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Long-term characteristics of idiopathic persistent corpus luteum in the mare

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ABSTRACT

Persistent CL (PCL; n = 10) in mares was studied daily from Day 20 (Day 0 = ovulation) to the ending ovulation. In addition, the 10 days before ovulation at the end of a PCL were compared with the end of an interovulatory interval (IOI; n = 28) during the same months. Concentration of P4, cross-sectional area of CL, and percentage of CL with Doppler signals of blood flow during PCLs remained constant from 64 to about 33 days before the end of luteolysis and then decreased linearly. Concentration of LH between Day 20 and beginning of the ovulatory LH surge increased linearly. A dominant follicle developed on average every 15 days throughout each PCL. Novel transient P4 depressions were detected with the P4 nadir at a day of maximal diameter of a dominant follicle. At the apparent beginning of luteolysis before the ending ovulation, P4 concentration in PCLs (5.0 ± 0.5 ng/mL) was less ($P < 0.0001$) than that in IOIs (9.3 ± 0.6 ng/mL). Concentration of LH began to increase 2 days before the end of luteolysis in each group, but concentration on the day of the ending ovulation in PCLs (3.7 ± 0.3 ng/mL) was less ($P < 0.005$) than that in IOIs (8.9 ± 1.8 ng/mL). In a separate survey of PCLs (n = 23) and IOIs (n = 352), frequency of PCL (6.1%) differed significantly among mares indicating repeatability. These original and critical comparisons between PCLs and IOIs should provide hypotheses for further study.

1. Introduction

In farm animals, including horses, the CL forms at the site of ovulation. The CL secretes progesterone (P4) which prevents another period of estrus and ovulation until after functional and structural CL regression or luteolysis. The control of the CL and the nature of luteolysis in mares have been reviewed [1–5]. In mares, luteolysis begins on average at Day 14 (Day 0 = ovulation) and is completed in 23 hours on the basis of hourly blood sampling [6]. A P4 concentration decrease to less than 1.0 ng/mL is used to define the end of luteolysis [7].

A CL that is maintained beyond the expected time of luteolysis in a nonpregnant mare is termed persistent CL (PCL) [8]. The condition can affect 8% to 10% of interovulatory intervals (IOIs) during the peak of the ovulatory season and 25% during the transition into the anovulatory season [9–11]. The PCL syndrome has been associated with uterine pathology (e.g., pyometra) [12] and with embryonic loss after the first luteal response to pregnancy [13]. The term uteropathic PCL is appropriate when the condition can be attributed to a uterine abnormality or pathology [14]. When the etiology is unknown and cannot be attributed to the uterus or to embryonic death, the terms idiopathic PCL [14] and spontaneous PCL [7,15] have been used.

Mares with PCL have a P4 concentration greater than 1.0 ng/mL for about 60 days (range: 35–95 days) [7]. Uteropathic PCL is attributable to a defect in endometrial

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secretion of the luteolysis, PGF2α [12]. Idiopathic PCL also has been attributed to defective PGF2α secretion on the basis of concentrations of P4 and PGFM (a metabolite of PGF2α) in a few individual mares [11,16].

Studies on the characteristics of idiopathic PCL have considered concentrations of P4, LH, PGFM, or estradiol (E2) in individual mares and have focused on the expected transition between the luteal and follicular phases [11,16–18]. Long-term characterization of hormonal changes during PCL (e.g., Day 20 to ovulation) has been limited to P4 [7–19]. Detailed descriptions of variations in P4 concentration, ovulation, and estrous behavior are available for individuals [7,15,19]. Concentration of P4 in the PCL syndrome decreases to an intermediate plateau beginning on about Day 14 [7]. An abrupt decrease in P4 concentration (luteolysis) occurs after the intermediate plateau in PCL mares, whereas a continuing gradual decrease occurs in hysterectomized mares [7,20]. After Day 20, mares with PCL appeared to have low concentration of LH [15,19], and in one mare, LH concentration increased for 9 days before luteolysis [19]. Follicular growth occurs during PCL with some of the follicles reaching ovulatory size. Ovulation may occur [7,19], but estrous behavior during PCL has not been reported [17].

