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Analyzing the impact of age, body condition, scrotum circumference testicular size, and hormone levels in pre-pubertal kundhi buffalo bull calves.

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Abstract: This study investigates and compares the physiological and behavioral characteristics of Kundhi buffalo bull calves focusing on the impact of age and size. Ten bull calves, aged between 14 to 18 months were selected and categorized into two groups: Group A: young (up to 14 months), with 210kg average body weight, and Group B: smaller (up to 18 months) with 305kg average body weight. The body weight of Kundhi buffalo calves linearly increased from the 1st to the 7th fortnight, with significant differences ($p<0.05$) observed between the two groups. The final body weight was higher in Group B (344.72 kg) when compared to Group A (262.04 kg). Similarly, scrotal circumference, girth, and length exhibited linear increases throughout the experiment with significant differences between the groups ($p<0.05$). Group B consistently showed larger scrotal circumferences (21.96 cm), girths (66.28 cm), and lengths (51.54 cm) compared to Group A (18.38 cm, 60.36 cm, and 47.44 cm, respectively). While testosterone and estradiol concentrations were non-significant ($p>0.05$) between the groups, they were higher in Group B (0.1504 ng/ml and 2.4724 pg/ml) than in Group A (0.1082 ng/ml and 2.2724 pg/ml). Behavioral observations, including nuzzling, sniffing, licking, bellowing, protrusion, and mounting, indicated higher scores in Group B. In both groups, mean nuzzling scores were (3.71 and 5.00), sniffing (0.28 and 1.28), licking (3.14 and 4.14), bellowing (0.85 and 2.14), protrusion (0.00 and 0.42), and mounting (0.00 and 1.28) in the "yes" category. These findings offer comprehensive insights into the physiological and behavioral differences influenced by age and size in Kundhi buffalo bull calves.

Keywords: Body Condition, Testicular Size, Hormone Levels, Pre-Pubertal, Kundhi Buffalo.

1. Introduction

In the Sindh region of Pakistan, the Kundhi buffalo, a breed of domestic buffalo, is recognized for its medium to large stature, characterized by a long, wide head and prominent, drooping ears. While the typical coat colour is black, variations in brown or grey may occur. Renowned for its high milk yield and suitability to the hot and humid local environment (Memon and Khushk, 2007; Anas et al., 2023), the Kundhi buffalo faces challenges such as genetic erosion, low fertility, and susceptibility to diseases, impacting its population (Wynn et al., 2021). Critical to the long-term sustainability of the Kundhi buffalo is its genetic diversity, necessitating studies to identify causes of genetic erosion and assess current genetic variability. Techniques like genetic marker-assisted breeding and the preservation of unique genetic lines should be explored to address these concerns (Devendra et al., 2000). Moreover, low reproductive rates in Kundhi buffalo herds demand investigative studies to identify root causes and develop strategies for enhanced reproductive efficiency through improved breeding practices, diet, and management methods (Hegde, 2019; Rout et al., 2021).

The prepubertal development of male buffalo (*Bubalus bubalis*) involves complex physiological and morphological changes. During the first few months of age, male buffalo calves experience rapid growth and weight development, with scrotal testicles starting to mature. Signs of sexual maturity, including increased testosterone levels and spermatogenesis, typically emerge between 6 to 8 months of age. Substantial increases in testosterone occur between 10 and 18 months, leading to the development of secondary sex traits like a thick neck, wide shoulders, and a deeper voice. Sexual maturity is generally achieved at approximately 18 to 24 months (Sharma and Purohit, 2014; Mason et al., 2020). The prepubertal and pubertal development of male buffalo involves morphological and physiological changes crucial for reproductive readiness. Hormonal orchestration, particularly increased testosterone levels, drives the expansion of testicles, formation of the vas deferens and epididymis, and initiation of spermatogenesis. Various factors, including breed, genetics, diet, and environment, influence the timing of sexual maturation (Yousef and Abdou, 2020).

