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**—Original Article—**

# Vulvar width and rima length as predictors of the ovarian follicular reserve in bovine females

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**Abstract.** The aim of this study was to investigate possible relationships between morphometric characteristics of the bovine reproductive tract and measures of fertility, such as antral follicle counts (AFCs) and the number and quality of recovered cumulus oophorus complexes (COCs). First, the genital tracts of 360 abattoir Zebu/Holstein crossbred cows were studied. Rima vulvae length (RL), vulvar width (VW), ovarian size, pelvic fat, number of aspirated COCs, and AFCs were recorded. An index of COC quality (CQI) was established, which weighed overall gamete quality based on the IETS classification. Second, the same external measurements and AFCs (by ultrasound) were analyzed in live Tabapuã (Zebu) cows (n = 48). Relative RL and vulvar width were defined as the original measurements divided by the body weight of each cow for statistical analyses. In abattoir tracts, the AFC was smaller ($P < 0.05$) in animals with large VW (34.88 ± 3.50) than in animals with small (45.71 ± 2.57) and intermediate (42.25 ± 2.45) VW. The CQI was higher ($P < 0.05$) when the pelvic fat score was intermediate (3.22 ± 0.06) than that in lean (2.99 ± 0.08) and obese (2.90 ± 0.12) animals. The CQI was higher ($P < 0.05$) in the intermediate ovary group (3.19 ± 0.08) than in the small (2.96 ± 0.08) and large (2.95 ± 0.09) ovary groups. In live cows, the AFC was higher ($P = 0.035$) in females with large relative rima length than in those with small relative rima length (18.96 ± 1.97 and 14.76 ± 1.51, respectively). Similarly, AFC was greater ($P = 0.0001$) in females with large relative VW than in those with small relative VW (20.08 ± 1.36 and 11.16 ± 1.60, respectively). In conclusion, larger external genitalia relative to body size were good predictors of the ovarian follicular reserve in live animals.

**Key words:** Antral follicle count, Bovine fertility, Cumulus oocyte complex, Genitalia measurements, Tabapuã breed

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Direct selection for fertility in cows can become impractical due to factors such as the long performance evaluation period as a result of the species inherent long generation interval and the low heritability of reproductive characteristics [1]. The evaluation of reproductive index comprising age at first calving and calving interval provides an accurate diagnosis of reproductive efficiency in cows [2]. However, these indicators are greatly influenced by non-genetic factors such as nutrition and management [3, 4]. Therefore, the search for indicators of fertility characteristics can be of great value in the establishment of consistent phenotypes that may be used in the selection of reproductively higher animals.

Ovarian characteristics such as antral follicle count (AFC), identifiable via ultrasonography (US), are associated with various indirect measurements of fertility in cows [5]. Ovarian size, for example, is a well-defined indicator of greater AFC in cattle [6, 7]. Ovarian follicular development is a characteristic of easy access for phenotype classification even in large herds. Cows and heifers may be grouped according to their AFC [6, 8] and ranked for reproductive selection. Furthermore, these overall assumptions are well established in *Bos taurus taurus* breeds, but further research is still required, especially in Zebu breeds.

In addition to the characteristics of the reproductive tract in cows, the nutritional and metabolic status may interfere with carcass fat deposition, growth patterns of ovarian follicles, and hormonal balance, which significantly impact oocyte quality [9]. Thus, the aim of this study was to investigate possible relationships between morphometric characteristics of the reproductive tract, antral follicle, and cumulus oocyte complex (COC) counts. It is hypothesized that superior external and internal genital measurements indicate higher AFCs. Additionally, it is hypothesized that pelvic fat amount influences COC quality.

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## Materials and Methods

The experiment was conducted in accordance with the Animal Experimentation Ethics Committee of the Federal University of Lavras (Protocol No: 022/2013).

The genital tracts of 360 crossbred Zebu/Holstein cows from a local abattoir were collected for the study. Rima vulvae length (RL), vulvar width (VW), lengths and diameters of the cervix, uterine horn, and oviduct were measured using a digital caliper. RL was determined as the distance between the dorsal and ventral commissures of the vulvar rima (Fig. 1). VW was determined as the distance between the lateral borders of the vulva placed at a 90-degree angle from the rima vulvae midpoint (Fig. 1). Based on frequency distributions, RL, and VW, the following groups were established for statistical analyses: small ≤ 7 cm (n = 75), medium > 7 and ≤ 10 cm (n = 131), and large > 10 cm (n = 32) for RL and small ≤ 5 cm (n = 75), medium > 5 and ≤ 7.5 cm (n = 131), and large > 7.5 cm (n = 32) for VW.

