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Exploring Morphometric Features of the Female Reproductive Tract in Brazilian Pantanal Cattle

Un Análisis de las Características Morfométricas del Tracto Reproductivo Femenino en el Ganado del Pantanal Brasileño

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SUMMARY: The cattle in the Pantanal region show a notable influence from Bos indicus breeds and their crossbreeds. However, a comprehensive biometric assessment of the reproductive system in these animals is currently lacking. This study evaluated the effects of breed, age, carcass weight, and estrous cycle phase on female reproductive system morphometry. A total of 124 healthy, non-pregnant reproductive tracts (83 Nelore and 41 Crossbred) were collected at a slaughterhouse. Neither the volume and weight of the ovaries nor the majority of uterine dimensions were affected by breed. Compared to heifers, cows showed longer uterine horns, a larger external caudal diameter of the uterine horns and body, and a greater external cranial diameter and internal caudal diameter of the cervix. The carcass weight (below vs. within commercial weight) affected the length of the uterine horns, uterine body, and cervix. Ovaries with a corpus luteum presented higher volume and weight than those with only follicles. Although the uterine measurements in the luteal phase were increased, the cycle stage did not affect the uterine morphometry. The average number of cervical rings was 5.0, independent of weight or breed. Crossbred animals presented a higher first cervical ring. Age didn´t influence the number, height, or distance between cervical rings. Deviation from the normal alignment of the cervix was recorded in 14.29 % of the animals, with a higher occurrence among Nelore cattle; deviation from the median axis was the most common (30%). This study emphasizes various parameters influencing the morphometry of the female reproductive system in Nelore cattle and their crosses, which could be crucial for implementing reproductive biotechnology techniques better suited to the morphological characteristics of zebu breeds.

KEY WORDS: Bos indicus; Brazilian Pantanal; Cervix alignment; Morphometry; Reproductive tract.

INTRODUCTION

The cattle production system in the upper Pantanal region of Mato Grosso was established by introducing Iberian breeds into this area after 1730. The introduction of these breeds, used through absorbing crosses, is at the origin of Pantaneiro cattle (Mazza *et al*., 1994), which was the foundation of cattle farming in the Pantanal region for almost three centuries (Mazza *et al*., 1992). In the 20th Century, crosses with Zebu cattle, particularly Nelore (Issa *et al*., 2006), shifted the dominant genetic background of animals used nowadays in beef production in the region (Rosa *et al*., 2007), driving also a marked decline in Pantaneiro breed (Mazza *et al*., 1994; Issa *et al*., 2006). In the last available census (2022), this region had 3,397,326 head of cattle (Instituto Brasileiro de Geografia e Estatística, 2022). Today, the Pantanal cattle predominantly comprise the Nelore breed and its crosses. Despite the substantial number of animals and diversity of breeds within this unique ecosystem and biome, a comprehensive biometric assessment of breeds and their crosses is currently lacking.

Bos indicus breeds and their crossbreeds typically perform better compared to *Bos taurus* breeds in tropical or subtropical environments. They are better adapted to cope with various stressors, including high temperatures, humidity, ectoparasite infestation, and low-quality forage (Camargo *et al*., 2007). The greater thermotolerance of the *Bos indicus* breeds makes them more suitable for livestock production in this environment. Despite these adaptive advantages, *Bos indicus* and their crossbreds could still improve their reproductive performance (Perotto *et al*., 2006).

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Assisted reproduction technologies, such as artificial insemination (AI), have been introduced to increase the herd's reproductive efficiency and the sustainability of the beef production system. Experienced AI technicians in the field frequently report notorious anatomical differences between the reproductive tract of *Bos indicus* animals or their crossbreds compared to the *Bos taurus*. Hence, detailing the reproductive tract morphometry in these animals is crucial, bolstering reproductive management and facilitating the implementation of biotechnology techniques tailored to their specific morphological characteristics. Furthermore, this would enable the adoption of clinical and zootechnical procedures, including artificial insemination, pregnancy diagnosis, in vitro and in vivo follicular manipulation, and embryo transfer, ultimately reducing infertility rates (Carvalho *et al*., 2010).

Despite its relevance for reproductive efficiency, the available information regarding biometric measurements of the reproductive system in these animals is limited. The current literature fails to encompass various factors that may impact these parameters in *Bos indicus* cattle, including breed, age, weight, or the estrous cycle stage. These factors might affect the biometric measurements of the reproductive system, subsequently affecting the interpretation of clinical findings during gynecological examinations or the application of reproductive biotechnologies (Nascimento *et al*., 2003). An integrated assessment of morphological characteristics and their influencing factors can enhance animal breeding practices. This could enable the selection of superior animals based on traits that optimize reproductive efficiency, consequently leading to improved production rates (Dirksen *et al*., 1993).

Therefore, this study aimed to assess how breed, age, weight, and estrous cycle phases influence the morphometric parameters of the reproductive system in female cattle raised in the Brazilian Pantanal region.

Table I. Inclusion and exclusion criteria used in this study.

Inclusion Criteria
Nelore or Nelore crossbred animals
Originated from Cáceres region
Existing correspondence between the carcass identification and the female reproductive tract
Age determination achieved
Carcass weight recorded
No evidence of systemic disease
Carcass approval for human consumption
Exclusion Criteria
Evidence of pregnancy or postpartum involution in the reproductive tracts
Asymmetry of the uterine horns
Evidence of reproductive tract diseases

MATERIAL AND METHOD

Sample collection. One hundred sixty-six female genital tracts were collected in September 2018 at the 3M abattoir in Cáceres, Mato Grosso, Brazil (-16.130135566692953, -57.694969557192856, 136m), and preliminarily assessed according to predefined criteria (Table I). Of the 166 original tracts, only 124 pieces were included in this study. As the batch to be slaughtered is only known on the morning of the slaughter, the number and breed of the animals were randomly conditioned for the slaughter days.

