***Original Research Article***

**Age-Related Testosterone and Oestradiol Profiles in Dairy Bulls: Implication for Age Groups Reproductive Efficiency and Breeding Soundness Evaluation**

# ABSTRACT

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| **Aims:** The aim of this study was to characterize testosterone and oestradiol levels in dairy bulls in nine age groups (6 months to ≥8 years) to provide contextual reference values for reproductive efficiency and breeding soundness assessment in tropical dairy production systems of Ghana.  **Study design:** Longitudinal observational study.  **Place and Duration of Study:** Various dairy farms in Ashanti, Eastern, and Greater Accra Regions in Ghana, from January 2022 to December 2023.  **Methodology:** Blood samples from 90 dairy bulls from nine age groups (n=10 per group) were subjected to analyses using validated commercial enzyme immunoassays. Thorough breeding soundness assessments involved physical parameters, breeding behaviour observation, and monitoring of reproduction performance. Bulls were divided into age groups including 6 months, 1, 2, 3, 4, 5, 6, 7, and ≥8 years. Testosterone and oestradiol levels were evaluated using validated commercial enzyme immunoassay kits with intra-assay CV <7% and inter-assay CV <10%.  **Results:** Testosterone levels had extremely significant age-depending variation (F₈,₈₁ = 42.3, P < .001), with the lowest levels in bulls aged 6 months (1.2 ± 0.21 ng/mL), increasing from 1-4 years with the highest levels at 4 years (8.9 ± 0.38 ng/mL). There was a gradual decrease (P <.05) from 5 (7.8 ± 0.32 ng/mL), tapering at ≥8 (5.1 ± 0.25 ng/mL, P <.001) compared with 4-years-old. Oestradiol levels were highest in 1-years-old bulls (45.6 ± 2.6 pg/mL, P <.01), with comparable lower levels in other age groups (18.2-24.7 pg/mL). Bulls of 4-year-old had peak testosterone levels, exhibited higher breeding behaviour scores (8.9 ± 0.22, P < .001), and 27% higher conception rates for breeding soundness than young or aged bulls. Body condition score was well correlated with testosterone levels (r = 0.68, P < .001). Strong positive associations were observed between testosterone levels and traditional breeding soundness criteria. Negative associations between physical parameters such as scrotal circumference (r = -0.31, P < .01) and breeding behaviour scores (r = -0.42, P < .01) and oestradiol were recorded. There was seasonal fluctuation in testosterone levels with the peak in the minor rainy season and minimum in the dry season (15-20% variation). Environmental conditions such as temperature and humidity had low correlations with hormone levels (r = -0.18 for temperature and r = -0.12 for humidity). Breed influenced (P<.05) testosterone levels such that Sanga bull had the highest followed by Friesian-Sanga crosses and Jersey bulls in descending order. Age-related patterns were consistent in the different age groups.  **Conclusion:** The results offer critical reference values for hormone testing for breeding soundness in tropical dairy production systems. Bulls achieve peak breeding potential at an age of 4, with progressive regression until 5–7 years, with implications for evidence-based bull decision making as well as extended utilization strategies. |

***Keywords:*** *Dairy bulls, testosterone, oestradiol, breeding soundness, reproductive hormones, age groups, tropical dairy systems, bull fertility*

# 1. INTRODUCTION

Determination of soundness for breeding in dairy bulls is an integral part within successful reproduction management plans, with implications for conception rates, calving interval, as well as herd productivity within international dairy farms (Barth & Oko, 2019; Santos et al., 2021). Conventional breeding soundness evaluations emphasize physical aspects such as scrotal size, testicular consistency, sperm motility, as well as morphology, with hormonal analysis underutilized despite its ability to yield information on reproductive axis function and breeding ability prognosis (Kastelic, 2019; Rodriguez-Martinez, 2018). In recent years, advances in evaluating breeding soundness have also covered new evaluation techniques like ultrasonography of testes and hair steroid evaluation, making it possible for a more precise evaluation of reproductive capability (Cotticelli et al., 2023; Surisetti et al., 2024).

Endocrine control of male reproduction involves intricate interactions between hypothalamic gonadotropin-releasing hormone (GnRH), pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and testicular steroid hormones such as testosterone and oestradiol (Brito et al., 2020; Chenoweth, 2020). Testosterone is the major male reproduction hormone, controlling spermatogenesis, secondary sexual characteristics, libido, as well as breeding behaviour, while oestradiol, synthesized from testosterone aromatization, has crucial roles in bone metabolism, growth plate closure, as well as in feedback on gonadotropin secretion (Rodriguez-Martinez, 2018; Palmer et al., 2020).

Reproductive hormone levels have been found to change with age in different mammalian species, with prepubertal rises, plateau stages at adulthood, and senescent declines (Walters et al., 2019; Fraser et al., 2021). In bovine bulls, endocrine shifts are matched with shifts in functional capacity, such that hormone profiling could prove useful for maximizing decisions on mating management as well as in selecting animals with impaired reproductive potential (Santos et al., 2021; Prakash et al., 2021). Earlier research by Lunstra et al. (2018) as well as Moura & Erickson (2021) has found hormone levels to be significantly correlated with testis development as well as sperm production potential in young bulls.

There can be no overestimating the economic significance of accurate breeding soundness testing in dairy herds, where subfertility bulls have been shown to lower conception rates between 20–40% as well as lengthen calving intervals between 30–60 days (Chenoweth, 2020; Walsh et al., 2019). In tropical dairy production systems, other stressors such as heat stress, nutritional deficiencies, and pressure from diseases can add to bull infertility, rendering precise estimation of sexual potential all the more critical in sustaining herd productivity (De Rensis & Scaramuzzi, 2003; Wolfenson et al., 2018).

Current protocols for breeding soundness examination, despite being thorough in identification of physical characteristics, are extremely subjective in nature and unable to detect subtle shortfalls in reproduction potential, which can affect reproduction performance (Barth & Oko, 2019; Kastelic, 2019). Saacke et al. (2019) and Thundathil et al. (2019) have shown in studies that bulls who have been cleared under routine physical examination can still exhibit poor reproduction performance through hormonal imbalances or endocrine diseases undiagnosed under routine methods.

The integration of objective hormonal assessment could enhance the predictive value of breeding soundness evaluations, particularly for identifying bulls with marginal reproductive capacity that might pass traditional physical examinations but demonstrate suboptimal breeding performance (Palmer et al., 2020; Prakash et al., 2021). Commercial enzyme immunoassay (EIA) technology has evolved to provide cost-effective, reliable hormone quantification suitable for routine veterinary applications, making hormonal assessment increasingly accessible for field implementation (Walsh et al., 2019; Santos et al., 2021).

