

Effect of investigational kisspeptin/metastin analog, TAK-683, on luteinizing hormone secretion at different stages of the luteal phase in goats

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Abstract. This study aimed to examine the response of luteinizing hormone (LH) secretion and ovarian steroid profile to TAK-683, an investigational metastin/kisspeptin analog, through treatment during different stages of the luteal phase in goats. Nine cycling Shiba goats (4.4 ± 2.3 years old) were assigned to early luteal phase (ELP, $n = 4$), mid-luteal phase (MLP, $n = 4$), and control ($n = 5$) groups. The ELP and MLP groups were administered 50 μg of TAK-683 intravenously on either day 5 or between days 7–14 after ovulation, respectively. The control group received vehicle between days 7–14 after ovulation. Blood samples were collected at 10-min (2–6 h), 2-h (6–24 h), and 24-h (24–96 h) intervals after treatment. Significant increases in plasma LH concentration were detected during the periods of 3 to 5 h and 2 to 5 h in the ELP and MLP groups, respectively. Estradiol concentrations continuously increased with the rise of basal LH secretion after TAK-683 treatment in two goats of the ELP group with a surge-like release of LH, but not in the goats without LH surge, i.e. the MLP and control group ones. Plasma progesterone concentration and the lengths of estrous cycle in all groups did not change significantly from the time before and after treatment. Present findings indicate that the responses of LH and ovarian steroids to treatment with TAK-683 depend on the stage of the luteal phase of the estrous cycle. We suggest that the stimulatory effects of TAK-683 on LH secretion are reduced in the process leading to the mid-luteal phase in cycling goats.

Key words: Goats, Luteal phase, Luteinizing hormone (LH) secretion, TAK-683

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Kisspeptin is mainly produced in the hypothalamus and is biologically active at various lengths of 10 to 54 amino acids. Kisspeptin-54 or kisspeptin-10, either the full length or the C-terminal amidated 10-amino-acid sequence of kisspeptin, potently stimulates luteinizing hormone (LH) secretion in both male and female mammals [1–7]. Our previous study showed that the stimulatory action of kisspeptin-10 on LH secretion is attributable to the stimulation of gonadotropin-releasing hormone (GnRH) neurosecretion into the hypophyseal portal circulation in castrated goats [8]. Kisspeptin is also involved in the hypothalamic regulation of GnRH secretion by gonadal steroids. Several lines of evidence suggest that the hypothalamic kisspeptin neurons expressing receptors for androgens, estrogens, and progesterone are implicated in the negative feedback control of GnRH/gonadotropin secretion [1]. From the clinical point of view, the ability of kisspeptin to induce LH secretion makes it a suitable target for the pharmacological manipulation of the gonadotropic axis.

TAK-683 is an investigational kisspeptin analog, developed for

clinical use as a synthetic peptide consisting of nine amino acids and having similar activity to the full-length peptide [9]. Previous studies demonstrated that a bolus administration of TAK-683 was able to stimulate LH secretion in rats [10] and goats [11]. In contrast, continuous exposure to TAK-683 strongly suppressed pulsatile LH secretion in male rats [12], and castrated [11] and ovariectomized goats [13].

Recent studies indicated that the response of LH secretion to a bolus administration of kisspeptin analog depends on the ovarian status at different stages of the reproductive cycle. One of the studies reported that the increase in the absolute value of plasma LH concentration after an administration of C6, another investigational kisspeptin analog, is much higher in the breeding season than that in the non-breeding season, in ewes [14]. The secretory pattern of LH secretion after a single injection of TAK-683 is characterized by episodic increases with a relatively small amplitude in ovariectomized goats. On the other hand, when TAK-683 was injected in goats in the follicular phase of the estrous cycle, a surge-like release of LH followed by ovulation was induced after treatment [15]. Despite these findings, there is little information available regarding the effect of kisspeptin analogs on LH secretion during the luteal phase. Interestingly, small increases in the basal LH secretion were transiently observed for several hours after TAK-683 administration and then a surge-like release of LH occurred in female goats treated with progesterone-releasing controlled internal drug releasing (CIDR) devices [16].

