015). This mechanism is thought to affect the growth of Sertoli cells and the morphological evolution of Leydig cells postpartum to increase both testis volume (size and diameter) and steroidogenic support for spermatogenesis onset and continuation (Benton *et al.*, 1995; Mutembei *et al.*, 2005a; Makela and Hobbs, 2019). Earlier research reveal that the deprivation of GnRH is associated with testicular atrophy and a decline in sperm quality, encompassing reduced density, poor morphological features, and diminished viability, without a direct impact on spermatogenesis itself (Chang *et al.*, 2021). The goal of this study was to determine whether additional exogenous GnRH stimulation given to the buck from three months of age to one year would improve spermatogenesis and testicular characteristics.

MATERIALS AND METHOD

Study location

The research was done in the Teaching and Research Farm of the University of Port Harcourt, Choba, Port Harcourt, Nigeria. The farm is situated between the latitudinal coordinates 4o53' 14"N to 4o54' 42"N and longitudinal coordinates 6º 54' 00"E to 6º 55' 50"E, as indicated by Chima *et al.* (2015), with an altitude of 374 meters, and temperatures ranging from 21.5° C during the cold periods to 32.5° C during the hot periods (Falling Rain Software, Ltd. 1996-2021).

Experimental animals

Twelve apparently healthy 3-month-old West African dwarf bucks were purchased from rural household farmers. Peste des petits ruminants (PPR) vaccination was given to the experimental animals, and they were allowed a two-week acclimatization period before the study began. The bucks were randomly separated into two groups of six bucks each: the experimental group (n=6) and the control group (n=6). The sample size is influenced by animal welfare concerns. They were housed together, with each animal properly identified and carrying a tag depicting its experimental group, in a spacious zinc-roofed concrete structure with a wooden platform on the floor to keep them from coming into contact with the concrete. Except when it rained, the animals were taken to the fields to graze and take in the sunshine while the goat pen was cleaned each day. During the course of the experiment, they were fed pasture and concentrates. Water and food were provided *ad libitum.*

Experimental design

Every month, starting from the third month of life until the twelfth month, the experimental animals were weighed, and the diameter of their right testicle (as it is naturally bigger than the left) was measured using a caliper. On the next day, the six bucks in the experimental group received GnRH (Cystoreline®) via the intramuscular route at a dosage of 0.5µg/kg body weight as prescribed by the manufacturer, while the six bucks in the control group were given an equivalent volume of normal saline subcutaneously. At 6 months of age, two bucks amongst those subjected to experimental procedure and two bucks amongst those not subjected and serving as a basis for comparison were castrated, and at 12 months of age, the eight remaining bucks-four from the group undergoing experiment and four from the group serving as the control in the experiment-were castrated to investigate the histological changes in the testicles. The castration was done under local anesthesia.

Ethical Approval

The University of Port Harcourt's Research Ethics Committee granted its approval with the reference number UPH/CEREMAD/REC/MM75/110.

Sample preparation

The extracted testicles were cleaned with a gauze bandage, and the weight was determined with the aid of a digitally sensitive scale after the removal of the epididymis and tunica vaginalis. The fixation of the testicular tissues, divided longitudinally into two halves, was done in 10% phosphate-buffered formalin and immediately transported to the laboratory for histology.

Slide preparation

With the aid of a microtome (Reichard Jung AG, Heidelberg), sections of 5µm thickness were made from the tissue blocks and placed on Super Frost-plus microscopic slides (Menzel Glaeser, D-38116 Braunschweig). Before staining, the microscopic slides were dried by leaving them at 60°C for an hour.