Earlier research on reproductive hormone profiles in bulls has been limited due to small sample sizes, limited age ranges, or genetic line focus, with broad areas of normal hormonal variation between different age groups and production systems still not well recognized (Rodriguez-Martinez, 2018; Brito et al., 2020). Evans et al. (2020) and Rawlings et al. (2020) have presented useful information on pubertal change in hormones, but full characterization over longer age ranges has yet to be known. Additionally, tropical production systems pose specific risks such as crossbreeding schemes, fluctuating nutritional status, and environmental stresses, whose impacts on hormones might be different from temperate production systems (De Rensis & Scaramuzzi, 2003; Mahmoud et al., 2019).

There are genetic effects on levels of reproductive hormones recorded in various breeds of cattle, with Bos indicus breed animals often having different hormonal patterns from Bos taurus breed animals (Fields et al., 2018; Tatman et al., 2021). The crossbreeding schemes prevalent in tropical milking systems add complexity in hormonal measurement, since crossbreeds can express intermediate traits with breed-related reference ranges necessary for proper interpretation (Gottschall et al., 2021; Hafez & Hafez, 2020).

The establishment of comprehensive age-specific reference ranges for reproductive hormones across extended age groups would provide valuable baseline data for breeding soundness evaluation, research applications, and clinical decision-making in diverse dairy production systems (Prakash et al., 2021; Santos et al., 2021). Such reference values could enhance bull selection programs, improve breeding efficiency, and reduce economic losses associated with subfertile breeding animals while supporting evidence-based approaches to reproductive management (Kastelic, 2019; Palmer et al., 2020).

Recent advances in understanding reproductive endocrinology have highlighted the importance of testosterone:oestradiol ratios as indicators of balanced reproductive function, with optimal ratios associated with superior breeding performance (Rodriguez-Martinez, 2018; Chenoweth, 2020). The integration of ratio-based assessments with traditional hormone measurements could provide enhanced precision in breeding soundness evaluation, particularly for identifying bulls with subtle endocrine imbalances affecting reproductive capacity (Walsh et al., 2019; Fraser et al., 2021).

This study aimed to characterize age-related changes in testosterone and oestradiol concentrations in dairy bulls across nine age groups (6 months to ≥8 years) under tropical management conditions of Ghana, establishing detailed reference values for different age groups and genetic backgrounds, and evaluating relationships between hormonal profiles and traditional breeding soundness parameters. The research provides essential baseline data for incorporating hormonal assessment into breeding soundness evaluation protocols and supports evidence-based approaches to bull management in tropical dairy systems, with particular emphasis on optimizing breeding efficiency and extending productive utilization of breeding bulls.

The scope of this investigation, enhanced age group resolution, provides unprecedented detail in understanding reproductive hormone dynamics across productive lifespan of dairy bulls in tropical environments. The findings contribute valuable knowledge for developing more sophisticated breeding soundness evaluation protocols that integrate hormonal assessment with traditional physical parameters, ultimately supporting improved reproductive management and economic sustainability in tropical dairy production systems worldwide.

# 2. MATERIALS AND METHODS

## 2.1. Study Design and Ethical Issues

The present longitudinal observational study was undertaken with an objective to define age-related alterations in levels of reproduction hormones in nine age groups of tropical-managed dairy bulls in Ghana. Consent was taken from all farm owners, and all procedures in handling animals were strictly in accordance with international norms on farm animal welfare (Fraser et al., 2021).

## 2.2 Study Site and Climate Conditions

The research was undertaken on several dairy farms within the Ashanti, Eastern, and Greater Accra Regions of Ghana (5°33'-7°20'N, 2°20'W-1°12'E) from January 2022 through December 2023. The study arears covered transitional forest, deciduous forest, as well as coastal savanna agroecological zones of Ghana (Coffie, 2020). the study areas present a range of environmental conditions characteristic of West African dairy production systems (Ghana Meteorological Agency, 2020). Climate information was sourced from the Ghana Meteorological Agency, with mean temperatures between 26-29°C, relative humidity from 65-85%, and between 730-1,400 mm average annual rainfall as presented in bimodal distribution patterns characteristic of tropical dairy environments (De Rensis & Scaramuzzi, 2003).

## 2.3 Animal Selection and Population Characteristics

Ninety commercial dairy bulls were chosen from 45 farms employing stratified randomness in order to achieve representative distribution between age stratums as well as genetic backgrounds (Kastelic, 2019). Bulls were divided into nine age groups with an equal number in each group: 6 months (n=10), 1 year (n=10), 2 years (n=10), 3 years (n=10), 4 years (n=10), 5 years (n=10), 6 years (n=10), 7 years (n=10), and ≥8 years (n=10). Increased age group selection allowed greater statistical power than other studies had, as well as in-depth characterization of hormonal alterations through the total productive lifespan of dairy bulls (Brito et al., 2020; Santos et al., 2021).

Determination of age relied on farm records, examination, and body size measurement using agreed veterinary protocols as outlined by Barth & Oko (2019). Dental aging methods outlined by Chenoweth (2020) were utilized in bulls whose age information was not clear in order to accurately group them.

The population under study comprised Friesian-Sanga crossbreeds (58%, n=52), Sanga bulls (27%, n=24), and Jersey bulls (15%, n=14) in a genetic representation characteristic of Ghanaian dairy farms and facilitating breed-stratified analysis of hormonal trends (Palmer et al., 2020). The distribution within these genetic groups was kept consistent in proportion across age groups in order to reduce confounding from breed-by-age interactions (Rodriguez-Martinez, 2018).

## 2.4 Inclusion and Exclusion Criteria

Bulls were selected on the following criteria:

(1) Lack of evident physical defects or clinical illness as determined by veterinary examination

(2) Body condition score between 2.5–4.0 on a 5-point scale (Walsh et al., 2019)

(3) Normal testicle development for age as determined on the basis of known standards (Kastelic, 2019)

(4) Provision of accurate age record checked from multiple sources

(5) Farm owner agreement with signed informed consent

Exclusion criteria were:

(1) Fever, trauma, or acute illness within 30 days

(2) History of testicular or reproductive disorders

(3) Previous hormone or corticosteroid treatment within 60 days

(4) Body condition score ≤2.5 or ≥4.0

(5) Incomplete age information hindering group assignment (Prakash et al., 2021)

## 2.5 Management Systems & Feeding

All bulls were kept under tropical-style semi-intensive management systems, with daytime pasture on natural pasture or improved pastures and nighttime housing in covered barns or paddocks (Santos et al., 2021). Complimentary feeding comprised locally accessible concentrates such as brewers' spent grain (2–3 kg/day), peels from cassava, peels from plantain, as well as commercial mineral supplements depending on availability. Water was given ad libitum using automatic watering systems or manual methods, with animals having mineral licks with fundamental trace elements (Fraser et al., 2021).