Population sample. Animals in this study (n= 124) originated from rural properties around Cáceres (MT). Based on the dental chronology [9], animals were divided into two groups: heifers (≤ 2.5 years; n= 38) and cows (≥ 2.5 years; n= 86).

Of the 124 animals in this study, 83 were Nelore, and 41 were Crossbred. Nelore and Crossbred were phenotypically distinguished based on the racial characterization of zebu cattle (ACNB Associação dos Criadores de Nelore do Brasil, 2006), performed at the entrance of the slaughter room before dehiding. Selected animals were accompanied along the slaughter line until evisceration to collect the reproductive system. Matching card numbers were inserted into the vagina and placed in the mandibula to identify the selected animals. Photos of the animals' dentition were taken for age determination. The collected samples were refrigerated during transportation for analysis after being packaged.

Based on their carcass weight, animals were further categorized below commercial weight (BCW; n=33) or within commercial weight (CW; n=91). For this categorization, we used the classification implemented in slaughterhouses in Brazil [i.e., commercial weight established at >300 kg BW for heifers and >360 kg BW for cows representing respectively a 10@ and 12@) carcass yield.

Reproductive tract biometrics

The morphometric data was obtained using a digital pachymeter, a millimeter standardized measuring tape, a ruler, a protractor, and a precision digital scale (BEL Engineering® SSR 600). Data was collected after cleaning the pieces of the excessive tissue.

Each reproductive tract was weighed and measured; the cervix positioning in relation to the reproductive tract medial axis was also evaluated, placing it in its natural position. Whenever an eventual deviation existed, its degree and direction were recorded. With the reproductive tract in an anatomical position, its total length was also recorded, from the vulva to the site of inversion of the uterine horns. The following measurements were then obtained:

Uterine horns and body: the external diameter of the uterine body (collected at the middle of the uterine body); the length of the uterine body (measured from the internal cervical ostium to the internal separation of the uterine horns); the thickness of the uterine body wall; the length of the right uterine horn (from the division of the uterine horns to the oviduct beginning); the external and internal diameter of each uterine horn (collected at a caudal point, at the bifurcation, and a cranial point, collected at 3 cm of the utero-tubal transition);

Cervix: the external diameter of the cervix (at a caudal point, over the most caudal cervical ring, representing the widest area of the cervix; and at a cranial point, over the most cranial cervical ring, representing the narrowest portion of the cervix); the internal cranial and caudal diameter of the cervical canal (obtained by inserting the handles of the caliper into the cervical canal and opening it to the maximum point of the opening, without deforming the piece); the total length of the cervix (between the most caudal cervical ring and the beginning of the ring closest to the uterine body); the number of existing cervical rings (the cervical rings were numbered from caudal to cranial). For each cervical ring, was also determined the height of each ring and the distance between rings

Vagina: the depth of the vaginal fornix was recorded, from the inversion of the cull de sac to the top of the cervical projection.

The ovaries were separated from the reproductive apparatus, weighed individually, and measured (length, height, and width) at their maximum point in each measurement. The volume of the ovaries was estimated using the formula:

$$V= (4/3) *\pi*a*b*c$$

where a = length radius, b=height radius and c=width radius. The radius measurement was defined as half of the measurements obtained for length, height, and width.

The developing structures in the ovaries were identified by inspection (Ireland *et al*., 1980) and categorized as corpus luteum or follicles; the latter were further classified according to their size, as larger or smaller than 5 mm. To score for the stage of the estrous cycle, each pair of ovaries was assessed, and the sample was further categorized as follows:

- In anestrus - ovaries with only follicles below 5 mm and devoid of luteal structure;
- In the follicular phase – ovaries with follicles larger than 5 mm and without an active corpus luteum;
- In the luteal phase - ovaries with active corpus luteum (with and without follicles).

Statistical analysis. The program IBM SPSS Statistics Version 25 ® was used for statistical analysis. The descriptive statistics were plotted for each biometric parameter, age, and breed. The data obtained from animals with a carcass weight below the commercial weight were included only to evaluate the effects of weight.

The normality of data distribution was tested with Shapiro-Wilk tests. As data did not follow a normal distribution, group comparisons were performed through non-parametric tests. The Mann-Whitney's U test for independent samples was used to compare medians, while Fisher's exact test was performed for the statistical comparison of percentages. For the statistical analysis of cervix deviations, the Z test was used to compare proportions using the Social Science Statistics program (https://www.socscistatistics.com).

RESULTS

Out of the 124 collected specimens, thirty-three originated from BCW animals. The remaining 91 samples were obtained from CW animals and were used to assess the impact of breed, age, and the estrous cycle phase on the morphometry of the female genital tract (Table II). The CW animals included in the study ranged from 2 to 10.5 years of age (4.15± 2.40 years); both racial groups (Nelore vs. Crossbred) exhibited no differences in age (median = 3 in both groups) or weight (207 kg vs. 205.25 kg). The overall length and global weight of the reproductive tract did not vary by either age or breed (Table II).

The two weight groups were exclusively compared (BCW vs. CW) to assess the influence of carcass weight on genital morphometry (Table III).

Ovarian morphometry. The median ovarian volume and weight were 4.42 cm^3 and 5.07 g, respectively (Table III). Although the ovarian volume and weight were slightly higher in cows compared to heifers, this difference was not statistically significant. BCW animals displayed smaller ovaries than CW animals (Table III), although no statistical differences were observed between these groups. Regarding the type of structures observed in the ovaries, a higher percentage of animals in anestrus was noted in the BCW group compared to the CW group (18.2% vs. 9.99%; p=0.463). Conversely, a higher percentage of animals in the luteal phase was observed in the CW group (64.84% vs. 48.48% in the BCW group; p=0.236) (Table III).