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As in the follicular phase, these findings indicate that TAK-683 has the potential to induce LH surge even in the endocrine milieu of the luteal phase.

Therefore, in this study, we examined the endocrinal response to a single injection of TAK-683 during the luteal phase in goats. This paper reports changes in the LH concentrations after an administration of TAK-683 and their association with secretion of ovarian steroids at the different stages of the luteal phase, i.e. early- and mid-luteal phases, of the estrous cycle in goats.

Materials and Methods

Animals

Nine cycling Shiba goats (4.4 ± 2.3 years of age; 30.3 ± 5.9 kg in body weight) maintained at the Tokyo University of Agriculture and Technology were used. Shiba goats are non-seasonal breeders under the natural daylight conditions in Japan. The goats were housed in outdoor paddocks with sheltered areas and were fed alfalfa hay cubes (660 g of dry matter/day), with clean water and mineral salt blocks available *ad libitum*. They were kept individually in cages temporarily when they were subjected to treatment and frequent blood sampling. All procedures were approved by the University Committee for the Use and Care of Animals, of the Tokyo University of Agriculture and Technology (Nos. 22–67).

Experimental procedure

Cycling goats were assigned to three groups: early luteal phase (ELP, $n = 4$), mid-luteal phase (MLP, $n = 4$), and control ($n = 5$) groups. Two goats (#4 and #24) were utilized first in the ELP group and then in the control or MLP group, respectively. One goat (#22) was first utilized in the control group, then the ELP group, and lastly in the MLP group. The goats were kept untreated for at least one estrous cycle before they were assigned to the other groups.

After detection of ovulation by ultrasound, the ELP and MLP groups received an intravenous administration of 50 μ g of TAK-683 on either day 5 after ovulation or between days 7 and 14 (mean \pm SD, 9.3 ± 1.7 days) after ovulation, respectively. During the period between days 7 and 14 after ovulation, the corpus luteum was considered to have been fully developed, as described previously [17]. The dosage of TAK-683 was determined based on previous studies [15, 16], i.e. 50 μ g (35 nmol) diluted in 5 ml of physiological saline, for injection purposes. The chemical profile of TAK-683 has been described previously [9]. Meanwhile, the control group received 5 ml of physiological saline between day 7 and day 14 after ovulation (mean \pm SD, 9.4 ± 1.3 days).

Blood samples were taken via an indwelling catheter (18-gauge, 30 cm in length; Argyle™ Catheter Kit, Nippon Sherwood Medical Industries, Shizuoka, Japan) inserted into the jugular vein before the experiment. Two milliliters of blood samples were collected at 10-min (2–6 h), 2-h (6–24 h), and 24-h (24–96 h) intervals for analysis of LH, progesterone, and estradiol. Samples were centrifuged at 4°C 3000 rpm; then, the separated plasma was kept at –20°C until being assayed.

Transrectal ultrasounds (HS-1500V, Honda Electronics, Aichi, Japan) with a 7.5-MHz linear probe (HLS-375M, Honda Electronics) were conducted at 24-h intervals or every other day to detect ovula-

tion. Ovulation was defined as the disappearance of a large follicle, which was reconfirmed by the development of corpus luteum at the same location.

Hormone assay

Plasma LH was assayed in duplicate using radioimmunoassay, as described previously [18]. Intra- and inter-assay coefficients of variation were 7.8% and 14.3%, respectively, and sensitivity was 0.14 ng/ml. Plasma estradiol-17 β concentrations were measured using a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, Michigan, USA), following extraction by dichloromethane (Wako Pure Chemical Industries, Osaka, Japan). Intra-assay coefficients of variation averaged 9.2%, and sensitivity was 0.48 pg/ml. Plasma progesterone concentrations were measured using enzyme immunoassays [19]. The intra-assay coefficient of variation was 9.4%, and sensitivity of the assay was 0.34 ng/ml.