Male buffalo undergo a significant period of development and maturation in their early years, establishing the foundation for future reproductive capability. Testes grow noticeably during this period, and the epididymis and vas deferens mature simultaneously. These structures are essential for the production and movement of fully developed spermatozoa. Hormonal regulation, specifically an increase in testosterone levels, plays a crucial role in these complex developmental processes (Robaire and Hermo, 1988; Picul and Remick 2017). The age at which sexual maturity is attained in male buffalo is influenced by intrinsic factors such as breed and genetics, as well as extrinsic factors like nutrition, environment, health, and social dynamics within the herd. Understanding these complexities is essential for effective management and maximizing the reproductive potential of male buffalo (Sharma et al., 2014; Vale, 2007).

Upon reaching sexual maturity, male buffalo enter the post-pubertal stage, displaying reproductive capabilities that impact breeding programs and overall herd efficiency (Chauhan and Selokar, 2022; Arain et al., 2023). To enhance the sustainability and productivity of buffalo populations, a comprehensive understanding of the interactions between genetic, nutritional, environmental, and social factors is crucial (Presicce, 2017). Puberty in male buffalo initiates a noticeable rise in hormone levels, particularly testosterone and luteinizing hormone (LH), crucial for the development of secondary sexual traits. Post-pubertal development involves continued testicle growth, increased sperm production, and significant contributions to overall herd fertility and genetic development (Brito, 2021; Mahmood et al., 2018). Compared to post-pubertal males, prepubertal male buffalo exhibit considerably lower hormone levels, with estradiol and testosterone production being modest. The intricacies of hormone production during prepubertal development are influenced by factors such as age, genetics, diet, and environmental conditions (Pereira, 2019). This study addresses the knowledge gap regarding age-related growth patterns in body and scrotum circumference, as well as testosterone levels in pre-pubertal Kundhi buffalo bull calves.

2. Materials and Methods

2.1. Age-based categorization of ten bull calves for an experimental study

Ten bull calves, aged between 14 and 18 months, were chosen for the experiment and categorized into two distinct groups: Group A and Group B. Group A comprises five calves aged up to 14 months, with an average body weight of 210 kg representing the younger and smaller individuals, while Group B includes five calves aged up to 18 months with average body weight of 305 kg, representing the slightly older and larger ones. The classification of these groups was primarily determined by age and health status.

2.2. Management of animals

The animals were reared under the stall-feeding management system at the animal farm in the Department of Animal Reproduction, Sindh Agriculture University Tandojam. Each animal was equipped with Radio-frequency identification (RFID) tags and identified using an RFID reader. The feeding regimen followed the routine system, incorporating green fodder, wheat straw, wheat bran, and cottonseed cake. As a rough estimate, green fodder was supplied at 2-3% of the calf's body weight, divided into two feedings of 4-6 kilograms per day. Wheat straw, provided as roughage, amounted to approximately 1-2 kilograms per feeding, totaling 2-4 kilograms daily. The quantity of wheat bran, a concentrated feed, varied based on the calf's age and weight, typically ranging from 1-2 kilograms per day. Cottonseed cake, typically administered at 1 kilogram per day, was mixed with other feeds and given twice daily. All animals had access to water ad libitum throughout the research period. Every animal in the study was in good health and fully vaccinated, establishing a reliable baseline. Over the three-month research duration, we consistently recorded key parameters every two weeks, ensuring thorough and dependable data collection for our research goals (Korejo et al., 2019).

2.3. Body weight

Body weight was accurately calculated using Schaeffer's formula at precisely scheduled fifteen-day intervals. This process entailed precise measurements of two key parameters: heart girth (the circumference around the chest) and body length (the distance from the shoulder to the base of the tail), with all measurements recorded in centimeters (cm). The formula employed for calculating body weight in kilograms (kg) was as follows: (Heart girth in cm * Heart girth in cm * Body length in cm) / 660 (Korejo et al., 2019).

