Theriogenology 100 (2017) 42–49

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Blood flow and echotextural differences between the future dominant and subordinate follicles before the beginning of diameter deviation in heifers

O.J. Ginther [a, b, *], R.R. Domingues [a, b], M.A.R. Siddiqui [a, b], S.V. Dangudubiyyam [a]

[a] Eutheria Foundation, Cross Plains, WI 53528, USA
[b] Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form
25 May 2017
Accepted 30 May 2017
Available online 31 May 2017

Keywords:
Dominant follicle
Follicle anechoic layer
Follicle deviation
Follicle blood flow
Follicle selection

ABSTRACT

Diameter deviation is the beginning of a decrease in growth rate of the largest subordinate follicle (SF) and a continuing growth rate of the dominant follicle (DF). In wave 1 in cattle, deviation begins 2 or 3 days after ovulation when the future DF is about 8.5 mm. Gray scale and power-flow Doppler ultrasound examinations were done in experiment 1 (daily examinations, n = 13) and experiment 2 (examinations every 8 h, n = 15). Blood flow and an anechoic layer in the follicle wall were normalized to the beginning of diameter deviation (day 0 or hour 0). Only waves with conventional diameter deviation (68% of waves) were used as identified by: (1) future SF greater than 7.0 mm when DF was 8.5 mm and (2) future DF and SF did not switch in diameter rank. In experiment 1, deviations in the extent of blood-flow signals and in the extent of anechoic layer began on the same day as deviation in diameter. In experiment 2, deviations in diameter, surface area (πd^2), and anechoic layer began in synchrony, and deviation in blood-flow signals began 16 h earlier. Blood-flow deviation before diameter deviation was shown by (1) a first difference ($P < 0.02$) between follicles at hour −16 and (2) development during the hours −24 to 0 of a greater ($P < 0.05$) percentage difference between follicles in blood-flow signals (11.1 ± 2.3%) than in surface area (7.4 ± 0.7%) or diameter (4.5 ± 0.4%). Results supported the hypothesis that the extent of blood flow in the future dominant and subordinate follicles deviates before diameter deviates. A similar hypothesis for anechoic layer was not supported; diameter and anechoic layer deviated in synchrony.

1. Introduction

In *Bos taurus* cattle, the follicles of the periovulatory wave (wave 1) attain about 4.0 mm by the day of ovulation (review [1]). The two largest follicles undergo a common-growth phase for two or three more days until they begin to deviate in diameter when the future dominant follicle (DF) is 8.5 mm. Deviation is characterized by continued growth rate of the largest follicle to become the DF and a reduction or cessation in growth rate of the second largest follicle to become the largest subordinate follicle (SF). The mechanism of follicle deviation in cattle has been proposed to represent the theory of selection of the DF [1]. The process of deviation begins in the future DF 12 h before diameter deviation and involves an increase in estradiol and granulosa LH receptors and maintenance of free insulin-like growth factor 1 (IGF1). Thereby, only the future DF is developmentally prepared to use the declining FSH in the wave-stimulating FSH surge and to respond to a transient increase in LH to become the DF.

The 8.5 mm mean diameter of the future DF at the beginning of deviation [2–4] has been used to define the expected beginning of deviation in experiments that interfere or prevent judging the day of deviation by observation using diameter graphs of individual waves [5–7]. However, the use of 8.5 mm for expected deviation is useful only when the characteristics of deviation are standard or conventional. That is, it is not applicable if it is known that the future SF is undersized (<7.0 mm when DF is 8.5 mm) in that it will not be clear whether SF was subjected to inadequate development or to the deviation mechanism [4]. Also, switching in diameter ranking between the two largest follicles so that the second largest follicle becomes the future DF might confuse studies on the mechanism of deviation. In the recent study [4], expected deviation at an 8.5 mm future DF was examined after excluding waves with

* Corresponding author. Eutheria Foundation, Cross Plains, WI 53528, USA.
E-mail address: oj.ginther@wisc.edu (O.J. Ginther).

http://dx.doi.org/10.1016/j.theriogenology.2017.05.028
0093-691X/

an undersized SF and switching. In 81% of waves, the expected deviation occurred on the same mean day as for observed deviation that was based on inspection of the plotted diameter profiles of the DF and SF in individuals.

In mares, a color-Doppler study found that the blood-flow area (cm^2) within the follicle wall begins to differentially increase in the future DF compared to the future SF in an equivalent of one day (3.0 mm difference in diameter) before the beginning of diameter deviation [8]. In a color-Doppler study in cows, no difference in the percentage of follicles with detectable blood flow between the two largest follicles was found before follicle selection (deviation) but was found after selection [9] or when the DF reached 10 mm [10]. In another study, blood-flow differences between the two largest follicles were not found until two days after the beginning of diameter deviation [11]. However, a preliminary study indicated that the percentage of the follicle wall with blood-flow signals began to increase differentially in the future DF versus SF before the beginning of diameter deviation [12].