Procedure for staining

The H&E staining method, first introduced by A. Wissowzky in 1876 and detailed by Titford (2009), was employed. The fixation of the testis

sample blocks was done in a 10% formalin solution with buffering for 24 hours. Sections were soaked in xylene for 4 minutes to remove paraffin, then graded ethanol (99%, 95%, 70%, and 30%) was used for rehydration for 2 minutes before being rinsed for 5 minutes under flowing water. After 5 minutes of hematoxylin staining, the glass slides were rinsed for 5 minutes in flowing water until the sections exhibited a "blue" colouration. They were quickly immersed in 1% acid alcohol (1% HCl in 70% alcohol) to remove excess dye. The glass slides were washed in flowing water before being dipped in ammonia water until the parts turned blue, then rinsed in running tap water. The counterstaining took 10 minutes with 1% eosin, followed by 3 minutes under flowing water. Moisture was removed from the glass slides by washing them in increasing levels of alcohol concentrations (70%, 80%, 90%, and 100%). The slides were then cleaned with two xylene solutions. Finally, the slide was mounted on dibutylphthalate-polystyrene-xylene. A single slide was used to depict each testicular portion, as there is homogeneity across various areas inside the testis of the goat.

RESULTS

The testes diameters did not show any notable distinction ($p<0.05$) across the treatment groups at month 12. The testicular mass however differed significantly ($p<0.05$) (Table 1).

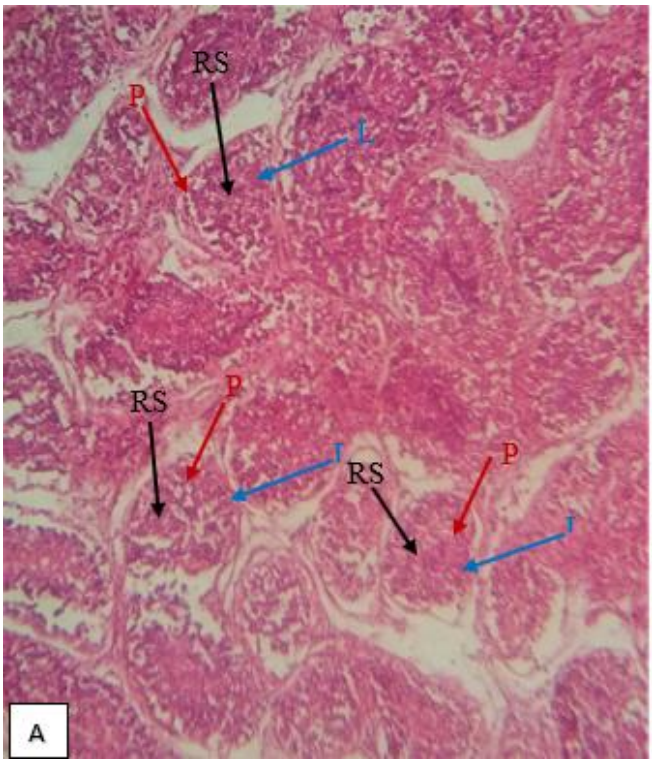
TABLE I: Independent sample t-test for average body weight and testicular diameter and in the GnRH-treated and control groups. (n=12).

Treatment	Buck weight (kg)	Testicular Diameter (mm)	Testicular mass (g)
GnRH	8.58±0.25	24.52±0.43	41.50±5.80[a]
Control	8.36±0.27	25.92±0.45	38.80±7.58[b]

a,b: Means with different superscripts are significantly ($p<0.05$) different.

Stages of Spermatogenesis in the GnRH-treated and control groups of the 6-Month-old testis.

Despite receiving GnRH treatment for four months, the testicular histology of a 6-month-old male deer still appeared immature. The majority of the seminiferous tubules were found to be in stage 1 of spermatogenesis, indicating the absence of any spermatid that is elongated or elongating. The most progressed spermatogenic germ cells observed were round spermatids, as shown in Figure 1.



