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SHORT COMMUNICATION

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Accuracy of ultrasound and visual inspection in antral follicular count in crossbred Holstein cows raised under grazing systems at high altitude

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Abstract

The objective of this research was to investigate the diagnostic accuracy of post-mortem ultrasound in antral follicle count (AFC) determination and compare it with visual AFC in grazing crossbred Holstein cows, at high altitude in Ecuador. Pre-mortem blood from 80 cows was collected, and AFC and ovarian characteristics were analysed post-mortem by ultrasound and visual techniques. AFC counts were stratified as high, medium or low by terciles. Mean AMH concentration in pre-mortem blood was 280.1 ± 15.53 pg/mL. The AFC obtained by visual inspection (26.9 ± 9.49 follicles) was 23.8% higher than by ultrasound (20.5 ± 7.53 follicles) in all ovaries. Body condition score, age and weight of the cattle did not interact with the count technique. In the low AFC group, visual inspection and ultrasound provided similar AFC results. However, in the Medium- and High-AFC groups, AFC by ultrasound was 14.9% lower than AFC by visual inspection. We confirm that ultrasound can be used with great accuracy for AFC >3 mm (close to the resolution limit) in grazing crossbred Holstein cows at high altitude.

KEYWORDS
antral follicle count, follicle development, oestrous cycle, ovary, post-mortem

1 | INTRODUCTION

Selecting animals with a high antral follicle count (AFC) has been shown to improve results during superovulation and embryo production (Guerreiro et al., 2014). However, conflicting findings exist regarding the relationship between AFC and reproductive efficiency (Alward et al., 2023). The interaction between AFC and fertility is affected by many other factors (Schwarzmann et al., 2023), including bovine species (*Bos indicus* or *Bos taurus*) or the concrete study (de Lima et al., 2020). Ultrasound can be used to identify animals with high AFC, reducing individual variability in superovulation results. However, ultrasound application has limitations, and serial ultrasound measurements must be performed during the oestrous cycle to obtain a reliable AFC (Perry & Cushman, 2016). AFC mean values vary between different studies, even among animals of the same breed (Guerreiro et al., 2014; Souza et al., 2014). This in vivo variability could be linked to the resolution of the ultrasound device used (Díez, 1992; Rico et al., 2012), which may be also influenced by the ovarian characteristics. Cows raised under grazing systems at high altitude show differences in the ovarian physiology (Ayala et al., 2019). As the AFC is heritable with this heritability being a much better selection tool than other traits (Alward et al., 2023) it is worthy to deepen the knowledge on this topic. Therefore, we considered it relevant to investigate whether the observed relationship between AFC post-mortem determined using ultrasound and visually remained consistent in crossbred Holstein cows. Additionally, we examined the morphological characteristics of the ovaries and the correlations with pre-mortem anti-müllerian hormone (AMH).

2 | MATERIALS AND METHODS

The study included the ovaries from 80 cyclic crossbred Holstein cows raised under extensive grazing systems at altitudes >2500 m above the sea level in Cuenca, Ecuador, slaughtered between January and June 2022. Body condition score ranged 2.5–3.25 (2.95 ± 0.02; scale 1–5), the age 4–8 years (5.92 ± 0.13 years) and weight 469–821 kg (556.5 ± 7.69 kg).

The research was conducted following the regulations outlined in the Terrestrial Animal Health Code, Chapter 7.8, 'Use of Animals in Research and Education' of the World Organisation for Animal Health. No animal experimentation procedures were applied at any stage of the study.

Blood samples were collected in Vacutainer® tubes with EDTA by puncturing the coccygeal vein of the cows in the pre-slaughter resting pen. Blood samples were kept at 5°C and transported to the laboratory. Upon arrival, samples were centrifuged at 1500*g* for 20 min to obtain the plasma, which was aliquoted and stored at −20°C until analysis. AMH was determined (Bovine AMH ELISA Al-114; Ansh Labs).

Animals could not be in vivo checked with ultrasound, because this assessment lasts more than 10 min per animal, inducing stress, circumstance to be avoided before slaughtering.

Ovaries were collected within 4 h of slaughter to assess the morphological characteristics of the ovaries post-mortem, as well as the AFC, stage of the oestrous cycle (metestrus, dioestrus, proestrus) and phase of the follicular wave (recruitment, selection, dominance) with two techniques: ultrasound (Aloka ProSound 2®, Tokyo, Japan, with a linear transducer of 7.5 MHz) and direct visual inspection.

