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Age-related changes in hormonal profiles and testicular diameter of West African Dwarf buck treated with gonadotropin-releasing hormone

IS Oruene[1]*, HM Mutembei[2] & AN Kipyegon[2]

1. *Department of Animal Science, Faculty of Agriculture, University of Port Harcourt, Port Harcourt, Nigeria*
2. *Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya*

*Correspondence: Tel.: +2348038880020; E-mail: drisoruene@gmail.com

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Abstract

This study aimed to investigate the impact of age-related peripheral and Gonadotropin Releasing Hormone (GnRH) -stimulated hormonal profiles on testicular size in West African dwarf bucks. Twelve 3-month-old bucks were randomly divided into two groups: the GnRH- treated group (n = 6) and the control group (n = 6). Over the course of 10 months, from month 3 to month 12, the testicular diameter of all bucks was measured monthly, and blood samples were collected monthly for hormone analysis. The results showed that testicular diameter increased with age and higher concentrations of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone (T). The GnRH-treated group reached its peak testicular diameter (28.36±1.44 mm) earlier at 8 months compared to the control group (30.50±1.44 mm) at 10 months, aligning with the peak concentrations of FSH, LH, and T. While the GnRH stimulation led to higher FSH, LH, T, and estrogen levels in the treated group, it didn't significantly affect the final testicular diameter ($p<0.05$). It can be concluded that hormonal levels rise with age and increasing testicular size, and GnRH stimulation can accelerate this process. Testicular size is an important factor in breeding soundness and is influenced by LH, FSH, and testosterone concentrations. These hormones can reliably indicate the reproductive capacity of the buck. Therefore, hormonal profiling should be an integral part of the breeding soundness examination.

Keywords: Buck, Estrogen, Follicle stimulating hormone, Luteinizing hormone, Testicular diameter, Testosterone

Introduction

The amounts of circulating hormones tend to vary with age in goats, similar to many other animal species whose studies have been documented in relation to testicular mass or diameter (Moura & Erickson, 1997; Patel *et al.,* 2017; Makela & Hobbs, 2019). During puberty, adult Leydig cells were demonstrated to multiply and grow exponentially, indicating a possible parallel pattern in the goat's testis (Tapaninen *et al.*, 1984; Li & Xie, 2005; Mutembei *et al.,* 2005).

Bucks can reach puberty as early as 4 to 8 months, although they might not be fully developed or have their best reproductive potential at that age (Noble, 2004). Hence, they are typically used for breeding around the age of 12 months. Their libido, fertility, semen quality, and volume are at their peak in late

summer and early autumn (Hillers *et al.,* 1984; Wildeus, 1999), which corresponds to the period when Testosterone (T) and Luteinizing Hormone (LH) levels are highest (Ritar *et al.,* 1990). Zarazaga *et al.* (2010) observed that when bucks experience a surge in plasma testosterone levels due to light exposure or the use of melatonin implants, it can lead to an elevation in their reproductive activity during the period of seasonal anestrus.

Testicular size is correlated with body growth (Ridler *et al.,* 2012), and according to Maroto-Morales *et al*. (2016), the testicular size of the buck is related to scrotal circumference, serving as a reliable gauge of sperm production.

Understanding the role of peripheral hormone activities in spermatogenesis is crucial for effective clinical utilization in breeding procedures for bucks. Therefore, this research aimed to investigate how age-related peripheral and GnRH-stimulated hormonal profiles influence testicular size patterns and mechanisms.

Materials and Methods

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 located at latitude 4º53' 14''N through 4º54' 42''N and longitude 6º 54' 00''E through 6º 55' 50''E (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.

Experimental animals

Twelve healthy apparently 3-month-old West African dwarf bucks were purchased from rural household farmers. *Peste des petits ruminants* (PPR) vaccination was given to the animals, and they were allowed a two-week acclimatization period prior to commencement of the study.

Ethical approval

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

The bucks were randomly divided into two groups of six bucks each: the experimental group and the control group. The properly tagged animals were housed together, in a spacious zinc-roofed concrete structure with a wooden platform placed 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. Throughout the period of the experiment, they were fed pasture and concentrates of 12 % crude protein. Water was 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 calliper. On the first day of each month, baseline pre-stimulated values of estrogen, FSH, LH, and testosterone were determined by collecting 2 ml of blood twice, with a 1-hour interval, through a jugular vein puncture for serum preparation.