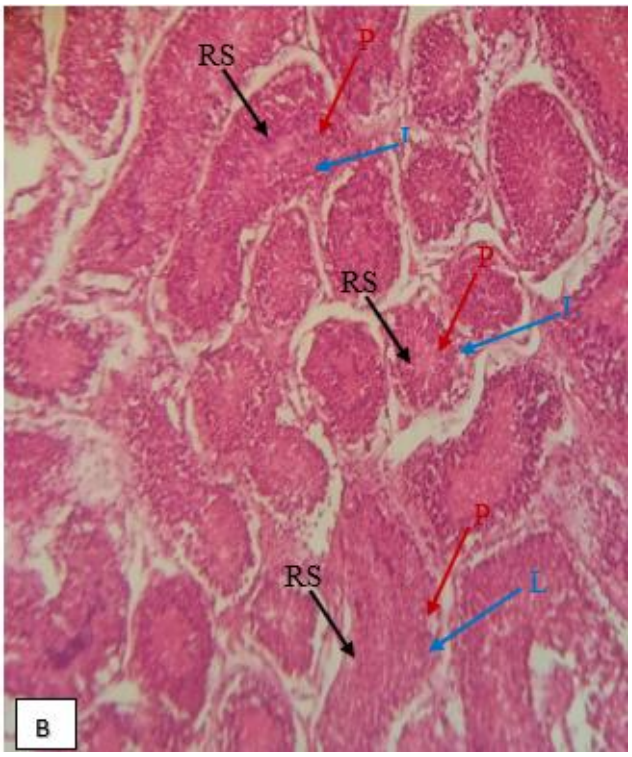


Fig. 1: (A, B) - Photomicrograph of a GnRH-stimulated testis from a 6-month-old buck, showing incomplete spermatogenesis. Round spermatids (RS) are observed closest to the lumen, while two generations of primary spermatocytes (L-leptotene and P-pachytene) are located at the basal region. Magnification x100.

Similarly, the control bucks exhibited testicular immaturity, with incomplete spermatogenesis noticed in a 6-month-old buck that had received saline treatment for 4 months. The bulk of the seminiferous tubules is in stage 1 of spermatogenesis, characterized by the absence of any elongated spermatids (ES). The most developed germ cells were round spermatids (Fig. 2).