Table II. Generic characterization (age and carcass weight) of the population of Nelore and Crossbred females evaluated in this study and of the global morphometry of the reproductive tract (length and total weight). Data are presented as mean ± standard deviation [median]

	Age		Breed		Total (n=91)	Significance	
	Heifer (n=36)	Cow (n=55)	Nelore (n=63)	Crossbred (n=28)		Age	Breed
Age (years)	2.04 ±0.14 [2.00]	5.54 ±2.15 [5.00]	4.07 ±2.27 [3.00]	4.34 ±2.69 [3.00]	4.15 ±2.40 [3.00]	**p≤0.001**	p=0.958
Carcass Weight (kg)	194.85 ±26.16 [187.50]	215.93 ±24.97 [211.50]	207.92 ±26.82 [207.00]	206.84 ±28.97 [205.25]	207.59 ±27.34 [206.50]	**p=0.011**	p=0.354
Length of reproductive tract (cm)	54.16 ±4.72 [53.80]	56.72 ±6.59 [56.00]	55.5 ±5.73 [55.00]	56.16 ±6.73 [55.70]	55.71 ±6.02 [55.20]	p=0.212	p=0.662
Global weight of the reproductive tract (g)	757.44 ±168.51 [722.50]	874.72 ±253.30 [779.00]	812.13 ±210.60 [739.00]	863.5 ±268.97 [792.00]	827.93 ±229.84 [769.00]	p=0.324	p=0.452

Length of the reproductive tract was obtained from the vulva to the point of the uterine horns' inversion, the reproductive tract in its anatomical position.

Table III. Morphometric data of the reproductive tract in animals below commercial weight (n= 33) and animals with commercial weight (n=91). Morphometric values are presented in the form of mean ± standard deviation [median].

	Animals below commercial weight (BCW)		Animals with commercial weight (CW)		Total		Significance
Breeds Nelore	60.61%	(n=20)	69.23%	(n=63)	66.94%	(n=83)	p=0.862
Crossbred	39.39%	(n=13)	30.77%	(n=28)	33.06%	(n=41)	p=0.370
Carcass Weight (kg)	161.20±12.30	[162.00]	207.59±27.34	[206.50]	195.24±31.78	[188.50]	**p≤ 0.001**
Age (years)	5.06±2.13	[5.00]	4.15±2.40	[3.00]	4.4±2.35	[3.50]	p= 0.151
Length of reproductive tract (cm)	52.18±4.86	[53.00]	55.71±6.02	[55.20]	54.77±5.93	[54.20]	p= 0.266
Total weight of the reproductive tract (g)	680.73±209.65	[694.00]	827.93±229.84	[769.00]	788.76±233.14	[740.50]	p= 0.223
Ovarian Length (cm)	2.82±0.60	[2.75]	2.93±0.60	[2.90]	2.90±0.60	[2.84]	p=0.086
Ovarian Height (cm)	1.35±0.33	[1.34]	1.45±0.36	[1.41]	1.43±0.36	[1.40]	**p= 0.016**
Ovarian Width (cm)	2.06±0.40	[2.08]	2.2±0.45	[2.20]	2.16±0.44	[2.14]	p=0.114
Ovarian Volume (cm^3)	4.4±2.23	[4.06]	5.31±3.03	[4.60]	5.07±2.87	[4.42]	p=0.315
Ovarian Weight (g)	5.24±2.66	[4.56]	5.69±2.61	[5.28]	5.57±2.63	[5.07]	p=0.062
Ovaries with F≤5mm (%)	18.18 %	(n=12)	19.78%	(n=36)	19.35%	(n=48)	p = 0.904
Ovaries with F> 5 mm and without CL (%)	30.30%	(n=20)	24.18%	(n=44)	25.81%	(n=64)	p= 0.608
Ovaries with corpus luteum (%)	51.52%	(n=34)	56.04%	(n=102)	54.84%	(n=136)	p = 0.647
Anestrus	18.18%	(n=6)	9.89%	(n=9)	12.10%	(n=15)	p= 0.653
Follicular Phase	33.33%	(n=11)	25.27%	(n=23)	27.42%	(n=34)	p= 0.629
Luteal Phase	48.48%	(n=16)	64.84%	(n=59)	60.48%	(n=75)	p= 0.236
Uterine Horn's Length (cm)	25.66±5.40	[26.70]	30.189±5.81	[29.70]	28.98±6.03	[29.15]	**p= 0.015**
Uterine Body Length (cm)	1.96±0.60	[2.01]	2.61±1.03	[2.48]	2.43±0.98	[2.35]	**p= 0.004**
Number of Cervical Rings	4.91±0.77	[5.00]	4.73±1.00	[5.00]	4.77±0.94	[5.00]	p=0.471
Cervix Length (cm)	6.42±1.43	[6.30]	7.13±1.53	[7.00]	6.94±1.53	[6.66]	**p= 0.042**
Depth of vaginal fornix (cm)	1.52±0.66	[1.45]	1.87±0.82	[1.70]	1.78±0.79	[1.58]	p= 0.104

The animals' breed did not affect the ovarian volume or weight (Nelore: 4.56 cm^3 and 5.22 g vs. Crossbred: 4.73 cm^3 and 5.32 g) (Table IV). Considering the BW group, the ovaries containing a corpus luteum were notably larger (6.86±3.68 cm^3) and heavier (7.06±2.90 g) than those with only follicles, regardless of the follicular size (4.16±2.25 cm^3 & 4.63±2.23 g in ovaries displaying follicles ≤ 5 mm, and 4.91±2.17 cm^3 & 5.41±1.93 g in ovaries with follicles > 5 mm and no corpus luteum; p<0.001; Table V). While not statistically significant, there were variations in ovary morphometry according to the estrous cycle phase, with ovaries being lighter and less voluminous during anestrus (Table VI).

Uterine morphometry. The length of the uterine horn, the external cranial diameter of the uterine horn, and the external diameter of the uterine body were the only measurements significantly larger in cows (31.34±5.97 cm, 1.64±0.40 cm, and 2.57±0.59 cm, respectively) compared with heifers (28.42±5.06 cm, 1.38±0.29 cm, and 2.29±0.41 cm, respectively) (Table VII). Breed had an impact solely on the external caudal diameter of the uterine horn (p=0.007), which was higher in Nelore females (Table VII). Crossbred animals presented a deeper vaginal fornix (2.16 vs. 1.75 cm) compared to Nelore, and a longer cervix (7.51 vs. 6.96 cm), although statistical significance was not reached (Table VIII).

