Theriogenology 82 (2014) 875–883

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of intraovarian proximity between dominant follicle and corpus luteum on dimensions and blood flow of each structure in heifers

O.J. Ginther [a,b,*], M.A.R. Siddiqui [a,b], J.M. Baldrighi [a], M.M. Hoffman [a]

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

ARTICLE INFO

Article history:
Received 15 April 2014
Received in revised form 27 June 2014
Accepted 30 June 2014

Keywords:
Blood flow
Corpus luteum
Dominant follicle
Intraovarian relationships
Ultrasonography

ABSTRACT

An intraovarian positive physiologic coupling between the extant CL and the ipsilateral preovulatory follicle (PF) or the future or established postovulatory dominant follicle (DF) was studied in 26 heifers. Ovaries were scanned by ultrasonic imaging from Day 16 (Day 0 = ovulation) of the preovulatory period until Day 6 of the postovulatory period. Hemodynamics of the follicles and CL were assessed by color-Doppler ultrasonography. When the PF and CL were ipsilateral compared with contralateral, blood-flow resistance in wall of the PF was lower ($P < 0.04$) on Days –2 and –1, and percentage blood-flow signals in the CL approached being greater ($P < 0.08$) on Days –4 to –1. During the postovulatory period, percentage of DF wall with blood-flow signals (44.1 ± 1.2% vs. 31.4 ± 2.8%) and percentage of CL with blood-flow signals (51.8 ± 1.2% vs. 42.5 ± 3.1%) were each greater ($P < 0.05$) when the two ipsilateral structures were adjacent (distance between antrum and CL wall, ≤ 3 mm) than when separated. On Day 0, the distance between follicle and ipsilateral CL was less ($P < 0.02$) for the future DF than for the future largest subordinate. Growth rate between Days 0 and 2 averaged over all growing follicles was greater ($P < 0.01$) when the follicles were ≤3 mm from the CL (1.1 ± 0.1 mm/day) than when farther from the CL (0.9 ± 0.1 mm/day). Results supported the hypotheses that (1) a positive intraovarian coupling occurs between the PF or postovulatory DF and the extant CL and (2) the coupling is enhanced when the ipsilateral DF and CL are in close proximity.

1. Introduction

In the *Bos taurus* breeds of cattle, two or three follicular waves develop every 8 or 9 days during an interovulatory interval (IOI) [1,2]. The first wave of the IOI is commonly termed wave 1. Wave 1 in two-wave IOIs and waves 1 and 2 in three-wave IOIs are anovulatory. Wave 1 emerges with 4- or 5-mm follicles on the day of ovulation [3,4]. However, the wave emerges with 1 to 3 mm follicles before ovulation, during the ascending portion of the wave-stimulating FSH surge [5,6]. Due to preovulatory emergence, when small follicles (1–3 mm) are considered, wave 1 may also be termed the periovulatory follicular wave. The follicle and hormone characteristics of a wave have been studied most extensively for wave 1 using ovulation or emergence at 4 mm as a reference. Therefore, follicle emergence is commonly described as occurring at 4 mm [6]. However, emergence or first detection can be based on any diameter (e.g., 2 mm, 6 mm) depending on the experimental protocol. Most of the follicles of wave 1 that emerge at 4 mm develop during a common growth phase for 2 or 3 days and then deviate into a dominant follicle (DF) and several subordinate follicles. Deviation during consecutive examinations has been defined as continued growth rate of the

* Corresponding author. Tel.: +1 608 798 3777; fax: +1 608 798 3722.
E-mail address: ginther@vetmed.wisc.edu (O.J. Ginther).

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.06.022

largest follicle (future DF) and the beginning of a reduction in growth rate of the second largest follicle (future largest subordinate) [6].

In single ovulators, the following four permutations or intraovarian patterns can occur: ovary with DF and CL in an ipsilateral relationship (pattern term, DF–CL), ovary with DF alone (term, DF), ovary with CL alone (term, CL), and ovary with neither DF nor CL (term, devoid) [7–10]. During the preovulatory period, the DF is the preovulatory follicle (PF), and similar terminology can be used by replacing DF with PF; the CL is from the previous IOI. During wave 1, the ipsilateral relationship or the intraovarian pattern of DF–CL and the requisite pattern in the opposite ovary (devoid) occur more frequently than the contralateral relationship with the DF in one ovary and the CL in the other [11].

