



Novel Insights into Androgen Hormone Dynamics and Semen Quality Response to Herbal Feed Supplementation in Breeding Bulls

J.K. Singh¹, P. Singh², M. Pande¹, A. Sharma², S. Kumar¹,
N. Chand¹, R. Prasad², S. Saha¹, A.S. Sirohi¹, Sarika¹

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ABSTRACT

Background: Androgens, encompassing testosterone, dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA) and androstenedione, serve as pivotal regulators of semen quality in bulls. Despite their recognized efficacy in enhancing sexual performance and fertility in humans, herbal feed supplements remain relatively unexplored in the realm of animal husbandry. Moreover, the prevailing issue of suboptimal semen quality among crossbred bulls poses a significant challenge to the cattle industry. This study aimed to study the impact of herbal feed supplementation on the dynamics of androgen hormones in breeding bulls and its potential to enhance semen quality.

Methods: Eighteen Frieswal breeding bulls were divided into three groups: a control cohort (G-I, n=6) provided with a standard diet and two treatment groups (G-II and G-III; n=6 each) enriched with herbal feed supplementation. The supplementation regimen comprised a mixture of Safed musli, Kali musli, Ashwagandha, Shatawar, Vidhara, Bidarikand, along with Gokhru and Sahajan seeds, administered at doses of 35 g and 70 g in G-II and G-III, respectively, over a duration of four months. Semen quality and hormonal levels (testosterone, DHT, DHEA and androstenedione) were assessed and blood/semen was collected from individual animal at day 0 (initial), 60 (first cycle) and 120 (second cycle) of experimental feeding.

Result: The results obtained revealed that DHT levels were significantly ($p<0.05$) lower in both G-II and G-III after 60 and 120 days of supplementation compared to those recorded in the control group. However, testosterone, DHEA and androstenedione levels did not vary significantly among groups. The inclusion of herbal supplements significantly ($p<0.05$) improved semen volume and initial progressive motility, suggesting enhanced sperm functionality. Additionally, sperm viability increased and abnormality decreased significantly ($p<0.05$) in supplemented groups, further indicating improved semen quality. It was concluded that herbal feed supplementation improves sperm motility and semen volume in breeding bulls and the concurrent decrease in blood dihydrotestosterone levels suggests a potential mechanism underlying these enhancements.

Key words: Androstenedione, Dehydroepiandrosterone, Dihydrotestosterone, Herbs, Semen quality, Testosterone.

INTRODUCTION

Androgens, a group of male sex hormones, play a pivotal role in regulating various physiological processes in male animals, including bulls. The collective influence of androgens, including testosterone, dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA) and androstenedione, is paramount in governing semen quality in bulls (Hafez and Hafez, 2013). These hormones regulate various aspects of sperm development, motility and fertility. A balanced androgen profile is essential for optimal reproductive function and, consequently, the success of breeding programs in the cattle industry.

Herbal feed supplements are known for their potential to enhance sexual performance and fertility and are a traditional remedy in Ayurveda and other traditional systems of medicine throughout history. Although their efficacy has been shown in humans, scientific literature offers limited information on their use in animals to enhance fertility and sexual performance. This gap persists despite the significant potential of herbal preparations to influence ruminal fermentation and improve nutrient utilization in ruminants (Karnani *et al.*, 2021).

¹Division of Cattle Physiology and Reproduction, ICAR-Central Institute for Research on Cattle, Meerut-250 001, Uttar Pradesh, India.

²Division of Cattle Nutrition and Management, ICAR-Central Institute for Research on Cattle, Meerut-250 001, Uttar Pradesh, India.

Corresponding Author: M. Pande, Division of Cattle Physiology and Reproduction, ICAR-Central Institute for Research on Cattle, Meerut-250 001, Uttar Pradesh, India.

Email: Megha.Pande@icar.gov.in

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The semen of crossbred bulls often exhibits poor quality of semen (Kumersan *et al.*, 2021), which can hinder artificial insemination and breeding programs. This has been attributed to genetic factors and differences in semen quality in crossbred animals (Yáñez-Ortiz *et al.*, 2022).

Presently, the bovine semen industry faces a significant challenge regarding seminal attributes in crossbred breeding bulls (Pande *et al.*, 2019). While there is some emerging evidence suggesting that specific herbal feed supplements may positively impact semen quality in animals (Pamungkas *et al.*, 2019; Jamili *et al.*, 2021), this area of research remains relatively unexplored. The present study was undertaken with the following objectives: to assess the impact of herbal feed supplementation on the dynamics of androgen hormones in breeding bulls and to evaluate the effect of herbal feed supplementation on the improvement of semen quality.

