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SHORT COMMUNICATION

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Morphometric characterization of Lidia cow (*Bos taurus*) reproductive apparatus

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Abstract

To study Lidia cow reproductive apparatus traits, a total of 90 organs were collected after slaughtering the cows from different *Bos taurus* breeds: (i) Lidia cattle breed - Brava dos Açores population (n=10) and Domecq lineage (n=11); (ii) Holstein Friesian females – 10-14-month-old heifers (n=15); 15-20-month-old heifers (n=10), 21-19-month-old heifers (n=18), and (iii) cows ≥ 30 months (n=26). The length and width were measured for five portions of the female reproductive apparatus (vulva and vagina, cervix, uterine body, uterine horns and ovaries). One-way ANOVA was performed with Tukey test. The level recognized to assume differences was $p<0.05$ to less. Differences were not shown between Lidia groups. In general, the Lidia cow reproductive apparatus was small in size that that of the matured cows in terms of all traits, with the exception of cervix rings (5.10 ± 0.17 rings) with $p\leq0.01$ for all the groups (averages ranged from 3.33 ± 0.11 rings to 3.50 ± 0.15 rings). The vulva and vagina (L= 27.31 ± 0.53 cm; W=2.07 ± 0.14 cm), the uterine body width (3.01 ± 0.18 cm) and the uterine horns (L= 12.24 ± 0.32; W= 1.13 ± 0.10) showed were smaller in size than those of the evaluated heifers from HF breed that ranged in age from 10 to 14 months ($p\leq0.01$). This study was the first to perform a morphometric characterization on the Lidia cow reproductive apparatus, and the results provide useful information for understanding reproductive approaches to be used with this breed.

Additional keywords: Lidia cattle breed; bovine female reproductive apparatus.

Abbreviations used: AI (artificial insemination); ART (assisted reproductive techniques); ET (embryo transfer); HF (Holstein Friesian breed); L (length); SEM (standard error of the mean); W (width).

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Introduction

The application of reproductive technologies varies widely in livestock production, especially in terms of its use in bovines. However, some particular breeds, such as the Lidia breed (fighting bull breed), present particular morphological traits, such as the smaller size, which pose challenges to the application of assisted reproductive techniques (ART) (Gomez, 2006), such artificial insemination (AI) or embryo transfer (ET). Moreover, as Lidia breed animals are selected for aggressiveness (Silva *et al.*, 2006), this behavioral trait may also be a complicating factor in ART procedures.

The anatomy of the female reproductive apparatus is one of the basic issues in some livestock cattle production operations. Most of the reproductive structures can be palpated through the rectum; this is the basis of routine fertility work performed through

the application of ART, pregnancy diagnoses and clinical examinations (Mukasa-Mugerwa, 1989; Jackson & Cockroft, 2002). The characteristics of the female bovine reproductive apparatus differs among subspecies, or even among breeds. Reports in old references, related to the reproductive apparatuses of *Bos indicus* (Megale & Couto, 1959) and *Bos taurus* (Perkins *et al.*, 1954; Roberts, 1971; Filho, 1982; Hafez, 1982; Sisson & Grossman, 1986), state that, in general, the reproductive tract of *Bos indicus* cattle is smaller than that of taurine cattle (Mukasa-Mugerwa, 1989). Recent studies have evaluated specific characteristics of the reproductive apparatus of the female bovine, such as the ovaries in pregnant and non-pregnant *Bos indicus* cows (Chacur *et al.*, 2006; Ramos *et al.*, 2008) and, more recently, Bărdaş *et al.* (2014), characterized the female reproductive apparatus of the Bălţată Românească breed (*Bos taurus*). As far as the Lidia bovine reproductive apparatus is concerned, only two reports described their small size and the nonexistence of uterine body (Goméz 2006; Patrón & Télles, 2013). Additionally, the number of Lidia cattle oocytes per ovary was low when compared to the recovery rates from other breeds (Demyda-Peyrás *et al.*, 2013). The same authors connected this finding with the moderate fertility in Lidia breed (Jiménez *et al.*, 2007). They also attributed it to the fact that their selection process is focused on aggressiveness because hostile animals have lower reproductive performances (Phocas *et al.*, 2006), as previously described. Nevertheless, the lower oocytes recovery rates after slaughtering animals, observed by Demyda-Peyrás *et al.* (2013) cannot be directly related with reproductive performance impairment, but rather to the small size of Lidia ovaries.

The aim of the present study was characterize the Lidia cow reproductive apparatus through morphometric assessment, and compare the results with those obtained from the Holstein Friesian (HF) breed.