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. Blood samples were collected serially via the jugular vein at one and two hours after treatment to determine the levels of GnRH and normal saline-stimulated estrogen, FSH, LH, and T.

Sample preparation

The blood samples were collected into red-top plastic vacutainers manufactured by Jiangsu HXRT MD Co., Ltd, China, and serum was extracted after leaving the blood sample overnight in a slant position for hormonal analysis using ELISA kits.

Hormonal analysis

Following the collection of serum samples, the concentrations of FSH, LH, T, and estrogen were determined using the Colorimetric Enzyme Immunoassay method. This technique relies on the Streptavidin Biotin sandwich assay principle, which was originally developed by Kato *et al*. (1977). The sensitivity of this method was 0.8 mIU/mL, and it detects changes in colour to yellow at a wavelength of 450nm. The analysis was carried out with the AutoElisa P. Elisa Plate Reader, a product of Labtech Company based in the United States of America.

Data analysis

The statistical package for the social sciences (SPSS) was used to analyze the data. The independent T-test was utilized to distinguish between the means that showed statistical significance. The values were presented as means ± standard errors. These values were entered in Excel sheets and represented as figures and graphs.

Results

The control group showed a pre-stimulated testosterone concentration increase from 1.40±0.62 ng/mL at 3 months to 4.33±0.44 ng/mL at 12 months. There was no significant increase ($p<0.05$) in testosterone concentration across the months after stimulation. Pre-stimulated testosterone concentration in the GnRH-treated group, ranged from 0.84±0.39 ng/mL at 3 months to 4.03±0.44 ng/mL at 12 months, and there was a significant increase ($p<0.05$) in testosterone concentrations across the months after stimulation from 2.90±0.47 ng/mL at 3 months to 5.59±0.52 ng/mL at 12 months, except at month 5 (2.79±0.43 ng/mL) and month 10 (5.14±0.52 ng/mL) (Figure 1).

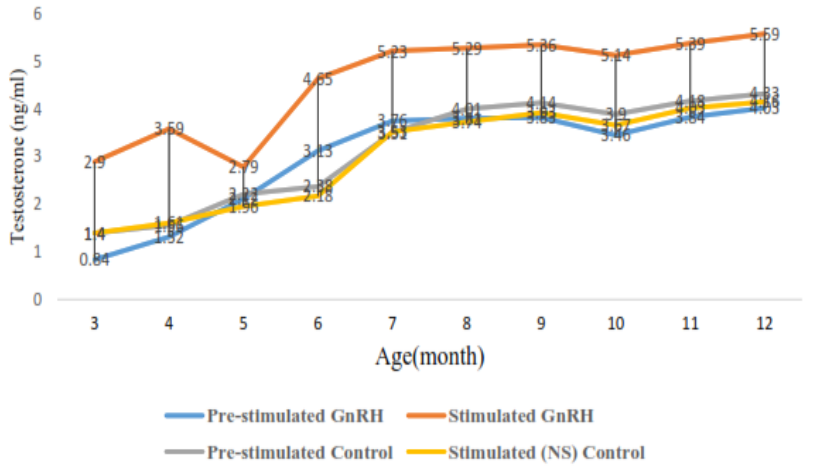

Figure 1: Testosterone concentrations following stimulation with GnRH in West African dwarf buck

In the control group, the pre-stimulated concentration of estrogen gradually increased with age, starting from the 3rd month where it was 77.50±30.15 pg/mL, and reaching 127±21.32 pg/mL at month 12. Pre-stimulated estrogen concentrations were higher in the GnRH-treated group at 140.00±19.07 pg/mL (month 3) and 138.63±21.32 pg/mL (month 12) (Figure 2).

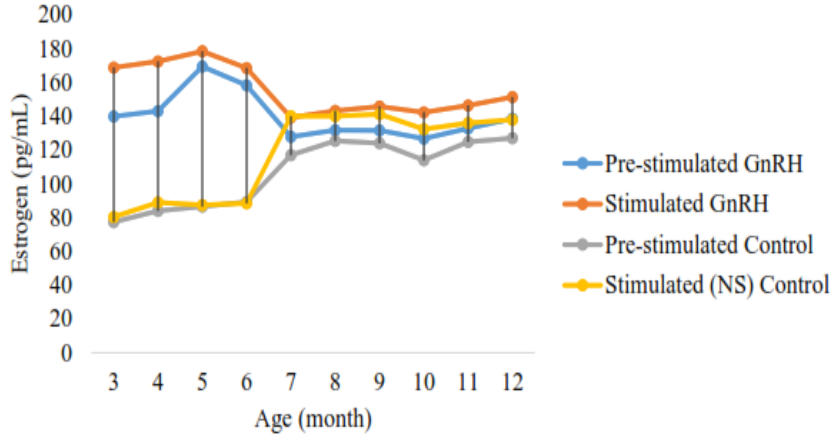

Figure 2: Estrogen concentrations following stimulation with GnRH in West African dwarf buck