2.4. Scrotum circumference

Scrotal circumference measurements were systematically conducted at fifteen-day intervals using a vernier caliper and an inch tape strip. To measure the circumference of the scrotum, an inch tape strip was employed. Carefully, the tape was wrapped around the broadest part of the scrotum, ensuring it was level and without any overlap between the starting and ending points. The recorded measurement, in inches, was noted where the two ends of the tape met, for future reference. When using the vernier caliper, it was adjusted until both jaws contacted the scrotum without excessive force, and the measurement displayed on the caliper scale was meticulously recorded (Korejo et al., 2019).

2.5. Sexual behavior

Scrotal circumference measurements were systematically conducted at fifteen-day intervals using a vernier caliper and an inch tape strip. To measure the circumference of the scrotum, an inch tape strip was employed. Carefully, the tape was wrapped around the broadest part of the scrotum, ensuring it was level and without any overlap between the starting and ending points. The recorded measurement, in inches, was noted where the two ends of the tape met, for future reference. When using the vernier caliper, it was adjusted until both jaws contacted the scrotum without excessive force, and the measurement displayed on the caliper scale was meticulously recorded (Korejo et al., 2019).

2.6. Analyzing the concentration of plasma testosterone and estradiol level

Blood samples were meticulously collected to measure plasma testosterone and estradiol levels, ensuring utmost professionalism, and prioritizing the welfare of the animals and the scientific integrity of the research. The Radioactive Immuno Explorers RIA kit was used to detect and analyze plasma testosterone and estradiol levels of the blood samples. To maintain the efficiency of the procedure, all reagents were brought to room temperature before pipetting. The wash solution was prepared by mixing the vial content with 950ml of distilled water, homogenizing the diluted solution, and storing it between 2-8°C. For sample

preparation, 50 µl of calibrator, control, or sample was added to the antibody-coated tube, followed by mixing with 300 µl of tracer solution. The solution was incubated for one hour at a controlled temperature between 18 to 25 degrees Celsius while gently shaking at 400 rpm. For counting, the contents of all tubes, excluding two tubes, were carefully aspirated. The total counts per minute (CPM) were washed once with 2 ml of the wash solution, and after aspirating the solution, counting was performed for 1 minute. Additionally, 300 µl of tracer was added to two additional tubes to obtain total CPM. Results were obtained from the Standard curve (Devkota et al., 2008). Samples were processed at the National Institute for Agriculture and Biology, Faisalabad, Pakistan.

3. Statistical analysis

The data were tabulated in Microsoft Excel, statistical analysis was done through Statistics 8.1 version. One-way ANOVA was applied, and LSD was used to compare the significant results.

4. Results

4.1. Body weight (kg) of pre-pubertal Kundi buffalo calf

The body weight of pre-pubertal Kundhi buffalo calves was recorded fortnightly, and the outcomes are presented in Table 1. The body weights of Kundhi buffalo calves in the 1st to 7th fortnights were significantly different ($p<0.05$) between Groups A and B. The body weights of Kundhi buffalo calves exhibited a linear increase from the 1st to the 7th fortnight in both Group A and Group B. The final body weight of Kundhi buffalo calves was higher in Group B (344.72 kg) compared to the body weight of Kundhi buffalo calves in Group A (262.04 kg).

Fortnight	Group A	Group B	LSD 0.05	P-value
1st	215.9±7.69 ^{b}	275.5±17.82 ^{a}	44.772	0.0154
2nd	223.1±7.39 ^{b}	286.3±17.60 ^{a}	44.042	0.0107
3rd	231.6±8.19 ^{b}	297.6±17.85 ^{a}	45.326	0.0099
4th	239.3±8.21 ^{b}	308.5±18.49 ^{a}	46.674	0.0091
5th	246.7±8.18 ^{b}	320.7±19.17^{a}	48.078	0.0075
6th	254.3±8.00 ^{b}	332.4±20.34^{a}	50.406	0.0073
7th	262.0±7.67 ^{b}	344.7±21.16^{a}	51.917	0.0063

Table 1 – Body weight (kg) of pre-pubertal Kundi buffalo calf, mean ± standard error for each group per fortnight, along with the LSD at a 0.05 significance level and the associated p-values.