The measurements of the external cranial and internal caudal diameters of the cervix were significantly larger in cows compared to heifers (p=0.023) with values of 2.20 cm and 1.64 cm vs. 1.99 cm and 1.41 cm, respectively (Table VIII). Additionally, there was a trend suggesting a larger external caudal diameter in adult cows than in heifers (4.11 cm vs. 3.6 cm; p=0.065).

The morphometry of the uterine horns and body remained consistent throughout the estrous cycle phases, with an overall increase in most measurements during the luteal phase (Table IX). However, animals in the follicular phase of the cycle exhibited a higher internal diameter of the cervix

Table IV. Influence of age and breed on morphometric parameters of the ovaries. Data are presented in the form of mean±standard deviation [median].

Ovarian morphometry	Age		Breed		Total (n=182)	Significance	
	Heifers (n=72)	Cows (n=110)	Nelore (n=126)	Crossbred (n=56)		Age	Breed
Length (cm)	2.85±0.50 [2.82]	2.97±0.65 [2.95]	2.92±0.58 [2.90]	2.94±0.63 [2.90]	2.92±0.60 [2.90]	p=0.210	p=0.594
Height (cm)	1.43±0.36 [1.40]	1.47±0.37 [1.43]	1.45±0.35 [1.43]	1.46±0.40 [1.40]	1.45±0.36 [1.41]	p=0.649	p=0.872
Width (cm)	2.14±0.42 [2.10]	2.24±0.47 [2.20]	2.17±0.44 [2.20]	2.26±0.48 [2.25]	2.20±0.45 [2.20]	p=0.230	p=0.291
Volume (cm^3)	4.92±2.95 [4.32]	5.55±3.07 [4.93]	5.19±2.78 [4.56]	5.56±3.55 [4.73]	5.30±3.03 [4.60]	p=0.448	p=0.872
Weight (g)	5.37±2.46 [4.90]	5.89±2.69 [5.70]	5.62±2.51 [5.22]	5.85±2.85 [5.32]	5.69±2.61 [5.28]	p=0.172	p=0.872

Table V. Ovarian morphometry according to the categorized ovarian activity [follicles under 5mm in diameter; follicles larger than 5 mm in absence of a corpus luteum (F>5); ovaries with corpus luteum]. Data are presented in the form of mean ± standard deviation [median].

Ovarian morphometry	Follicles ≤ 5 mm (N= 68)	Follicles > 5 mm without corpus luteum (N= 51)	Ovaries with corpus luteum (with and without follicles) (N= 63)	Total (N= 182)	Significance
Length (cm)	$2.78±0.60^{a}$ [2.80]	$2.85±0.47^{ab}$ [2.84]	$3.15±0.63^{b}$ [3.10]	2.93±0.60 [2.90]	**p= 0.026**
Height (cm)	$1.30±0.30^{a}$ [1.30]	$1.48±0.31^{b}$ [1.50]	$1.60±0.41^{b}$ [1.60]	1.45±0.36 [1.41]	**p≤ 0.001**
Width (cm)	$2.03±0.40^{a}$ [2.09]	$2.12±0.32^{a}$ [2.10]	$2.45±0.49^{b}$ [2.47]	2.20±0.45 [2.20]	**p≤ 0.001**
Volume (cm^3)	$4.16±2.25^{a}$ [3.90]	$4.91±2.17^{a}$ [4.19]	$6.86±3.68^{b}$ [6.33]	5.31±3.03 [4.60]	**p≤ 0.001**
Weight (g)	$4.63±2.23^{a}$ [4.51]	$5.41±1.93^{ab}$ [5.25]	$7.06±2.90^{b}$ [7.00]	5.69±2.61 [5.28]	**p≤ 0.001**

Different superscript letters indicate statistical difference (p<0.001).

Table VI. Ovary morphometry according to the phases of estrous cycle. Data are presented in the form of mean ± standard deviation [median].

Ovarian morphometry	Anestrus (N=9)	Follicular Phase (N= 22)	Luteal Phase N= 60)	Total (N= 91)	Significance
Length (cm)	2.86±0.71 [2.90]	2.88±0.65 [2.86]	2.95±0.56 [2.93]	2.93±0.60 [2.90]	p=0.253
Height (cm)	1.34±0.28 [1.30]	1.45±0.33 [1.42]	1.47±0.38 [1.43]	1.45±0.36 [1.41]	p=0.329
Width (cm)	2.08±0.43 [2.15]	2.08±0.46 [2.10]	2.26±0.45 [2.21]	2.20±0.45 [2.20]	p=0.171
Volume (cm3)	4.56±2.46 [4.51]	4.85±2.44 [4.30]	5.59±3.28 [4.87]	5.31±3.03 [4.60]	p=0.572
Weight (g)	5.00±2.46 [4.82]	5.22±2.29 [4.95]	5.97±2.72 [5.67]	5.69±2.61 [5.28]	p=0.409

compared to those in anestrus (p= 0.030) (Table IX).

Carcass weight influenced the uterine morphometric measurements. In the BCW group, the uterine horns and the uterine body were shorter than in the CW group (medians of 26.7 cm vs. 29.7 cm, p=0.015, and 2.01 cm vs. 2.48 cm, p=0.004, respectively) (Table III). Additionally, animals in the CW group had longer cervices compared to those in the BCW group (7.00 cm vs. 6.30 cm; p=0.042) (Table III).