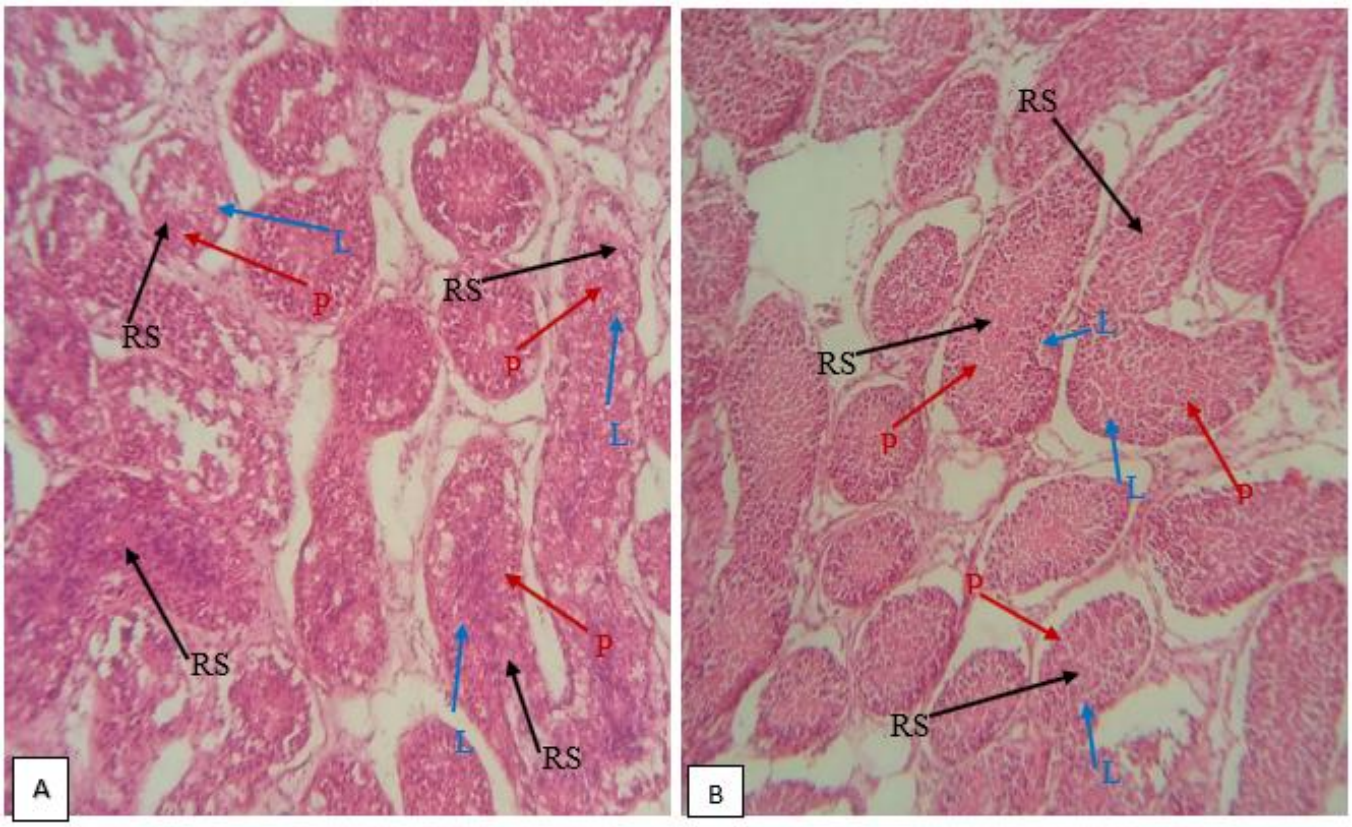


Fig. 2: (A, B):Testicular tissue photomicrograph of a 6-month-old control buck: RS are found closest to the lumen, and two generations of primary spermatocytes (L-leptotene and P-pachytene) are located basally. Magnification x100.

Stages of Spermatogenesis in the GnRH-treated and controlled groups of the yearling testis.

Complete spermatogenesis was visible in the histology of a yearling buck that had received GnRH therapy for 10 months, as indicated by the existence germ cells of all ages. The bulk of the seminiferous tubules is in stage 8 of the spermatogenic process, and most seminiferous tubules have undergone full and complete spermatogenesis. After detaching from their residual bodies (Rb), spermatozoa (Spz) move from the tubular epithelium. Residual bodies and spermatozoa can be detected in the tubule lumen (Figs. 3 and 4).