MATERIALS AND METHODS

The research was conducted at the Division of Cattle Physiology and Reproduction, ICAR-Central Institute for Research on Cattle, Meerut, India. A total of eighteen healthy Frieswal breeding bulls (5/8 Holstein Friesian and 3/8 Sahiwal, recently recognized as the first synthetic cattle breed of India), aged between 3 and 8 years, were selected for this study during the period 2020-2022. These bulls were maintained under identical conditions. The research activities were conducted in strict accordance with the guidelines and approvals provided by the Animal Ethics Committee of the Institute.

Feeding and grouping

A herbal feed, comprising ingredients traditionally documented in Ayurveda for their potential to enhance sexual performance and semen quality in humans was prepared. It included Safed Musli (*Chlorophytum borivillianum*), Kali Musli (*Curculigo orchoides*), Ashwagandha (*Withania somnifera*), Shatavari (*Asparagus racemosus*), Vidhara (*Argyreia speciosa*), Bidarikand (*Ipomoea digitata*), Gokhru Seeds (*Tribulus terrestris*), Sahajan Seeds (*Moringa oleifera*). The precise ratio of these ingredients remains undisclosed due to patent considerations.

During the feeding trial, three distinct groups were established, each consisting of six animals. The control group (G-I) received a standard diet, whereas, individual animals in the second (G-II) and third (G-III) groups were daily administered 35 g and 70 g of the herbal feed, respectively, incorporated into the concentrate mixture. This supplementation regimen was maintained over a duration of 4 months.

Semen collection, evaluation and processing

Semen samples were obtained from the bulls during the early morning hours, between 8 AM and 9 AM, utilizing an artificial vagina and adhering to established standard protocols (DAHD, 2022). The initial progressive motility (IPM) and semen volume were recorded. Sperm concentration (million/mL) was quantified using a bovine photometer. The assessment of IPM was conducted under a light microscope equipped with a thermo-stage at a magnification of 200x. Viability, abnormality, acrosome integrity and membrane stability (HOS) tests were conducted on fresh semen

samples. The methods followed were detailed previously (Pande *et al.*, 2022).

Androgen assay

Blood was collected from individual animal at day 0 (initial), 60 (first cycle) and 120 (II cycle) of experimental feeding and plasma was separated immediately after centrifugation at 4°C. The blood plasma was collected into microtubes, followed by storage at a temperature of -20°C until the time of analysis. For the assessment of steroid hormones, Testosterone, Androstenedione, Dihydrotestosterone and Dehydroepiandrosterone, bovine-specific enzyme-linked immunosorbent assay (ELISA) kits were employed. These ELISA kits were sourced from GBiosciences®, USA and the manufacturer's instructions were followed.

Statistical analyses

Data were analysed using SPSS 20.0 (SPSS Inc., Chicago, USA) statistical software. The graphs were designed using GraphPad Prism software (San Diego, CA, USA).

RESULTS AND DISCUSSION

This study is the first of its kind, offering novel insights into the dynamics of androgen hormones and the response of semen quality to herbal feed supplementation in breeding bulls.

Effects of herbal feed supplementation on plasma androgen dynamics

The testosterone levels, whether within individual treatment groups (different cycles) or across all groups, did not reach statistical significance (Fig 1). This suggests that the administered herbal treatment did not induce substantial alterations in testosterone concentrations during the course of the study.

Testosterone is a primary androgen hormone crucial for regulating spermatogenesis, the process of sperm production. It stimulates the proliferation and maturation of sperm precursor cells into functional spermatozoa (Smith and Walker, 2014). Adequate testosterone levels are essential for maintaining sufficient sperm count and semen quality (Grande *et al.*, 2022). While some evidence suggests that certain herbs and extracts can increase testosterone levels in men (Smith *et al.*, 2021), our study found no significant changes in blood testosterone levels in bulls, possibly due to variations in dosages and types of herbs used.

Dihydrotestosterone (DHT) levels exhibited a significant reduction ($p < 0.05$) in groups G-II and G-III following 60 days of herbal feed supplementation at doses of 35 and 70 g/day/bull, respectively, corresponding to the first sperm cycle (Fig 2). This significant reduction was sustained at 120 days compared to the baseline levels at day 0; however, non-significantly higher values were observed at 120 days as compared to 60 days.