The median number of cervical rings recorded was 5.0, and this count remained consistent regardless of breed or animal weight (Table III). Animals with five rings represented 49.2% of the total, 26.6% presented four rings, 17.7% had six rings, 8% exhibited two or seven rings, 3.2% had three rings, and 1.6% only evidenced one cervical ring. Crossbred animals had greater ring heights than Nelore; however, statistically significant differences were observed only for the height of the first cervical ring (2.05 vs. 1.74 cm; p=0.035). Additionally, the first cervical ring tended to be higher in cows than in heifers (1.89 vs. 1.70 cm; p=0.065). No differences were observed in the number, height, or distance of the remaining cervical rings based on age or breed (Table VIII).

Deviation of the cervix from the usual rectilinear alignment was observed in close to 14.29% (n=13) of the evaluated reproductive tracts (Table X). However, none of the deviation parameters analyzed showed statistical significance.

Table VII. Influence of age (heifer and cow) and breed (Nelore and Crossbred) on the measurements of uterine horns and body. Data are presented in the form of mean ± standard deviation (n) [median].

Measurements (in cm)		Age		Breed			Significance	
		Heifers	Cows	Nelore	Crossbred	Total	Age	Breed
Uterine Horns	Length	28.42±5.06 (36) [29.00]	31.34±5.97 (55) [30.6]	29.93±6.17(63) [29.7]	30.76±4.83 (28) [29.6]	30.19±5.79 (91) [29.7]	**p≤0.023**	p=0.875
	External Caudal Diameter	1.38±0.29(72) [1.37]	1.64±0.40(110) [1.57]	1.53±0.40(126) [1.46]	1.55±0.33(56) [1.44]	1.54±0.38(182) [1.45]	**p≤0.0001**	p=0.838
	External Cranial Diameter	0.69±0.17(72) [0.67]	0.71±0.18(110) [0.68]	0.73±0.18(126) [0.69]	0.65±0.16(56) [0.64]	0.70±0.18(182) [0.67]	p=0.550	**p=0.007**
	Internal Caudal Diameter	0.31±0.22(68) [0.23]	0.32±0.13(98) [0.31]	0.31±0.17(114) [0.28]	0.33±0.17(52) [0.30]	0.31±0.17(166) [0.28]	p=0.788	p=0.556
	Internal Cranial Diameter	0.17±0.14(68) [0.13]	0.15±0.06(98) [0.15]	0.16±0.11(114) [0.14]	0.14±0.07(52) [0.14]	0.16±0.10(166) [0.14]	p=0.214	p=0.219
Uterine Body	Length	2.61±1.19(36) [2.40]	2.6±0.91 (55) [2.50]	2.51±1.09(63) [2.35]	2.83±0.84(28) [2.56]	2.61±1.03 (91) [2.48]	p=0.413	p=0.986
	External Diameter	2.29±0.41(36) [2.28]	2.57±0.59(55) [2.53]	2.46±0.55(63) [2.36]	2.47±0.54(28)[2.42]	2.46±0.54(91) [2.40]	**p≤0.018**	p=0.903
	Internal Diameter	0.33±0.29(34) [0.27]	0.38±0.20(49) [0.32]	0.36±0.24(57) [0.29]	0.37±0.24(26)[0.36]	0.36±0.24(83) [0.30]	**p=0.014**	p=0.120

Table VIII. Influence of age and breed on morphometric measurements of the cervix and vaginal fornix. Data are presented in the form of mean±standard deviation (n) [median].

Morphometry		Age		Breed			Significance	
		Heifers	Cows	Nelore	Crossbred	Total	Age	Breed
Cervix (in cm)	Length	7.05±1.39(36) [6.68]	7.17±1.63(55) [7.08]	6.96±1.41(63) [6.66]	7.51±1.73(28) [7.53]	7.13±1.53(91) [7.00]	p=0.897	p=0.228
	External Caudal Diameter	3.60±0.69(36) [3.60]	4.11±1.04(55) [4.16]	3.91±0.97(63) [3.79]	3.88±0.89(28) [3.83]	3.90±0.95(91) [3.79]	p=0.065	p=0.875
	External Cranial Diameter	1.99±0.40(36) [2.02]	2.20±0.50(55) [2.27]	2.10±0.43(63) [2.10]	2.16±0.56(28) [2.21]	2.12±0.47(91) [2.10]	**p= 0.023**	p=0.766
	Internal Caudal Diameter	1.41±0.40(36) [1.36]	1.64±0.61(55) [1.57]	1.53±0.54(63) [1.40]	1.60±0.56(28) [1.3]	1.55±0.55(91) [1.47]	**p= 0.023**	p=0.452
	Internal Cranial Diameter	0.76±0.25(36) [0.80]	0.82±0.30(55) [0.80]	0.78±0.28(63) [0.76]	0.83±0.28(28) [0.90]	0.80±0.28(91) [0.80]	p=0.834	p=0.137
Number of cervical rings		4.97±0.70(36) [5.00]	4.56±1.13(55) [5.00]	4.71±0.97(63) [5.00]	4.75±1.08(28) [5.00]	4.73±1.00(91) [5.00]	p=0.802	p=0.944
Depth of vaginal fornix (in cm)		1.71±0.72(36) [1.54]	1.98±0.86(55) [1.77]	1.75±0.76(63) [1.51]	2.16±0.88(28) [2.03]	1.87±0.82(91) [1.70]	p=0.212	p=0.072
Height of the cervical rings (in cm)	1st cervical ring	1.77±0.59(36) [1.70]	2.17±0.96(55) [1.89]	1.89±0.86(63) [1.74]	2.26±0.83(28) [2.05]	2.01±0.86(91) [1.82]	p=0.065	**p=0.035**
	2nd cervical ring	1.15±0.36(36) [1.08]	1.27±0.67(55) [1.11]	1.17±0.52(63) [1.04]	1.34±0.65(28) [1.33]	1.22±0.57(89) [1.09]	p=0.699	p=0.040
	3rd cervical ring	0.96±0.42(36) [0.91]	0.99±0.41(54) [0.93]	0.94±0.39(62) [0.90]	1.06±0.45(28) [1.04]	0.98±0.41(88) [0.93]	p=0.969	p=0.286
	4th cervical ring	0.74±0.36(36) [0.68]	0.72±0.34(51) [0.70]	0.72±0.33(61) [0.70]	0.76±0.33(26) [0.78]	0.73±0.33(85) [0.70]	p=0.899	p=0.307
	5th cervical ring	0.60±0.25(28) [0.51]	0.64±2.23(34) [0.59]	0.62±0.23(41) [0.53]	0.64±0.26(21) [0.58]	0.62±0.24(62) [0.54]	p=0.202	p=1.000
	6th cervical ring	0.50±0.25(6) [0.45]	0.72±0.35(9) [0.67]	0.72±0.33(10) [0.64]	0.46±0.26(5) [0.41]	0.63±0.33(15) [0.53]	p=0.119	p=0.282
Distance between rings (in cm)	1st and 2nd cervical ring	1.33±0.43(36) [1.26]	1.21±0.46(53) [1.20]	1.24±0.43(62) [1.21]	1.31±0.51(27) [1.23]	1.26±0.45(89) [1.21]	p=0.462	p=0.944
	2nd and 3rd cervical ring	0.94±0.36(36) [0.89]	1.05±0.34(52) [1.00]	0.95±0.33(61) [0.93]	1.14±0.36(27) [1.04]	1.01±0.35(88) [0.98]	p=0.515	p=0.644
	3rd and 4th cervical ring	0.90±0.27(35) [0.83]	0.80±0.32(49) [0.80]	0.83±0.30(58) [0.80]	0.86±0.31(26) [0.82]	0.84±0.30(84) [0.80]	p=0.847	p=0.928
	4th and 5th cervical ring	0.65±0.32(31) [0.70]	0.70±0.37(37) [0.73]	0.65±0.36(45) [0.63]	0.72±0.34(23) [0.73]	0.67±0.35(68) [0.73]	p=0.626	p=0.608
	5th and 6th cervical ring	0.54±0.17(6) [0.56]	0.64±0.23(9) [0.60]	0.59±0.23(10) [0.58]	0.62±0.19(5) [0.55]	0.60±0.21(15) [0.56]	p=0.287	p=0.580