Statistical analyses

All data are represented as mean \pm SD; the difference between groups was determined using Kruskal-Wallis followed by Dunn's test. Time-course analysis of the LH and ovarian steroid concentration changes before and after injection, were compared using analysis of variance (ANOVA), followed by Tukey's or Dunnett's test, or Kruskal-Wallis followed by Dunn's test, according to the distribution. A confidence level of $P < 0.05$ was considered as statistically significant. The LH surge was defined according to a previous study that determined spontaneous LH surge in cycling goats [18]; i.e., an increase in LH concentration over the level of 10 ng/ml in the plasma.

Results

In all the animals in the ELP and MLP groups, the LH concentration underwent a sustained increase with a small amplitude from 0 to 6 h after injection. In the ELP group, two patterns of LH responses were found (Fig. 1). A surge-like release of LH was detected in 2 out of the 4 goats, but not in the other two goats. The mean LH concentrations from 0 to 6 h after injection were 1.1 ± 0.4 , 1.0 ± 0.3 , and 0.6 ± 0.2 ng/ml in the ELP, MLP, and control groups, respectively. Increase in the LH concentration with a relatively small amplitude occurred within 6 h after treatment, with mean peak values of 2.7 ± 2.8 and 1.9 ± 0.6 ng/ml in the ELP and MLP groups, respectively. In both ELP and MLP groups, significant increases in LH concentration were detected during the periods of 3 to 5 h and 2 to 5 h, respectively, compared with that at 0 h relative to injection (Fig. 2).

Changes in plasma estradiol and LH concentrations from –2 to 24 h in all the goats in the ELP and MLP groups are shown in Fig. 3. The concentrations of estradiol in the two goats (#3, #4) showing the LH surge, continued to increase in parallel with the rise of basal LH concentration until 8 h after injection, with peak values of 18.7 and 12.5 pg/ml, respectively. The continuous elevation of estradiol levels were followed by robust increases in LH levels, and reached peak values of 18.4 and 12.8 ng/ml, at 12 and 14 h respectively (left panels, Fig. 3). Meanwhile in the other two goats of the ELP group, estradiol concentration did not increase after injection; hence, a surge-like release of LH did not occur. The continuous increase in estradiol concentration after TAK-683 injection was not observed in

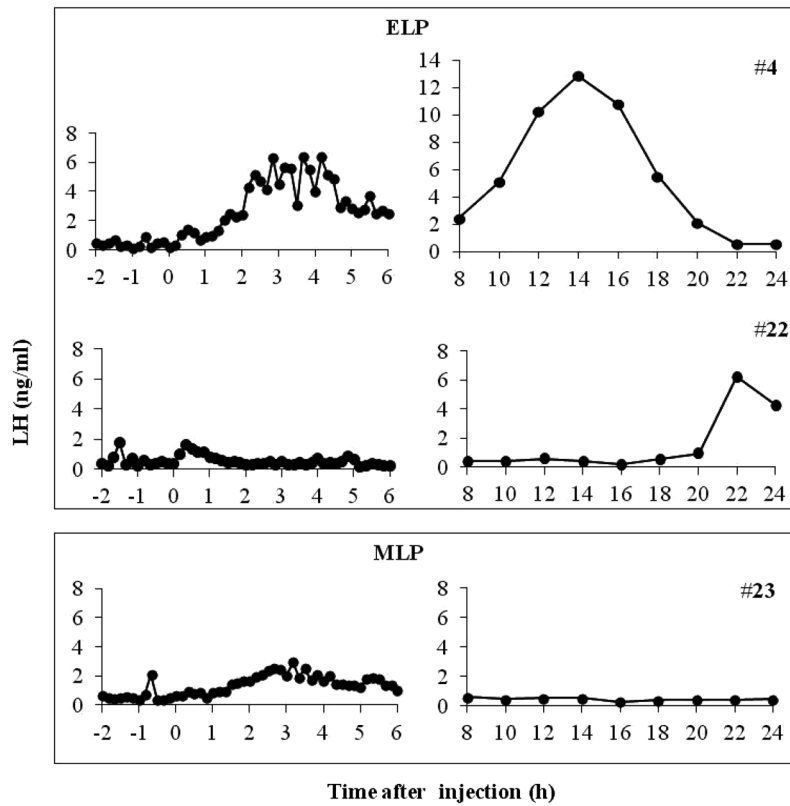


Fig. 1. Representative profiles of LH after TAK-683 treatment in ELP (upper box) and MLP (lower box) groups. A surge-like release of LH was detected at around 14 h after treatment in goat #4 from the ELP group, whereas no surge occurred in goat #22 (ELP) and #23 (MLP).

