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Gross anatomical and biometrical studies of the uterine body during different phases of estrus cycle in buffalo (*Bubalus bubalis*)

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Abstract
A gross anatomical and biometric investigation was undertaken to analyze the buffalo uterus at various stages of the estrus cycle. A discernible escalation in uterine tone was observed a few days prior to the commencement of estrus, reaching its peak during proestrus and estrus, and subsequently subsiding post-estrus. The average length of the uterine body exhibited variations during proestrus (1.67±0.08 cm), estrus (1.86±0.09 cm), metestrus (1.65±0.07 cm), diestrus (1.68±0.08 cm), anestrus (1.68±0.04 cm), and early pregnancy (1.65±0.08 cm). Likewise, the average diameter of the cervix demonstrated fluctuations across different phases: proestrus (4.85±0.13 cm), estrus (4.80±0.08 cm), metestrus (4.92±0.21 cm), diestrus (5.33±0.18 cm), anestrus (5.19±0.24 cm), and early pregnancy (5.74±0.34 cm). Concurrently, the average circumference of the cervix displayed variations during proestrus (17.70±0.24 cm), estrus (18.09±0.27 cm), metestrus (17.72±0.28 cm), diestrus (17.93±0.37 cm), anestrus (17.18±0.32 cm), and early pregnancy (17.82±0.56 cm). This research contributes valuable insights into the dynamic anatomical changes in the buffalo uterus throughout the estrus cycle.

Keywords: Cervix, biometry, estrus cycle, early pregnancy

Introduction
The estrus cycle encompasses four distinct phases-proestrus, estrus, metestrus, and diestrus—each representing cyclic changes in the uterus influenced by pituitary hormones acting on the ovary (Deshpande, 1994) [4]. The uterus undergoes structural and functional variations depending on the reproductive status (Shukla *et al*., 1973) [14]. It is imperative to comprehend these changes for a comprehensive understanding of buffalo reproductive physiology. Notably, there is a dearth of comprehensive information on the biometrical alterations in sexual organs during pregnancy in buffaloes, a gap that holds significant clinical importance, especially in early pregnancy diagnosis (Khan, 1989) [10].

Materials and Methods
The current investigation involved 60 buffaloes, with samples categorized into six groups corresponding to different phases of the estrus cycle, namely proestrus, estrus, metestrus, diestrus, anestrus, and early pregnancy. Each group comprised ten samples, and meticulous cleaning was performed under running tap water to eliminate tissue debris, fascia, and blood clots. Various gross anatomical observations and biometrical parameters, including length, maximum circumference, and maximum diameter of the uterine body, were measured in centimeters using a weighing balance, non-stretchable thread, scale, and vernier caliper. The obtained results were subjected to statistical analysis following the methodology of Snedecor and Cochran (1967) [15], and the findings are discussed below.

Results and Discussion
The uterine body, characterized by its relatively short length, presented an appearance of extended length owing to the connection of the caudal parts of the horns through connective and muscular tissue, enveloped by a common peritoneal layer.

This anatomical configuration could potentially create the illusion that these caudal parts are integral components of the uterine body. The body displayed a flattened shape from its upper to lower aspects and was suspended by broad ligaments affixed to the lateral regions of the pelvic cavity. At the cranial end, the body bifurcated, giving rise to uterine horns, while at the caudal end, it extended seamlessly into the cervix. These observations closely correspond with descriptions provided by Getty (1975) [7], Ghosh (2006) [8], Hafez (1980) [9], and Frandson *et al*. (1994) [6] in the context of cows. The unique features of the buffalo uterine anatomy, as delineated above, highlight similarities with the structural characteristics reported in the literature for bovine counterparts, further reinforcing the generalizability and consistency of these anatomical features across related species.

On the luminal surface of the body, elongated oval prominences were identified as caruncles, providing attachment points for fetal membranes. The shapes and sizes of these caruncles varied slightly throughout the uterine body. These observations are consistent with findings by Asdell (2002) [1] in cows, Schmidt *et al*. (2006) [13] in African buffalo, and Bandyopadyay *et al*. (2007) [3] in bovines.

Length of the body of uterus (cm)

The length of the uterine body throughout various phases of the estrus cycle and early pregnancy exhibited a range from 1.42 to 2.21, 1.50 to 2.31, 1.42 to 2.10, 1.50 to 2.30, 1.50 to 2.00, and 1.40 to 2.10 cm, respectively. The corresponding average values were 1.67±0.08, 1.86±0.09, 1.65±0.07, 1.68±0.08, 1.68±0.04, and 1.65±0.08 cm (refer to Table 1 and 2). Notably, statistical analyses indicated that the differences in average values were not statistically significant. However, upon comparing these findings with previous studies conducted by Drennan and Macpherson (1966) [5], Getty (1975) [7], Hafez (1980) [9], Laing *et al*. (1988) [11], Chauhan and Adamu (1990) [2], Peters and Ball (1995) [12], Ghosh (2006) [8], and Asdell (2002) [1] in diverse cattle breeds, variations in reported uterine body lengths were observed. These differences might be attributed to factors such as breed, size, age, and reproductive status. Of particular note, Khan (1989) [10] reported an increase in uterine body length during early pregnancy in Nili Ravi buffalo, positing that this augmentation could be attributed to the accommodation of fetal membranes. However, the present study did not observe any statistically significant increase in uterine body length during early pregnancy. The divergence in findings emphasizes the potential influence of breed-specific characteristics and reproductive parameters on the observed variations in uterine dimensions.