4.2. Scrotal circumference (cm) of pre-pubertal Kundi buffalo calf

The scrotal circumference of pre-pubertal Kundhi buffalo calves was measured fortnightly, and the outcomes are detailed in Table 2. The scrotal circumference of Kundhi buffalo calves showed significance ($P<0.05$) from the 1st to the 7th fortnight between Groups A and B. The scrotal circumference of Kundhi buffalo calves exhibited a linear increase from the 1st to the 7th fortnight in both Group A and Group B. The final scrotal circumference of Kundhi buffalo calves was higher (21.96 cm) in Group B compared to the scrotal circumference of Kundhi buffalo calves (18.38 cm) in Group A.

Fortnight	Group A	Group B	LSD 0.05	P-value
1st	16.6±0.21 ^{b}	19.7±1.16 ^{a}	2.7382	0.0311
2nd	16.8±0.22 ^{b}	20.1±1.15 ^{a}	2.7203	0.0252
3rd	17.2±0.19 ^{b}	20.6±1.15 ^{a}	2.7082	0.0195
4th	17.5±0.18 ^{b}	20.8±1.10 ^{a}	2.5408	0.0177
5th	17.7±0.22 ^{b}	21.3±0.99 ^{a}	2.3650	0.0087
6th	18.0±0.22 ^{b}	21.6±0.98 ^{a}	2.3376	0.0082
7th	18.3±0.22 ^{b}	21.9±0.98 ^{a}	2.3198	0.0074

Table 2 – Scrotal circumference (cm) of pre-pubertal Kundi buffalo calf, mean ± standard error for each group per fortnight, along with the LSD at a 0.05 significance level and the associated p-values.

4.3. Girth (cm) of pre-pubertal Kundi buffalo calf

Results on the girth of pre-pubertal Kundhi buffalo calves on a fortnightly basis were recorded, and the outcomes are detailed in Table 3. The girth of Kundhi buffalo calves showed significance ($P<0.05$) from the 1st to the 7th fortnight between Groups A and B. The girth of Kundhi buffalo calves exhibited a linear increase from the 1st to the 7th fortnight in both Group A and Group B. The final girth of Kundhi buffalo calves was higher (66.28 cm) in Group B compared to the girth of Kundhi buffalo calves (60.36 cm) in Group A.

Fortnight	Group A	Group B	LSD 0.05	P-value
1st	56.8±0.87 b	61.9±1.42 a	3.8511	0.0160
2nd	57.4±0.85 b	62.7±1.37 a	3.7295	0.0117
3rd	58.1±0.93 b	63.4±1.33 a	3.7582	0.0121
4th	58.7±0.92 b	64.0±1.34 a	3.7596	0.0115
5th	59.1±0.82 b	64.8±1.33 a	3.6293	0.0068
6th	59.8±0.86 b	65.5±1. 40 a	3.8067	0.0085
7th	60.3±0.83 b	66.2±1.42 a	3.8157	0.0072

Table 3 – Girth (cm) of pre-pubertal Kundi buffalo calf, mean ± standard error for each group per fortnight, along with the LSD at a 0.05 significance level and the associated p-values.

4.4. Length (cm) of pre-pubertal Kundi buffalo calf

Results on the length of pre-pubertal Kundhi buffalo calves on a fortnightly basis were recorded, and the outcomes are detailed in Table 4. The length of Kundhi buffalo calves showed significance (P<0.05) from the 1st to the 7th fortnight between Groups A and B. The length of Kundhi buffalo calves exhibited a linear increase from the 1st to the 7th fortnight in both Group A and Group B. The final length of Kundhi buffalo calves was higher (51.54 cm) in Group B compared to the length of Kundhi buffalo calves (47.44 cm) in Group A.

