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Circadian influence on the preovulatory LH surge, ovulation, and prolactin concentrations in heifers

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ABSTRACT

A novel circadian study of the effect of clock hours on the preovulatory LH surge, ovulation, and maximal PRL concentration was done in 13 nontreated Holstein heifers. Hourly blood sampling and hourly ultrasound examinations to detect the hour of ovulation began at 8 and 48 hours, respectively, after CL area (cm^2) had decreased 15% from the area at 15 days postovulation. The resulting experimental period began at the beginning of postluteolysis (progesterone, <1 ng/mL) and encompassed a mean of 3.5 days until ovulation. The frequency of the peak of the preovulatory LH surge for the three 8-hour periods of a 24-hour day was different ($P < 0.02$) between 2:00 AM to 9:00 AM ($N = 9$), 10:00 AM to 5:00 PM ($N = 3$), and 6:00 PM to 1:00 AM ($N = 1$). The median was 6:00 AM. The frequency of ovulations for 8-hour periods was different ($P < 0.02$) between 3:00 AM to 10:00 AM ($N = 9$), 11:00 AM to 6:00 PM ($N = 3$), and 7:00 PM to 2:00 AM ($N = 1$). The median was 7:30 AM. Two or three clusters of PRL pulses occurred during the 3.5 days. Based on all available PRL pulse clusters ($N = 36$), the clock hours of the maximal concentration/cluster was greater ($P < 0.0001$) for 9:00 AM to 2:00 PM ($N = 33$ clusters) than for each of the three other 6-hour periods ($N = 0$, 1, or 2 per period). The median was 11:30 AM. The hypothesis was supported that the peak of the preovulatory LH surge, ovulation, and maximal PRL concentration during pulse clusters occur with greater frequency during certain clock hours in heifers.

1. Introduction

Daily rhythms in reproductive events have been described as diurnal [1–4] or circadian [5–7]. Circadian regulation of reproductive events has been described in detail in rats and hamsters [5–7]. Administration of a barbiturate during a few critical clock hours delays the LH surge for 24 hours. The LH surge is timed by a clock in the suprachiasmatic nucleus of the hypothalamus. The importance of the hypothalamic nucleus has been demonstrated by lesion experiments [8]. Estradiol acts permissibly to prime the hypothalamic release of gonadotropin-releasing hormone [3,8]. The effect of environmental light on the retina is the predominant external cue for the endogenous timing system that regulates the rhythmicity of seasonal and daily reproductive mechanisms [7,8]. The light information is processed by the suprachiasmatic nucleus which then signals the pineal gland to begin nocturnal production of melatonin. In addition to the response to light, the hypothalamic nucleus is also a self-sustained oscillator. Therefore, in the absence of a rhythmic environmental factor (e.g., continuous darkness) an endocrinological rhythm might be only partially altered.

The incredibly complex endogenous circadian timekeeping system has been studied in rodents in detail [5–8]. At the other extreme in farm animals, even descriptions of circadian patterns of reproductive hormones and events are limited. In ewes at 10 days postmating, progesterone

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concentration is lesser and LH concentration is greater during the day (10:00 AM to 6:00 PM; 10:00 AM to 4:00 PM) than at night (10:00 PM to 4:00 AM) [4]. In mares, based on blood samples collected each day at 1:00 AM, 7:00 AM, 1:00 PM, and 7:00 PM throughout the estrous cycle, a circadian pattern occurs on Days 4 to 12 (Day 0 = ovulation) in progesterone and on Days 4 to 5 in LH [1]; the lowest concentration for both hormones was at 1:00 AM. In women, the preovulatory LH surge occurs between midnight and 8:00 AM, but there have been few additional studies on circadian rhythms in human reproduction [7]. In two cows, diurnal variation in progesterone and LH was not detected in blood samples collected hourly during estrus and until ovulation [9]. Blood sampling in heifers every 6 hours indicated that the concentration of LH on Days 5 to 9, but not on Days 10 to 14, is lower at 7:00 AM than at each of the other three time periods [2]. The effect of hour of the 24-hour day on the hour of the preovulatory surge and ovulation in cattle apparently has not been reported.