Ovarian length, width, and height were measured using a digital caliper and AFCs were performed using a manual counter and a pen to mark already counted follicles to avoid redundancy. Ovarian size was defined as the multiplication of the three measurements. Ovarian size was classified for analyses as: small ≤ 7 $mm^3$ (left n = 65 and right n = 58), medium > 7 and ≤ 11 $mm^3$ (left n = 73 and right n = 57), and large > 11 $mm^3$ (left n = 88 and right n = 122), regardless of animal.

Follicles were aspirated and isolated as previously described [10, 11]. COCs were classified from I (excellent) to V (degenerate) [12]. An index for COC quality (CQI), adapted from a previous study [13], was determined for each animal. It was calculated as the sum of the number of COCs within each grade multiplied by a descending series of numbers (from 5- best grade to 1- worst grade), divided by the total number of COCs for each cow, such that, CQI = (COC grade I × 5 + COC grade II × 4 + COC grade III × 3 + COC grade VI × 2 + COC grade V × 1)/Total COCs.

A mean (from three independent observers) score for pelvic fat (around ligaments and genital structures) was proposed. For this analysis, 65 genital tracts were classified as: lean (denoting absence of fat), intermediate (denoting some fat), or obese (denoting large widespread fat deposits).

In a second experiment, the external genitalia of 48 Tabapuã cows (eight to four years old) was measured similarly as in the first experiment, except for the internal genitalia, which was measured using a 5.0 MHz linear transrectal probe and an Aloka 500 ultrasound unit in B-mode. Cows were between 30 and 90 days post-partum and mostly in diestrus. Images were recorded (Play TV USB 2.0- Prolink Computer, 2000) and measurements and counts performed afterwards by two observers. The mean was then calculated for analysis. To adjust the external genitalia measurements to animal size, RL and VW were divided by the BW and termed relative rima length (RRL) and relative VW (RVW), respectively. Based on frequency distributions, RRL, and RVW, the following groups were established for analyses: low ≤ 1.90 and high > 1.90; low ≤ 1.30 and high > 1.30, respectively.

All statistical procedures were performed using the SAS® statistical program (SAS, Cary, NC, USA). Data were tested for normality by the PROC UNIVARIATE and necessary conversions were made when variables were non-normally distributed. Additional correlation analysis was performed between RL/VW and live weight in experiment II. Continuous data, AFCs, and CQI were also analyzed and compared between the different ovarian size and pelvic fat groups by PROC GLM. Means were compared using the Tukey test considering a 5% probability for significance.

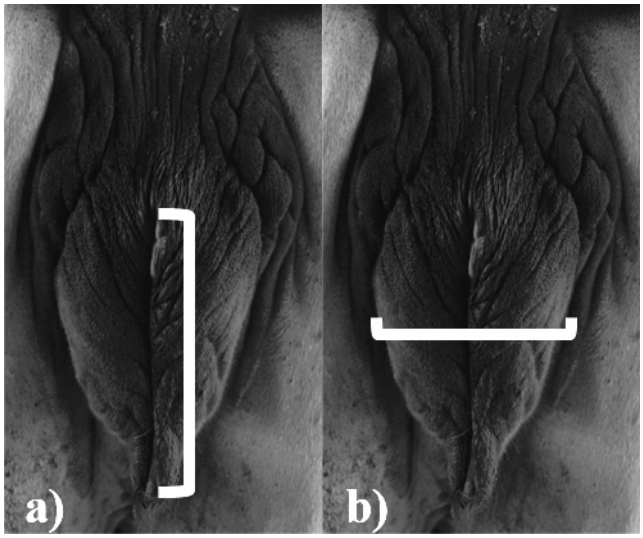


**Fig. 1.** Rima length (a) and vulvar width (b) in bovine females.

## Results and Discussion

The lengths and diameters of the cervix, uterine horn, and oviduct did not influence AFC. AFCs were similar between the different RL groups and between the small and medium VW groups. However, AFC was lower ($P < 0.05$) in the large VW group than in the small and medium VW groups (Table 1). The number of antral follicles counted directly on the abattoir ovaries was not associated with any of the RL groups. To the best of our knowledge, there is no report in the literature demonstrating similar association for comparisons. It is possible, however, to relate similar AFC, as previously reported in Nelore cows [14, 15]. The Tabapuã breed has the Nelore breed as one of its main components, therefore similarity between their AFC is expected.