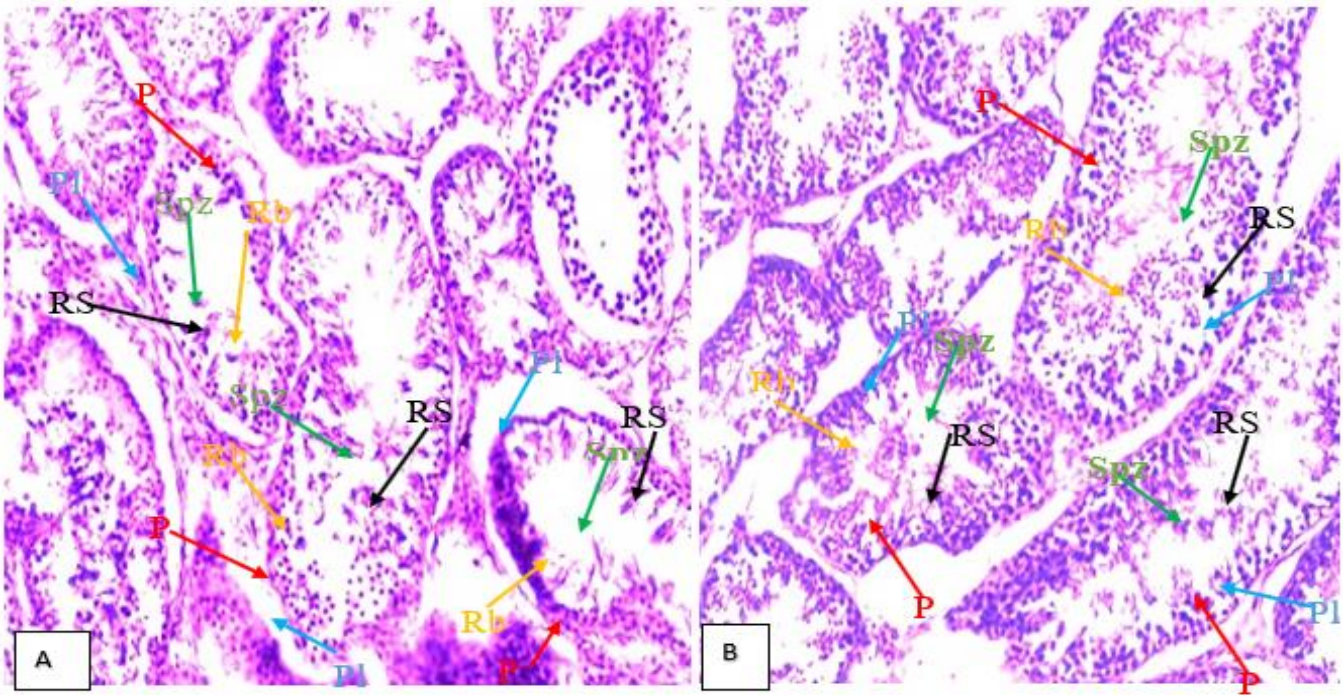


Fig. 3: (A, B): GnRH-stimulated testicular tissue photomicrograph of a yearling buck: RS with two generations of primary spermatocytes (L-leptotene and P-pachytene) are still present in the epithelium. Magnification x200.

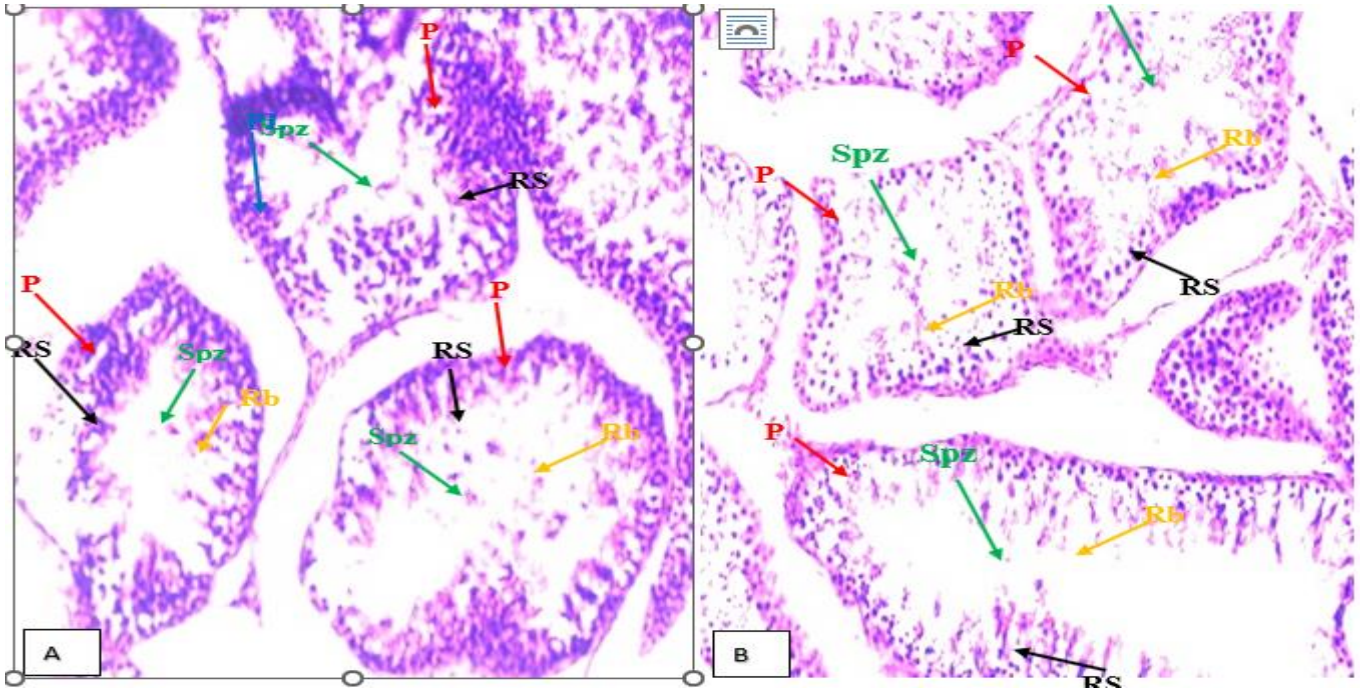


Fig. 4: (A, B): GnRH-stimulated testicular tissue photomicrograph of a yearling buck: RS with two cohorts of primary spermatocytes (L-leptotene and P-pachytene) are still present in the epithelium. Magnification x200.