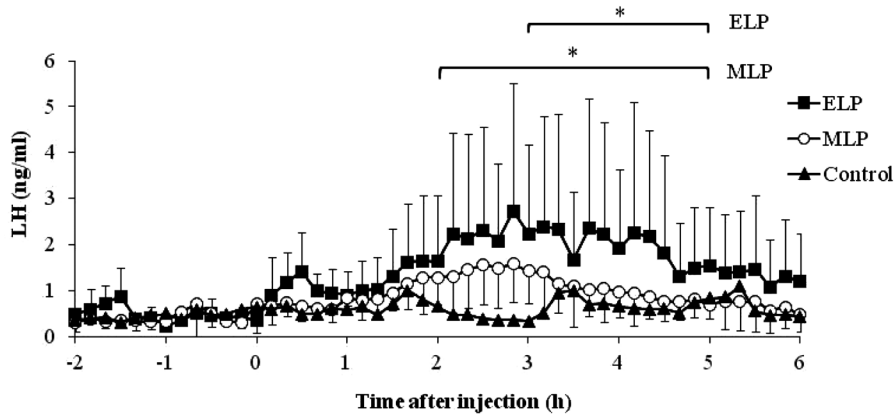


Fig. 2. Mean plasma LH concentration from -2 to 6 h after TAK-683 treatment in the early luteal phase (ELP) and mid-luteal phase (MLP), compared with the control group. Asterisks indicate significant differences, compared with the value at 0 h after treatment ($P < 0.05$).

any of the goats in the MLP group (right panels, Fig. 3).

In the MLP group, the mean plasma progesterone concentration did not change significantly at 24 h after injection, i.e. 12.3 ± 2.4 ng/ml, compared with that at 0 h (just before TAK-683 injection), i.e. 10.9 ± 4.7 ng/ml. Although the mean progesterone concentration of the ELP group on the day of treatment was relatively lower than that

of the MLP group (6.5 ± 2.3 vs. 10.9 ± 4.7 ng/ml; $P = 0.06$), there was no significant difference between the ELP, MLP, and control (9.5 ± 2.3 ng/ml) groups.

Ovulations were detected within 48 hours after injection in the two goats with LH surge in the ELP group, but not in the other goats. The average length of estrous cycle after treatment in the