There was a significant increase ($p<0.05$) in the values of FSH from the 7th month to the 12th month in the GnRH-treated group post-stimulation (Figure 3).

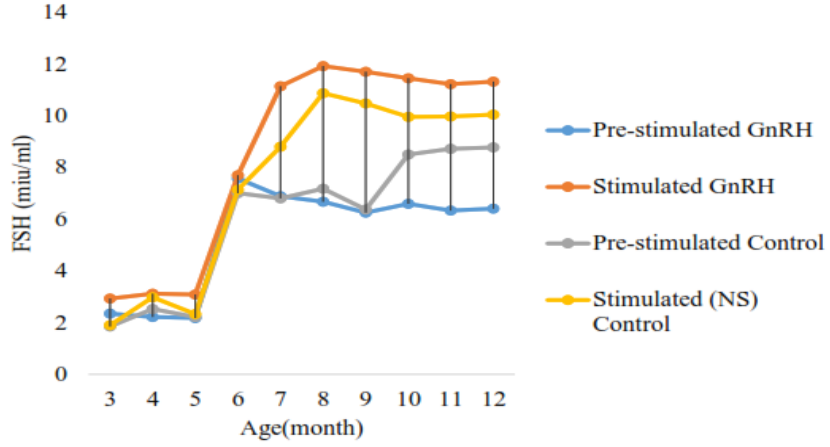

Figure 3: Follicle stimulating hormone FSH concentrations following stimulation with GnRH in West African dwarf bucks

LH pre-stimulated and stimulated concentrations were generally higher in the control group. After stimulation, the GnRH-treated group exhibited a significant increase ($p<0.05$) in LH values from month 9 to month 12 (Figure 4).

At month 3, the testicular diameter was 14.18±1.29 mm in the GnRH-treated group and 14.90 ± 0.00 mm in the control group. By month 4, it had increased to 19.94±1.17 mm in the GnRH-treated group and 20.78±1.38 mm in the control group. In month 5, the testicular diameter further increased to

20.89±3.94 mm in the GnRH-treated group and 23.84±3.15 mm in the control group. In month 6, the testicular diameter was 23.18±2.40 mm in the GnRH-treated group and 23.77±5.07 mm in the control group. At month 7, the GnRH-treated group (28.25±1.83 mm) had a larger testicular diameter than the control group (27.25±5.31 mm). The GnRH-treated group reached its peak at month 8 (28.36±1.99 mm) and maintained a relatively stable testicular diameter through months 9 (28.20±1.85 mm), 10 (27.95±1.87 mm), 11 (27.27±1.13 mm), and 12 (27.25±1.30 mm). In contrast, the control group reached its peak testicular diameter in month 10 (30.50±2.27 mm), slightly decreased in month 11 (29.60±3.05 mm), and slightly increased in month 12 (29.83±3.44 mm) (Figure 5).

After GnRH injection, testosterone levels peaked in the GnRH-treated group at two hours (4.90±0.15ng/mL), while the control group reached its peak before normal saline delivery (3.16±0.16ng/ml) (Figure 6). Similarly, estrogen concentration peaked 2 hours after GnRH treatment (161.15±7.00 pg/mL), whereas the control group showed its peak one hour after normal saline administration (123.42±7.11 pg/mL) (Figure 7).