Material and methods

Morphometric assessment

A total of 90 reproductive apparatuses from non-pregnant bovine female were collected and measured immediately after they had been slaughtered for commercial purposes. Animals were selected without any reproductive problem and in non-pregnant stages. The estral cycle stage was not detected. Afterwards, animals were divided into six groups: Group A with 10 heifers belonging to Brava dos Açores population; group B with 11 Lidia cattle heifers belonging to the Domecq lineage. Animals belonging to these groups were aged between 30 to 35 months. In the group C, D, E and F, all animals were from the HF breed and divided according their age: HF heifers from 10 to 14 months (n=15); HF heifers from 15 to 20 months (n=10); HF heifers from 21 to 29 months (n=18) and Holstein cows more than 30 months old (n=26).

The parameters chosen for the morphometry were the length and the width of the different components of the female reproductive apparatus: vulva and vagina, cervix, uterine body, uterine horns and ovaries. Parts were measured using a caliper and a ruler ribbon, fixed on a table. All of the widths of the different parameters and the ovary lengths were measured by caliper. All the other measures were performed using the ruler ribbon. To facilitate measuring, samples were first cleaned and connective tissues and ligaments were detached. For the uterine horns, the measurements were made from the end of the uterine body to the beginning of the bifurcation until the utero-tubal junction. For the vulva and the vagina portion, the distance between the vulva and the beginning of cervix was measured. The width measurements were taken in the middle of vagina, cervix and uterine body. Uterine horns widths were taken at the beginning of the horns bifurcation, and the caliper was positioned in the extreme faces of the ovaries to make the width and length assessments. The number of antral follicles was counted in all 180 ovaries.

Statistical analysis

All the statistical analyses were performed using the SPSS vers. 21 software. Before statistical differences analysis, the normality and homogeneity evaluations were obtained for the different variables. To assess normality distribution of the different variables, the Kolmogorov-Smirnov test was employed. Levene statistics were performed to test the equal homogeneity, and a one-way ANOVA was performed using the Tukey test to assess the statistical differences among groups. The level of significance accepted was from 5% to less. Results are presented as mean ± standard error of the mean (SEM).

Results and discussion

The Lidia cattle breed is constituted by several lineages, with different morphological traits (Montesinos, 2002). This fact has been not confirmed by the present study as heifers belonging to Lidia cattle showed similar values for all parameters measured among Brava dos Açores and Domecq cows (Table 1), both lineages without genetic relationships (Correia *et al.*, 2014). For almost

Table 1. Morphometric measures of female reproductive apparatus. A, B: Lidia cattle groups; C, D, E, F: Holstein breed. A: Brava dos Açores population (n=10); B: Domecq lineage (n=11); C: 10-14 month Heifers (n=15); D: 15-20 month Heifers (n=10); E: 21-29 month Heifers (n=18); F: cows ≥ 30 months (n=26). The results are presented as mean ± standard error of the mean (SEM). Statistical differences performed by ANOVA are presented between Lidia cattle and other groups ([a] $p \leq 0.05$; [b] $p \leq 0.01$).

Female reproductive apparatus traits		Lidia cattle			Holstein breed			
		(A)	(B)	(A and B)	(C)	(D)	(E)	(F)
Vagina	Lenght	26.15 ± 0.76	28.36 ± 0.61	27.31 ± 0.53	32.11 ± 0.82[b]	32.82 ± 1.41[b]	32.69 ± 0.64[b]	37.52 ± 0.71[b]
	Width	1.68 ± 0.15	2.42 ± 0.18	2.07 ± 0.14	3.32 ± 0.11[b]	3.36 ± 0.16[b]	3.39 ± 0.20[b]	3.99 ± 0.13[b]
Cervix	Lenght	4.34 ± 0.21	4.97 ± 0.24	4.67 ± 0.17	4.74 ± 0.25	5.26 ± 0.28	6.27 ± 0.30[b]	7.34 ± 0.31[b]
	Width	1.42 ± 0.17	1.42 ± 0.16	1.42 ± 0.11	1.73 ± 0.10	1.70 ± 0.11	1.85 ± 0.10[a]	2.88 ± 0.10[b]
	Ring number	4.90 ± 0.23	5.27± 0.24	5.10 ± 0.17	3.40 ± 0.19[b]	3.50 ± 0.17[b]	3.33 ± 0.11[b]	3.50 ± 0.15[b]
Uterine body	Lenght	2.32 ± 0.36	1.42 ± 0.23	1.85 ± 0.23	2.10 ± 0.30	2.00 ± 0.34	1.90 ± 0.23	4.50 ± 0.31[b]
	Width	1.26 ± 0.20	1.54 ± 0.22	1.40 ± 0.67	3.01 ± 0.18[b]	3.06 ± 0.41[b]	3.16 ± 0.16[b]	4.09 ± 0.08[b]
Uterine horn	Lenght	11.91 ± 0.53	12.55 ± 0.38	12.24 ± 0.32	18.58 ± 0.78[b]	18.48 ± 1.27[b]	19.07 ± 0.62[b]	20.16 ± 0.62[b]
	Width	0.89 ± 0.13	1.35 ± 0.11	1.13 ± 0.10	1.64 ± 0.08[a]	1.74 ± 0.19[a]	1.75 ± 0.09[a]	2.44 ± 0.11[b]
Ovary	Lenght	2.53 ± 0.10	2.82 ± 0.10	2.68 ± 0.08	2.64 ± 0.144	2.78 ± 0.11	3.02 ± 0.12	3.31 ± 0.1[b]
	Width	1.67 ± 0.11	2.05 ± 0.08	1.87 ± 0.08	1.39 ± 0.11[a]	1.44 ± 0.12[a]	1.64 ± 0.07	2.03 ± 0.09[b]
	Follicle number	11.05 ± 1.25	10.95 ± 1.94	11.00 ± 1.15	17.9 ± 2.55	14.15 ± 3.17	23.97 ± 4.46[a]	26.71 ± 3.20[b]

