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Differential regulation of the secretion of luteinizing hormone and follicle-stimulating hormone around the time of ovulation in the bitch

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Abstract

Plasma concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined 3–6 times daily in six Beagle bitches from the start of the follicular phase until 5 d after the estimated day of ovulation. The aim of the study was to gain more detailed information regarding the changes in and the temporal relation between these hormones around the time of ovulation. In all bitches, the pre-ovulatory LH surge was accompanied by a pre-ovulatory FSH surge. The mean duration of the pre-ovulatory FSH surge (110 ± 8 h) was significantly longer than that of the pre-ovulatory LH surge (36 ± 5 h). The FSH surge started concomitantly with the pre-ovulatory LH surge in four bitches, and 12 h before the start of the LH surge in the other two bitches. The pre-ovulatory LH surge had a bifurcated pattern in four bitches. The mean plasma LH concentration before (1.9 ± 0.4 μg/L) and after (1.9 ± 0.3 μg/L) the pre-ovulatory LH surge were similar. The mean plasma FSH concentration during the period 72–28 h before the pre-ovulatory LH surge (1.6 ± 0.3 U/L) was lower ($P < 0.001$) than that during the period 100–144 h after the pre-ovulatory LH surge (3.1 ± 0.2 U/L). In conclusion, this study demonstrated concurrent pre-ovulatory surges of FSH and LH and provided more evidence for differential regulation of the secretion of FSH and LH.

Keywords: Canine; Gonadotropins; Estrous cycle; Progesterone; Dog

1. Introduction

The domestic dog, a mono-estrous species, has an estrous cycle which is considerably longer than that of most other domestic species. Spontaneous ovulations are followed by a luteal phase, which lasts about 75 d in the non-pregnant bitch, and a non-seasonal anestrus of about 2–10 mo [1]. Proestrus and early estrus, characterized by bloody vaginal discharge, constitute the follicular phase, which varies in length from 6 to 20 d. The follicular phase lasts until ovulation, which usually takes place within 3 d after the start of estrus behavior. The duration of estrus varies from 3 to 21 d. The occurrence of the pre-ovulatory luteinizing hormone (LH) surge and ovulation cannot be predicted reliably by determining the start of estrus [2].

Gonadotropins play an essential role in the induction of the follicular phase and ovulation. In dogs, follicle stimulating hormone (FSH) pulses appear to occur

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concomitantly with LH pulses in all stages of the estrous cycle and anestrus [3]. The pre-ovulatory LH-surge is also associated with a surge in FSH secretion [4]. The reported duration of the canine pre-ovulatory LH surge, ranging from 1 to 5 d, is relatively long [1,2,5], compared to other domestic species. In cattle, for example, the duration of the pre-ovulatory LH surge is only 8 h [6]. In humans, however, the duration of the pre-ovulatory LH surge is about 2 d [7]. In dogs, ovulation is assumed to take place approximately 2–3 d after the pre-ovulatory LH surge and is accompanied by a strong increase of plasma progesterone concentration [2,8,9]. Detailed information about the temporal relation between the pre-ovulatory LH and FSH surges in the bitch is limited. The aim of this study was to increase the knowledge of changes in and temporal relations between plasma concentrations of LH and FSH around the time of ovulation in the bitch.

2. Materials and methods

Six healthy Beagle bitches, 4–6 y of age, were used in this study. All had been born and raised in the Department of Clinical Sciences of Companion Animals and were accustomed to the laboratory environment and procedures such as collection of blood. They were housed singly or in pairs in indoor–outdoor runs, fed a standard commercial dog food once daily, and given water ad libitum. All dogs were examined three times weekly for the presence of swelling of the vulva and serosanguineous vaginal discharge, which were used as markers of the onset of proestrus. From the first day of observed proestrus, vaginoscopy was performed once daily until shrinkage of the vaginal mucosa was seen for the first time. To estimate the time of ovulation, plasma concentrations of progesterone were determined three times weekly from the start of proestrus. Ovulation was assumed to occur when the plasma progesterone concentration exceeded 16 nmol/L [9–11]. Blood samples were collected from the jugular vein, immediately placed in chilled lithium heparin-coated tubes and centrifuged at 4 °C for 10 min at 1500 × *g*. Plasma was stored at −25 °C until assayed. Blood samples were collected at 8-h intervals during the early follicular phase, starting on the first day of proestrus, and lasting until the initial observation of shrinkage of the vaginal mucosa. Blood samples were collected at 4-h intervals during the late follicular phase and the early luteal phase, i.e., from the first day of shrinkage of the vaginal mucosa until 5 d after the estimated day of ovulation.

