Characterization of the Follicular Dynamic Patterns in a Red Flemish Herd in Southern Brazil

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ABSTRACT

Background: The Flemish Red is one of the oldest breeds of French cattle and, in France, the crosses with other red breeds and replacing them with more productive breeds caused a severe reduction in the number of purebred Flemish Red animals. Due to this drastic decrease in the Flemish Red population, this breed is at risk of extinction. The objective of the present study is to describe follicular development and serum progesterone (P4) profiles in Flemish Red cows raised in Southern Brazil for the improvement of reproductive management of this breed, considering that these animals may exhibit traits which disappeared in European animals due to genetic dilution.

Materials, Methods & Results: The estrus cycles of two groups of post-pubertal non-lactating Flemish Red (FR, n = 7) and Holstein (HOL, n = 7) cows were synchronized with a Prostaglandin F₂α analog. From the day of estrus, the ovaries were evaluated by transrectal ultrasound every 24 h for 21 days or until the detection of the second ovulation. The diameters of the dominant follicle (DF) and subordinate follicles (SF) were recorded according to the day of the estrus cycles and analyzed based on the diameter of both DF and SF on the day of follicular emergence, the day and diameter of DF on the day of divergence, daily growth rates of DF and FS, and the maximum diameter of both the ovulatory follicle and the dominant non-ovulatory follicle (DNOF). Samples of blood were collected every five days for P4 measurement (RIA). During emergence of the first follicle wave, the DF measured 3.97 ± 0.19 mm for FR and 4.00 ± 0.35 mm for HOL, while the SF reached 3.40 ± 0.22 mm in FR and 3.07 ± 0.26 mm in HOL. The daily follicle growth rates was 1.10 ± 0.04 mm for DF and 0.67 ± 0.06 mm for the SF in FR, and were 1.01 ± 0.05 mm for DF and 0.72 ± 0.09 mm for the SF in HOL. Since the emergence DF was larger than the SF and divergence occurred on the third day, with the DF measuring 8.04 ± 0.37 mm and 8.39 ± 0.47 in FR and HOL, respectively. The maximum diameter of the ovulatory follicle in FR cows was 13.16 ± 0.33 mm and the average maximum diameter of the DNOF was 13.01 ± 0.48 mm. Holstein cows showed values of 14.20 ± 0.60 and 13.00 mm ± 0.59 mm, respectively, for the same measurements. The P4 concentration was adjusted to a cubic regression, and in FR group ranged from 0.155 ± 0.016 ng/mL to 6.651 ± 1.868 ng/mL. In HOL group, P4 ranged from 0.300 ± 0.048 ng/mL to 5.957 ± 1.233 ng/mL.

Discussion: None of the variables assessed in FR cattle differed significantly from HOL cattle. Both the DF and SF were detected at the same moment, in disagreement with other studies that show that the DF may be identified earlier than the SF. However, the interval between evaluations of the present study was larger than that of these other studies, which likely explains this discrepancy. Although daily follicle growth rates did not differ between groups, the DF and the SF had different growth rates. The growth rates of the DF are constant since its emergence until the beginning of the static growth phase or ovulation. The SF growth rate is similar to that of the DF only until the moment of divergence and from that moment on its growth decreases or ceases, showing that the difference between DF and SF sizes observed after divergence is caused by the cessation of SF growth and not to increase in FD growth rate. The findings on P4 profiles agree with values previously established for Holstein cows. Even though serum P4 concentrations were similar between groups in all evaluations, it varied significantly during the estrus cycle, in a pattern similar to that described in dairy and beef cows. It is concluded that Flemish Red and Holstein cows have a similar follicular development and P4 profiles, suggesting that the use of biotechnologies applied to Holstein cows should work in Flemish Red animals.

Keywords: conservation, reproduction, follicular dynamics.
INTRODUCTION

The Flemish Red is one of the oldest breeds of French cattle and is considered both a dairy and beef breed although is used mainly for milk production. In France, cross-breeding with Belgian and Danish red breeds and replacing them with more productive breeds like the Holstein has caused such a severe reduction in the number of purebred Flemish Reds that this breed is considered endangered [11,20].

The conservation of animal genetic resources is essential to contain the rapid loss of varieties and breeds through the genetic dilution or replacement of some breeds for others that are more productive [16,17]. Thus, current conservation practices aim to maintain the maximum genetic diversity of each species, predicting the needs for the development of sustainable production systems [9].

It is common knowledge that reproductive biotechnologies have been applied to several species and have clear benefits in conservation of wild and domestic animals [29]. In particular, a good understanding of follicular growth is fundamental for the optimization of reproductive technologies [4] and this information for Flemish Red cattle is absent.