Rhythmic pulses of PRL have been described in heifers for 12 hours before the beginning of the luteolytic period to 36 hours after the end of the luteolytic period [10]. The interval between the peaks of adjacent pulses is 4 or 5 hours. In lactating cows, serum PRL is greatest at 4:00 PM; however, the stage of the estrous cycle and time of year were not reported [11]. Based on a preliminary study in July, multiple pulses of PRL form clusters during the follicular phase or during 3.5 days before ovulation in heifers. Using PRL means that were normalized to ovulation, the maximum concentration of a cluster was approximately 24 hours from the maximum of an adjacent cluster. The 24-hour cycle indicated that PRL clustering was influenced by environmental factors, but the clock hours associated with clustering and with ovulation were not determined.

The effect of daylength in cattle has been studied more for its role in seasonal or circannual hormonal rhythms than for its role in daily or circadian rhythms. Circannual rhythms occur in circulating concentrations of LH [12] and PRL [13,14]. The circannual rhythm in LH has been demonstrated in ovariectomized heifers, and the PRL rhythm occurs in intact cattle. Prolactin increases fourfold in prepubertal heifers exposed to photoperiods of 16 hours light:8 hours dark compared with daily photoperiods of <12 hours [15].

The current study in heifers used hourly blood sampling and hourly ovulation detection to test the hypothesis that the peak of the preovulatory LH surge, ovulation, and maximal PRL concentration during pulse clusters occur with greater frequency during certain clock hours in heifers.

2. Materials and methods

2.1. Heifers and clock hours

Holstein dairy heifers (N = 13) aged 18 to 24 months and weighing 450 to 580 kg were used during July 2011 in the northern temperate zone (latitude: 43°12′N). Only natural estrous cycles were used without induced luteolysis, induced ovulation, or synchronization of estrus or ovulation. The heifers were kept under natural light in an open shelter and were maintained by ad libitum access to water, trace mineralized salt, and primarily grass hay. Heifers were selected that had docile temperament and were acclimated to the chute system. The day of ovulation was detected by transrectal ultrasonography, and the first day of the experiment (Day 15 postovulation) occurred for the first of the 13 heifers on June 26. The last ovulation at the end of the experiment occurred on July 20. The experiment therefore was initiated shortly after the summer solstice of June 20. Sunrise and sunset for the summer solstice are 5:19 AM and 8:42 PM (16 hours light:8 hours dark).

2.2. Protocol

Cross-sectional area (cm^2) of the CL was determined every 8 hours by transrectal ultrasound using the maximum CL image, beginning on Day 15 (Day 0 = ovulation). Hourly blood sampling and hourly ultrasound examinations to detect the hour of ovulation began 8 hours and 48 hours, respectively, after the CL area had decreased 15% from the area on Day 15. Progesterone concentrations for these samples indicated that the hourly sampling began at the beginning of postluteolysis (progesterone, <1 ng/mL) and encompassed a mean of 3.5 days until ovulation. Mean day and diameter of the future ovulatory follicle at the beginning of the hourly examinations for ovulation were Day 19.7 ± 0.6 and 14.6 ± 0.6 mm, respectively. To minimize animal stress, hourly examinations for ovulation detection were made quickly (<5 minutes) with a simple search for disappearance of the preovulatory follicle. Blood was collected from an indwelling catheter that was inserted 8 hours before the beginning of the hourly blood sampling. Heifers were released from the holding chute after every blood sampling or ultrasound examination and given access to feed and water.

Clusters of PRL pulses were recognized as illustrated (Fig. 1). A cluster consists of 2 to 6 pulses of PRL. The maximum value in a cluster was represented by the pulse peak with the greatest concentration. The clock hour when ovulation occurred was determined from the recorded hourly ultrasound records. The clock hour for concentrations of LH and PRL were determined from the recorded clock hour of ovulation and the known number of hours from ovulation retroactively to each hourly sample. For example, if ovulation in an individual occurred at 7:00 AM and the peak of the preovulatory LH surge occurred 25 hours before ovulation, the LH peak was assigned to 6:00 AM of the previous day. A day encompassed midnight 11:00 PM. The frequency of an event was compared among sets of hours that divided evenly into 24 hours (2, 3, 4, 6, 8, and 12 hours). The hour-set that appeared to best encompass the hours of an event was then used for comparisons over the 24 hours. For the peak of the LH surge and ovulation, the duration of the selected hour set was 8 hours. For maximal concentration in a PRL cluster, the duration of the selected set was 6 hours. In addition, the LH and PRL concentrations for the two complete days (midnight to 11:00 PM for each day) that preceded the day