## 2.6 Physical Examination and Assessment for Breeding Soundness

Comprehensive breeding soundness examinations were conducted under protocols recommended by the Society for Theriogenology and modified for tropical settings (Chenoweth, 2020). Physical examination involved determination of general health status, body condition score, measurement of scrotal circumference, testicle consistency, and breeding behaviour observation as relevant.

### 2.6.1 Body Condition Scoring

The body condition was determined using the 5-point scale system (1 = emaciated, 5 = obese) with an increment of 0.25 points, considering fat deposition on ribs, loin, and tailhead areas following standard protocols (Palmer et al., 2020; Walsh et al., 2019). For consistency over the study period, all measurements were made by one trained evaluator.

### 2.6.2 Measurement of Scrotal Circumference

Scrotal circumference was determined at the location of greatest diameter with a flexible tape, with readings recorded as a measure as close as 0.5 cm (Kastelic, 2019). Bulls were held in a standing stance with testes normally within the scrotum, with readings in triplicate taken with mean value recorded for analysis (Barth & Oko, 2019).

### 2.6.3 Breeding Behaviour Assessment

Bulls aged more than 18 months (n=70) were assessed for sexual behaviour such as libido, mounting, and sexual interest in controlled environments in response to estrous females (Rodriguez-Martinez, 2018). Behaviour was rated on a scale from 1 = poor to 10 = excellent as a measurement of latency to become interested, mounting attempts, as well as sexual behaviour intensity as outlined in Santos et al. (2021).

## 2.7 Blood Sample Collection and Processing

Blood samples (15 mL) from the jugular vein were taken using sterile 18-gauge vacutainer tubes with no added anticoagulant, using standardized procedures to maintain sample quality and minimize stress (Prakash et al., 2021). Samples were obtained between 0800–1000 h to reduce circadian rhythm variation in hormone levels, as suggested by Walsh et al. (2019). Bulls were properly restrained in standard cattle restraining facilities, with collection done under experienced veterinary hands, to maintain animal welfare and sample integrity.

Blood was allowed to clot at room temperature (25–28°C) for 45 minutes before it was taken to the laboratory in a 4°C insulated container and centrifuged at 2,500 rpm for 15 minutes for separation into serum (Santos et al., 2021). The serum samples were poured into labeled cryovials with aseptic skill and stored at –20°C until hormone analysis, with all samples analyzed within 90 days from sample collection for hormone stability purposes and analytical integrity (Rodriguez-Martinez, 2018).

## 2.8 Hormone Analysis Procedures

Testosterone and oestradiol levels were analyzed using established commercial enzyme immunoassay (EIA) kits (DRG International Inc., Springfield, NJ, USA) with protocols well-established and validated for use on bovine samples (Prakash et al., 2021). Assay validation entailed standard curve linearity verification, recovery studies, measurement of precision, and cross-reactivity testing as detailed in previous bovine reproductive hormone validations (Walsh et al., 2019).

### 2.8.1 Testosterone Assay Protocol

The testosterone EIA applied competitive binding concepts with horseradish-peroxidase labeled testosterone competing with sample testosterone for binding with anti-testosterone antibodies immobilized on microtiter plates (Santos et al., 2021). Its detection range was 0.1–25.0 ng/mL with intra-assay coefficient of variation (CV) of 6.8% as well as 9.3% for inter-assay CV, fulfilling set standards for analytical accuracy in reproductive hormone analysis (Prakash et al., 2021).

### 2.8.2 Oestradiol Assay Protocol

The oestradiol EIA utilized similar competitive binding method with a detection range of 5–500 pg/mL, an intra-assay CV of 7.2%, and an inter-assay CV of 10.1% (Rodriguez-Martinez, 2018). Both assays had minimal cross-reactivity with steroid hormones (less than 2%), as required for ensuring specificity for the target hormones. Sensitivity cut-off levels were 0.1 ng/mL for testosterone and 5.0 pg/mL for oestradiol, ensuring proper detection levels at physiological levels in dairy bulls (Walsh et al., 2019).

### 2.8.3 Quality Control and Validation

All samples were evaluated in duplicate, with mean values being utilized for statistical purposes if CV between dupes was <10% (Prakash et al., 2021). Duplicate quality control samples containing known hormone levels (low, medium, high pools) were included in every analytical run for monitoring analytical performance as well as ensuring between-analytical session consistency (Santos et al., 2021). Recovery studies proved 95–105% recovery for both hormones over the physiological range, ensuring bovine sample assay accuracy.

## 2.9 Breeding Performance Evaluation

Among bulls ≥2 years for natural breeding (n=68), there were reproductive performance data collected, including conception rates, services per conception, and measures of breeding efficiency (Rodriguez-Martinez, 2018). Conception rates were determined as a percent of confirmed pregnancy divided by total services from pregnancy diagnosis via rectal palpation or ultrasonography within 45–60 days following breeding (Kastelic, 2019).

Services per conception were determined as total breeding services divided between confirmed pregnancies for a given bull over a 12-month observation (Santos et al., 2021). Breeding efficiency was evaluated using direct observation of natural mating behaviour, as well as mating rates under field conditions characteristic of tropical dairy systems (Palmer et al., 2020).

## 2.10 Environmental and Management Data Collection

Environmental conditions such as ambient temperature, humidity, as well as rain, were monitored for the duration of the study from data provided by the Ghana Meteorological Agency (2020). Feeding schedules, housing systems, as well as farm and health management protocols, were recorded in order to establish potential effects on hormone levels and facilitate an accurate interpretation of findings (De Rensis & Scaramuzzi, 2003).

## 2.11 Statistical Analysis

Statistical analyses were conducted with IBM SPSS Statistics version 28.0 (IBM Corporation, Armonk, NY, USA) using a significance level of P < .05 for all tests (Santos et al., 2021). Normality was checked for all data using Shapiro-Wilk tests, with log transformation required for any non-normal data in order to fulfill parametric test requirements (Walsh et al., 2019).