Advanced spermatogenesis was noticed in the testes of a 12-month-old control buck given saline treatment. Within the lumen of the seminiferous tubules, residual bodies, round spermatids, and elongated spermatids were observed. Round and elongated spermatids can be found within the inner space of the seminiferous tubules (Figs. 5 and 6).

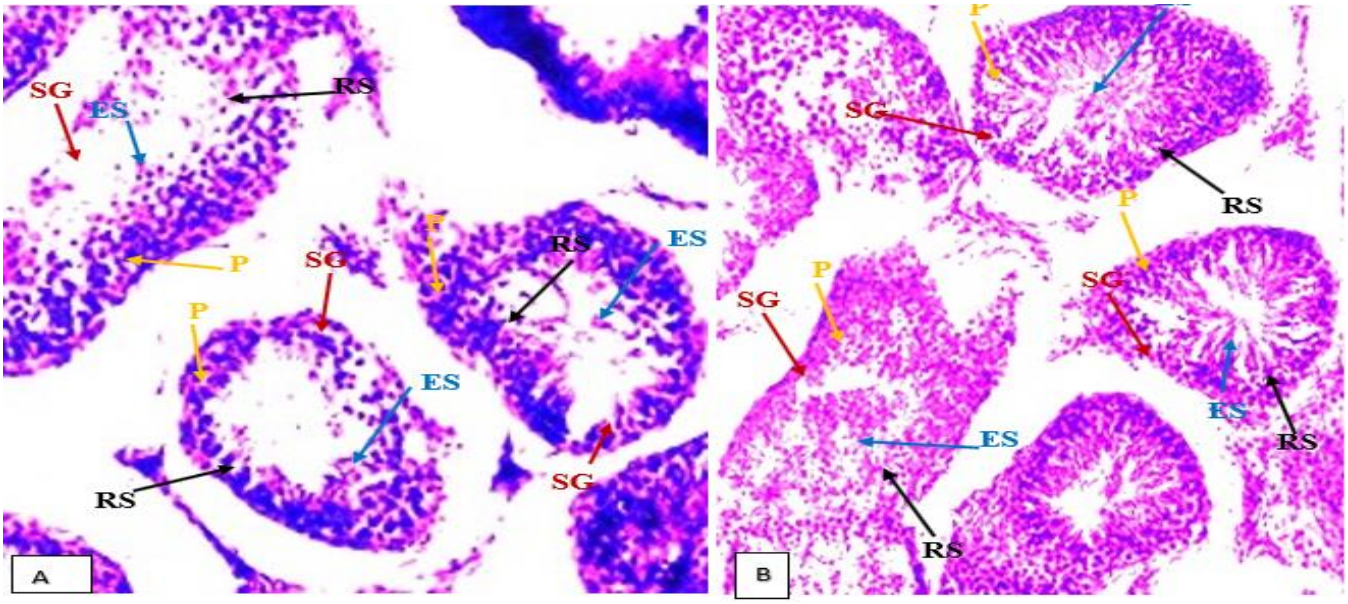


Fig. 5: (A, B): Testicular photomicrograph of a control yearling buck: Seminiferous tubules are mostly in stage 6 of spermatogenesis. RS, P, and SG) are present close to the basal lamina, and bundles of ES have shifted away from the SC with their tails sticking out into the tubule lumen. Magnification×200.