Maximum circumference of the body of uterus (cm)

The maximum circumference of the uterine body across various phases of the estrus cycle and early pregnancy displayed a range from 16.83 to 19.30, 17.20 to 19.50, 16.50 to 19.00, 16.00 to 19.50, 16.00 to 19.50, and 15.00 to 21.00 cm, respectively. The corresponding average measurements were 17.70±0.24, 18.09±0.27, 17.72±0.28, 17.93±0.37, 17.18±0.32, and 17.82±0.56 cm (refer to Table 1 and 2). Importantly, statistical analyses indicated that the differences in average values were not statistically significant. The existing literature does not provide available data for comparison in terms of the uterine body circumference. This absence of comparative data makes it challenging to draw parallels or contrasts with findings from other studies. As such, this study contributes valuable insights into the specific biometric aspects of the buffalo uterus during different reproductive phases, adding a novel dimension to the existing body of knowledge in the field.

Maximum diameter of body of the uterus (cm)

The maximum diameter of the uterine body across various phases of the estrus cycle and early pregnancy demonstrated a range from 4.13 to 5.42, 4.52 to 5.30, 4.00 to 6.10, 4.30 to 6.00, 4.00 to 6.50, and 4.29 to 7.00 cm, respectively. Correspondingly, the average measurements were 4.85±0.13, 4.80±0.08, 4.92±0.21, 5.33±0.18, 5.19±0.24, and 5.74±0.34 cm (refer to Table 1 and 2). Significantly, statistical analyses indicated that the differences between the average values were statistically significant at a 1% level. However, when means were compared with at least one common superscript, no significant differences were observed, whereas the maximum diameter of the uterine body with no common superscript showed significant differences. In contrast, Chauhan and Adamu (1990) [2] reported a uterine body diameter in African zebu cattle as 3.24±6.03 cm. The observed disparity could potentially be attributed to species variation, emphasizing the importance of considering species-specific anatomical variations when interpreting and comparing biometric data. These findings underscore the need for comprehensive species-specific investigations to discern and appreciate the nuances in reproductive anatomy across different animal species.

Table 1: Range of the biometrical observations of various parameters of the uterine body of buffalo (*Bubalus bubalis)* in different phases of estrus cycle

Sr. No.	Parameters	Proestrus	Estrus	Metestrus	Diestrus	Anestrus	Early Pregnancy
1	Length of the uterine body (cm)	1.42-2.21	1.50-2.31	1.42-2.10	1.50-2.30	1.50-2.00	1.40-2.10
2	Circumference of the uterine body (cm)	16.83-19.30	17.20-19.50	16.50-19.00	16.00-19.50	16.00-19.50	15.00-21.00
3	Diameter of the uterine body (cm)	4.13-5.42	4.52-5.30	4.00-6.10	4.30-6.00	4.00-6.50	4.29-7.00

Table 2: Statistical analysis of the biometrical observations of various parameters of the uterine body of buffalo (*Bubalus bubalis)* in different phases of estrus cycle

Sr. No.	Parameter	Mean±SE						Coefficient of Variation	F value
		Proestrus	Estrus	Metestrus	Diestrus	Anestrus	Early Pregnancy		
1	Length of the uterine body (cm)	1.67±0.08	1.86±0.09	1.65±0.07	1.68±0.08	1.68±0.04	1.65±0.08	14.73	1.046^{NS}
2	Circumference of the uterine body (cm)	17.70±0.24	18.09±0.27	17.72±0.28	17.93±0.37	17.18±0.32	17.82±0.56	6.69	0.682^{NS}
3	Diameter of the uterine body (cm)	4.85$^{b\pm}$0.13	4.80$^{b\pm}$0.08	4.92$^{b\pm}$0.21	5.33$^{ab\pm}$0.18	5.19$^{ab\pm}$0.24	5.74$^{a\pm}$0.34	13.08	2.833*
*: (p≤0.05), **: (p≤0.01) and NS: Non-significant.									
Note: Mean with at least one common superscript do not differ significantly.									

Conclusion

The uterine body was of increased length compared to its actual measurement, attributed to the fusion of the caudal portions of the horns by connective and muscular tissue, enveloped by a shared peritoneal layer. Extending caudally, the body seamlessly transitioned into the cervix. Along the luminal surface of the body, distinct elongated oval prominences were observed on the mucosa, identified as caruncles, displaying variations in size and shape throughout the uterine body. Notably, the average values obtained from all biometrical observations recorded in the present study were found to be statistically non-significant. However, it is essential to highlight that differences in the average values of the maximum diameter of the uterine body reached statistical significance at a 5% level of significance. These findings underscore the intricate anatomical features of the buffalo uterus and emphasize the importance of considering optical illusions in the interpretation of uterine dimensions. Furthermore, the statistical significance in the maximum diameter differences underscores the need for meticulous attention to specific biometric parameters in reproductive anatomy studies.

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