Fortnight	Group A	Group B	LSD 0.05	P-value
1st	44.0±0.39 b	47.1±0.90 a	2.2763	0.0134
2nd	44.6±0.39 b	47.8±0.86 a	2.1908	0.0092
3rd	45.1±0.39 b	48.6±0.89 a	2.2176	0.0072
4th	45.7±0.35 b	49.3±0.90 a	2.2464	0.0057
5th	46.3±0.37 b	50.2±0.95 a	2.3517	0.0051
6th	46.8±0.37 b	50.8±0.94 a	2.3494	0.0045
7th	47.4±0.36 b	51.5±0.95 a	2.3575	0.0039

Table 4 – Length (cm) of pre-pubertal Kundi buffalo calf, mean ± standard error for each group per fortnight, along with the LSD at a 0.05 significance level and the associated p-values.

4.5. Testosterone and estradiol concentration of pre-pubertal Kundi buffalo calf

Results on the testosterone and estradiol concentrations of pre-pubertal Kundhi buffalo calves were determined. Testosterone and estradiol concentrations of Kundhi buffalo calves showed non-significance (P>0.05) between Groups A and B. The testosterone and estradiol concentrations of Kundhi buffalo calves were higher (0.1504 ng/ml and 2.4724 pg/ml) in Group B than in Group A (0.1082 ng/ml and 2.2724 pg/ml), respectively.

4.6. Physical behavior of pre-pubertal Kundi buffalo calf

Results regarding mean scores of physical behavior of Groups A and B, for nuzzling, sniffing, licking bellowing, protrusion and mounting in the "yes" category of pre-pubertal Kundhi buffalo calves are presented in Table 5.

Physical behavior	Response	Group A	Group B
Nuzzling	Yes	3.71	5.00
	No	1.28	0
Sniffing	Yes	0.28	1.28
	No	4.71	3.71
Licking	Yes	3.14	4.14
	No	1.85	0.85
Bellowing	Yes	0.85	2.14
	No	4.14	2.85
Protrusion	Yes	0.00	0.42
	No	5.00	4.57
Mounting	Yes	0.00	1.28
	No	5.00	3.71

Table 5 – Physical behavior of pre-pubertal Kundi buffalo calf, means score were shown.

5. Discussion

The observed differences in body weight between Group A and Group B Kundhi buffalo calves throughout the experiment provide valuable insights into the growth patterns of these animals. In Group B (344.72 kg) and Group A (262.04) showing body weight from the 1st to the 7th fortnight in both groups underscores the growth potential during this developmental period, a phenomenon reported in various studies on buffalo calves (Singh et al., 2015). The significant differences ($p<0.05$) in body weight between Group A and Group B Kundhi buffalo calves during the 1st to 7th fortnight of the experiment indicate that age and size play a crucial role in determining the growth rate of these animals. Similar findings have been reported in studies comparing the growth performance of buffalo calves in different age groups (Kumar et al., 2018). The physiological changes associated with age and size likely contribute to the variations in body weight observed in this study. The final body weight of Kundhi buffalo calves further emphasizes the impact of age on growth, with Group B exhibiting a significantly higher average body weight (344.72 kg) compared to Group A (262.04 kg). This discrepancy aligns with previous research demonstrating that older buffalo calves tend to reach higher body weights than their younger counterparts due to increased feed intake and improved metabolic efficiency (Sharma et al., 2019). The observed findings are consistent with the general understanding of buffalo calf growth, where age-related factors such as hormonal changes and physiological development significantly influence body weight (Patel et al., 2021). Additionally, the importance of size as a contributing factor to the observed differences cannot be overlooked, as larger-sized individuals in Group B likely had a greater capacity for nutrient utilization and energy storage, leading to the observed higher final body weight. In conclusion, this study highlights the significant influence of age and size on the growth patterns of Kundhi buffalo calves. The linear increase in body weight, coupled with the consistent differences between Group A and Group B, underscores the importance of considering both age and size factors in the management and nutrition of these animals for optimal growth and development.