Although not statistically significant, cervix deviations were more prevalent in heifers than cows (19.44% vs. 10.91%; p=0.254).

Deviation of the cervix with less than 30º was the most frequently observed, with no significant differences between heifers and cows (85.71% vs. 83.33%; p=0.904). While more prevalent in Nelore, crossbred females exhibited only cervical deviations with less than 30º (80% vs. 100%; p=0.400) (Table X). Cervical deviations of more than 30° were observed in only two Nelore animals, one heifer and one cow. The most commonly observed deviation was medial, occurring more frequently in cows than in heifers (83.33% vs. 57.14%; p=0.307). Conversely, lateral angulation of the cervix was detected more often in heifers than in cows (42.86% vs. 16.67%; p=0.307; refer to Table X). Considering the direction of deviation, it is worth noting that deviations along both axes (median and transverse) were more prevalent in the Nelore breed (Table X).

Table IX. Differences in uterine morphometry in animals in different estrous cycle phases. Data are presented in the form of mean±standard deviation [median].

Uterine morphometry (in cm)		Anestrus (n= 9)		Follicular Phase (n=22)		Luteal Phase (n=60)		Total (n=91)		Significance
Uterine Horns	Length	26.98±4.70	[27.5]	29.08±5.63	[29.1]	31.07±5.80	[30.3]	30.19±5.79	[29.7]	p=0.099
	External Caudal Diameter	1.44±0.57	[1.40]	1.52±0.35	[1.41]	1.56±0.36	[1.48]	1.54±0.38	[1.45]	p=0.409
	External Cranial Diameter	0.69±0.18	[0.67]	0.69±0.17	[0.63]	0.71±0.18	[0.68]	0.7±0.18	[0.67]	p=0.517
	Internal Caudal Diameter	0.31±0.23	[0.22]	0.34±0.19	[0.29]	0.31±0.15	[0.29]	0.31±0.17	[0.28]	p=0.592
	Internal Cranial Diameter	0.21±0.21	[0.17]	0.17±0.12	[0.14]	0.15±0.06	[0.14]	0.16±0.10	[0.14]	p=0.165
Uterine Body	Length	2.29±0.64	[2.34]	2.73±1.40	[2.41]	2.61±0.91	[2.53]	2.61±1.03	[2.48]	p=0.316
	External Diameter	2.22±0.74	[2.22]	2.38±0.40	[2.34]	2.53±0.54	[2.41]	2.46±0.54	[2.4]	p=0.568
	Internal Diameter	0.45±0.41	[0.28]	0.32±0.19	[0.27]	0.36±0.23	[0.31]	0.36±0.24	[0.30]	p=0.096
Cervix	Length	7.04±1.08	[7.00]	7.16±1.62	[7.19]	7.13±1.56	[6.69]	7.13±1.53	[7.00]	p=0.706
	Internal Cranial Diameter	1.39±0.53	[1.09]	1.62±0.62	[1.44]	1.55±0.52	[1.48]	1.55±0.55	[1.47]	p=0.905
	Internal Caudal Diameter	0.65±0.21[a]	[0.70]	0.83±0.31[b]	[0.86]	0.81±0.27[b]	[0.80]	0.8±0.28	[0.80]	**p=0.030**

Different superscript indicates statistical difference (p<0.05).

Table X. Changes in the positioning of the cervix. considering the alignment, degree and direction of deviation.

