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Blood flow to follicles and CL during development of the periovulatory follicular wave in heifers

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ABSTRACT

The hemodynamics of the developing CL and the future dominant follicle (DF) was studied in 22 heifers during wave 1 on Days 0 to 5 (Day 0 = ovulation). Color-Doppler ultrasonography was used to determine the resistance index (RI) at the most prominent Doppler signal in an ovarian arterial branch before entry into the ovary; a decrease in RI indicates a downstream increase in vascular perfusion. The RI for each of four intraovarian patterns averaged over days was different ($P < 0.05$) from each of the other patterns as follows: DF–CL (DF and CL in the same ovary), 0.52 ± 0.02; CL alone, 0.60 ± 0.01; DF alone, 0.67 ± 0.01; neither DF nor CL, 0.78 ± 0.01. The differences in RI among intraovarian patterns began on Day 0 or 1, indicating that the extent of vascular perfusion on Days 0 to 5 for the various patterns may have been influenced by events that occurred before ovulation. The percentage of the DF wall with color-flow signals was greater ($P < 0.05$) in the DF–CL pattern than in the DF pattern on each of Days 2 to 5 and was greater ($P < 0.0001$) in the DF–CL pattern when the DF was adjacent to the CL ($40.2 \pm 2.0\%$) than when separated ($24.5 \pm 1.9\%$). Dimensions of DF ($P < 0.01$) and CL ($P < 0.02$) were greater when adjacent to each other. The results supported the hypotheses for wave 1 that (1) vascular perfusion is greater for the DF–CL intraovarian pattern than for the DF or CL pattern and (2) the extent of blood-flow Doppler signals in the wall of the developing DF is greater for the DF–CL pattern than for the DF pattern. Our preferred interpretation is that a change in vascular perfusion of the CL is accompanied by a similar change in perfusion of the DF when the two structures are in the same ovary especially adjacent.

1. Introduction

Waves 1, 2, and 3 are commonly used to represent the two or three follicular waves that occur during an interovulatory interval (IOI) in cattle [1]. Wave 1 in two-wave IOIs and waves 1 and 2 in three-wave IOIs are anovulatory. Wave 1 is by far the most extensively studied anovulatory wave [2]. In wave 1, diameters of the follicles are 4 or 5 mm at ovulation. The individual follicles increase in diameter during a common-growth phase until deviation in growth rates begins about 2.5 days after ovulation [3]. Deviation occurs when the largest follicle is about 8.5 mm and is characterized by continued growth of the largest follicle to become the dominant follicle (DF) and the beginning of a decrease in growth rate and regression of the remaining subordinate follicles. Before deviation, all growing follicles of the wave have the capacity for dominance [4]. The location (left or right ovary) of the DF and the CL is represented by four intraovarian patterns: DF and CL in the same ovary, DF alone, CL alone, and neither DF nor CL.

The anatomy of the artery and its branches that supply an ovary in cattle has been described [5]. The ovarian artery originates from the aorta and divides into a uterine branch and an ovarian branch about 6 cm from the ovary.

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The ovarian branch divides in the ovarian pedicle into two or three branches, and each branch subdivides into two or three smaller branches. As a result, four to nine arteries enter the hilus of the ovary. The arterial branch that supplies the CL in cattle increases in diameter two- or threefold by the time the CL matures [6]. Only one arterial branch in the pedicle delivers the primary if not the entire arterial supply to the CL [5,6]. Presumably, this same arterial branch previously supplied the preovulatory follicle. It is expected that the arterial branch supplying the preovulatory follicle also enlarges throughout the ovarian pedicle; however, this apparently has not been studied, specifically. The arterial branches in the pedicle are interconnected by prominent anastomoses [5]. However, it is not known if the intraovarian presence of one structure (DF or CL) directly affects the blood supply to the other structure either through the arterial anastomoses in the pedicle or by intraovarian arterial branches from a common extraovarian or intraovarian larger branch. If so, a signal from either the DF or CL to the arterial architecture for an increase or decrease in blood flow may affect the blood supply to both structures. In this regard, a drawing of the intraovarian arterial architecture in humans indicates that a branch of a single intraovarian artery has branches to both the CL and a neighboring mature follicle [7].