Following the administration of GnRH and normal saline, FSH values peaked at two hours in the GnRH-treated (9.13±0.28miu/ml) and control groups (7.91±0.3miu/ml),

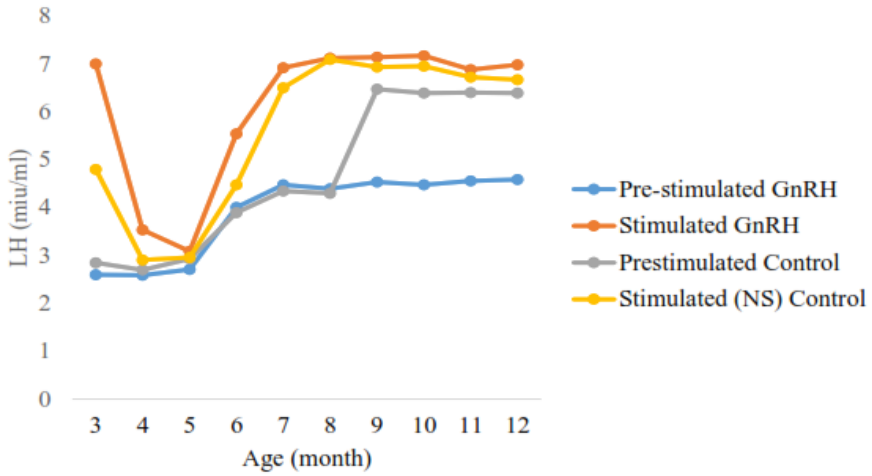

Figure 4: Luteinizing hormone concentrations following stimulation with GnRH in West African Dwarf buck

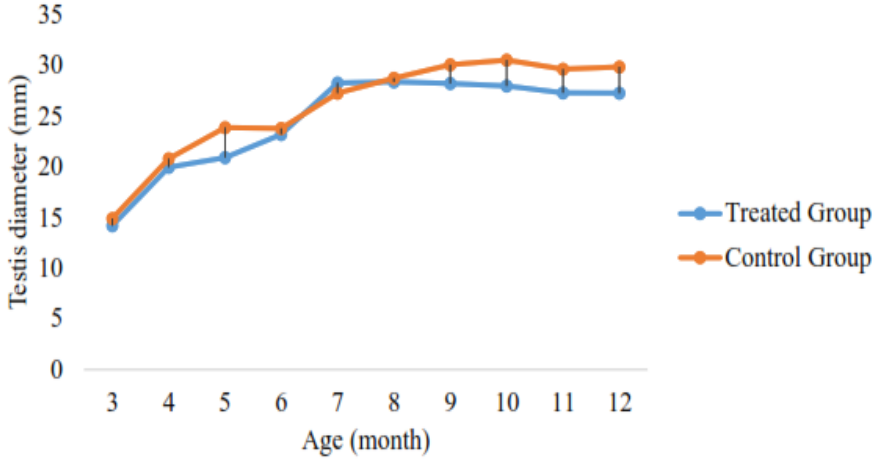

Figure 5: Testicular diameter of West African dwarf buck following stimulation with GnRH

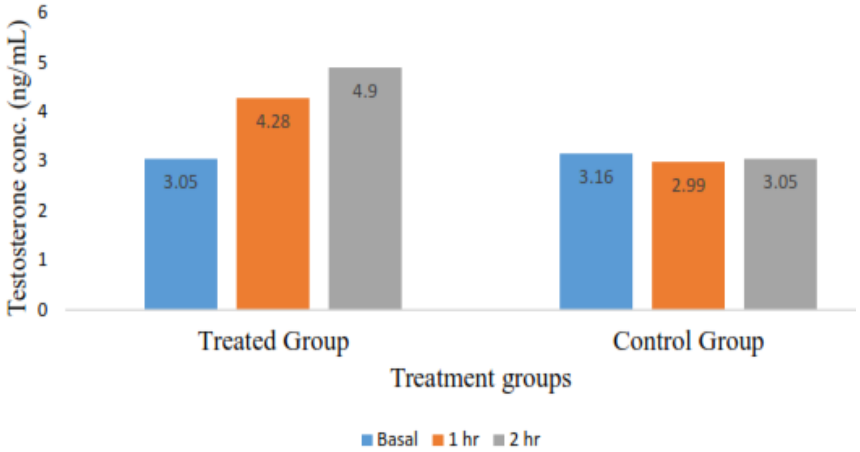

Figure 6: Time-related changes in testosterone levels following stimulation of West African dwarf buck with GnRH

