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Hormone concentrations temporally associated with contralateral and ipsilateral relationships between the CL and preovulatory follicle during the third follicular wave in heifers

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ABSTRACT

Concentrations of circulating hormones after Day 14 (Day 0 = ovulation) were determined daily in 87 interovulatory intervals (IOIs) in heifers. The IOIs were grouped into four permutations according to an ipsilateral (Ipsi) or contralateral (Contra) relationship between the CL and the preovulatory follicle and two (2W) or three (3W) follicular waves per IOI. The number of IOIs per group differed ($P < 0.005$) from equality among the Ipsi-2W ($n = 27$), Contra-2W ($n = 31$), Ipsi-3W ($n = 9$), and Contra-3W ($n = 20$) groups. A continuous decrease in progesterone (luteolysis) began later ($P < 0.05$) in the Contra-3W group (Day 18.0 ± 0.4) than in each of the Ipsi-2W (15.4 ± 0.2), Contra-2W (15.6 ± 0.2), and Ipsi-3W (16.2 ± 0.5) groups. Concentrations of LH and estradiol began to increase near the beginning of luteolysis in each group. A minor FSH surge that did not stimulate a major follicular wave developed in about 50% of the IOIs in each group, except that none were detected in the Ipsi-3W group. The minor FSH surge reached a peak about 4 days before ovulation and several days after wave 3 had emerged. The hypothesis that luteolysis begins earliest in two-wave IOIs, intermediate in three-wave IOIs with an ipsilateral CL/follicle relationship, and latest in three-wave IOIs with a contralateral relationship was supported. The hypothesis that a minor FSH surge occurs most frequently in association with three follicular waves was not supported.

1. Introduction

The lengths of the interovulatory interval (IOI) and luteal phase in *Bos taurus* cattle are about 3 days longer when the extant CL and the preovulatory follicle are located in opposite ovaries (contralateral) than when they are in the same ovary (ipsilateral) [1–4]. A 3-day extension of the IOI and luteal phase is also characteristic of three follicular waves per IOI [5,6]. The interval from ovulation to the beginning of a continuous decrease in progesterone and to the beginning of postluteolysis (progesterone <1 ng/mL) was prolonged for the combination of a contralateral CL/follicle relationship and three waves (Contra-3W) in 4/4 IOIs but not in any IOI with an ipsilateral relationship and two waves (Ipsi-2W, 0/5) or contralateral relationship and two waves (Contra-2W, 0/5) [1]. Only 1 of the 14 IOIs had an ipsilateral relationship and three waves (Ipsi-3W), precluding its inclusion in the statistical analyses.

A recent survey used the diameter of the future ovulatory follicle on Day 16 (Day 0 = ovulation) for classification into two-wave or three-wave IOIs [4]. The percentage of IOIs for the four permutations of the CL/follicle relationship (ipsilateral or contralateral) and number of waves (two or three) was as follows: Ipsi-2W, 34%; Contra-2W, 35%; Ipsi-3W, 8%; and Contra-3W, 23%. The ipsilateral and contralateral CL/follicle relationships occurred with similar frequency in two-wave IOIs. In some IOIs, the interval from

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ovulation to progesterone <1 ng/mL (end of luteolysis and beginning of postluteolysis) was known. The interval was similar for the two CL/follicle relationships in two-wave IOIs; however, in three-wave IOIs compared with two-wave IOIs, the interval was about 2 and 3 days longer for the ipsilateral and contralateral relationships, respectively.

Both major and minor follicular waves occur in heifers [7]. When the maximum diameter of the largest follicle of a wave reached <10 mm or ≥10 mm, the wave was defined as a minor wave (no dominant follicle) or major wave, respectively. A minor wave occurred in 7 of the 14 heifers and emerged with 4-mm follicles on a mean of 5.7 days before ovulation. The largest follicle reached a maximum diameter of 5.0 to 8.7 mm and then regressed. The emergence of a minor wave seemed to be associated with an FSH surge in 5 of the 7 heifers, but the change in FSH concentration was not significant. It is not known whether a minor FSH surge in heifers is involved in the reported greater frequency of the contralateral than ipsilateral CL/follicle relationship in three-wave IOIs [4].

