Available online at www.sciencedirect.com
ScienceDirect
Theriogenology
Theriogenology 66 (2006) 1419–1422
www.journals.elsevierhealth.com/periodicals/the

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

J. de Gier [a,*], H.S. Kooistra [a], S.C. Djajadiningrat-Laanen [a], S.J. Dieleman [b], A.C. Okkens [a]

[a] *Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 8, P.O. Box 80154, 3508 TD Utrecht, The Netherlands*
[b] *Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, P.O. Box 80151, 3508 TD Utrecht, The Netherlands*

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

* Corresponding author. Tel.: +31 30 2531477; fax: +31 30 2518126.
E-mail address: J.deGier@vet.uu.nl (J. de Gier).

0093-691X/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2006.02.001

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