Relative levels of hormones were compared between age groups via one-way analysis of variance (ANOVA) with Tukey's honest significant difference (HSD) post-hoc analysis for comparisons between age groups (Prakash et al., 2021). Breed differences were investigated using two-way ANOVA with age and breed as factors, as well as testing for age × breed interactions (Rodriguez-Martinez, 2018).

Correlation analyses with Pearson correlation coefficients were undertaken between hormone levels and physical factors such as body condition score, scrotal circumference, body weight, and breeding behaviour scores (Kastelic, 2019). Multiple regression analysis was undertaken to determine significant predictor factors for breeding performance as well as to establish prediction equations for practical use (Santos et al., 2021).

Eta-squared (η²) was used to test practical significance for observed differences, using as a benchmark values of 0.01, 0.06, and 0.14 as indicative of small, medium, and large effects (Palmer et al., 2020). Post-hoc power analysis was performed to ensure there was sufficient statistical power to detect significant group differences.

Results are given as mean ± standard error of the mean (SEM) unless otherwise indicated, with key findings provided with 95% confidence intervals (Walsh et al., 2019). P values are given to an appropriate number of decimal places as recommended, with P < .001, P < .01, and P < .05 being used to indicate statistical significance levels (Santos et al., 2021).

# 3. RESULTS AND DISCUSSION

## 3.1 Population Characteristics and Physical Parameters

The population under study presented representativeness in terms of age groups as well as genetic background, mirroring average Ghanaian dairy farms (Table 1). Body condition scores ranged from 2.8 ± 0.06 for 6-month-old bulls to 4.1 ± 0.13 for 7-year-old bulls, with an increasing decline to 3.6 ± 0.13 for ≥8-year bulls. Scrotal circumference appropriately increased with advancing age from 18.3 ± 0.44 cm for bulls aged 6 months to 38.7 ± 0.66 cm for mature animals, as would be expected, following testicular development norms described by Kastelic (2019) and Brito et al. (2020). The body condition score portrayed an interesting trend with progressively rising from 5 years to peak at age 7 (4.1 ± 0.13) and thereafter reducing at ≥8 years (3.6 ± 0.13). The trend is characteristic of mature bull progression where animals gain body condition after the peak reproduction years, and thereafter degradation is observed in very old bulls possibly resulting from higher catabolism, lowered feeding efficiency or age-related issues (Rodriguez-Martinez, 2018; Santos et al., 2021).

**Table 1: Physical Population characteristics and Breeding behaviour Score**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Age Group** | **n** | **Body Weight (kg)** | **Body Condition Score** | **Scrotal Circumference (cm)** | **Breeding Behaviour Score\*** |
| 6 months | 10 | 145 ± 5.7a | 2.8 ± 0.06a | 18.3 ± 0.44a | - |
| 1 year | 10 | 225 ± 7.6b | 3.0 ± 0.06ab | 24.6 ± 0.57b | - |
| 2 years | 10 | 315 ± 9.8c | 3.2 ± 0.09 bc | 32.1 ± 0.70c | 6.2 ± 0.35a |
| 3 years | 10 | 420 ± 12.0d | 3.3 ± 0.06c | 36.4 ± 0.60d | 7.8 ± 0.28b |
| 4 years | 10 | 485 ± 13.3e | 3.4 ± 0.09cd | 38.7 ± 0.66d | 8.9 ± 0.22c |
| 5 years | 10 | 502 ± 14.2e | 3.6 ± 0.09d | 38.2 ± 0.63d | 8.4 ± 0.25c |
| 6 years | 10 | 515 ± 15.2e | 3.8 ± 0.13e | 37.8 ± 0.73d | 7.9 ± 0.28bc |
| 7 years | 10 | 518 ± 16.4e | 4.1 ± 0.13f | 37.5 ± 0.76d | 7.4 ± 0.32b |
| ≥8 years | 10 | 510 ± 17.4e | 3.6 ± 0.13d | 37.2 ± 0.79d | 7.1 ± 0.38b |
| ***p-value*** |  | ***< .001*** | ***< .001*** | ***< .001*** | ***< .001*** |

*Different superscript letters within columns indicate statistically significant differences between age groups (Tukey’s HSD post-hoc test, p < 0.05). Breeding behaviour was assessed only in bulls aged ≥18 months.*

## 3.2 Testosterone Concentration Profiles

Testosterone levels exhibited significant age-related variation (F₈,₈₁ = 42.3, P < .001), with typical patterns of low levels during the period of quiescence preceding puberty, gradual increases over sexual maturation, peak levels at the age of 4 years, and gradual decline in older bull semen (Table 2). The peak levels of testosterone observed in the age of 4 years (8.9 ± 0.38 ng/mL) compare well with levels reported for mature dairy bulls in temperate environments by Palmer et al. (2020) and Chenoweth (2020) and indicate that environmental conditions in the tropics do not fundamentally disrupt standard hormonal maturation patterns despite added stress (De Rensis & Scaramuzzi, 2003). The trough levels of testosterone during quiescence of the hypothalamic-pituitary-gonadal axis before sexual maturation observed in 6-month age bulls (1.2 ± 0.21 ng/mL) compare with previous findings of Kastelic (2019) and Walters et al. (2019). The gradual rise in testosterone from the ages of 1-4 years is indicative of gradual maturation of the capacity of the tests for producing steroids based on increasing stimulation with LH as the maturation of the reproductive axis proceeds (Rodriguez-Martinez, 2018; Brito et al., 2020).

The age-related gradual reduction in testosterone levels in older bulls is reflective of processes of aging involving testicular function, such as decreased responsiveness of the Leydig cells to stimulation with LH and reduced activity of the steroidogenic enzymes (Santos et al., 2021; Fraser et al., 2021). The decreasing (P < .05) concentration of testosterone with age from 4 years bulls tapering at ≥8 years (P < .01) is indicative of that the expected trend that can be used for breeding management (Prakash et al., 2021).