The number of COCs was not associated with any of the RL groups, similar to the AFC. Total COC means in the present trial were smaller to those reported in Zebu cows [16, 17]. The number of oocytes was always lower than the AFC, which may reflect losses due to aspiration techniques and methodology, although this is commonly found in the literature [18, 19]. The same is true in relation to embryo recovery and corpora lutea counts in embryo transfer reports [20, 21]. The number of COCs recovered was higher than those reported in *Bos taurus taurus* [6], and a wider range was observed (from 1 to 120 oocytes) [17]. Further comparisons with other results are quite difficult due to the unique approach of this trial.

With respect to VW, the only difference identified was the smaller AFC in the larger VW class, and no relation to total COC counts

**Table 1.** Rima vulvae length, vulvar width, antral follicle count, and total cumulus oocyte complexes in bovine abattoir genital tracts

| | Groups | n | AFC ± SEM | n | COC ± SEM |
|---|---|---|---|---|---|
| Rima length | Small | 75 | 42.63 ± 2.85 | 73 | 10.27 ± 1.13 |
| | Medium | 131 | 40.18 ± 2.15 | 127 | 12.55 ± 0.85 |
| | Large | 32 | 48.03 ± 4.23 | 32 | 14.85 ± 1.68 |
| Vulvar width | Small | 75 | 45.71 ± 2.57$^{A}$ | 73 | 12.41 ± 1.02 |
| | Medium | 131 | 42.25 ± 2.45$^{A}$ | 127 | 13.09 ± 0.98 |
| | Large | 32 | 34.88 ± 3.50$^{B}$ | 32 | 9.80 ± 1.40 |

Values are least squares means ± standard error of the means. Means followed by superscripts within the column indicate differences ($P < 0.05$, Tukey test). n, number of cows; AFC, antral follicle count; COC, total cumulus oocyte complexes; SEM, standard error of the mean. Rima length classification: small ≤ 7 cm; medium > 7 and ≤ 10 cm; large > 10 cm. Vulvar width classification: small ≤ 5 cm; medium > 5 and ≤ 7.5 cm; large > 7.5 cm.

**Table 2.** Ovarian size and antral follicle count in bovine abattoir ovaries (left and right)

| | Groups | Left ovary | | Right ovary | |
|---|---|---|---|---|---|
| | | n | AFC ± SEM | n | AFC ± SEM |
| Ovarian size [a)] | Small | 65 | 17.46 ± 1.50 | 58 | 17.18 ± 1.76 $^{A}$ |
| | Medium | 73 | 22.73 ± 1.42 | 57 | 21.35 ± 1.81 $^{B}$ |
| | Large | 88 | 23.00 ± 1.29 | 122 | 24.90 ± 1.24 $^{B}$ |

Values are least squares means ± standard error of the means. Means followed by superscripts within the column indicate differences ($P < 0.05$, Tukey test). n, number of ovaries; AFC, antral follicle count; SEM, standard error of the mean. [a)] Ovarian size classification for the left and the right ovaries: small ≤ 7 $mm^3$, medium > 7 and ≤ 11 $mm^3$, and large > 11 $mm^3$.

**Table 3.** Index of cumulus oocyte complex quality, pelvic fat score, and ovarian size in abattoir bovine genital tracts

| | Groups | n | CQI ± SEM |
|---|---|---|---|
| Fat pelvic score | Lean | 20 | 2.99 ± 0.08 $^{B}$ |
| | Intermediate | 36 | 3.22 ± 0.06 $^{A}$ |
| | Obese | 9 | 2.90 ± 0.12 $^{B}$ |
| Total ovarian size | Small | 21 | 2.96 ± 0.08 $^{B}$ |
| | Medium | 25 | 3.19 ± 0.08 $^{A}$ |
| | Large | 19 | 2.95 ± 0.09 $^{B}$ |

Values are least squares means ± standard error of the means. Means followed by superscripts within the column indicate differences ($P < 0.05$, Tukey test). Pelvic fat score classification: Lean, absence of fat; Intermediate, some fat; Obese, large widespread fat deposits. n, number of observations; CQI, index of cumulus oocyte complex quality; SEM, standard error of the mean. Total ovarian size classification: small ≤ 15 $mm^3$, medium > 15 and ≤ 25 $mm^3$, and large > 25 $mm^3$.

