SHORT COMMUNICATION

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Progesterone circulation and follicular growth in adapted *Bos taurus* cows

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Abstract

The creole breed Blanco Orejinegro (*Bos taurus* adapted) is an important animal genetic resource in Colombia. However, very little is known about the reproductive physiology of the breed. The objective was to determine the dynamics of progesterone circulation and follicular growth in Blanco Orejinegro cows throughout the estrous cycle. Fifteen cyclic Blanco Orejinegro cows were used and subjected to hormonal protocol for estrous and ovulation synchronization. Once the time of ovulation was identified by monitoring the ovarian dynamics with ultrasonography equipment. For description of the results, the mean was used as a measure of central tendency and the standard error of the mean. Statistical analysis was longitudinal descriptive. Blood samples were collected every 24 hr throughout the estrous cycle for serum quantification of progesterone (P4), monitoring of corpus luteum (CL) growth dynamics and follicular dynamics. It was possible to verify that 76.92% of the Blanco Orejinegro cows presented two follicular waves during the estrous cycle and the highest circulating levels of P4 (> 6.00 ng mL^{-1}) were observed on the 14th day of the estrous cycle. It was concluded that the creole cows of the Blanco Orejinegro breed presented two follicular waves per estrous cycle. In addition to, the size of the CL was consistent with the secretion of progesterone presenting higher circulating levels at the end of the luteal phase.

Introduction

The traditional livestock systems in Colombia are characterized using locally adapted bovine breeds (*Bos taurus*) and their crossbreeds. However, industrialization of livestock allowed the entry of improved breeds and the autochthonous breeds were gradually displacing. It is estimated that, the national inventory of creole breeds does not exceed 15,000 animals.[1]

Since their introduction at the time of the conquest, they were subjected to a long process of natural selection that provided them with unique characteristics of adaptation such as tolerance to the hot and humid tropics, maintenance capacity in low quality pastures, tolerance to diseases and parasites and high reproductive efficiency.[2] This last characteristic has allowed them to remain over time, however, very little is known about the reproductive physiology of these breeds.

According to Lonergan and Sánchez,[3] reproductive efficiencies in cattle is a multifactorial mechanism, which is largely associated with progesterone (P4). The hormone has a direct effect on oocyte quality, early embryonic and fetal development, maintenance of the luteal phase and the uterine environment.

One study determined that progesterone was related to LH pulse frequency, estradiol, growth hormone (GH), insulin and insulin-like growth factor-1 (IGF-1) synthesis. These hormones and factors are associated with cell proliferation, steroidogenesis, follicular growth, oocyte quality and ovarian reserve.[4]

The objective of the current study was to determine the dynamics of progesterone circulation and follicular growth in Blanco Orejinegro cows throughout the estrous cycle.

Materials and Methods

The current study was developed in accordance with the Colombian Statute for the Protection of Animals with Resolution No. 8430 of 1993, which contemplates biomedical research with animals.

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Animal selection and location. Fifteen cyclic cows of the Blanco Orejinegro breed, non-lactating, non-pregnant and aged between 4 - 8 years, average weight of 410 ± 22.54 kg and body condition status of 4.00 ± 0.41 on the scale of 1 to 5 points were selected. During the experimental period, the animals were fed under grazing conditions with *Brachiaria decumbens* and *Brachiaria brizantha* grass, in addition, they received a commercial supplementation (1.00 kg per cow, daily) composed of 20.00% crude protein, plus mineral supplementation (60.00 g per cow, daily) and water *ad libitum*.[5]

Estrous and ovulation synchronization protocol. All selected females received two sequential applications of 150 µg cloprostenol (Ciclase DL®; Syntex, Buenos Aires, Argentina), a synthetic analogue of prostaglandin F2α (PGF2α), 12 days apart, intramuscularly (IM).[6] The estrous and ovulation synchronization protocol were initiated eight days after the last $PGF_2\alpha$ application. On day 0 (D0) all animals received an intravaginal device of 1.30 g progesterone (CIDR®; Zoetis, Kalamazoo, USA) plus 2.00 mg IM of estradiol benzoate (Syntex). Eight days later (D8), the progesterone device was removed and a 150-µg dose of cloprostenol was administered IM. Twenty-four hr later (D9), 1.00 mg of estradiol benzoate was administered IM as an ovulation inducer.[7]