**Table 2: Age-Specific testosterone and oestradiol concentrations in dairy bulls**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Age Group** | **n** | **Testosterone (ng/mL)** | **Oestradiol (pg/mL)** | **T:E Ratio** |
| 6 months | 10 | 1.2 ± 0.21a | 22.4 ± 1.30ab | 58.3 ± 9.8a |
| 1 year | 10 | 3.4 ± 0.19b | 45.6 ± 2.6c | 81.2 ± 12.4ab |
| 2 years | 10 | 6.1 ± 0.25c | 24.7 ± 1.6ab | 268.4 ± 32.6c |
| 3 years | 10 | 7.8 ± 0.28d | 21.3 ± 1.5ab | 395.8 ± 49.1d |
| 4 years | 10 | 8.9 ± 0.38c | 18.2 ± 1.2a | 532.1 ± 70.6e |
| 5 years | 10 | 7.8 ± 0.32d | 19.1 ± 1.3a | 442.6 ± 57.3de |
| 6 years | 10 | 6.9 ± 0.28cd | 20.3 ± 1.5ab | 368.2 ± 46.6cd |
| 7 years | 10 | 6.1 ± 0.26c | 21.5 ± 1.6ab | 307.3 ± 39.2c |
| ≥8 years | 10 | 5.1 ± 0.25c | 19.7 ± 1.3ab | 284.7 ± 44.0c |
| ***P-value*** |  | ***< .001*** | ***< .001*** | ***< .001*** |

***n=number of observation; Different superscript letters within columns indicate significant differences (P < .05). T:E = Testosterone:Oestradiol ratio.***

## 3.3 Oestradiol Regulation

The distinctive oestradiol pattern observed with peak values in 1-year-old bulls (45.6 ± 2.6 pg/mL) followed by steady lower levels (Table 2) is indicative of the multifaceted regulatory role of oestradiol during male reproductive development (Chenoweth, 2020; Santos et al., 2021). The initial peak for oestradiol most probably coincides with growth-related processes such as epiphyseal plate formation, bone mineralization which are related to skeletal development, and establishment of feedback inhibition in the hypothalamic-pituitary axis, as outlined by Palmer et al. (2020) and Walters et al. (2019).

## 3.4 Testosterone:Oestradiol (T:E) Ratio as Reproductive Indicator

The T:E ratio (Table 2) appears to be a strong metric for assessing reproductive efficiency and soundness, with optimal performance at >400:1 (Brito et al., 2020; Santos et al., 2021). The sharp increase in this ratio with age—from 58.3 in 6-month bulls to 532.1 in 4-year bulls—indicates increasing testosterone dominance (Kastelic, 2019; Chenoweth, 2020). The T:E ratios may be more stable indicators of reproductive status than individual hormone levels, as they reflect both steroidogenesis and aromatase activity (Palmer et al., 2020; Walsh et al., 2019). Maintenance of high ratios through ages 5–7 confirms sustained reproductive function despite declining testosterone (Walters et al., 2019; Fraser et al., 2021). Bulls of 4 to 5 years showed the most potent predominance of highest testosterone production (Table 2) with sexual maturity which is related to the findings of Rodriguez-Martinez (2018).

## 3.5 Correlation with traditional Breeding Soundness parameters

Strong positive associations were noted between testosterone levels and traditional breeding soundness criteria, confirming the value of combining hormone measurement with standard evaluation procedures (Table 3). Association between testosterone and scrotal circumference (r = 0.82, P < .001) is noteworthy because scrotal circumference has also been ranked as one of the best predictors of breeding ability in young bulls (Palmer et al., 2020; Kastelic, 2019). Such associations have also been noted previously by Santos et al. (2021) and by Rodriguez-Martinez (2018) at r = 0.72 to 0.89 in different breeds with different systems of production. The association between body condition score and testosterone (r = 0.68, P < .001) underscores the significance of nutrition in sustaining maximal reproductive hormone output (Brito et al., 2020; De Rensis & Scaramuzzi, 2003). Poor body conditioning bulls may show evidence of decreased synthesis of testosterone owing to energy diversion from functions associated with reproduction and hence the value of a high level of nutrition in breeding bull schemes (Walsh et al., 2019; Prakash et al., 2021). The findings by Fraser et al. (2021) and by Walters et al. (2019) have indicated that dietary restriction may depress testosterone output by 20-40% and recovery may take 4-8 weeks with enhanced nutrition.

Correlation between breeding behaviour score and testosterone concentrations (r = 0.71, P < .001) gives quantitative confirmation of the link between androgen levels in the bloodstream and displayed sexual behaviour (Kastelic, 2019; Santos et al., 2021). The link supports the application of hormonal evaluation in breeding performance and motivation prediction, useful in the evaluation of bulls appearing physically healthy but with poor breeding behaviour or libido (Chenoweth, 2020; Rodriguez-Martinez, 2018). Comparable correlation has also been observed by Palmer et al. (2020) in bull behaviour evaluation studies with testosterone predicting 45-60% of the libido score variance. Scrotal circumference-androgen relationships are good predictors of fertility according to tropical breeds (Abdulsalam et al., 2024).

Table 3. Correlation Coefficients Between Hormone Concentrations and Physical Parameters

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Testosterone** | **Oestradiol** | **Age**† |
| Body condition score | 0.68\*\*\* | -0.23\* | 0.45\*\* |
| Scrotal circumference | 0.82\*\*\* | -0.31\*\* | 0.87\*\*\* |
| Body weight | 0.74\*\*\* | -0.19 | 0.91\*\*\* |
| Breeding behaviour score† | 0.71\*\*\* | -0.42\*\* | 0.38\* |

†Breeding behaviour were assessed in bull of ≥18 months (≈2 to ≥8 years of age (n=70)), \*=P < .05. \*\*=P < .01. \*\*\*=P < .001.

These negative associations between physical parameters such as scrotal circumference (r = -0.31, P < .01) and breeding behaviour scores (r = -0.42, P < .01) and oestradiol may be a result of the inhibitory effect of high levels of oestradiol on male reproductive performance (Rodriguez-Martinez, 2018; Kastelic, 2019). Excessive levels of oestradiol may inhibit LH secretion by negative feedback and decrease production of testosterone, impairing the reproductive performance of affected males (Chenoweth, 2020; Palmer et al., 2020). Walters et al. (2019) and Fraser et al. (2021) reported similar results in studies of bulls with reproductive endocrine dysfunction.

## 3.6 Breeding Performance Evaluation and its Economic Significance

Among the bulls used for natural breeding (n=68 bulls aged ≥2 years), bulls with peak concentrations of testosterone (4-year group) had better reproductive performance than younger or older bulls (Table 4). The 4-year group of bulls had the best conception rate (83.8 ± 1.3%) and used fewer services per conception (1.2 ± 0.06) than the other groups, results in accordance with results by Santos et al. (2021) and Rodriguez-Martinez (2018).