Plasma LH concentrations were determined by heterologous radioimmunoassay (RIA), as described previously [12]. Plasma FSH concentrations were determined applying a human immunometric sandwich assay (Amerlite, Amersham, UK) as described previously [3]. Plasma concentrations of progesterone were determined by a previously validated RIA [13].

The highest plasma LH concentration detected during the pre-ovulatory LH-surge was taken as $T = 0$. The start of the pre-ovulatory LH and FSH surges was defined as the first of two consecutive measurements that exceeded the mean plasma hormone concentration plus one standard deviation of the period 72–28 h before $T = 0$. Similarly, the end of the pre-ovulatory LH and FSH surges was defined as the last measurement that exceeded the mean plasma hormone concentration plus one standard deviation, during the period 100–144 h after $T = 0$ and was preceded by a measurement which met the same requirement. The pre-ovulatory surges were considered to have bifurcated patterns when the decline between two consecutive plasma hormone concentrations was more than two standard deviations of the mean plasma hormone concentration in the period 72–28 h before $T = 0$. Differences in basal plasma LH and FSH concentrations before and after the pre-ovulatory LH/FSH surge were analyzed with a paired Student's *t*-test (two-tailed). The difference in duration of the mean pre-ovulatory LH and FSH surges was analyzed with an independent Student's *t*-test. Results are given as mean ± S.D. Differences at $P < 0.05$ were considered significant.

3. Results

In all bitches, a pre-ovulatory LH surge, with a mean duration of 36 ± 5 h, was detectable. The mean peak plasma LH concentration was 18.7 ± 5.8 μg/L. The mean plasma LH concentrations during the period 72–28 h prior to $T = 0$ and during the period 100–144 h after $T = 0$ were 1.9 ± 0.4 and 1.9 ± 0.3 μg/L, respectively. In four of six bitches, the LH surge showed a bifurcated pattern (Fig. 1).

In all bitches a pre-ovulatory FSH surge was present. The mean duration of the FSH surge (110 ± 8 h) was longer than that of the LH surge ($P < 0.001$). The mean peak plasma FSH concentration was 13.8 ± 2.0 U/L. The mean plasma FSH concentration during the period 72–28 h before $T = 0$ (1.6 ± 0.3 U/L) was lower ($P < 0.001$) than that during the period 100–144 h after $T = 0$ (3.1 ± 0.2 U/L). The pre-ovulatory LH surge started concomitantly with the pre-ovulatory FSH surge

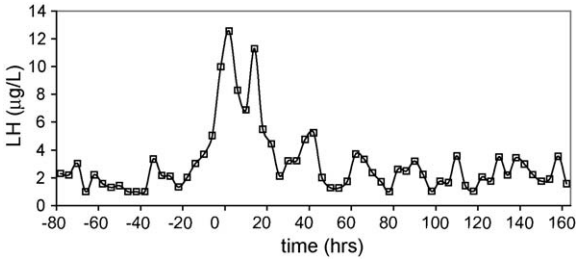

Fig. 1. The plasma concentration of LH (□) of a 6-y-old beagle bitch during the late follicular and early luteal phase. $T = 0$ is the highest plasma LH concentration during the pre-ovulatory LH surge. Note the bifurcated pre-ovulatory LH surge.

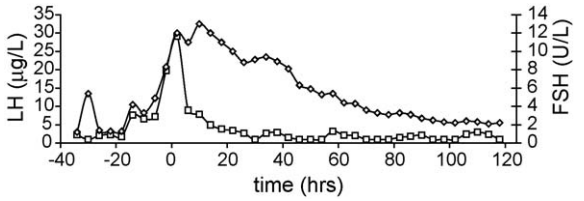

Fig. 2. Plasma concentrations of LH (□) and FSH (◇) of a 6-y-old beagle bitch during the peri-ovulatory period. $T = 0$ is the highest plasma LH concentration during the pre-ovulatory LH surge.

in four bitches (Fig. 2), and 12 h after the FSH surge in the other two bitches.