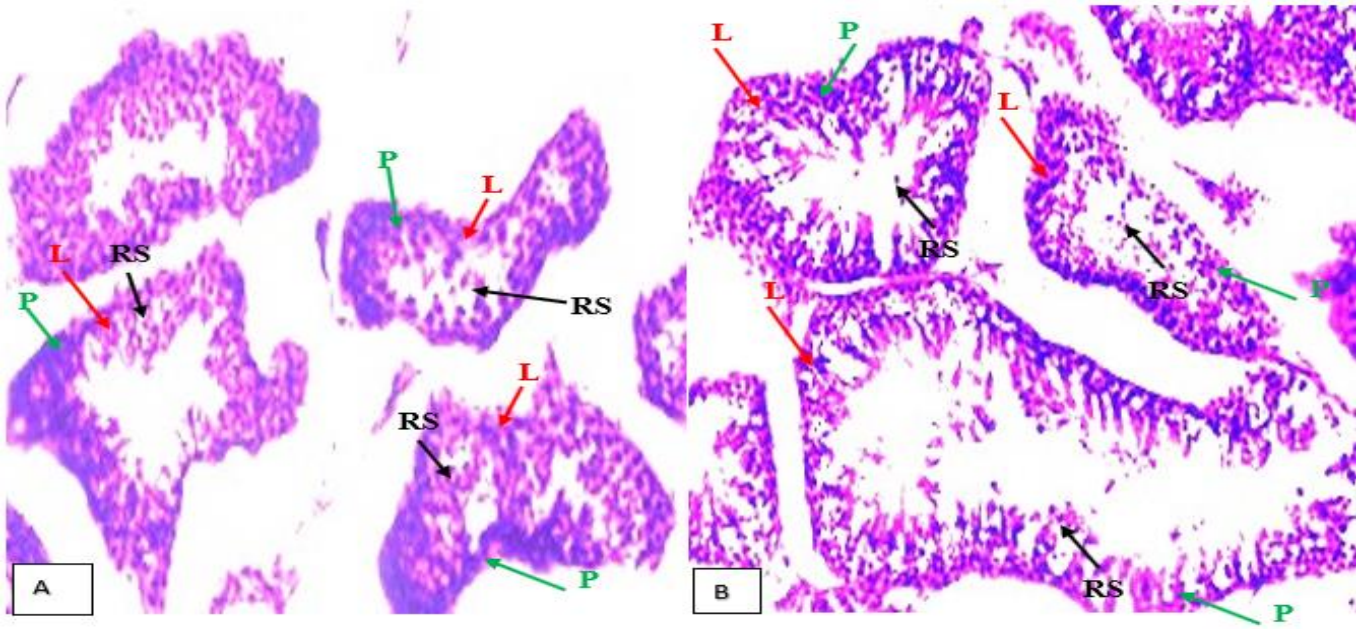


Fig. 6: (A, B): Testicular tissue photomicrograph of a control yearling buck: The majority of the seminiferous tubules are in stage 1 of spermatogenesis. RS reside nearest the lumen in two

generations of primary spermatocytes. This stage is distinguished by the absence of any ES. Magnification×200.