The Flemish Red breed has existed in Brazil since 1945, at which time a nucleus of animals was established in Santa Catarina State [10] and the remaining animals from the original group comprise a herd of about 50 animals [23] which have not been crossed with other breeds. These animals represent a unique genetic pool for the study of reproductive traits of this breed, which may have been altered in France due to genetic dilution. The aim of this study was to characterize follicle development and progesterone (P4) levels during a complete estrous cycle in purebred Flemish Red cattle in Southern Brazil.

MATERIALS AND METHODS

Animals

The experiment was conducted at Lages Experimental Station (EEL) of Santa Catarina State Rural Extension and Agricultural Research Enterprise (EPAGRI) from April to May (Autumn) 2012. EEL is located at 27º48’30”S latitude, 50º19’52”W longitude at an altitude of 916 m above sea level.

Seven Flemish Red (FR) and seven Holstein (HOL) multiparous, cycling non-lactating non-pregnant cows were used in this study. Body condition scores were between 3 and 3.5 (1 = emaciated; 5 = obese) and ages varied from 4 to 7 years. Mean bodyweights were 566.7 ± 18.16 and 598.0 ± 24.60 kg for FR and HOL, respectively. They were kept on natural pasture (Axonopus spp. and Paspalum spp.) with free access to mineral supplements and water. All animals were evaluated by rectal palpation and ultrasound examination in order to identify signs of cycling and of any reproductive abnormality.

Synchronization and estrus detection

Estrous cycles were synchronizes by two intramuscular injections of 500 µg Sodium Cloprostenol1 administered 11 days apart. One day after the second dose, the animals were observed three times daily for one week during a one-hour period at 7 AM, 3 PM and 11 PM for estrus detection.

Ultrasound evaluation

The ultrasound examination of the ovaries started on the day following the detection of estrus. These exams were always accomplished by the same operator with the use of a 7.5 mHz linear transrectal probe, and were repeated every 24 h for 21 days or until the evidence of the second ovulation. To follow follicular dynamics, the ovaries were mapped at each evaluation and follicle diameters were measured; the largest follicle was retrospectively identified as the dominant follicle (DF) and the second largest as the subordinate follicle (SF).

Blood collection and serum P4 assay

In order to analyze P4 profiles, blood samples were collected from each animal immediately before the ultrasound examination starting on the day of estrus and every 5 days thereafter until the day of second estrus. The blood was collected through puncture of the coccygeal vein or artery with vacuum tubes without anticoagulant, and kept on ice until the collection has finished in all animals. Serum was separated by centrifugation at 1600 g for 15 min, and stored in plastic tubes at -20°C. Progesterone concentrations were measured by solid phase radioimmunoassay1. The intra-assay coefficient of variation for low P4 was 0.35% and for high P4 was 5.1%. The sensitivity of assay was 0.05 ± 0.002 ng/mL.

Statistical Analysis

The growth rates (mm/day) of the follicles were determined through linear regression as described [28]. The diameter of the follicles on the day of follicle wave emergence and the maximum diameter of the follicles were compared between breeds with Student’s t test.
The daily variations in follicle diameter and in serum P4 between and within groups were evaluated by analysis of variance for repeated measures with breed and day as classes and animal as repetition factor. The day of divergence was determined through segmented regression as proposed by Bergfelt [5], with the absolute difference between the diameter of the two major follicles as a dependent variable. Continuous variables associated with follicular dynamics and with serum P4 concentrations that were not normally distributed (Shapiro-Wilk test) or which had homogeneous variances (Bartlett test) were subjected to BoxCox transformation. Statistical analyses were performed using R [24] and JMP [27] software. The data are presented as mean ± SEM, and probabilities of < 5% were considered significant.

RESULTS

Two animals (one Flemish Red and one Holstein) were excluded from the analysis as they did not present signs of estrus and another three (one Flemish Red and two Holsteins) were excluded because they developed luteal cysts during the experiment.

The results obtained in the present study are shown in Figure 1. No differences in follicular growth patterns between Flemish Red and Holstein cows were noticed ($P \geq 0.05$). The diameter of DF and SF in each group during the estrus cycle is shown in Figures 1A and 1B. Serum P4 levels were also similar between groups (Figure 1C).

When the follicles were detected for the first time, the diameter of the largest follicle was 3.97 ± 0.19 mm for FR and 4.00 ± 0.35 mm for HOL. At the same moment, the second largest follicle measured 3.40 ± 0.22 mm in FR and 3.07 ± 0.26 mm in HOL, and there was no difference between breeds ($P \geq 0.05$), although the future DF was already larger than the future SF in both breeds ($P < 0.05$).