		Age		Breed			Significance	
		Heifer	Cows	Nelore	Crossbred	Total	Age	Breed
Cervix Alignment	Rectilinear	29 (80.56%)	49 (89.09%)	53 (84.13%)	25 (89.29%)	78 (85.71%)	p=0.254	p=0.515
	Deviation	7 (19.44%)	6 (10.91%)	10 (15.87%)	3 (10.71%)	13 (14.29%)		
	Total	36	55	63	28	91	-	-
	Significance	**p ≤0.0001**	**p ≤0.0001**	**p ≤0.0001**	**p ≤0.0001**	**p ≤0.0001**	-	-
Degree of Cervix Deviation	Up to 30°	6 (85.71%)	5 (83.33%)	8 (80.00%)	3 (100.00%)	11(84.61%)	p=0.904	p=0.400
	Between 31° to 60°	1 (14.29%)	1 (16.67%)	2 (20.00%)	0 (0.00%)	2 (18.18%)		
	Total	7	6	10	3	13	-	-
	Significance	**p = 0.008**	**p = 0.002**	**p = 0.007**	**p=0.014**	**p ≤0.0001**	-	-
Direction of Cervix Deviation	Transverse Deviation	3 (42.86%)	1 (16.67%)	4 (40.00%)	0 (0.00%)	4 (30.77%)	p=0.307	0.186
	Median Deviation	4 (57.14%)	5 (83.33%)	6 (60.00%)	3 (100.00%)	9 (69.23%)		
	Total	7	6	10	3	13	-	-
	Significance	p = 0.596	**p = 0.020**	p = 0.373	**p=0.014**	**p = 0.050**	-	-

DISCUSSION

Nelore genetics predominates in the Pantanal cattle production in Mato Grosso region (Barbosa *et al*., 2014). It is commonly used in crosses with various European breeds to enhance beef and milk production. Difficulties have been reported in employing purebred European animals for natural mating. As an alternative, breeders utilize bulls of composite breeds (such as Brangus, Braford, and Canchim) or resort to artificial insemination using semen from European breeds to enhance productivity in the Pantanal region (Rosa *et al*., 2007). In this production system, it is common for small producers to breed Nelore with crossbred animals to produce calves with good weight gain, even if it diminishes the milk potential of their farm. This backcrossing strategy dilutes the prevalence of European genetics and increases the proportion of Nelore genes in the crossbred animals in the Pantanal region. It allows them to retain some reproductive performance traits while maintaining higher thermal and parasitic tolerance. The specific degree of crossbreeding or the breeds involved remains unspecified, but one could infer that it reflects the reality of the Pantanal beef production system. Although somewhat greater in crossbred animals, most morphometric data collected in Nelore and crossbred females exhibited notable similarity, hinting at a high level of adsorption of Nelore genes respecting the reproductive tract biometrics.

Compared to the existing information on ovarian morphometry in *Bos indicus* cattle raised in Brazil, our study has addressed several previously unexplored parameters. The primary morphometric parameters of the ovaries (length, height, width, and weight) observed in this study align with findings reported by other authors (Neves *et al*., 2002; Monteiro *et al*., 2008; Carvalho *et al*., 2010; Dias Junior *et al*., 2016). However, the estimated ovary volume in this study is slightly lower than the one Neves *et al*. (2002) reported. Although these authors did not specify the method used to estimate ovarian volume, a methodology disparity could account for this difference. In comparison to animals of different zebu breeds in other regions, the morphometric parameters of the ovaries obtained in our study are notably higher (Kunbhar *et al*., 2003; Bello *et al*., 2012; Perumal *et al*., 2013; Kouamo *et al*., 2017; Islam *et al*., 2019). These disparities could stem from local selection practices focusing on superior genotypes and phenotypes for enhancing production efficiency, animal management, and feeding (de Souza *et al*., 2018). This selection process likely contributes also to varying conformations and mature weights among the animals.

Age did not appear to impact ovarian morphometry, likely due to the uncommon practice of marketing pre-pubertal animals for slaughter in this region. This effect might have been more noticeable if this study had included younger animals.

We observed a slight increase in ovarian morphometric measurements during the cycle's luteal phase, consistent with findings from Nascimento *et al*. (2003). In our study, the presence of a corpus luteum (CL) in the ovary notably augmented ovarian weight and volume, irrespective of the presence of other structures. This observation suggests that the denser luteal tissue might exert a more pronounced influence on the overall weight of the ovary.

The female body condition, categorized by commercial weight after slaughter (BCW vs. CW), demonstrated an impact on ovarian morphometric data. Generally, animals below the commercial weight exhibited lower values. Additionally, this group displayed a reduced proportion of animals with ovarian activity or corpus luteum, indicating decreased ovarian activity. The importance of body condition in reproductive function is widely acknowledged. Animals with adequate body condition are more likely to maintain regular cyclic ovarian activity than lean or obese animals (Possa *et al*., 2015). Furthermore, nutritional deficiencies can reduce to reduced ovarian size and functionality (Sakate *et al*., 2013).

In terms of uterine morphometry, the average lengths of the uterine horns found in this study were higher compared to those reported by Monteiro *et al*. (2001, 2008) but closer to the values reported by Carvalho *et al*. (2010), both of which utilized Brazilian zebu animals. However, studies involving zebu from diverse geographical origins depict lower values than those reported here (Kunbhar *et al*., 2003; Bello *et al*., 2012; Perumal *et al*., 2013; Kouamo *et al*., 2017; Islam *et al*., 2019). These disparities may arise due to variations in the conformation and weight of zebu females across different geographic regions, suggesting a considerable variability in the reproductive tracts within the *Bos indicus* breed.

Age primarily influenced the length of the uterine horns and the external cranial diameter, with all uterine parameters typically higher in cows compared to heifers. These differences might be attributed to prior pregnancies in the cow group.

The CW group presented longer uterine horns than the BCW group. Montanholi *et al*. (2004) reported a positive correlation between weight gain and an increase in the reproductive tract score. Additionally, it was noted that heifers with higher rates of weight gain exhibit earlier development of the reproductive tract (Montanholi *et al*., 2004).