DISCUSSION

The testicular diameter of the GnRH-treated and control groups did not show a significant difference ($p<0.05$). However, their yearling testicular mass varied significantly ($p<0.05$). These variations can be attributed to the age and body weight of the West African dwarf buck, which influence testicular diameter, and the fact that exogenous GnRH stimulates testicular volume, as indicated by Vickery *et al*. (1985).Chang *et al.* (2021) reported that GnRH-deprivation resulted in testicular atrophy and reduced sperm quality, density, morphological characteristics, and viability. It is important to note that testicular mass is a more accurate measure of the sperm-producing parenchyma in the testis compared to testicular diameter, which only serves as a proxy for testicular mass (Tomlinson *et al.,* 2017)). At the age of 6 months, both the treated and controlled animals exhibited similar seminiferous epithelia, containing spermatogonia, spermatocytes, and round spermatids, but they lacked elongated spermatids, spermatozoa, and residual bodies. This indicated incomplete spermatogenesis and potentially testicular immaturity. However, at 12 months of age, after receiving sustained GnRH stimulation for 10 months, the seminiferous epithelia of the treated animals showed significant advancements. They now contained spermatogonia, spermatocytes, round spermatids, elongated spermatids, spermatozoa, and residual bodies. Similar cell types were also observed in the seminiferous epithelia of the controlled animals, but in lower quantities compared to the treated group. The GnRH stimulation resulted in a faster rate of germ cell maturation and increased spermatozoa production, suggesting a beneficial effect on spermatogenesis.This finding aligns with a study by Lin *et al.* (2019), which observed that GnRH pulse subcutaneous infusion can accelerate spermatogenesis and testicular maturation in humans. Similar positive effects of supplementary GnRH were also reported by Zirkin and Chen (2000) in yearling bulls. GnRH was found to increase Sertoli cells, improve testicular steroidogenesis, and enhance spermatogenesis. Nayak *et al.* (2022) also discovered significant beneficial effects of supplementing exogenous GnRH during the pre-monsoon season in Ganjam goat bucks, leading to improvements in various aspects of reproductive parameters in the goats. Regarding the stages of spermatogenesis, the results were consistent with previous studies by Leblond and Clermont (1952), Swierstra (1968), Clermont (1972), Parvinen *et al.* (1986), Hess (1990), Russell (1993a), Franca *et al.* (1999), and Mutembei *et al.* (2005). The majority of seminiferous tubules in the GnRH-treated animals showed two ages of round spermatids and primary spermatocytes at 200X magnification. Young leptotenes and aged pachytenes were still present in the epithelium, while spermatozoa detached from their residual bodies and progressed from the tubular epithelium. In the control group, elongated spermatids had moved out of the nuclei of Sertoli cells, with their tails protruding into the tubule lumen. Some animals showed spermatogonia, pachytenes, and round spermatids closest to the basal lamina in their tubules. In other animals, round spermatids were closer to the lumen, succeeded by two successive generations of primary spermatocytes at the basal region. According to earlier researchers, each individual interrelation of germ cells with Sertoli cells is termed a stage in

the cycle of seminiferous epithelium. The duration of a cycle in spermatogenesis depends on how long it takes for the same stage to return in a specific tubule segment. Additionally, species-specific variation exists in the proportion of seminiferous tubules that are actively undergoing spermatogenesis. According to Franca et al. (1999), a seminiferous cycle in goats lasts for 10.6 days and comprises 8 stages. Based on their description and the observed germ cell interaction in the testicular histology slides, the GnRH-treated bucks were in stage 8, while the control bucks were in stages 1 and 6. The spermatogenesis process in goats takes about 47.7 days before a spermatozoon is formed from a type A1 spermatogonium over the course of 4.5 cycles. Furthermore, Foster (2016) noted that a single segment of a tubule usually corresponds to a specific spermatogenic stage. A separate stage, but not always the following one, can be seen in an adjacent length of the same tubule. Thus, the seminiferous epithelium cycle develops over time as a comprehensive set of phases, and not every tubule is in its full state of spermatogenesis at any given time.According to Franca *et al.* (1999), Hess (1990), Kohler (2004), and Mutembei (2005b), the 8 stages of spermatogenesis can be categorized into three distinct phases: Phase I, which occurs right after spermiation and is marked by the complete lack of fully elongated spermatids (stage 1); Phase II, observed during the elongation of spermatids and defined by the presence of spermatids at different maturation stages (stages 2–6); and Phase III, corresponding to stages 7-8, visible before sperm release and distinguished by the existence of completely lengthened spermatids. According to these additional divisions, the control buck was in phases I and II, while the GnRH-treated buck was in phase III. These results suggest that the GnRH treatment possibly enhanced spermatogenesis in the treated group.

CONCLUSION

The anticipated outcome of super-stimulation is an increased spermatogenesis output, which is supported by the observed rise in germ cell numbers from round spermatids to elongated spermatids.

RECOMMENDATION

To enhance spermatozoa production during breeding in the West African dwarf goat, farmers may administer GnRH. Consequently, they might choose to give GnRH to selected breeding bucks with desirable traits before collecting semen for artificial insemination, preservation, and commercial storage. This approach aims to increase both the quantity and quality of spermatozoa available.

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