Study of echotextural changes in the wall of follicles of mares by gray-scale ultrasonic imaging has demonstrated the greater development of an anechoic layer beneath the granulosa in the future DF than in the SF before the beginning of diameter deviation [13,14]. An anechoic layer indicates the relative absence or scarcity of reflecting tissue interfaces such as in areas with a high proportion of fluids as in edema or in the lumen of blood and lymph vessels and the antrum of follicles [15]. The first day of a difference between the two largest follicles in the extent of an anechoic layer in mares occurred about one day earlier than the beginning of diameter deviation. The presence of an anechoic layer in the wall of follicles and deviation in gray-scale echotextural changes have not been reported in cattle.

Antral follicles are being stimulated for superovulation and for transvaginal oocyte recovery in embryo transfer programs indicating a need for more knowledge on the nature of the deviation process. The present projects characterized the temporal relationships preceding and during deviation between the future DF and SF of wave 1 in follicle diameter, surface area (three dimensional), blood-flow Doppler signals in the follicle wall, and anechoic layer beneath the granulosa. Hypothesis 1 was that the extent of blood flow in the future dominant and largest subordinate follicles deviates before diameter deviates. The rationale was from a positive finding in mares [8] and conflicting positive [12] and negative [9–11] findings in cattle. Hypothesis 2 was that the extent of an anechoic layer beneath the granulosa deviates before diameter deviates. The rationale was from a study in mares that found that the anechoic layer deviated before the diameter deviated [13].

2. Materials and methods

2.1. Heifers

Holstein dairy heifers (*Bos taurus*) weighing 380–470 kg were used in the northern temperate zone in two experiments in May to June and September to October. The heifers were kept in an open shelter with natural light and were provided *ad libitum* access to primarily grass hay, water, and trace-mineralized salt. The heifers were not bred at any time. Length of the interovulatory interval (IOI) was consistently 17 days or greater. A heifer was not used if two ovulations occurred at the beginning of the IOI or if two DFs (>10 mm [16]) developed in the periovulatory wave (wave 1). Animals were handled in accordance with the US Department of Agriculture Guide for Care and Use of Agricultural Animals in Research.

2.2. Ultrasound scanning

A duplex B-mode (gray scale) and pulsed-wave color Doppler ultrasound instrument (Aloka SSD 3500; Aloka America, Wallingford, CT, USA) with a 7.5-MHz transducer was used. Identity of each follicle (tracking) was maintained from examination to examination as described [15,17]. Follicle end points were: (1) diameter from an average of height and width of the follicle at the apparent maximal area from two separate frozen gray-scale images, (2) surface area calculated from diameter for a sphere (πd^2), (3) estimated percentage of the cross-sectional circumference with Doppler blood-flow signals, and (4) estimated percentage of cross-sectional circumference with an anechoic layer beneath the granulosa. Follicle diameter refers to antral diameter in that the scanner's cursors were placed at the distinctive periphery of the antrum. Blood flow was identified by Doppler signals using power-flow mode [18]. The power-flow mode is three to fivefold more sensitive to blood flow than conventional color-flow imaging. The anechoic layer is a gray or black area beneath the granulosa and was studied in B-mode (gray scale) [15] (Fig. 1). Estimations were made of the percentage of the two-dimensional circumference of the follicle wall with an aggregate or spot of color signals of blood flow and the percentage of circumference with an anechoic layer. Upon location of a color spot or an anechoic layer, the transducer was manipulated when needed so that the spot or area was viewed during an estimated maximal cross-section of the follicle.

2.3. Data handling

For each heifer, diameter of each of the two follicles that became the DF or SF were plotted retrospectively over time. The beginning of observed diameter deviation or the time when the growth rate of the future DF continued but the growth rate of the future SF began to decrease was judged by inspection of the graphs [1–3]. Means for observed deviation were normalized to ovulation (Day 0; uppercase D) or to the beginning of diameter deviation (day 0; lowercase d). Also, the beginning of the expected deviation was determined based on the time the largest follicle was closest to 8.5 mm [4]. Determining time of diameter deviation was not attempted for waves in which the established DF (diameter, ≥

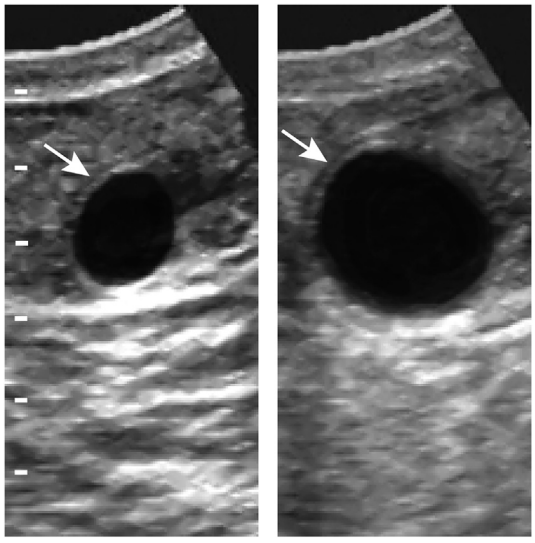

Fig. 1. Gray-scale ultrasonograms showing an anechoic layer (arrow) beneath the granulosa of a 7.6 mm and an 11.2 mm follicle. The graduation marks (left) are in 5 mm increments.