Ovarian ultrasonography. For corpus luteum (CL) growth dynamics and follicular dynamics, all cows underwent transrectal ultrasound examinations using a DP 30 Vet ultrasound machine (Mindray Vet, Shenzhen, China), equipped with a linear transducer with a frequency of 7.50 MHz. Ultrasounds were initiated at the time of insertion of the progesterone implant, repeated at 24hr intervals during the permanence of the device. After the withdrawal of progesterone and until ovulation, ultrasound scans were performed at 12-hr intervals. The time of ovulation was defined as the absence of visualization of the dominant follicle followed by formation of the corpus hemorrhagicum and then CL in the same position. After ovulation. Ultrasound scans were performed at 24-hr intervals, recording all ovarian dynamics during the entire estrous cycle.

Serum progesterone quantification. For the serum quantification of P4, samples of 7.00 mL of blood were collected in tubes without anticoagulant from the coccygeal vein. This procedure was performed during ultrasound examinations. The samples were centrifuged at 3,000 *g* for 15 min. Once the serum was obtained, it was stored in cryotubes at – 20.00 ˚C until processing. The concentration of P4 was determined by the radioimmunoassay technique (RIA) in solid phase using the commercial kit Coat-A-Count® 17α-OH progesterone (Siemens Health Care Diagnostics Inc., Los Angeles, USA). All the procedures were performed in accordance with the methodology proposed by the manufacturer. Three assays were performed, each with a sensitivity of 0.03 ng mL^{-1}. The inter-assay coefficient of variation was 4.10% and the intraassay coefficients of variation were 5.40, 4.30 and 4.90% for assays 1, 2 and 3, respectively.

Statistical analysis. The follicular diameter evaluated by ultrasound was analyzed by ANOVA and the mean was used as a measure of central tendency and the standard error of the mean. The statistical analysis was descriptive longitudinal, using the daily averages of the 15 cows for the evaluation of dominant and subordinate follicle diameter, follicular growth rate and CL growth rate and serum P4 concentration. The data were analyzed in the Statgraphics Centurion Software, (Version 18.1.16; StatPoint Technologies Inc., Warrenton, USA).

Results

Out of the 15 cows subjected to estrous and ovulation synchronization, 86.66% (13/15) ovulated after removal of the progesterone device. Out of these cows that underwent ovulation, 76.92% (10/13) showed two follicular waves and 23.07% (3/13) showed three follicular waves.

The diameter of the dominant follicle (DF) and the largest subordinate follicle (SF) of the first follicular wave were 9.10 ± 0.44 mm and 7.50 ± 0.27 mm, respectively. In the second follicular wave the sizes were 7.00 ± 0.24 mm and 6.70 ± 0.32 mm, respectively (Fig. 1).

The timing of follicular divergence was evaluated by the visual method, where this mechanism occurred on the 4th day of the first follicular wave and on the 13th day of the second follicular wave (Fig. 1).

The preovulatory follicle diameter was 14.20 ± 0.20 mm and the growth rate during the last 96 hr before ovulation was 1.05 ± 0.40 mm per day.

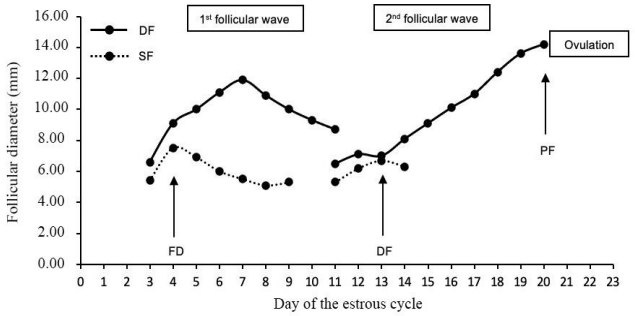

Fig. 1. Follicular growth dynamics of Blanco Orejinegro cows subjected to estrous and ovulation synchronization protocol. DF: Dominant follicle, SF: Subordinate follicle, FD: Follicular divergence and PF: Preovulatory follicle.