In all bitches, plasma progesterone concentrations increased after the pre-ovulatory LH surge to concentrations measured at the time of ovulation. Five days after the estimated day of ovulation, the mean plasma progesterone concentration in these six bitches was 101 ± 31 nmol/L.

4. Discussion

The results of this study show the temporal relation between the plasma concentrations of LH and FSH during the peri-ovulatory period. In all bitches, ovulation was determined by measuring plasma progesterone concentration and the start of the luteal phase progressed normally, since high plasma progesterone concentrations were measured within 5 d after the pre-ovulatory LH surge [2,8,9].

In all bitches, ovulation was preceded by a LH surge. The mean duration of the pre-ovulatory LH surge was 36 h, which is similar to the findings of Onclin et al. [5], but shorter than observed in the study of Wildt et al. [2]. Different sample frequencies and different cut-off points may explain these differences. The mean plasma LH concentration before the pre-ovulatory LH surge was not significantly different from that after the pre-ovulatory LH surge, which corresponded to the findings of Wildt et al. [2].

In four of the six bitches, a bifurcated pattern of the pre-ovulatory LH surge was observed and in two of these four bitches, the dip had a duration of at least 4 h. This bifurcated pattern of the pre-ovulatory LH surge has also been described by Wildt et al. [2] in 4 out of 25 bitches, using a twice-daily sample frequency. Obviously, taken into account the relatively short duration of the dip, the bifurcated pattern of the pre-ovulatory LH surge can easily be missed when a low-frequency sample schedule is used. In sheep, it has been shown that an elevated GnRH level in the pituitary portal blood is required for initiation and maintenance of the LH surge [14]. From the observed significant dip during the pre-ovulatory LH surge in the bitch, it may be hypothesized that the GnRH surge also shows a temporary decline. In other species, in which the duration of the LH surge is often much shorter, a similar bifurcated pattern has not been described.

The mean duration of the peri-ovulatory FSH surge was about three times as long as that of the pre-ovulatory LH surge; this may, at least in part, be ascribed to a longer half-life of FSH than LH. In other mammals, a pattern of glycosylation of FSH that differs from that of LH has been described [15]. It is because of this difference in glycosylation patterns that LH is eliminated from the circulation faster than FSH [16].

The mean plasma FSH concentrations before and after the pre-ovulatory LH surge were significantly different. Furthermore, the pre-ovulatory FSH surge did not show a bifurcated pattern in any of the bitches. Additionally, in two bitches, the FSH surge started 12 h earlier than the LH surge. These observations suggested a differential regulation of FSH and LH secretion in the dog. The main regulator of FSH and LH secretion by the pituitary gonadotropes is GnRH [17]. Differential regulation of FSH and LH secretion can at least partly be explained by the frequency and amplitude of GnRH pulses [18–21]. In addition, a specific hypothalamic FSH-releasing factor [22] and gonadal feedback may play a role in the differential or non-parallel secretion of FSH and LH [18,19].

In conclusion, this study demonstrated concurrent preovulatory surges of FSH and LH and also provided evidence for differential regulation of the secretion of FSH and LH in the bitch.