The observed linear increase in scrotal circumference among Kundhi buffalo calves from the 1st to the 7th fortnight in both Group A and Group B is indicative of progressive testicular development during this critical growth phase. This finding aligns with established literature highlighting the importance of monitoring scrotal circumference as an essential parameter for assessing reproductive maturity and potential breeding performance in male animals (Kumar et al., 2018. The significant differences ($P<0.05$) in scrotal circumference observed between Group A and Group B Kundhi buffalo calves during the 1st to 7th fortnight of the experiment underscore the influence of age and size on testicular growth. Comparable studies have reported similar age-related variations in scrotal circumference, emphasizing the significance of physiological changes associated with increasing age in male animals (Patel et al., 2021). The higher scrotal circumference observed in Group B aligns with the general trend of increased testicular development in older and larger-sized individuals. The final scrotal circumference of Kundhi buffalo calves further supports the influence of age and size on reproductive development, with Group B exhibiting a significantly higher average scrotal circumference (21.96 cm) compared to Group A (18.38 cm). This difference may be attributed to the advanced age and larger body size of Group B, which could lead to enhanced hormonal activity and testicular growth (Sharma et al., 2019).

The observed linear increase in girth among Kundhi buffalo calves from the 1st to the 7th fortnight in both Group A and Group B reflects a consistent pattern of growth and development during the experimental period. This finding aligns with established knowledge regarding the natural progression of body dimensions in growing animals, highlighting girth as a reliable indicator of overall body size and muscular development (Gupta et al., 2019). The significant differences ($P<0.05$) in girth observed between Group A and Group B Kundhi buffalo calves during the 1st to 7th fortnight of the experiment underscore the impact of age and size on the growth rate. Similar studies have reported comparable age-related variations in girth, emphasizing the significance of physiological changes associated with increasing age in buffalo calves (Patel et al., 2021). The higher girth observed in Group B may be attributed to the advanced age and larger body size of the individuals in this group. The final girth of Kundhi buffalo calves further supports the influence of age and size on overall body dimensions, with Group B exhibiting a significantly higher average girth (66.28 cm) compared to Group A (60.36 cm). This difference likely stems from the cumulative effects of age-related growth and increased body mass in the older and larger-sized individuals in Group B (Williams 2020). The findings of this study contribute to the understanding of the dynamic nature of girth as a comprehensive measure of body size and development in Kundhi buffalo calves. The observed linear increase and significant differences between the groups provide valuable insights into the physiological changes associated with age and size in these animals. In conclusion, the study highlights the importance of monitoring girth as a key parameter in assessing the growth and development of Kundhi buffalo calves. The observed linear increase and significant differences between Group A and Group B, along with the higher final girth in Group B, emphasize the role of age and size factors in shaping the overall body dimensions of buffalo calves during the early stages of growth.

The observed linear increase in the length of Kundhi buffalo calves from the 1st to the 7th fortnight in both Group A and Group B indicates a consistent and progressive growth pattern during the experimental period. This finding follows established knowledge of the natural development of body dimensions in growing animals, where length serves as a crucial indicator of skeletal and overall body growth (Gupta et al., 2020). Significant differences ($P<0.05$) in length were observed between Group A and Group B Kundhi buffalo calves during the 1st to 7th fortn.

6. Conclusion

The study revealed that body weight, scrotal circumference, girth, and length exhibited a linear increase from the 1st to the 7th fortnight in both groups. Final measurements in Group B, comprising older and larger-sized calves, consistently surpassed those in Group A. These findings underscore the substantial impact of age and size on the physical development of Kundhi buffalo bull calves. Notably, testosterone and estradiol concentrations did not exhibit significant differences between the two groups. However,

concentrations were generally higher in Group B, hinting at a potential trend that merits exploration in future studies. Behavioral observations, encompassing nuzzling, sniffing, licking, bellowing, protrusion, and mounting, revealed variations between the two groups. Group B displayed higher mean scores in most categories, indicating potential behavioral differences associated with age and size.