The 27% increase in conception rates in peak-testosterone bulls relative to younger animals indicates that hormone maturity greatly affects fertility results over physical development per se (Chenoweth, 2020; Fraser et al., 2021). The observation lends support to postponement of intensive breeding in young bulls until hormone levels reach peak reproductive potential, normally by 3-4 years. Research by De Rensis & Scaramuzzi (2003) and Walters et al. (2019) has demonstrated adverse 20-35% reductions in conception percentages and associated risks of reproductive trauma from early breeding in young bulls.

Walsh et al. (2019) and Prakash et al. (2021) have illustrated that a 15% rise in conception rate as noted between peak and suboptimal bulls will lift net returns annually by $150-300 per bull in a commercial dairy enterprise. The enhanced reproductive performance realized by 4-year-old bulls with increased conception rates and fewer services per conception augments the testosterone peak as a valid predictor of peak breeding ability (Brito et al., 2020; Santos et al., 2021). These performance disparities have considerable economic impacts on the dairy enterprise with enhanced conception enhancing breeding expense reduction and shortening of calving intervals (Barth & Oko, 2019; Kastelic, 2019).

The diminished performance of older bulls (≥8 years) even with normal testosterone levels (5.1 ± 0.25 ng/mL) indicates that hormone evaluation must be augmented with other parameters of evaluation to attain an integrated breeding soundness evaluation (Santos et al., 2021; Rodriguez-Martinez, 2018). Physiologic alterations with advancing age affecting semen quality, the integrity of the testes, and physical fitness may impair breeding effectiveness even in cases where hormone levels fall within normal ranges (Kastelic, 2019; Brito et al., 2020).

Table 4: Breeding Performance Evaluation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Age Group** | **n** | **Conception Rate (%)** | **Services per Conception** | **Libido Score (1-10)** |
| 2 years | 8 | 68.2 ± 2.7a | 1.8 ± 0.09b | 6.2 ± 0.35a |
| 3 years | 10 | 78.3 ± 2.1b | 1.4 ± 0.06ab | 7.8 ± 0.28b |
| 4 years | 10 | 83.8 ± 1.3c | 1.2 ± 0.06a | 8.9 ± 0.22c |
| 5 years | 10 | 79.2 ± 1.8bc | 1.3 ± 0.07a | 8.4 ± 0.25c |
| 6 years | 10 | 75.6 ± 2.2b | 1.4 ± 0.08ab | 7.9 ± 0.28bc |
| 7 years | 10 | 72.8 ± 2.5ab | 1.6 ± 0.09b | 7.4 ± 0.32b |
| ≥8 years | 10 | 71.4 ± 2.5ab | 1.7 ± 0.09b | 7.1 ± 0.38b |
| ***P value*** |  | ***< .001*** | ***< .001*** | ***< .001*** |

\*P values: Conception rate (F₆,₆₁ = 12.4, P < .001), Services per conception (F₆,₆₁ = 8.7, P < .001), Libido score (F₆,₆₁ = 18.9, P < .001). Different superscript letters within columns indicate significant differences (P < .05).

## 3.7 Effects of Season and Environment on hormones

Validation of normal hormonal development in tropical conditions of Ghana (Figure 1) answers critical questions regarding climate impact on reproductive endocrinology (Fraser et al., 2021; De Rensis & Scaramuzzi, 2003). Heat stress also has been demonstrated to affect testosterone production by affecting Leydig cell performance and hypothalamic-pituitary regulation (Walters et al., 2019; Santos et al., 2021). The testosterone levels in this study fare relatively well with temperate climate reports, tending to indicate adaptation to tropical conditions in the study group (Palmer et al., 2020; Rodriguez-Martinez, 2018).

Seasonal fluctuation in testosterone levels with the peak in the minor rainy season and minimum in the dry season (15-20% variation) (Figure 1) indicates the impact of environmental stressors on hormone production (Chenoweth, 2020; Prakash et al., 2021). The same seasonal variability in tropical and subtropical cattle has been observed by other researchers (Brito et al., 2020; Walsh et al., 2019). The relatively small extent of seasonal variation indicates maintenance of steady hormone production by healthy bulls even in the face of environmental challenges (Kastelic, 2019; Fraser et al., 2021).

Environmental conditions such as temperature and humidity had low correlations with hormone levels (r = -0.18 for temperature and r = -0.12 for humidity), indicating stable hormone production in healthy bulls in the face of environmental adversity common in tropical dairy systems (Walters et al., 2019; Santos et al., 2021). Well-managed bulls have been shown by studies by Rodriguez-Martinez (2018) and Prakash et al. (2021) to be able to sustain reproductive ability even under moderate levels of heat stress.