DHT is an active metabolite of testosterone and acts as a potent androgen in the male reproductive system,

contributing to the development and maintenance of secondary sexual characteristics (Cai *et al.*, 1994). It is primarily synthesized from testosterone by the action of the enzyme 5 α -reductase. It is possible that certain phytochemicals within these herbal supplements might have influenced the activity of 5 α -reductase or the conversion of testosterone to DHT, leading to decreased DHT concentrations in the bloodstream. Elevated levels of DHT are known to stimulate oxidative stress (Ji *et al.*, 2022) and thus may have negative impacts on semen quality.

Herbal medicines have been studied for their potential to reduce DHT levels. Some herbal remedies have antiandrogenic properties, which may inhibit DHT production or activity (Dhariwala and Ravikumar, 2019; Csikós *et al.*, 2021). However, it is important to note that research on this topic is limited to androgenetic alopecia and the effectiveness of herbal medicine in reducing DHT levels and improving semen quality parameters remains limited.

Dehydroepiandrosterone (DHEA) levels among all groups of bulls showed no significant difference within treatment groups or across different sperm cycles (Fig 3).

DHEA, a precursor hormone synthesized by the adrenal glands, can be enzymatically converted into both testosterone and estrogen within the body. Elevated levels of circulating plasma DHEA have been proposed as an indicator of human longevity, with diminished DHEA levels associated with various pathophysiological conditions such as adrenal insufficiency, severe systemic illnesses, acute stress and anorexia (Nenezic *et al.*, 2023). While the research surrounding DHEA's impact on semen quality is less extensive compared to testosterone and DHT, it is plausible that DHEA may indirectly influence fertility by contributing to the hormonal environment necessary for normal reproductive function.

The levels of Androstenedione remained relatively stable among the groups following 60 days (I cycle) and

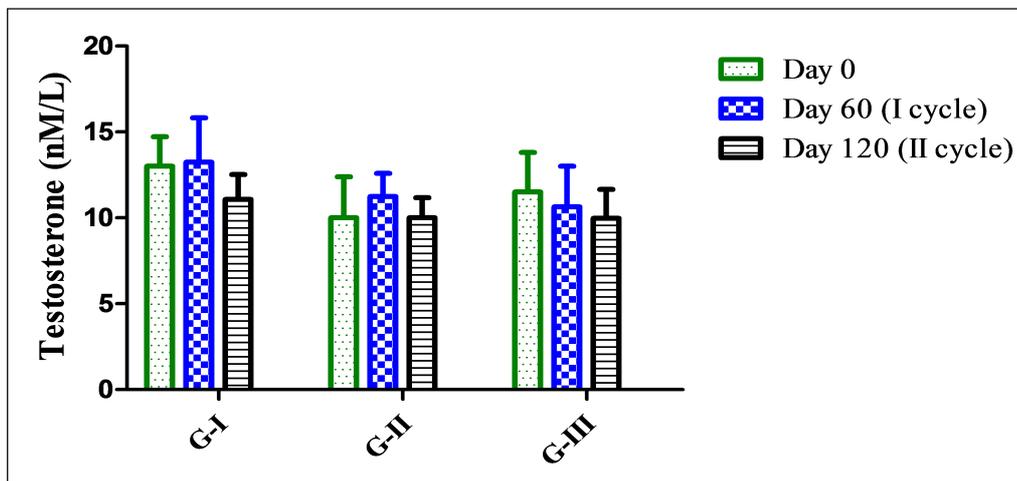


Fig 1: Blood plasma Testosterone levels in Frieswal Breeding Bulls (n=6) following herbal feed (HF) supplementation (G-I, Control; G-II, HF @ 35 gm/day/bull; G-III, HF @ 70 gm/day/bull).