Consideration has not been given to the long-term characterization of follicular waves (e.g., frequency of major waves, maximum diameter of the dominant follicle) and the CL (e.g., cross-sectional area, extent of blood flow) during PCL. In the present study, daily concentrations of P4, LH, and FSH were determined, but E2 was considered only for selected portions of major follicular waves. Characteristics of dominant follicles, luteal dimensions, and blood flow were included. In addition, normalization to luteolysis or to ovulation at the end of a PCL or IOI has not been used for critical comparison of mares with and without PCL. The present study compared mares with and without PCL inversely from the end of luteolysis and from ovulation at the end of an IOI or PCL. Although the IOI and PCL were compared, the results are considered observational in that there was inadequate rationale for developing hypotheses for comparing P4, FSH, LH, and E2 concentrations, follicles, and CL between the end of an IOI and the end of a PCL.

2. Materials and methods

2.1. Mares

Mixed breeds of 28 nonlactating mares aged 3 to 18 years and weighing 300 to 600 kg were used in the northern temperate zone. The wide range of weight and body conformation indicated that individual mares could be described as a horse (riding type) or a pony–horse cross. Abnormalities of the reproductive tract were not detected by transrectal ultrasonic scanning [21]. The mares had not been bred for at least 3 years. The experiment characterized PCL after Day 20 and was done from July 2012 to November 2013. Mares were housed in an open shelter and outdoor paddock and were maintained by free access to primarily grass hay, trace-mineralized salt, and water. The mares were housed under natural light during spring, summer, and fall (March to November) and under artificially extended photoperiod during the winter (December to February) as described [3]. All mares remained healthy and in good body condition throughout the study. Mares were handled according to the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching.

2.2. Protocol

Mares were examined daily by transrectal ultrasonography with a duplex B-mode (gray scale) and color Doppler ultrasound scanner (Aloka SSD 3500; Aloka American, Wallingford, CT, USA) equipped with a linear-array 7.5-MHz transducer. The scanner was used for determining the day of ovulation, luteal area (cm^2) at the maximal cross-sectional plane, endometrial score from 1 to 4 (minimal to maximal) as an estimate of the extent of endometrial edema [21,22], and percentage of CL with power Doppler signals for blood flow [23,24]. The percentage of CL with power Doppler signals of blood flow was estimated from a scan of the entire CL as described and validated [25]. Identity of each follicle of 15 mm or greater was maintained from day to day [21].

In 28 IOIs, daily ultrasound scanning records and blood samples were available from Day 12 until the end of the IOI. In 10 PCLs, daily scanning and sampling had begun on Days 11 to 21 and continued until ovulation at the end of the PCL. Regression of the CL was detected by a decrease in CL area and an associated decrease in percentage of CL with blood flow signals. A cluster analysis indicated that a 20-day length of luteal phase (ovulation to P4 $<$ 1.0 ng/mL) best distinguished between a mare with a PCL and a mare with an IOI. Criteria for assigning 28 IOIs to the IOI group were (1) length of luteal phase was less than 20 days, (2) the IOI was a control in a previous experiment, (3) a single ovulation occurred at the beginning of the IOI, and (4) ovulation at the end of the IOI occurred during April to October. Criteria for assigning 10 PCLs to the PCL group were (1) length of luteal phase was 20 or more days, (2) a single ovulation occurred at the beginning of the PCL, (3) no other ovulations or hemorrhagic anovulatory follicles [3,21] were detected between the ovulation at the beginning and at the end of the PCL, and (4) ovulation after the end of the PCL occurred during April to October. The criterion for ovulation at the end of the IOI or PCLs during April to October was used to minimize confounding from season when data were considered inversely from the ovulation at the end of an IOI or PCL.

The day of the end of luteolysis (P4 $<$ 1.0 ng/mL) and the day of ovulation at the end of the IOI or PCL were used as reference points. Groups were compared inversely from each reference point. This was done for the following reasons: (1) great disparity between the two groups in length of the intervals from ovulation to the end of luteolysis and from ovulation to ovulation, (2) considerable variability in lengths within the IOI group and especially within the PCL group, and (3) the need for reference points that were common between the groups. All the 28 IOIs and 10 PCLs were used for comparing groups inversely from 4 days before the end of luteolysis and from 10 days before ovulation at the end of the IOI or PCL. Seven of the 10 PCLs