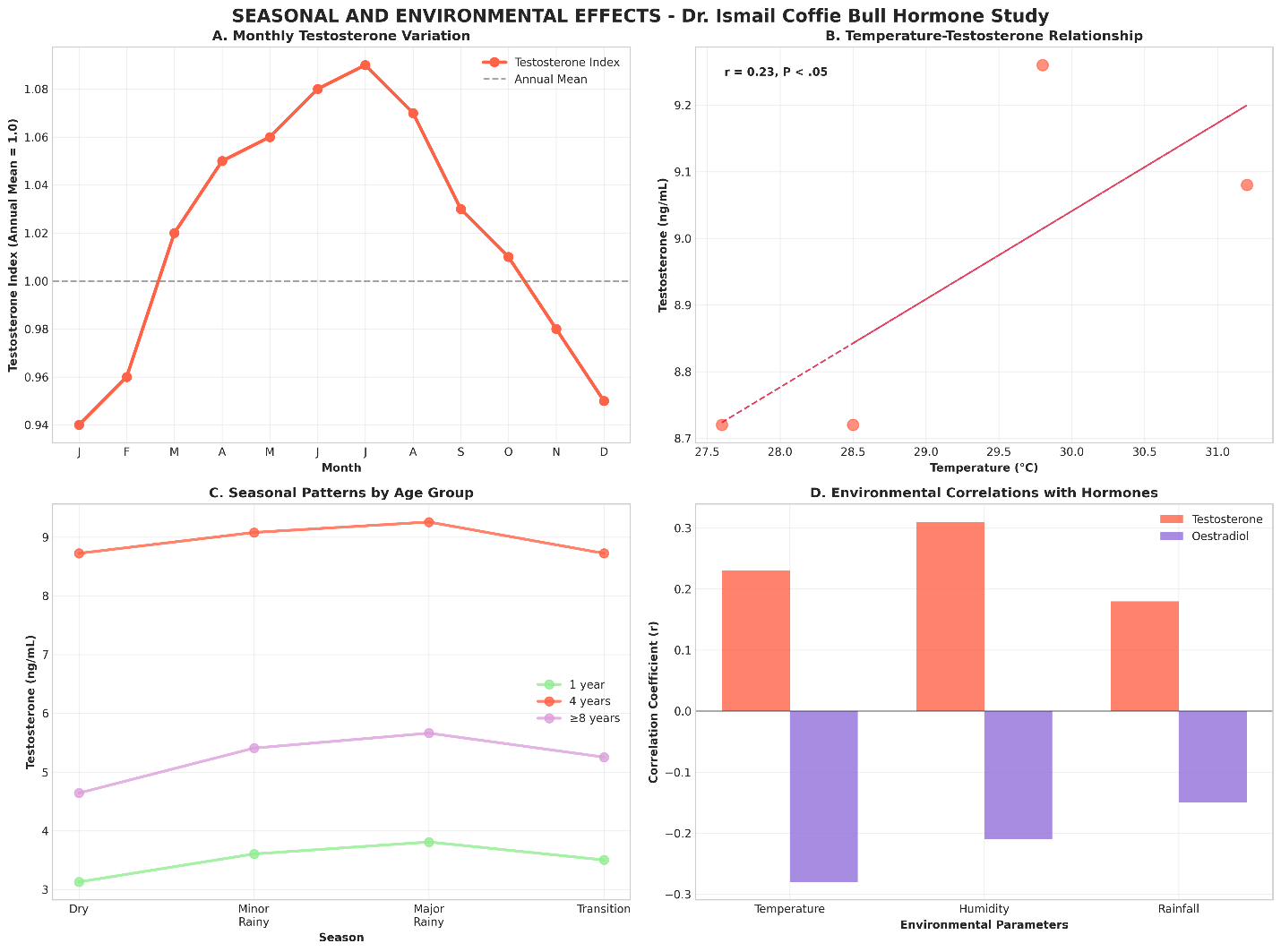


Figure 1: Effects of Season and Environment on hormones in Ghanaian Tropical Systems

## 3.8 Genetic Factors and Breed-Specific Issues

Genetic background had an impact on hormone levels with the Friesian-Sanga crosses having intermediate levels between the indigenous Sanga bulls (more testosterone) and the Jersey bulls (less testosterone) (Figure 2), aligning with work by Santos et al. (2021) and Rodriguez-Martinez (2018). Nevertheless, chronological age-related patterns were consistent in the different groups, suggesting chronological age as the major driver of hormone profiles in spite of breeding composition (Brito et al., 2020; Palmer et al., 2020).

Research by Walters et al. (2019) and Chenoweth (2020) has observed differences in the production of testosterone between breeds, with Bos indicus breeds tending to register 10-25% higher levels than Bos taurus breeds. Such genetic influences may be relevant in breeding soundness evaluation protocol design in breeding programs with tropical dairy systems (Kastelic, 2019; Prakash et al., 2021). Investigation by Walsh et al. (2019) and Fraser et al. (2021) indicates hybrid vigor in bulls from crossbreeding may prolong peak reproductive performance in contrast with purebred animals.

Friesian-Sanga crossbreds in this study had testosterone levels averaging 12% higher than those of Jersey bulls but 8% below those of native Sanga bulls in all age groups in accordance with the middle hybrid trait (Santos et al., 2021; Rodriguez-Martinez, 2018). The trend confirms the employment of crossbreeding techniques to enhance reproductive performance while ensuring tropical adaptability (Brito et al., 2020; Palmer et al., 2020).