The average diameter (mm) of each ovary (right and left) and the corpus luteum were determined using a calliper with the following formula: (major diameter + minor diameter)/2. In the second step, the right and left ovaries were weighed separately using a precision balance.

For AFC determination using ultrasound, the ovaries were placed in a tray with water and held with forceps to facilitate scanning. The protocol described by Ayala et al. (2019) was followed, which recommends performing a lateral-medial, dorsal-ventral and cranial-caudal scan of the right ovary followed by the left ovary, counting all the follicles on the ovarian surface. Each follicle was measured to obtain its average diameter using the formula: width + length/2.

AFC determination by visual inspection was performed by counting each antral follicle (>2 mm) on the ovary surface. The size of the antral follicle (mm) was determined using a calliper by applying the formula: width + length/2. Based on these values, the animals were grouped by follicle size as follows: antral follicle <3 mm; between 3–5 mm; over 5 and up to 10 mm; and > 10 mm. Finally, the corpus luteum was enucleated to determine its weight using a precision balance, and its diameter was measured using a calliper, as described before.

The same experienced technician performed all determinations.

The results were analysed using SPSS v.25 (IBM Corp.), normality assessed with Kolmogorov–Smirnov test. AFC data were grouped into high (>30 follicles), medium (16–30 follicles) and low (≤15 follicles) AFCs. The effect of the variables Body Condition Score, Age and Body Weight on the AFC was analysed. Mean comparisons among AFC groups were analysed with ANOVA and Tukey's test and Pearson's correlation (*p*-value < .05 for significance).

3 | RESULTS AND DISCUSSION

The average AMH concentration in plasma was 280.1 ± 15.53 pg/mL, similar value to earlier works (Souza et al., 2014). The individual characteristics age, BCS or weight did not significantly influence the count results by the techniques implemented to assess AFC. The average AFC are resumed in Table 1. Ultrasound could not detect 23.8% of AFC, which may be attributed to the limited capacity of the ultrasound device (<2 mm; Díez, 1992) or to the person performing the ultrasound examination. However, the same experienced veterinarian, with over 10 years ultrasound experience, performed all determinations in our study.

The AFCs obtained align with previous findings in cows under similar grazing management (Ayala Guanga et al., 2022). However, Holstein cows in tropics, fed total mixed rations exhibited between 11% (Gobikrushanth et al., 2017) and 25% (Furukawa et al., 2020) more antral follicles. These differences can be related to the type of feeding since increased dietary intake was associated with 37% increased AFC (Gutiérrez et al., 1997). Hypobaric hypoxia in high-altitude areas compromised folliculogenesis and fertility (Parraguez et al., 2013) and changed ovarian physiology in Criollo cattle (Ayala et al., 2019).

Values in AFC groups by visual and ultrasound inspection are depicted in Figure 1. Ultrasound detected 85.1% AFC visually observed in the medium and high AFC groups.

Visual inspection and ultrasound yielded similar values for the diameter of both ovaries within the same AFC group (low, medium or high AFC), with a larger size of the right ovary in the high AFC group

TABLE 1 Anti-müllerian hormone (AMH) and antral follicle count (AFC) assessed by visual inspection or ultrasound in Holstein cows.

	Average ± SD	Range	*r**
AMH (pg/mL)	280.1 ± 15.53	53.7–580.1	
AFC by visual inspection	$26.9^{a} \pm 9.49$	6–53	.85
AFC by ultrasound	$20.5^{b} \pm 7.53$	5–37	.81

Note: ^{a,b}For significantly different values with *p* < .001.

* Significant Pearson's coefficient between AMH and AFC.