The current study used IOIs from the previous experiments in which daily blood samples were available on some days from Day 14 to ovulation. The goal was to find previously unknown temporal relationships that may account for the reported differential extension of the luteal phase between ipsilateral and contralateral CL/follicle relationships in three-wave IOIs and the greater frequency of the contralateral than ipsilateral relationship during the third wave. The study was unique, owing to the availability of blood samples from an adequate number of IOIs with the ipsilateral relationship and three waves. The hypotheses were as follows: (1) luteolysis begins earliest in two-wave IOIs, intermediate in three-wave IOIs with an ipsilateral CL/follicle relationship, and latest in three-wave IOIs with a contralateral relationship, and (2) a minor FSH surge occurs most frequently in association with three follicular waves. The rationale for hypothesis 1 was the survey report [4] that luteolysis ended 2 and 3 days later in the three-wave ipsilateral and contralateral groups, respectively. The rationale for hypothesis 2 was the report [7] of the presence of a minor follicular wave in some heifers several days before ovulation.

2. Materials and methods

2.1. Heifers and sources of records and blood samples

A total of 87 IOIs in 48 Holstein dairy heifers (*Bos taurus*) was used from the controls in six reported experiments [1–3,8–10] and four unpublished experiments conducted during the past 3 years. These 87 IOIs and 109 additional IOIs were used previously in surveys [4] of CL/follicle relationships and number of follicular waves per IOI and their relationships to length of the IOI.

The heifers were provided *ad libitum* access to water, trace-mineralized salt, and primarily grass hay with grain supplementation during the winter and were kept under natural light in an open shelter. The IOIs were natural in that they were not preceded by artificially altered length of the IOI or by induced luteolysis, induced ovulation, or synchronization of estrus or ovulation. The heifers were not bred. Heifers were handled in accordance with the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Research. Follicle diameter, CL/follicle relationship, day of ovulation, and apparent number of follicular waves were obtained from the routine and experimental transrectal ultrasound records [11]. Diameter of the future ovulatory follicle was recorded for all IOIs from Day 15 to the day before ovulation. An IOI was not used if two ovulations occurred either at the beginning or end of the IOI.

The four permutations of CL/follicle relationship and number of follicular waves per IOI were assigned as follows: ipsilateral relationship between the CL from the ovulation at the beginning of the IOI and the preovulatory follicle at the end of the IOI with two waves (Ipsi-2W), contralateral CL/follicle relationship with two waves (Contra-2W), ipsilateral CL/follicle relationship with three waves (Ipsi-3W), and contralateral CL/follicle relationship with three waves (Contra-3W). The number of follicular waves per IOI was judged by the diameter of the retrospectively identified ovulatory follicle on Day 16. When the future ovulatory follicle was >10 mm or ≤10 mm on Day 16, the number of waves per IOI was recorded as two and three, respectively. The reliability of distinguishing between two and three follicular waves by this method is based on a reported preliminary study and on results of a survey [4] that were similar to the results of the previous reports that were based on day-to-day identity of follicles [5,6].

Daily blood samples were collected from Day 14 until ovulation, but the number of days with samples was not uniform among the previous projects. The number of samples per day was 32 for Days 14 and 15, 73 for Day 16, 83 for Day 17, 62 for Days 18 and 19, and 44 for Day 20. The occurrence of ovulation on different days contributed to the reduced number of days with samples after Day 18. Therefore, analyses were done from Days 14 to 18 and retroactively from ovulation at the end of the IOI (Day 0) to Day –6. In addition, IOIs with complete data from Day 14 to ovulation were used for hormone comparisons between the Ipsi-3W group ($n = 5$) and the Contra-3W group ($n = 9$).

2.2. End points

For all 87 IOIs, the concentrations of progesterone, FSH, LH, and estradiol-17β (estradiol) were determined for Days 14 to ovulation and were compared among the four permutations. Progesterone concentrations were examined for each IOI to determine the day of the first continuous progressive decrease and the day progesterone reached <1 ng/mL (end of luteolysis or beginning of postluteolysis). Progesterone was analyzed from Days 14 to 21, for 3 days before and 2 days after the end of luteolysis, and for 5 days before the end of the IOI. Concentrations of FSH and LH were analyzed only until Day 18, owing to irregularities after Day 18 from the occurrence of the preovulatory surges on different days. The preovulatory FSH and LH concentrations were analyzed from Day –6 to 0. Concentrations of estradiol were assayed and analyzed from Days 14 to 20 in seven randomly selected IOIs per group, but