The findings in this study evidenced a longer uterine body average compared to previous studies (Kunbhar *et al*., 2003; Bello *et al*., 2012; Perumal *et al*., 2013; Kouamo *et al*., 2017), yet lower than those reported by Islam *et al*. (2019) in *Bos indicus* females in Bangladesh. Additionally, larger uterine body diameters were found herein compared to Kunbhar *et al*. (2003) and Islam *et al*. (2019) but lower than the values presented by Bello *et al*. (2012) or Perumal *et al*. (2013). Such differences may result from existing core *Bos taurus* influences found in Nelore animals in the Pantanal region. These outcomes also suggest that animals from the Brazilian Pantanal exhibit a longer and narrower uterine body compared to most zebu breeds. This information may be relevant for technicians conducting artificial insemination in these animals.

Existing data on other zebu breeds from various regions suggest significant variability in the cervix length. Some studies report higher values compared to those presented here, both for cervix length (Kunbhar *et al*., 2003; Carvalho *et al*., 2010; Bello *et al*., 2012; Perumal *et al*., 2013; Kouamo *et al*., 2017) and external diameter (Bello *et al*., 2012; Kouamo *et al*., 2017; Islam *et al*., 2019). Nevertheless, the values obtained in our study were higher for cervix length compared to those by Islam *et al*. (2019), and lower for outer diameter compared to Kunbhar *et al*. (2003) and Perumal *et al*. (2013).

In this study, a larger number of cervical rings were observed compared to those reported in animals of *Bos taurus* lineages (Hafez, 1988) and other zebu breeds (Carvalho *et al*., 2010; Perumal *et al*., 2013). The number of cervical rings typically determines the length of the cervix, which is generally slightly longer in *Bos indicus* than in *Bos taurus* animals (Correia *et al*., 2018). A higher number of rings might pose challenges during artificial insemination due to increased barriers (rings) to overcome when accessing the uterine body.

This study marks the first detailed description of cervical ring morphometric measurements in cattle. Significant breed effects were solely observed for the height of the first cervical ring, although rings also tended to be higher in cows. The increased measurements of the first cervical ring may facilitate its localization and the insertion of the insemination gun. This assertion is supported by the author's (HAF) field experience during AI technical courses, mainly when working with zebu and crossbred cows in field classes.

The estrous cycle phase impacted the length and internal cranial diameter of the cervix, with higher measurements observed during the follicular phase. This finding aligns with the observations of da Silva (1981) and Hafez (1988) regarding the influence of estrogen on cervix swelling during estrus. Additionally, carcass weight category also affected cervix length, showing higher values in CW animals, suggesting a positive association between body condition and the development of the cattle reproductive tract.

It is commonly accepted that the cervix is relatively linear. Deviations from the normal alignment on bovines are rare, and little information is available on the impact or prevalence of such variations in cattle. In the present study, 14.3% (n=13) of the animals showed cervix deviation, an incidence higher in heifers than in cows. Deviations lower than 30º and in the median plane prevailed. The lower prevalence in cows suggests this is not an acquired condition, secondary to calving problems or dystocia that may damage the cervix. Since an effect of age or breed on the incidence of cervix deviations was not observed, it is hypothesized that they may have a genetic background. Otte *et al*. (2016), describing a case of cervix deviation, suggested that severe changes in the alignment of the cervix may impair reproductive performance and be associated with low pregnancy rates in animals submitted to artificial insemination. Data gathered herein does not allow a conclusion in this regard. However, increased difficulty may be encountered when performing artificial insemination in these animals, even though it is often possible to insert the AI gun through the cervix without causing any injury with a bit of patience and correct handling.

CONCLUSION

The comprehensive data presented here could aid in developing anatomical models depicting the reproductive apparatus of *Bos indicus*. Such models, designed to be more adapted, reliable, and realistic, could enhance the training of technicians in reproductive biotechnology procedures. The knowledge of morphometric measurements and their combined variations may significantly support the implementation of effective reproductive management and biotechnology techniques tailored to the morphological characteristics of zebu breeds.

AZUAGA FILHO, H.; PAYAN-CARREIRA, R. & COLAÇO, B. Un análisis de las características morfométricas del tracto reproductivo femenino en el ganado del Pantanal brasileño. *Int. J. Morphol., 42(3)*:795-804, 2024.

RESUMEN: El ganado de la región del Pantanal muestra una notable influencia de las razas *Bos indicus* y sus cruces. Sin embargo, actualmente falta una evaluación biométrica exhaustiva del sistema reproductivo de estos animales. Este estudio evaluó los efectos de la raza, la edad, el peso de la canal y la fase del ciclo estral sobre la morfometría del sistema reproductivo de la hembra. En un matadero se recogieron un total de 124 tractos reproductivos sanos y no preñados (83 Nelore y 41 cruzados). Ni el volumen ni el peso de los ovarios ni la mayoría de las dimensiones del útero se vieron afectados por la raza. En comparación con las novillas, las vacas mostraron cuernos uterinos más largos, un diámetro caudal externo más grande de los cuernos y del cuerpo uterino, y un diámetro craneal externo y un diámetro caudal interno más grandes del cuello uterino. El peso de la canal (por debajo *versus* dentro del peso comercial) afectó la longitud de los cuernos uterinos, el cuerpo uterino y el cuello uterino. Los ovarios con cuerpo lúteo presentaron mayor volumen y peso que aquellos con solo folículos. Aunque las medidas uterinas en la fase lútea aumentaron, la etapa del ciclo no afectó la morfometría uterina. El número promedio de anillos cervicales fue de 5,0, independientemente del peso o la raza. Los animales mestizos presentaron un primer anillo cervical más alto. La edad no influyó en el número, altura o distancia entre anillos cervicales. La desviación de la alineación normal del cuello uterino se registró en el 14,29 % de los animales, con mayor ocurrencia en el ganado Nelore; la desviación del eje de la mediana fue la más común (30%). Este estudio enfatiza varios parámetros que influyen en la morfometría del sistema reproductor de las hembras en el ganado Nelore y sus cruces, lo que podría ser crucial para implementar técnicas de biotecnología reproductiva más adecuadas a las características morfológicas de las razas cebú.

PALABRAS CLAVE: *Bos indicus*; **Pantanal brasileño; Alineación del cuello uterino; Morfometría; Aparato reproductor.**

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