**Table 4.** Relative indexes of antral follicle count, rima vulvae length, and vulvar width in the Tabapuã Zebu breed cows

| | Groups | n | AFC ± SEM |
|---|---|---|---|
| Relative Rima Length (RRL) | Low | 29 | 14.76 ± 1.51 $^{B}$ |
| | High | 19 | 18.96 ± 1.87 $^{A}$ |
| Relative Vulvae Width (RVW) | Low | 20 | 11.16 ± 1.60 $^{B}$ |
| | High | 28 | 20.08 ± 1.36 $^{A}$ |

Values are least squares means ± standard error of the means. Means followed by superscripts within the column indicate difference ($P < 0.05$, Tukey test). n, number of cows; AFC, antral follicle count; SEM, standard error of the mean. RRL classification: low ≤ 1.90 and high > 1.90. RVW classification: low ≤ 1.30 and high > 1.30.

could be detected. In fact, this finding was unexpected, based on our proposed hypothesis. No such results and comparisons were found in the literature for discussion. Perhaps, as later confirmed in this paper, external genitalia measurements taken without considering other external traits related to body size, may not be sufficient to define them correctly.

In left ovaries, AFCs were similar between all the 3 size groups. In right ovaries, AFCs were similar between the medium and large ovary groups, and were higher than that of the small group (Table 2). This may reflect a possible higher functional activity of the right ovary, which is commonly observed in the literature [22, 23]. The finding that larger ovaries yielded more follicles was expected, and is similar to other reports in *Bos taurus taurus* [6, 24].

The CQI was greater in the intermediate pelvic fat group than in the lean and obese groups. The CQI was higher in the medium ovary group than in the large and small ovary groups (Table 3). The CQI indices were higher in the intermediate ovarian size and the intermediate pelvic fat groups than in the respective remaining groups. These results correlate with a series of publications and express a direct link between excessive fat and likely low fertility in cows. High body fat deposition has been associated with low oocyte quality [9, 25]. This indicates that the nutritional and metabolic status may interfere with the growth patterns of ovarian follicles and hormonal synthesis, which impact on oocyte quality. In a similar manner, the results indicate that medium ovaries may be more functional compared to those in the extreme size groups. On the other hand, Mossa [26] and Ireland [5, 6, 27] showed that cows with low AFC have smaller ovaries and reduced numbers of follicles and morphologically sound oocytes. Additionally, those cows have reduced superovulation responses, low transferable embryo yield, chronically elevated gonadotropins secretion with low circulating AMH, and progesterone during their estrous cycles.

These also indicate that the proposed CQI may be a more refined predictor of the metabolic status of cows in comparison to the stratification (by oocyte grade) method that is usually found in the literature.

The AFC was larger in the higher RL and VW relative indices (Table 4). Through these data it can be verified that the elaboration of indices that convey more precisely that cow size relationships is necessary in order to make better predictions related to fertility traits. It is clear that when relative size indices were used, the external genitalia morphometry was easily associated with AFC and probably reflects fertility. These data correlate with the ovarian size/AFC findings [6], which is the concept that external genitalia measurements are also associated with AFC, as long as, their relation to cow size is taken into account.

VW was more strongly associated with AFC than RL. However,

its measurement, according to the method applied in this study, depends on the rima mid-point, which was used to determine VW. Unfortunately, in a few cows, the external limits of the vulva are not easily identified, adding a minor limitation to the uniformity of this method. RL would be the ideal variable because it is repeatable and unequivocal. Independent of these arguments, within our data limits, VW was a better predictor of AFC than RL. This approach has proven to be useful and relatively feasible to be applied in the selection of cows with higher AFC under field conditions.

In conclusion, external genitalia measurements in abattoir reproductive tracts, which did not take into account the size of the cow, are not appropriate for predicting ovarian follicular reserve. In Tabapuã cows vulva measurements relative to body size were good predictors of the ovarian follicular reserve, especially VW. Additional studies to further verify the possible associations of the external reproductive tract morphology with AFC, as well as, studies to improve our proposed methodology in Zebu cows are warranted.

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