Transrectal color-Doppler ultrasonic imaging and color-Doppler ultrasonography have become useful technologies for noninvasive study of the vascularity of the internal genitalia in large animals [8–11]. In ultrasonic imaging, the extent and direction of blood flow in tissues is displayed in color. In ultrasonography, placement of a sample-gate cursor on the image of an artery provides a graphic display of arterial pulsation and includes the resistance index (RI). Decreased RI indicates increased vascular perfusion of the tissues supplied by the artery downstream from the RI assessment. As an example of research findings in follicle function by color-Doppler technologies, the percentage of the wall of the preovulatory follicle with blood-flow signals is greater in cattle [12] and mares [13] that subsequently become pregnant. In mares, blood flow in the follicle wall begins to increase in the future DF compared with other follicles about 1 day before diameter deviation; deviation occurs when the largest follicle is about 22 mm [14]. In cows, the preovulatory follicle has been studied by color-Doppler imaging [8,10,12], but smaller follicles (e.g., $<$10 mm) have been given only limited attention. In an initial study, there was no significant difference between the future dominant and subordinate follicles in the number of follicles with detectable blood-flow Doppler signals in the follicle wall before diameter deviation [8]. After deviation, the number of subordinate follicles with detectable blood flow decreased. In addition, small follicles that had detectable blood flow the day before the occurrence of diameter deviation grew to larger diameters than those without detectable blood flow.

The present study of wave 1 used the following approaches: (1) determining the effect of intraovarian patterns on the extent of ovarian vascular perfusion based on RI assessment in an artery in the ovarian pedicle and (2) determining the effect of intraovarian patterns on the extent of blood-flow Doppler signals in follicle walls. Hypotheses were (1) vascular perfusion is greater for the DF–CL intraovarian pattern than for the DF or CL pattern, (2) the extent of blood-flow Doppler signals in the wall of the developing DF is greater for the DF–CL pattern than for the DF pattern, and (3) deviation in blood-flow signals between the future DF and largest subordinate follicle occurs earlier than deviation in diameters.

2. Materials and methods

2.1. Heifers

Holstein (*Bos taurus*) dairy heifers aged 18 to 24 months were used (n = 22). The heifers were kept in natural light in an open shelter and were provided *ad libitum* access to water, trace-mineralized salt, and primarily grass hay. The IOIs were natural in that they were not preceded by induced luteolysis, induced ovulation, or synchronization of estrus or ovulation. The heifers were not bred. The day of ovulation was determined by transrectal ultrasound examinations [15]. An IOI was not used if two ovulations occurred at the beginning of the IOI or if two DFs ($\geq$10 mm [3]) developed during wave 1. The heifers were in an experimental herd and not in a dairy herd and were handled in accordance with the US Department of Agriculture Guide for Care and Use of Agricultural Animals in Research.

2.2. Follicle diameter and blood-flow assessment

The identity of individual follicles $\geq$6 mm of wave 1 was maintained from Day 0 (day of ovulation) until Day 5. Subordinate follicles remaining from the preceding ovulatory wave were identified by the decreasing diameters after Day 0 and were not considered. The ovary containing the CL and DF and side of ovary (left or right) were recorded. The future DF was defined retroactively throughout Days 0 to 5 as the largest follicle on Day 5 [2].

Ovaries were divided into four intraovarian patterns on the basis of whether an ovary contained the DF or CL as follows: DF and CL (DF–CL), DF alone, CL alone, or neither DF nor CL. For the DF–CL pattern, the narrowest distance between the wall of the follicle and the wall of the CL was recorded. When the distance between the walls of the DF and CL was greater than 2.0 mm on Day 5, the ovary was assigned to a DF–CL-separated subpattern; earlier during the wave (Day 3), the mean distance was 5.0 mm. When apparent intervening tissue was not detected between the walls of the DF and CL, or the distance between walls was less than 2.0 mm, the ovary was assigned to a DF–CL-adjacent subpattern. The partitioning into subpatterns was based on adjacency or separation on Day 5.

A duplex B-mode (gray scale) and pulsed-wave color-Doppler ultrasound instrument (Aloka SSD 3500; Aloka America, Wallingford, CT, USA) equipped with a linear-array, 7.5-MHz transducer was used for transrectal scanning. The resistance index (RI) of the most prominent blood-flow color spot within 1 cm of the base of the ovary was determined in color-Doppler mode as described [10,16]. A cursor gate with a 1-mm opening was placed on the signal spot. The RI was determined by the scanner and