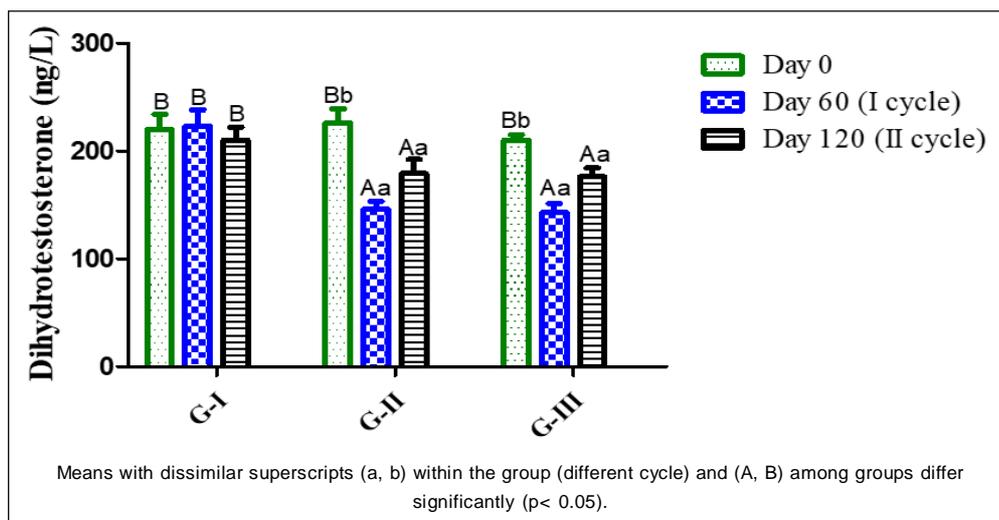


Fig 2: Blood plasma Dihydrotestosterone levels in Frieswal Breeding Bulls (n=6) following herbal feed (HF) supplementation (G-I, Control; G-II, HF @ 35 gm/day/bull; G-III, HF @ 70 gm/day/bull).

120 days (II cycle) of herbal feed supplementation (Fig 4). Statistical analysis revealed no significant variations in Androstenedione levels, suggesting that the herbal feed had minimal impact on this particular hormone over the specified durations.

Androstenedione is a precursor hormone that serves as a crucial intermediate in the biosynthesis of both testosterone and estrogen. It plays a significant role in regulating secondary sexual characteristics, muscle development and bone density (Badawy *et al.*, 2021).

Importantly, the assessment of reproductive hormones, holds a central role in the diagnosis and management of male fertility disorders. However, the correlation between androgens and sperm quality in bulls has produced inconclusive findings across various studies (Javed *et al.*, 2000; Rajak *et al.*, 2014). Therefore, it is imperative to conduct further research to establish a comprehensive framework for discerning hormone-associated bull infertility, thereby facilitating the formulation of efficacious treatment protocols.

Effects of herbal feed supplementation on semen quality parameters of breeding bulls

The effects of herbal feed supplementation on quality parameters of fresh semen in Frieswal breeding bulls are presented in Table 1. After 60 days (Cycle I) and 120 days (Cycle II) of supplementation, a statistically significant ($p < 0.05$) increase in semen volume was observed in G-II and G-III, in comparison to the control group (G-I). However, no significant changes were observed between in G-II and G-III.

Similarly, a notable trend of significant ($p < 0.05$) increase was observed in the initial progressive motility of spermatozoa. This finding indicates that, following 60 days and 120 days of herbal feed supplementation, bulls in G-II and G-III exhibited a statistically significant ($p < 0.05$) improvement in the forward movement compared to the control group.

After 60 days (Cycle I) and 120 days (Cycle II) of supplementation, bulls in G-II and G-III exhibited a