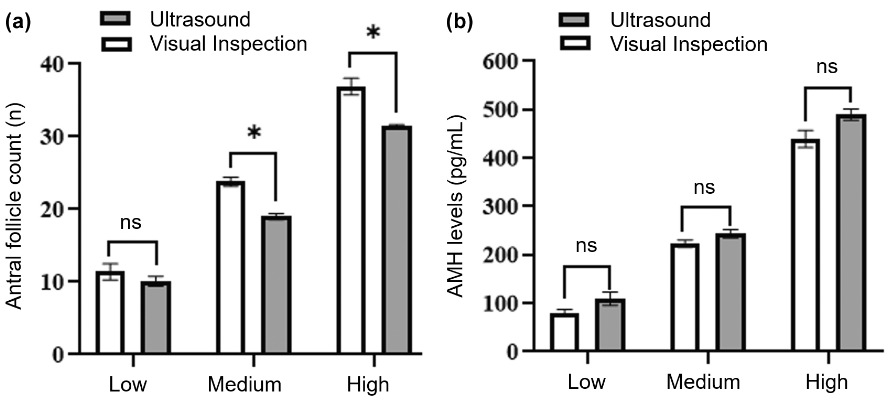

FIGURE 1 Antral follicle counts (a) and anti-müllerian hormone (b) determined visually or ultrasound in animals classified as having low, medium or high antral follicle counts by terciles. *, $p < .001$; ns, not significant.

TABLE 2 Follicles by size (<3, 3–5, >5–10 and >10 mm) in grazing crossbred Holstein cows at altitudes >2500 m, determined visually or by ultrasound, according to groups with low (≤15), medium (16–30) or high (>30 follicles) antral follicle count (AFC).

	Number of follicles					
	Visual inspection			Ultrasound		
Follicle size	Low AFC	Medium AFC	High AFC	Low AFC	Medium AFC	High AFC
<3 mm	8.4 ± 3.64^{a}	16.8 ± 4.21^{b}	$27.7 \pm 5.59^{d*}$	6.2 ± 2.24^{A}	12.9 ± 3.52^{B}	$20.6 \pm 6.10^{C*}$
3–5 mm	1.8 ± 1.30^{a}	5.3 ± 3.90^{b}	6.8 ± 4.08^{b}	1.8 ± 1.07^{A}	4.0 ± 1.90^{B}	7.4 ± 4.14^{C}
>5–10 mm	0.3 ± 0.70^{a}	1.1 ± 1.14^{ab}	1.8 ± 1.72^{b}	1.6 ± 1.43^{A}	1.7 ± 1.44^{A}	1.7 ± 1.56^{A}
>10 mm	0.9 ± 0.60^{a}	0.6 ± 0.68^{a}	0.5 ± 0.50^{a}	0.8 ± 0.55^{A}	0.6 ± 0.53^{A}	0.6 ± 0.59^{A}
Total	11.4 ± 3.39^{a}	23.8 ± 4.14^{b}	$36.9 \pm 6.00^{c*}$	10.1 ± 2.49^{A}	19.0 ± 2.84^{B}	$31.4 \pm 1.12^{C*}$

Note: $^{a–d; A–C}$Comparison among low, medium and high AFC groups in the Visual and Ultrasound inspection, by Anova and Tukey's test with $p < .001$.
* AFC by Visual versus Ultrasound and by Student's *t*-test with $p < .001$.

compared to the low and medium AFC groups. Moreover, right ovaries weighed more than the left ovaries only in the high AFC group (data non-shown). These results are consistent with those previously reported by Ireland et al. (2011) showing higher AFC linked to larger ovaries; or similar to that found in Morris et al. (1993) with the right ovary being confirmed to be the heaviest, while ovarian weight correlated with AFC in de Lima et al. (2020).

Follicle numbers by size stratified by high, medium or low AFCs are detailed in Table 2.

Ultrasound proved reliable in detecting follicles <3 mm in animals with low AFC. However, it failed to correctly identify 25.6% of follicles <3 mm in the high AFC group compared to the AFC visually observed. Assessing small follicles is more challenging by ultrasound due to the resolution limit. Therefore, equipment with higher resolution and cautious interpretation is required when assessing small follicles in cows with large follicle amount. To select animals with more AFC may be important to differentiate cattle by AFC groups (small follicle amount, visually counted, in the high AFC group was 3.3 times higher than in the low AFC group).

In conclusion, the assessment of AFC for follicles >3 mm can be accurately performed using ultrasound in Holstein cow at altitudes >2500 m. However, if quantifying small follicles in animals with many follicles, a high-resolution ultrasound device should be used. Our findings confirm the larger right ovaries size in high AFC animals. Results are helpful for selecting crossbred cows raised at high altitudes for reproduction programs.

AUTHOR CONTRIBUTIONS

LA, JD, JS, AJ, GG and RR performed the experimental work and analyses; LA, JD and SA designed the experiments; LA and SA wrote the initial draft; all authors revised the manuscript.

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CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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