7. References

Memon A, Khushk AM. Economic analysis of buffalo dairy farms in Sindh. Indus J. Bio Sci. 2007;8(2):117-123.

Anas M, Farooq M, Asif M. Et al. A Novel Insight into the Identification of Potential SNP Markers for the Genomic Characterization of Buffalo Breeds in Pakistan. Animals, 2023; 13(15): 2543.

Wynn PC, Warriach HM, Iqbal H, McGill DM. The future of smallholder farming in developing countries in the face of climate change: a perspective with a focus on Pakistan. Anim. Prod. Sci. 2021 doi: 10.1071/AN20496.

Devendra C, Thomas D, Jabbar MA, Kudo H. Improvement of livestock production in crop-animal systems in rainfed agro-ecological zones of South-East Asia. ILRI (aka ILCA and ILRAD). 2000.

Hegde NG. Buffalo husbandry for sustainable development of small farmers in India and other developing countries. Asian J. Res. Anim. Vet. Sci. 2019;3(1):1-20.

Rout PK, Behera BK, Rout PK, Behera BK. Cattle and Buffaloes Farming. Sustainability in Ruminant Livestock: Management and Marketing. 2021;77-115.

Sharma RK, Jerome A, Purohit, GN. Reproductive physiology of the male and female buffalo. Bubaline. Theriogenol. 2014;5702, 0614.

Mason KA, Schoelwer MJ, Rogol AD. Androgens during infancy, childhood, and adolescence: physiology and use in clinical practice. Endocrine Reviews, 2020; 41(3): bnaa003.

Yousef MS, Osman AM, Abdou M. et al. Effects of age and season on serum testosterone level in male buffaloes. J. Adv. Vet. Res. 2020;10(3):154-158.

Robaire B, Hermo L. Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. The physiology of reproduction. 1998;1:999-1080.

Picul CA, Remick AK. Impact of age on the male reproductive system from the pathologist's perspective. Toxicol. Pathol. 2017;45(1):195-205.

Vale WG. Effects of environment on buffalo reproduction. Ital. J. Anim. Sci. 2007;6:130-142. doi:10.4081/ijas.2007.s2.130

Chauhan MS, Selokar N. Biotechnological applications in buffalo research. Springer. 2022; doi: 1007/978-981-16-7531-7

Arain S, ur Rehman Q, Azeem M, Jahanzaib M, Waheed A, Riaz A, Shazinosh, MB. Biotechnological therapies for animal reproduction in the livestock sector. Pure. Appl. Bio. 2023;12(2):1269-1285. doi:10.19045/bspab.2023.120130

Presicce GA. The Buffalo (*Bubalus bubalis*)-Production and Research. Bentham Science Publishers. 2017

Brito LF. Endocrine control of testicular development and initiation of spermatogenesis in bulls. Bovine. Repro. 2021;47-57. doi:10.1002/9781119602484.ch5

Mahmood S, Kumar A, Singh R, et al. Scrotal circumference: A predictor of testosterone concentration and certain attributes of seminal vesicles influencing buffalo male fertility. Vet. world. 2018;11(6):v739. doi: 10.14202/vetworld.2018.739-747

Pereira NN. Factors Affecting Testicular Growth and Development in the Beef Bull (Doctoral dissertation, North Dakota State University). 2019

Korejo NA, Parveen F, Memon MI, et al. Age-related changes in body weight, body conformation and scrotal circumference and prepubertal sexual behavior of Kundhi buffalo bull calves. Sindh Uni. Res. J. 2019; 51(01): 75-80.

Devkota B, Koseki T, Matsui M, et al. Relationships among age, body weight, scrotal circumference, semen quality and peripheral testosterone and estradiol concentrations in pubertal and postpubertal Holstein bulls. J. Vet. Med. Sci. 2008;70: 119-121.

Singh A, Malik R, Kumar D. Testosterone and its role in male reproductive function in buffaloes: A review. Vet. 2015;8(3):307-312.