The hemodynamics of the developing CL and the future DF have been studied on Days 0 to 5 (Day 0 = ovulation) [7]. Color-Doppler ultrasonography was used to determine the blood-flow 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 indicated that the vascular perfusion for each of four intraovarian patterns averaged over days decreased in the following descending order: DF–CL, CL, DF, and devoid. 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 was a reflection of events that occurred during the preovulatory period. It was observed [7], but not critically tested, that the percentage of the DF wall with color-flow signals was greater in the DF–CL pattern when the DF was adjacent to the CL than when separated. Dimensions of DF and CL were greater when adjacent to each other. An interpretation was that the CL and future or established DF are coupled in that a change in vascular perfusion of either the CL or DF is accompanied by a similar change in perfusion of the other structure when the two structures are in the same ovary, especially when the DF and CL are adjacent. The finding of a positive intraovarian two-way influence or coupling between the DF and CL when adjacent to each other was observational and critical confirmation is needed. The observations were made postovulation and no information is available on whether similar intraovarian relationships occur between the PF and the extant CL.

The hypotheses in the current study were as follows: (1) a positive intraovarian coupling occurs between the PF or postovulatory DF and the extant CL; and (2) the coupling is enhanced when the ipsilateral DF and CL are in close proximity.

2. Materials and methods

2.1. Heifers

Holstein dairy heifers (a *Bos taurus* breed) aged 16 to 20 months were used (n = 28). The heifers were kept in an open shelter with natural light and were provided *ad libitum* access to water, trace-mineralized salt, and primarily grass hay. The IOIs were not preceded by induced luteolysis, induced ovulation, or synchronization of estrus or ovulation, and the heifers were not bred. An IOI was not used if two ovulations occurred at the beginning of the IOI or if two DFs (≥10 mm [12]) developed during wave 1. Heifers were handled in accordance with the US Department of Agriculture Guide for Care and Use of Agricultural Animals in Research.

2.2. Follicle and CL dimensions and blood-flow assessment

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. Identity of individual follicles was maintained (follicle tracking) as described [13] for the periovulatory wave (wave 1) from Day 16 (Day 0 = ovulation) of the preovulatory period until Day 6 of the postovulatory period. The preovulatory period was defined as Days –4 to –1 and the postovulatory period as Days 0 to 6. An adequate number of waves was not available for Day –5 due to the initiation of the experiment on Day 16 of the previous IOI. The PF and the prevailing CL during the preovulatory period and the DF, CL, and largest subordinate follicle during the postovulatory period were the principle targets, but all growing follicles ≥2 mm were tracked and measured. The four defined intraovarian patterns during the preovulatory period were PF–CL, PF, CL, and devoid. During the postovulatory period, the intraovarian patterns were DF–CL, DF, CL, and devoid. The DF was identified as the largest follicle on Day 6 [1]. Although the DF was not firmly identifiable until after deviation in growth rates between the two largest follicles, the identity of the future DF began at 2 mm based on the retroactive tracking records. The term “DF” is used for both the future DF and the postdeviation or established DF.

The DF of the DF–CL pattern was evaluated for distance between DF and CL. The two cursors were set at the junction of the anechoic antrum of the follicle and at the anechoic thin line representing blood vessels at the periphery of the CL. These two cursor settings were used because of the distinct boundaries compared with the indistinct delineation of the theca interna, theca externa, and stroma. In addition, distances ≤3 mm were defined as an adjacent category due to the difficulty of measuring distances of 1 or 2 mm. Distances were recorded in millimeters when ≥3 mm. The entire ovary was scanned in a slow continuous motion. The scanner’s sine-memory function was used for controlled replay of the scan for selecting gray-scale images when needed for determining follicle identity and for measuring distance between DF and CL. The sine memory stored 10 seconds of the scan using 290 sequential frames. The frames were visually examined and two frames with the most distinct and minimal distance between the follicle antrum and CL boundary were selected. The distance within each of the two frames was averaged.

The RI of the most prominent blood-flow color spot in the ovarian pedicle within 1 cm of the base of the ovary was determined in color-Doppler mode as described [14,15]. The RI was also obtained from a prominent color-Doppler spot in the wall of the PF or DF. A color spot was used