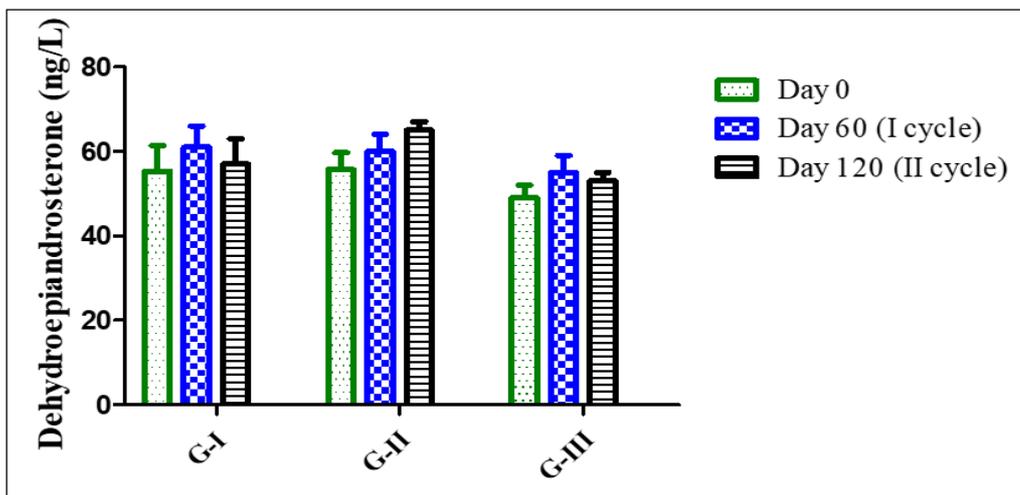


Fig 3: Blood plasma Dehydroepiandrosterone levels in Frieswal Breeding Bulls (n=6) following herbal feed (HF) supplementation (G-I, Control; G-II, HF @ 35 gm/day/bull; G-III, HF @ 70 gm/day/bull).

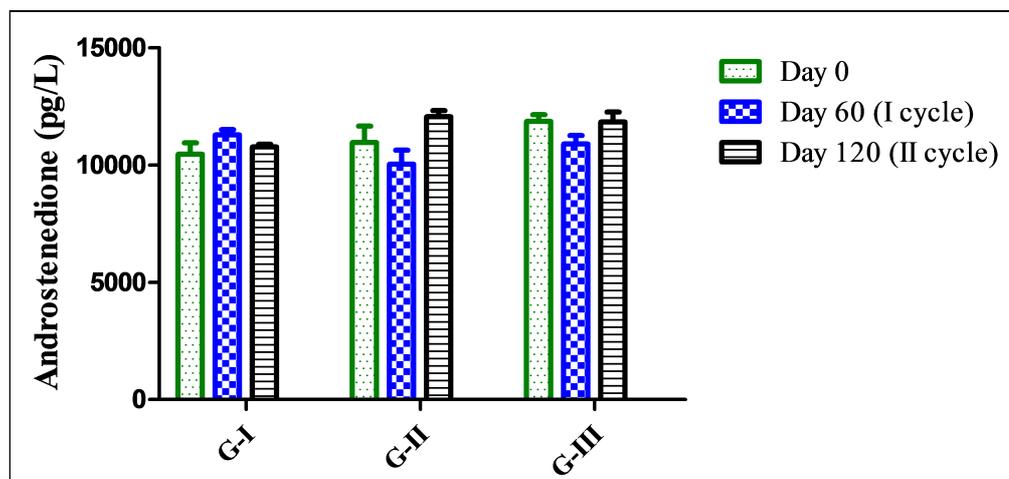


Fig 4: Blood plasma Androstenedione levels in Frieswal Breeding Bulls (n=6) following herbal feed (HF) supplementation (G-I, Control; G-II, HF @ 35 gm/day/bull; G-III, HF @ 70 gm/day/bull).

Table 1: Semen quality parameters in frieswal breeding bulls following herbal feed (HF) supplementation.

Group	Time point	N	Vol (mL)	Concentration (10 ⁶ /mL)	IPM (%)	Vaibility (%)	Abnormality (%)
G-I	T0	36	4.59±0.24 ^a	1087.45±95.16	40.25±3.78 ^a	45.65±2.45 ^a	10.36±1.49 ^a
	C I	36	4.87±0.89 ^a	1145.78±87.23	42.48±2.49 ^a	47.54±4.51 ^a	10.56±1.54 ^a
	C II	36	5.14±0.14 ^a	1189.95±55.48	43.10±1.56 ^a	48.16±3.20 ^a	9.23±2.66 ^a
G-II	T0	34	4.89±0.17 ^a	1184.59±93.52	41.26±2.45 ^a	46.71±3.23 ^a	10.46±1.46 ^a
	C I	39	5.79±0.89 ^b	1278.47±88.12	49.27±3.14 ^b	53.15±4.52 ^b	7.78±2.59 ^b
	C II	32	5.63±0.48 ^b	1179.54±95.33	50.65±2.47 ^b	55.12±3.45 ^b	8.94±1.62 ^b
G-III	T0	37	4.08±0.34 ^a	1345.05±92.71	40.25±3.78 ^a	43.66±2.71 ^a	11.57±0.66 ^a
	C I	36	5.91±0.45 ^b	1248.67±67.78	48.48±2.49 ^b	54.11±3.59 ^b	9.07±1.86 ^b
	C II	33	5.82±0.51 ^b	1118.57±67.16	53.10±1.56 ^b	57.66±3.72 ^b	8.24±2.15 ^b

G-I, Control; G-II, HF@35 gm/day/bull; G-III, HF@ 70 gm/day/bull; T0, Initial (0 day); C I: First Cycle (60 days); C II: Second Cycle (120 days); N, number of semen ejaculates; Vol, volume; IPM, Initial progressive motility.