The interval between follicle emergence and divergence, determined through segmented regression, was three days and did not differ between breeds. The diameter of the DF at the beginning of follicular divergence in Flemish Red cows (8.04 ± 0.37 mm) was similar ($P \geq 0.05$) to that in Holsteins (8.39 ± 0.47 mm). The daily follicle growth rate in the FR cows was 1.10 ± 0.04 mm/day for DF and 0.67 ± 0.06 mm/day for SF, whereas in the HOL cows, daily growth rates were 1.01 ± 0.05 mm/day for DF and 0.72 ± 0.09 mm/day for SF. These values were not significantly different between breeds.

Figure 1. Follicular dynamics and serum progesterone profiles in Flemish Red and Holstein cows. PANEL A) Growth profile of dominant follicle (DF) in Flemish Red (FR, n = 5) and Holstein (HOL, n = 4) cows during estrus cycle. PANEL B) Growth profile of the largest subordinate follicle (SF) in Flemish Red (FR, n = 5) and Holstein (HOL, n = 4) cows during estrus cycle. PANEL C) Serum progesterone concentration in Flemish Red (FR) and Holstein (HOL) cows during the estrus cycle. PANEL D) Difference (mm) between the two largest follicles present in the ovary of Flemish Red and Holstein cows per day the of estrus cycle. D0: day of estrus detection. Data are expressed as mean ± SEM. In Panel C, different letters indicate significant difference between times ($P < 0.05$).
The ovariatory follicle had mean maximum diameter of 13.16 ± 0.33 mm in FR while the dominant non-ovariatory follicle (DNOF) had a mean maximum diameter of 13.01 ± 0.48 mm, and Holstein cows these follicles had diameters of 14.20 ± 0.60 mm and 13.00 ± 0.59 mm, respectively. There were no differences between breeds.

The lowest levels of P4 were 0.155 ± 0.016 ng/mL for FR and 0.300 ± 0.048 ng/mL for HOL during estrus, and maximum levels of 6.651 ± 1.868 ng/mL and 5.957 ± 1.233 ng/mL were reached during diestrus for FR and HOL groups, respectively.

**DISCUSSION**

The similarities of follicular development between breeds observed in the present study were expected since the animals studied were from European breeds, agreeing with previous studies on Holsteins [15, 28]. This is demonstrated by the data presented in Figure 1C, in which there is an absence of difference (P ≥ 0.05) in serum P4 levels between FR and HOL breeds. As the control of follicular growth is indirectly influenced by P4 levels [30], a difference between the two groups was not expected.

Both the largest and the second largest follicles were detected at the same moment, in disagreement with other studies that show that the DF may be identified earlier than the SF [8,14,15]. However, the interval between evaluations of the present study (24 h) was larger than that of these other studies (8 h), which likely explains this discrepancy.

The interval between follicle emergence and divergence in the present experiment is in accordance with the values published for European animals [5,12,19]. Again, if the interval between evaluations was shorter, the timing of divergence could have been estimated more precisely but this was not the main objective of the present study.

The values obtained on the diameter of the DF at the beginning of follicular divergence in Flemish Red cows are comparable to those previously established [1,5,15,21]. Although daily follicle growth rate did not differ between breeds, the DF and the SF had different growth rates (P < 0.05). The mean growth rate of the DF in this study is similar to data published by others [2,6,7,28]. The growth rates of the DF are constant between wave emergence and the beginning of the static growth phase or ovulation. The SF showed a distinct behavior, as its growth rate is similar to that of the DF only until the moment of divergence and from that moment on its growth decreases or ceases [13,18]. Such a pattern occurred in this study as illustrated in Figure 1D showing that the difference between DF and SF sizes observed after divergence is caused by the cessation of SF growth and not to an increase in the growth rate of the FD.

The values found for the mean maximum diameter of the ovariatory follicle and DNOF in FR agree with values of 13 - 20 mm reported in other European breeds [2,3,25,28]. The comparison between groups for this variable is in agreement with a previous study that described the ovarian activity in Holstein cows and crossbreed of Holstein/Zebu (*Bos indicus*) in the Midwest of Brazil [3].

The P4 profiles observed in the present study are consistent with those previously established for Holstein cows [26]. Even though serum P4 concentrations were similar between breeds at all evaluations (P ≥ 0.05; Figure 1C), and were not influenced by breed-time interactions (P ≥ 0.05), it varied significantly during the estrus cycle (P ≥ 0.05) and adjusting to a cubic model represented by the equation , in a pattern similar to that described on diary and beef cows [22].

**CONCLUSION**

Flemish Red cows have similar patterns of follicular development and serum progesterone profiles compared with Holstein cows, suggesting that reproductive biotechnologies that are employed in Holsteins should be applicable to Flemish Red.

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