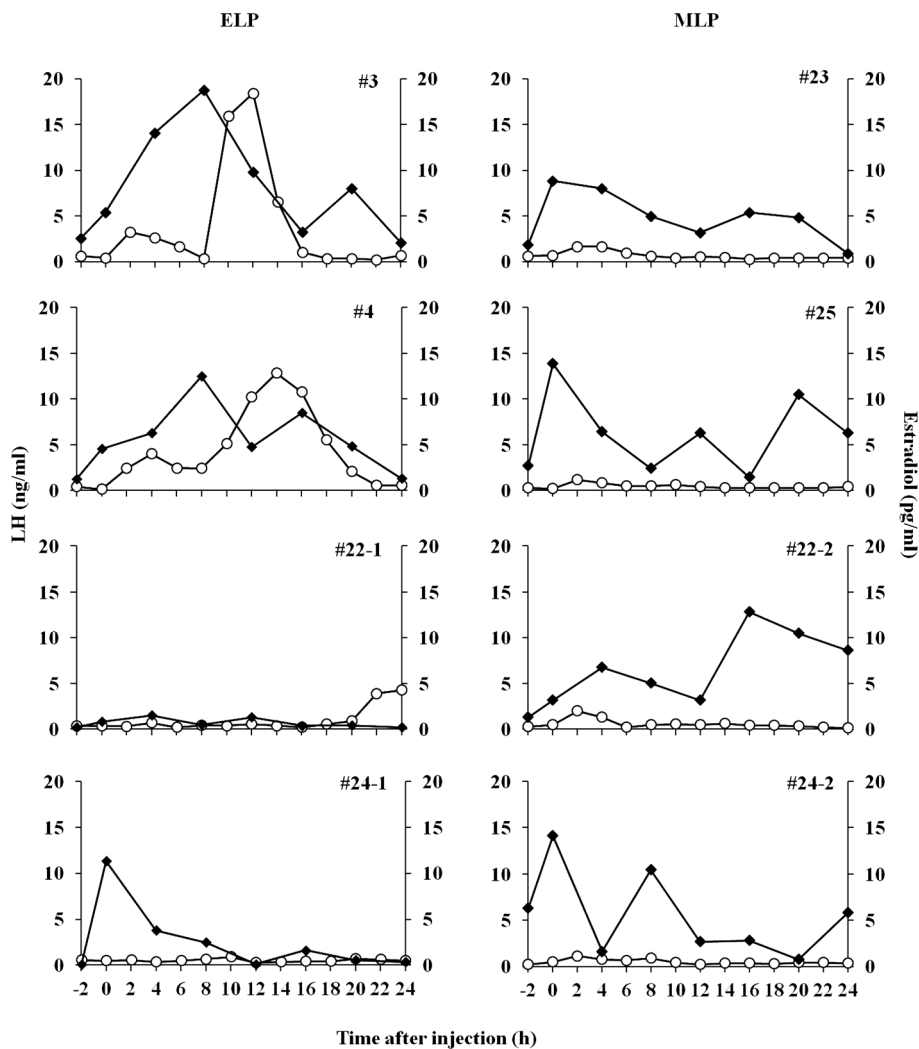


Fig. 3. Individual profiles of plasma LH (open circle) and estradiol (closed square) in goats from the ELP (left panels) and MLP (right panels) groups. The 0 h indicates the time just before injection of TAK-683.

ELP, MLP, and control groups were 21.5 ± 3.0 , 19.8 ± 1.0 , and 18.8 ± 1.8 days, respectively. There was no significant difference in the average length of estrous cycle among all groups.

Discussion

Contrary to our previous findings in the follicular phase [16], no LH surge was observed after a bolus administration of TAK-683 during the mid-luteal phase in goats. On the other hand, half of the four goats in the ELP group showed a response similar to that of goats treated with progesterone-releasing CIDR; LH surge occurred, with the peak time around 12 h after TAK-683 administration [16]. A small amplitude increase in LH secretion was observed within the initial 6 h after administration, in both the ELP and MLP groups. It can be summarized that the responses of surge mode secretions of LH to the treatment with TAK-683 depend on the stage of the luteal phase and that the stimulatory effects of TAK-683 on LH secretion

are reduced in the process leading to the mid-luteal phase.

As described above, our previous study demonstrated that TAK-683 treatment induces a surge-like release of LH in goats treated with CIDR [16]. CIDR is well-known as a standard tool used to mimic the endocrine milieu of the luteal phase in cows [20], ewes [21], and goats [22]. A number of studies demonstrated that plasma concentrations of progesterone during the CIDR treatment were maintained at a level similar to that in the natural luteal phase in ruminant species, including goats [23, 24]. Therefore, the different reaction of LH secretion to the TAK-683 treatment between the previous (LH surge occurred) and the present (no LH surge occurred) studies was unexpected. The profile of LH secretion after TAK-683 treatment in CIDR-treated goats was characterized by a small amplitude increase followed by a significant rise in estradiol concentration in the initial 6 h after treatment [16]. This is similar to the present finding in the two goats showing an LH surge. Interestingly, in these goats from the ELP group, estradiol levels continued to increase gradually until 8 h after injection,