The serum concentration of P4 during the estrous cycle is represented in Figure 2. The maximum concentration of P4 was observed around day 14 of the estrous cycle. It remained at high levels between the 10th and 15th days of the estrous cycle (Fig. 2).

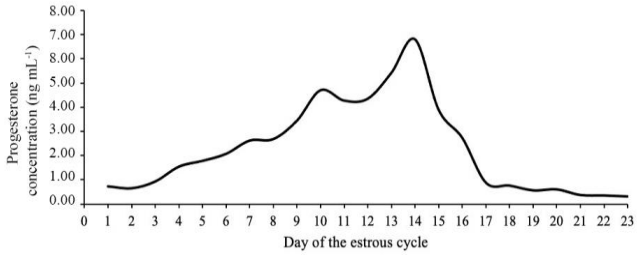

Fig. 2. Serum progesterone concentration during the estrous cycle of Blanco Orejinegro cows.

Discussion

The results of the current study presented evidence on the dynamics of follicular growth in Blanco Orejinegro Creole cows, where the predominant number of follicular waves during the estrous cycle was two. This result agreed with report of Quezada-Casasola *et al*., in Mexican Criollo cows where 77.30% of the females presented two follicular waves.[8]

It was described that *Bos taurus* females presented two to four follicular waves during the estrous cycle, with a predominance of two follicular waves.[9] Other study in *Bos indicus* cows reported a predominance of three follicular waves in Nelore, Brahman and Gyr breeds.[10-12] Ginther *et al*., suggested that the number of waves of follicular development during the estrous cycle in cattle was regulated by the length of the luteal phase.[13] Therefore, these results suggested that Blanco Orejinegro (*Bos taurus* adapted) creole cows presented follicular wave patterns like those observed in *Bos taurus* females.

The growth dynamics of the follicles: dominant and subordinate, seem to present a common initial development and only after follicular divergence a decrease in the growth rate of the subordinate follicle is observed. This mechanism has been previously described by Ginther *et al*.,[14] in Holstein animals (*Bos taurus*) and Sartorelli *et al*.,[15] in Nelore heifers (*Bos indicus*). The diameter of the dominant follicle at the time of follicular deviation by the visual method was 7.00 ± 0.24 mm, that was consistent with reports of Castilho *et al*., which found a diameter of 7.20 ± 0.43 mm.[16]

According to Ginther *et al*., the moment of follicular divergence in Holstein cows occurs 2.80 days after wave emergence.[17] In Nelore heifers, the divergence occurs 2.50 to 2.70 days after the wave emergence. These differences between *Bos taurus* and *Bos indicus* are conditions specific to each genetic group that lead to variation in follicular growth rate.

According to Utt *et al*.,[18] the growth rate of the dominant follicle of Angus cows was 1.20 mm per day, that agreed with results of the present work. However, in another study it was described that the growth rate of the dominant follicle in Nelore heifers was 1.50 mm per day[15] that were contrary to those obtained in the present study, considering that the animals used here were of different genetic group, management and climatic conditions.

In the current study, it was observed that CL functionality and P4 release synthesis in Blanco Orejinegro cows showed similar dynamics to those reported in *Bos taurus*[19] and breeds. These results also indicated the ability of the CL of Blanco Orejinegro cows to secrete high levels of P4.

The high serum P4 levels presented at the end of the luteal phase (> 6.00 ng mL^{-1}), in the Blanco Orejinegro cows of the current study, were consistent with those presented in Mexican Criollo cows[8] and in Romosinuano breed Criollo cows,[20] indicating a pattern of increased hormonal secretion of P4 at the end of estrous cycle, favoring the growth of the dominant follicle of the ovulatory wave and probably oocyte quality.

The results of the present study allowed us to conclude that Creole cows of the Blanco Orejinegro breed present two follicular waves per estrous cycle and the highest circulating levels of progesterone occur at the end of the luteal phase.

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Conflict of interest

The authors declare that they had no conflict of interest in the conduct of the study.

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