traits, the reproductive apparatuses observed in Lidia heifers were smaller in size than heifers belonging to the HF breed (C, D and E). Compared to animals of about the same age both for Lidia and HF, Lidia cattle had a small vulvas and vaginas (length (L) = 27.31 ± 0.53 cm; width (W) = 2.07 ± 0.14 cm) compared to the HF group with 32.69 ± 0.64 and 3.39 ±, 0.20 respectively for length and width ($p \leq 0.01$). For the group of cows, values obtained by this parameter were higher (37.52 ± 0.71 cm), which supported previously published data (Roberts, 1971; Filho, 1982; Sisson & Grossman, 1986; Hafez, 1988). In these previous studies, measurements varied between 30 cm and 42 cm. Results obtained in the present study for the measurements of this parameter in Lidia cattle, are however similar to those published by Bărdaş *et al.* (2014), working with cows from the Bălţată Românească breed, in which the vaginal length was 27.22 cm.

In the present study, differences were also observed between Lidia and HF cattle for the cervix measurements, being respectively L = 4.67 ± 0.17 cm; W = 1.42 ± 0.11 cm and L = 6.27 ± 0.30 cm; W = 1.85 ± 0.10 cm for Lidia and HF, as well as for its number of annular rings (5.1 ± 0.17 *vs* 3.33 ± 0.11) ($p \leq 0.01$). Jackson & Cockroft (2002), analyzed heifers and reported that their cervix was about 2 cm in width and 4 cm in length. As far as the number of rings are concerned, our values for the HF ring numbers are not in agreement with those obtained by Roberts (1971), in which the number of cervix rings for *Bos taurus*, excluding Lidia, was similar to those obtained for the Lidia heifers by the present study. Nevertheless, it must be noted that the measurement criteria described in several studies are not clear and, for this reason, comparisons with other reports must be used only cautiously.

No statistical differences were observed among breeds related to uterine body length. However, for width, Lidia heifers presented a much thinner uterine body than that observed in the HF heifers (1.40 ± 0.67 cm *vs* 3.16 ± 0.16 cm; $p \leq 0.01$). For the uterine horns and ovary, data were collected separately for left and right horn and ovary. No statistical differences were found among them and, for this reason, data represent the average obtained for both. Lidia heifers showed lower lengths compared to the HF animals, with 12.24 ± 0.32 cm *vs* 19.07 ± 0.62 cm ($p \leq 0.01$), respectively. In terms of ovary size, no statistical differences were observed among heifers from different breeds. The only statistical difference was observed between the numbers of follicles, which was significantly higher in the HF as compared to the Lidia breed, respectively 11.00 ± 1.15 *vs* 23.97 ± 4.46. As the ovaries of Lidia cattle showed lower number of follicles when compared with HF animals, one would expect that the number of oocytes in Lidia ovaries is smaller, as suggested by Demyda-Peyrás *et al.* (2013).

This experiment was the first to carry out a morphemic characterization of the female reproductive apparatus of Lidia breed bovines, using Brava dos Açores population and Domecq lineage as model,

while also showing clear differences between Lidia and other cattle breeds, in this case the HF. The vulva and vagina, uterine body and the uterine horns of Lidia cattle showed lower sizes as compared with HF heifers from different ages, evidencing the small size of the Lidia heifer's reproductive apparatus with more than 30 months. The small uterine body and high number of cervix rings found in Lidia cattle is another uncommon trait, when compared with female bovines of other breeds. The results of this research provide useful information for understanding the reproductive approach to be used in the Lidia cattle breed.

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