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References

[1] Concannon PW. Biology of gonadotrophin secretion in adult and prepubertal female dogs. J Reprod Fert Suppl 1993;47:3–27.
[2] Wildt DE, Chakraborty PK, Panko WB, Seager SWJ. Relationship of reproductive behavior, serum luteinizing hormone and time of ovulation in the bitch. Biol Reprod 1978;18:561–70.
[3] Kooistra HS, Okkens AC, Bevers MM, Popp-Snijders C, van Haaften B, Dieleman SJ, et al. Concurrent pulsatile secretion of luteinizing hormone and follicle-stimulating hormone during different phases of the estrous cycle and anestrus in beagle bitches. Biol Reprod 1999;60:65–71.
[4] Olson PN, Bowen RA, Behrendt MD, Olson JD, Nett TM. Concentrations of reproductive hormones in canine serum throughout late anestrus, proestrus and estrus. Biol Reprod 1982;27:1196–206.
[5] Onclin K, Murphy B, Verstegen JP. Comparisons of estradiol, LH and FSH patterns in pregnant and nonpregnant beagle bitches. Theriogenology 2002;57:1957–72.
[6] Dieleman SJ. Steroids of preovulatory bovine follicles relative to the peak of luteinizing hormone Utrecht: Utrecht University; 1984
[7] Hoff JD, Quigley ME, Yen SSC. Hormonal dynamics at midcycle: a reevaluation. J Clin Endocrin Metabol 1983;57:792–6.
[8] Phemister RD, Holst PA, Spano JS, Hopwood ML. Time of ovulation in the Beagle bitch. Biol Reprod 1973;8:74–82.
[9] Concannon P, Hansel W, McEntee K. Changes in LH, progesterone and sexual behavior associated with preovulatory luteinization in the bitch. Biol Reprod 1977;17:604–13.
[10] Okkens AC, Dieleman SJ, Bevers MM, Willemse AH. Evidence for the non involvement of the uterus in the lifespan of the corpus luteum in the cyclic dog. Vet Quart 1985;7:169–73.
[11] Wildt DE, Panko WB, Chakraborty PK, Seager SW. Relationship of serum estrone, estradiol-17beta and progesterone to LH, sexual behavior and time of ovulation in the bitch. Biol Reprod 1979;20:648–58.
[12] Nett TM, Akbar AM, Phemister RD, Holst PA, Reichert LE, Niswender GD. Levels of luteinizing hormone, estradiol and progesterone in serum during the estrous cycle and pregnancy in the Beagle bitch. Soc Exp Biol Med 1975;148:134–9.
[13] Dieleman SJ, Schoenmakers HJN. Radioimmunoassays to determine the presence of progesterone and estrone in the starfish *Asterias rubens*. Gen Comp Endocrinol 1979;39:534–42.
[14] Karsch FJ, Bowen JM, Caraty A, Evans NP, Moenter M. Gonadotropin-releasing hormone requirements for ovulation. Biol Reprod 1997;56:303–9.
[15] Schwartz NB. The 1994 Stevenson Award Lecture. Follicle-stimulating hormone and luteinizing hormone: a tale of two gonadotropins. Can J Physiol Pharmacol 1995;73:675–84.
[16] Chappel SC, Ulloa-Aguirre A, Coutifaris C. Biosynthesis and secretion of follicle-stimulating hormone. Endocrinol Rev 1983;4:179–211.
[17] Rijnberk A. Hypothalamus-pituitary system. In: Rijnberk A, editor. Clinical endocrinology of dogs and cats. Kluwer Academic Publishers; 1996. p. 11–34.
[18] Mann GE, Campbell BK, McNeilly AS, Baird DT. The role of inhibin and oestradiol in the control of FSH secretion in the sheep. J Endocrinol 1992;133:381–91.
[19] Shupnik MA. Gonadal hormone feedback on pituitary gonadotropin genes. Trends Endocrinol Metabol 1996;7:272–6.
[20] Haisenleder DJ, Dalkin AC, Ortolano GA, Marshall JC, Shupnik MA. A pulsatile gonadotropin-releasing hormone stimulus is required to increase transcription of the gonadotropin subunit genes: evidence for differential regulation of transcription by pulse frequency in vivo. Endocrinology 1991;128:509–17.
[21] Vizcarra JA, Wettemann RP, Braden TD, Turzillo AM, Nett TM. Effect of gonadotropin-releasing hormone (GnRH) pulse frequency on serum and pituitary concentrations of luteinizing hormone and follicle-stimulating hormone, GnRH receptors, and messenger ribonucleic acid for gonadotropin subunits in cows. Endocrinology 1997;138:594–601.
[22] Yu WH, Karanth S, Walczewska A, Sower SA, McCann SM. A hypothalamic follicle-stimulating hormone-releasing decapeptide in the rat. Proc Natl Acad Sci USA 1997;94:9499–503.