^{a,b} Values bearing different superscripts in a column differ significantly (p<0.05).

statistically significant (p<0.05) improvement in sperm viability compared to the control group (G-I). Specifically, viability increased from 48.16±3.20% in G-I to 55.12±3.45% and 57.66±3.72% in G-II and G-III, respectively.

Similarly, there was a significant (p<0.05) reduction in sperm abnormality in G-II and G-III, with values dropping to 8.94±1.62% and 8.24±2.15%, respectively, compared to 9.23±2.15% in the control group. These findings suggest that the herbal feed supplementation not only enhances sperm motility and volume but also improves sperm quality by increasing viability and reducing abnormality.

Herbal feed supplements have been explored for their potential benefits in reproductive health. Safed Musli (*Chlorophytum borivilianum*), rich in steroidal saponins, alkaloids, proteins, carbohydrates, fiber, vitamins and minerals, has shown potential in enhancing sexual performance and supporting male reproductive health (Khanam *et al.*, 2013). Kali Musli (*Curculigo orchoides*), containing steroidal saponins, phenolic compounds, flavonoids, tannins and alkaloids, has been associated with improvements in conditions such as asthenozoospermia, oligospermia, hypospermia and teratospermia (Chauhan *et al.*, 2010). Ashwagandha (*Withania somnifera*), known for its adaptogenic properties and rich in withanolides, alkaloids, steroidal lactones, tannins, flavonoids and amino acids, reduces stress and anxiety, thereby indirectly supporting potency and reproductive health (Paul *et al.*, 2021). It also holds promise for improving growth performance in livestock under stall-fed conditions (Singla *et al.*, 2017). Shatavari (*Asparagus racemosus*), rich in saponins, polysaccharides, flavonoids, alkaloids, vitamins and minerals, is recognized for improving female reproductive health by addressing hormonal imbalance, follicular growth, oocyte quality and infertility through reduced oxidative stress and increased antioxidant levels (Pandey *et al.*, 2018). Additionally, supplementation with shatavari root powder has been shown to significantly enhance milk production in crossbred cows (Chavan *et al.*, 2023).

Vidhara (*Argyreia speciosa*), with alkaloids, steroidal saponins, flavonoids, tannins and resins, promotes sexual

health in both sexes (Riaz *et al.*, 2010). Bidarikand (*Ipomoea digitata*), rich in steroidal saponins, flavonoids, alkaloids, tannins and resins, is an effective promoter of spermatogenesis, especially when consumed with milk, demonstrating faster action (Dutta and Sengupta, 2018). Gokhru (*Tribulus terrestris*), containing saponins, alkaloids, flavonoids, steroids and resins, has been found to improve sperm parameters when taken orally (Sanagoo *et al.*, 2019). Sahajan (*Moringa oleifera*), with vitamins, minerals, essential amino acids, antioxidants and fatty acids, enhances antioxidant capacity, maintains semen functionality and reduces sperm abnormalities (Zeng *et al.*, 2019; Shokry *et al.*, 2020).

Our research focused on the combined effects of these herbs, revealing positive results and affirming their potential synergy and effectiveness. This pioneering study contributes to the growing body of knowledge on herbal supplementation and underscores the importance of exploring synergistic effects for enhanced reproductive well-being in breeding bulls. Previous studies have highlighted the potential of herbal medicines to enhance semen quality in humans and reproductive parameters in breeding boars (Tu *et al.*, 2023) and roosters (Qi *et al.*, 2019). Our study stands as the first exploration of utilizing a combination of Ayurvedic herbs in breeding bulls with the effects of these herbs on hormonal dynamics and semen quality.

CONCLUSION

Our study demonstrated that the herbal feed supplement comprising Safed Musli, Kali Musli, Ashwagandha, Shatavari, Vidhara, Bidarikand and seeds of Gokhru and Sahajan significantly improved sperm motility, semen volume and viability, while also reducing sperm abnormality in breeding bulls. Additionally, the decrease in blood dihydrotestosterone levels suggests a potential mechanism for these enhancements. This finding paves the way for further investigations into the specific mechanisms and consequences of this hormonal change on bull reproductive performance.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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