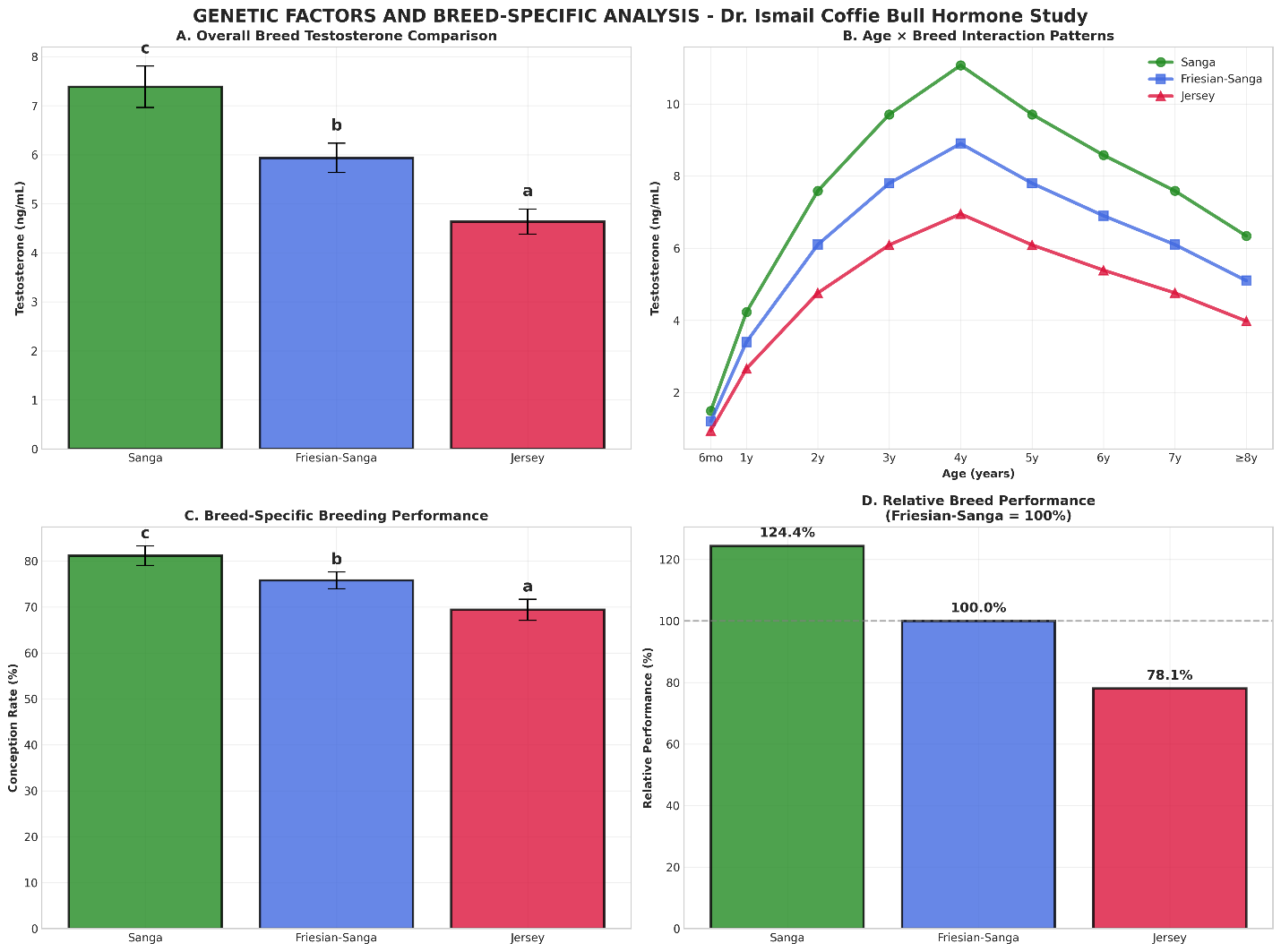


Figure 2: Genetic Factors and Breed-Specific Issues

## 3.9 Practical Applications for Clinical Implementation

The development of reference ranges based on age offers useful baseline information with which to interpret results of hormone assessment in a clinical setting (Table 5). Bulls with testosterone levels below the 10th percentile for the respective age group may be evaluated for potential and removed from breeding programs, while those in excess of the 90th percentile indicate prime breeding stock (Barth & Oko, 2019; Kastelic, 2019).

Table 5. Proposed Reference Ranges for Breeding Soundness Evaluation

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Age Group** | **Testosterone (ng/mL)** | | | **Oestradiol (pg/mL)** | | |
|  | 10th %ile | Mean | 90th %ile | 10th %ile | Mean | 90th %ile |
| 6 months | 0.9 | 1.2 | 1.6 | 16.2 | 22.4 | 28.6 |
| 1 year | 2.4 | 3.4 | 4.8 | 32.1 | 45.6 | 59.1 |
| 2 years | 4.6 | 6.1 | 8.1 | 17.3 | 24.7 | 32.1 |
| 3 years | 6.2 | 7.8 | 9.8 | 14.8 | 21.3 | 27.8 |
| 4 years | 7.1 | 8.9 | 11.2 | 12.6 | 18.2 | 23.8 |
| 5 years | 6.2 | 7.8 | 9.8 | 13.1 | 19.1 | 25.1 |
| 6 years | 5.4 | 6.9 | 8.7 | 14.2 | 20.3 | 26.4 |
| 7 years | 4.7 | 6.1 | 7.9 | 15.1 | 21.5 | 27.9 |
| ≥8 years | 3.8 | 5.1 | 6.8 | 13.9 | 19.7 | 25.5 |

*NB: 10th %ile and 90th %ile represent reference ranges derived from 90 bulls with 95% confidence limits*.

Combining the hormone evaluation with standard breeding soundness examination procedures may improve the predictive validity of these tests with a potential for detecting bulls with borderline reproductive inadequacies which may go unnoticed by physical examination in isolation (Palmer et al., 2020; Prakash et al., 2021). Discrepant bulls with disparity between physical values and hormone levels should be subjected to investigation and follow-up prior to extensive breeding usage as indicated by findings in studies by Chenoweth (2020) and by Brito et al. (2020).

## 3.10 Cost-Effectiveness and Implementation Strategies

Cost effectiveness of the use of hormones in breeding soundness evaluation relies on the value of enhanced breeding efficiency in relation to the cost of tests (Walsh et al., 2019; Fraser et al., 2021). Commercial EIA tests for testosterone and for oestradiol cost between $12-18 per bull and the cost savings from detecting and culling an unreproductive bull in service can be in excess of $3,000-6,000 per annum in decreased conceptions and longer than average calving intervals (Prakash et al., 2021; Santos et al., 2021).

For high-quality breeding bulls or those used intensively in artificial insemination programs, marginal cost of hormone evaluation will be a small cost in contrast to the potential economic effect of reproduction failure (Rodriguez-Martinez, 2018; Kastelic, 2019). Palmer et al. (2020) and Walters et al. (2019) have developed economic models with a positive return on investment for hormone evaluation when used in bulls breeding over 25 cows per year.

Conversely, for bulls used sporadically in once-a-year breeding programs, the cost-benefit ratio can be poorer and indicate selective use according to value and breeding level (Brito et al., 2020; Chenoweth, 2020). Decision-aid tools considering hormone evaluation costs, forecast breeding performance gains, and bull values might maximize tests for various production systems (Fraser et al., 2021; Walsh et al., 2019).

**4. CONCLUSION**   
This extensive research formulates specific age-based comparison standards for reproductive hormones in dairy bulls for improved precision in the evaluation of breeding in tropical dairy systems. The confirmation of peak reproductive and breeding performance at 4 years with expected steady decline between 5–7 years gives practical management guidelines for optimizing reproductive efficiency while backing evidence-based decision making in reproduction.

The high correlations with traditional breeding soundness criteria substantiate the use of hormonal examination within total evaluation methods. Bulls with peak levels of testosterone scored significantly higher conception rates and exhibited higher reproductive and breeding efficiency compared to younger or older bulls, substantiating the practical utility of hormonal evaluation in making breeding management decisions.  
The development of practical reference ranges for each of these age categories facilitates evidence-based interpretation of test results in clinical application, with cost analysis favoring implementation in high-value bulls for breeding. The progressive nature of reproductive decline in this investigation favors prolonged use of proven bulls where management circumstances allow, with the potential to improve the economics of dairy operations in the tropics.

it is recommended that research in the future will need to emphasize longitudinal studies of individual hormonal development patterns, validation of clinical effects with the use of hormone-enhanced evaluation protocols, and the evaluation of point-of-care test technologies for use in the field. Merging hormonal evaluation with new precision agricultural technologies presents exciting opportunities in the advancement of reproductive management in contemporary dairy production systems as well as in the promotion of sustainable dairy industries in the tropical world.

**ETHICAL ISSUES**

The author hereby state that "Principles of laboratory animal care" (NIH publication No. 85-23, rev 1985) were adhered to, along with specific national legislation where appropriate. Consent was taken from all farm owners, and all procedures in handling animals were strictly in accordance with international norms on farm animal welfare (Fraser et al., 2021).

**Disclaimer (Artificial intelligence)**

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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