then started to decrease after the LH surge occurred. On the other hand, none of the other goats in the ELP and MLP groups showed this continuous increase in the plasma estradiol concentration after TAK-683 treatment. Increase in estradiol concentration triggers the induction of a surge release of GnRH, which in turn induces an LH surge, as described previously [25, 26]. Another study demonstrated that the continuous infusion of kisspeptin to anestrus ewes increased the plasma level of estradiol and then induced an LH surge [27]. Kisspeptin acts as GnRH and LH stimulators, and its mechanism is regulated by estradiol [3, 28]. Another group of researchers revealed that kisspeptin itself plays a role as a mediator of estradiol-induced positive feedback in the hypothalamus [29]. A possible interpretation for the different response of LH secretion between our present and previous studies is that estradiol is a key mediator in the action of TAK-683 on LH secretion and the lack of continuous activation of estradiol production after TAK-683 administration resulted in the failure to induce an LH surge in the mid-luteal phase.

This hypothesis is supported by the reduction of the stimulatory response of LH in the initial 6 h after TAK-683 treatment in the MLP group, compared with the ELP group, particularly in the two goats showing LH surge. An administration of TAK-683 at the same dose as in the present study immediately induced a robust increase in LH secretion in the follicular phase of goats [16]. It seems that sensitivity of LH stimulation to TAK-683 decreases in the progression toward the luteal phase from the follicular phase. The previous study demonstrated that a single intravenous injection of Kisspeptin-10 (0.77 nmol/ μ g/kg) during the luteal phase was able to increase plasma LH levels from below 1 ng/ml at pre-treatment to reach a peak value of 1.5 ± 0.6 ng/ml, within 30 min after injection in goats [30]. Meanwhile, an intravenous injection of 50 μ g kisspeptin during natural luteal phase in sheep caused a small increase (< 1 ng/ml) of LH concentration within 120 min after injection [31]. We observed a similar LH response during the mid-luteal phase; however, a mean peak LH value of 1.3 ± 0.4 ng/ml was detected within 4 h after injection. It is well known that an increase in LH secretion in circulation promotes estradiol production from the follicle, suggesting that the stimulatory response of LH to the current TAK-683 treatment (50 μ g/head) in the mid-luteal phase may be insufficient to maintain the continuous stimulation of estradiol secretion, which gives rise to a lack of LH surge in the mid-luteal phase. In addition, the lack of pulsatile LH stimulation by TAK-683 in half of the goats from ELP group resulted in low estradiol production; hence, the surge-like release of LH was not induced.

The general consensus is that a high circulating-progesterone concentration inhibits LH secretion from the hypothalamus, resulting in a reduction of LH concentration in the circulation [32]. However, in our previous study, when the luteal phase milieu was successfully mimicked by CIDR insertion, the LH surges could be induced by TAK-683 treatment regardless of the presence of high levels of circulating progesterone [16]. The factor(s) attenuating the reaction of LH secretion to TAK-683 treatment in the natural luteal phase in the current study, is unknown. This weakening action may be associated with the presence of mature corpora lutea, particularly during the mid-luteal phase. A recent study demonstrated that GnRH treatment acted in a different manner toward LH secretion in cows having a corpus luteum compared with those without a corpus luteum [33].

A suppressed pattern of LH was detected in cows bearing a corpus luteum, compared to that in cows with no corpus luteum. Kojima *et al.* [34] proposed that the presence of a corpus luteum produces factors other than progesterone that may influence pulsatile LH secretion. Further studies are needed to determine the physiological factor(s) being involved in the regulation of TAK-683-induced LH secretion during the luteal phase.

In conclusion, the response of LH secretion to TAK-683 treatment in the mid-luteal phase is different from that observed in our previous study, which showed the effect of TAK-683 on LH secretion in the follicular phase or in the mimicked endocrine environment of the luteal phase produced by CIDR treatment in goats. Half of the goats given TAK-683 in the early luteal phase showed a small amplitude increase in LH secretion followed by LH surge. These findings indicate that responses of LH secretions to the treatment with TAK-683 depend on the stage of the luteal phase, and it is suggested that this effect is reduced in the progression from the follicular phase to the luteal phase in cycling goats.

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