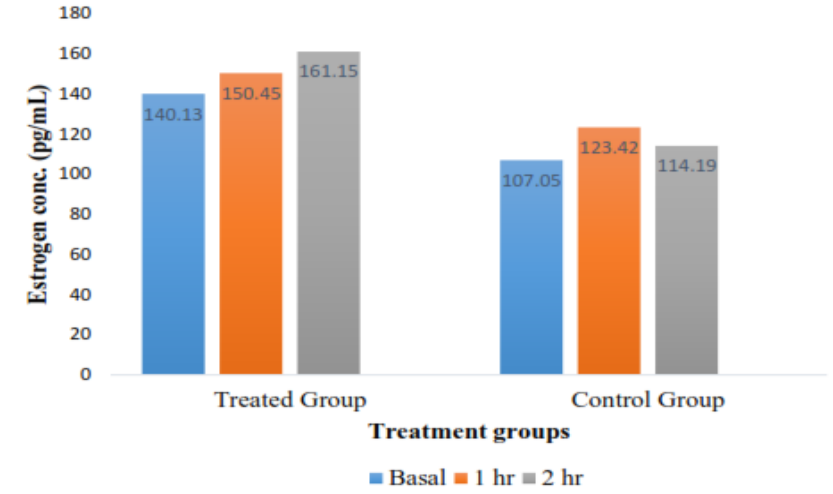

Figure 7: Time-related changes of estrogen in West African dwarf buck following treatment with GnRH

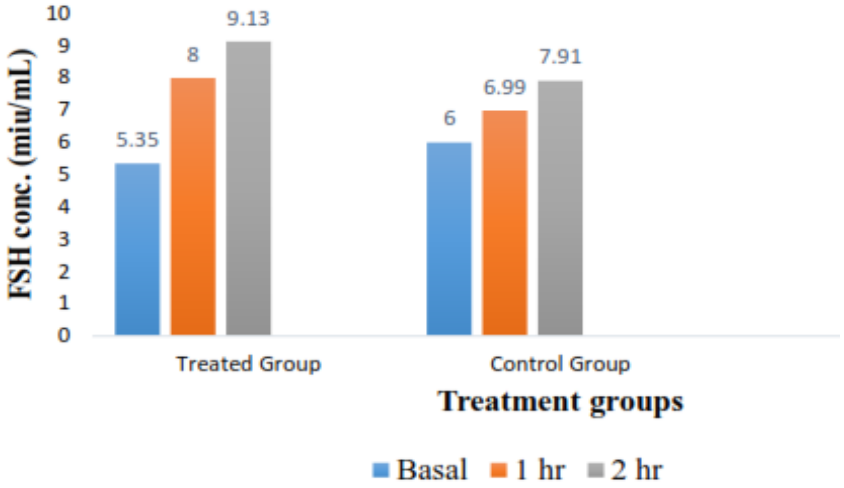

Figure 8: Time-related changes of follicular stimulating hormone (FSH) in West African dwarf buck following treatment with GnRH

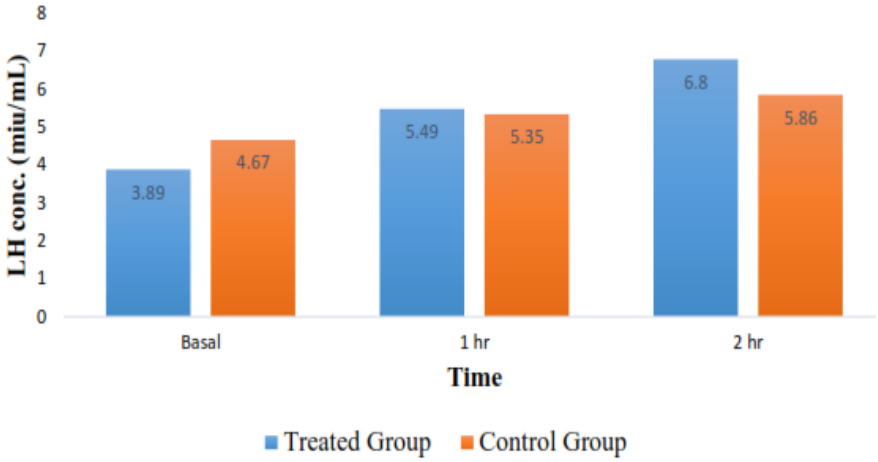

Figure 9: Time-related changes of luteinizing hormone (LH) in West African dwarf buck following treatment with GnRH

respectively (Figure 8). LH concentration exhibited a similar response, with the highest concentrations observed in the GnRH-treated (6.80±0.18miu/ml) and control groups (5.86±0.19miu/ml) two hours after injection (Figure 9).

Discussion

In general, as age advanced, the pre-stimulated LH, FSH, and testosterone levels showed a progressive increase in both the control and GnRH-treated groups. This is consistent with the typical rise in testosterone levels observed in intra-testicular tissues and peripheral blood, which is also positively associated with the rate of sperm production (Berndtson & Jones, 1989).

After GnRH stimulation, a significant increase in testosterone concentration was observed across different age groups. Additionally, the concentrations of LH and FSH also exhibited significant increases following GnRH stimulation across various ages. These findings are expected since GnRH stimulation leads to an increase in FSH and LH concentrations, and LH, in turn, stimulates testosterone production. Knol *et al.* (1993) reported similar observations, demonstrating that intravenous administration of GnRH induces notable and dose-dependent increases in plasma concentrations of LH and testosterone.

In an experiment to ascertain the link between endogenous testosterone and gonadotrophin production in humans, Bridges *et al*. (1993) found that LH and FSH are co-secreted, and that a surge of testosterone arrives 60 minutes after a surge of LH. There were declines in stimulated testosterone concentration across some of the months. The release of LH and FSH in a pulsatile manner by the anterior pituitary in response to GnRH stimulation could be responsible for the episodic nature of testosterone (Tsutsumi & Webster, 2009). These patterns across the months were irregular; these findings are consistent with those of Moura & Erickson (1997)

The variation in testicular diameter generally increased as age and hormonal concentrations rose, and reached its peak at 8 months old for the GnRH-treated group and 10 months of age for the control group. Similar patterns were observed in changes in scrotal circumference in West African dwarf bucks, indicating a positive relationship between scrotal circumference and age. Scrotal circumference progressively increases with age, and at around 8 months of age and older, a consistent measurement of 17cm–18cm is observed (Raji & Ajala, 2015). It appears that both testicular diameter and scrotal circumference were influenced by the animals' age, body weight, and hormonal profiles, aligning with the findings of Ajani *et al*. (2015).

Optimal testis function requires increased concentrations of LH and FSH to activate Leydig and Sertoli cells (Dufau & Catt, 1978; Plant, 2015). The GnRH-treated group showed a faster testicular growth rate and achieved peak testicular volume, possibly due to the effect of exogenous GnRH stimulation. According to Kuijper *et al*. (2008), humans experience rapid testicular growth from birth until the age of 5 months, which coincides with the peak value of LH and FSH observed during the early months of life, as noted by Andersson *et al*. (1998), Grumbach (2005), Hadziselimovic *et al.* (2005), and Kuiri- Hänninen *et al.* (2011). This enhancement of testis volume (size and diameter) and steroidogenic support facilitates the initiation and continuation of spermatogenesis by influencing the growth of Sertoli cells and morphological changes in Leydig cells (Benton *et al.,* 1995; Mutembei *et al.,* 2005; Makela & Hobbs, 2019).

Nayak *et al.* (2022) found significant beneficial effects of supplementing exogenous GnRH and the pre-monsoon season in their study on Ganjam goat bucks. These effects were observed in endocrinological profiles, scrotal circumference, testicular volume, testicular weight, sex behavioural profiles, sperm production, cryo-survivability, and fertility rate in Ganjam goats. While these findings largely align with the results of the present study, there was no substantial change in testicular diameter in this study The GnRH-treated group exhibited peak levels of FSH, LH, and estrogen two hours after the GnRH administration, while Moura & Erickson (1987) observed that peak levels of FSH, LH, and testosterone appeared three hours after the GnRH injection, except for estrogen, which reached its peak 4.5 hours after GnRH administration. These differences in the time of response may be attributed at least in part, to variances in animal species and the type of GnRH preparation used.

In conclusion, hormone concentrations show an unpredictable pattern of increase with age and testicular diameter, and they are accelerated by external stimulation using GnRH. Notably, the size of the testicles in goats, which usually reaches its peak at approximately 8 months, plays a vital role in breeding soundness examination and is influenced by LH, FSH, and testosterone concentrations. The concentrations of these hormones can effectively serve as an indicator of the reproductive capacity of the buck. It is recommended that in breeding soundness examination (BSE) of the buck, hormonal profiling could be a very reliable index, in addition to examining the age and testicular diameter, and hence should be included as an integral part of BSE.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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