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—Original Article—

An administration of TAK-683 at a minimally effective dose for luteinizing hormone stimulation under the absence of the ovary induces luteinizing hormone surge in ovary-intact goats

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Abstract. The present study aimed to evaluate hormonal responses and their association with the TAK-683 blood concentrations in goats administered TAK-683 at a low dose, which had been previously determined as the minimally effective dose for luteinizing hormone (LH) stimulation in ovariectomized goats. In Experiment 1, 5 μg of TAK-683 treatment had no significant stimulatory effect on LH secretion in ovariectomized Shiba goats (n = 4). In Experiment 2, cycling goats received the treatment of prostaglandin $F_{2\alpha}$ and progesterone-releasing controlled internal drug releasing (CIDR) to induce the follicular phase, then they were treated with 5 μg of TAK-683 (hour 0) intravenously (n = 4, IV) or subcutaneously (n = 3, SC) or with vehicle intravenously (n = 4, control) at 12 h after CIDR removal. Blood samples were collected at 10-min (–2–6 h), 2-h (6–24 h), or 6-h (24–48 h) intervals. Ovarian ultrasonographic images were assessed daily to confirm ovulation after the treatment. A surge-like release of LH was immediately observed after injection in all animals in the IV (peak time: 4.2 ± 0.6 h, peak concentration: 73.3 ± 27.5 ng/ml) and SC (peak time: 4.6 ± 0.4 h, peak concentration: 62.6 ± 23.2 ng/ml) groups, but not in the control group. Ovulation was detected within 3 days after TAK-683 injection in all animals in the IV and SC groups, and the interval period from TAK-683 administration to ovulation in the IV group was significantly ($P < 0.05$) shorter than that of the control group. No significant changes were observed between the IV and SC groups in terms of luteal diameter and blood progesterone levels after ovulation. The present findings suggest that the involvement of one or more ovarian factor(s) is indispensable for a TAK-683-induced LH surge leading to ovulation in goats.
Key words: Goats, Luteinizing hormone (LH) surge, Ovary, TAK-683

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The hypothalamic kisspeptin is considered to be a key neuroendocrine regulator for mammalian reproduction by controlling the pulsatile and surge mode secretion of gonadotropin-releasing hormone (GnRH) [1, 2]. TAK-683 has been developed for the purpose of clinical use as a potent analog of the C-terminal decapeptide of kisspeptin by Takeda Pharmaceutical Company Limited, Osaka, Japan [3, 4]. It has been confirmed that an intravenous injection of TAK-683 stimulates GnRH neurosecretion into hypophyseal portal circulation followed by a rise in the peripheral concentrations of luteinizing hormone (LH) in orchidectomized goats [5].

The specific action of TAK-683 on pulsatile and surge mode secretion of LH has been demonstrated as follows. Previous studies have reported that chronic administration of TAK-683 completely blocks pulsatile LH secretion in male rats [3] and orchidectomized goats [5]. The subsequent study showed that subcutaneous infusion of TAK-683 for 5 days did not influence the occurrence of estradiol-induced LH surge, while it completely suppressed pulsatile LH secretion in ovariectomized goats [6]. These findings suggest that the effects of continuous exposure to TAK-683 on the mechanism(s) that regulate the pulsatile and surge mode secretion of GnRH/LH are different.

More recent studies have indicated that the potential of a bolus administration of TAK-683 to GnRH/LH secretion differs between the ovariectomized [5, 7] and ovary-intact [8, 9] goats. The secretory pattern of GnRH/LH after a single intravenous injection of 50 μg of TAK-683 was shown to be characterized by episodic increases of relatively small amplitude in orchidectomized [5] and ovariectomized goats [5, 7]. On the other hand, the same TAK-683 treatment in cycling goats in the follicular phase immediately induced a surge-like release of LH reaching peak values within 10 h after administration [8]. Ovariectomized goats did not exhibit a surge-like release of LH in response to a single intravenous injection of the same dose of TAK-683 as in the above research [7], even though plasma concentration of LH after administration increased in a dose-dependent manner [5]. It is reasonable to assume that differences in the response of LH secretion

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after a bolus injection of TAK-683 between ovariectomized and ovary-intact goats are not due to the effect of the dose of TAK-683 treatment. The importance of hypothalamic kisspeptin signaling on surge mode secretion of GnRH/LH has been shown in many species [10, 11]. These findings suggest the possibility that the presence or absence of ovaries determines whether a bolus TAK-683 administration will promote the activity of the GnRH/LH surge-generating center in the hypothalamus. To validate this hypothesis, the exact mechanisms regulating the induction of the LH surge by TAK-683 should be explored.

In our previous study, the minimally effective dose of TAK-683 for LH stimulation was determined in ovariectomized goats [5], but not yet for stimulation of LH surge in ovary-intact goats. It is unclear whether TAK-683 administration at this minimum dose for LH stimulation under the absence of ovary induces surge-like release of LH in the presence of ovaries. Moreover, little information is available about the TAK-683 blood level profiles that are associated with the occurrence of LH surge after TAK-683 treatment in ovary-intact goats. Thus, the present study aims to evaluate hormonal and ovarian responses during the follicular phase in goats administered with TAK-683 the previously determined minimally effective dose for LH stimulation in ovariectomized goats. Profiles of plasma concentrations of TAK-683 after treatment and their association with endocrine responses were also evaluated for analysis of the efficacy of different routes for administration (intravenous *vs*. subcutaneous) of TAK-683 in ovary-intact goats.

Materials and Methods

All procedures were approved by the Committee for the Care and Use of Experimental Animals, the National Institute of Agrobiological Sciences, or the University Committee for the Use and Care of Animals of the Tokyo University of Agriculture and Technology.

Experiment 1

Four ovariectomized Shiba goats (5–7 years of age and 22–30.2 kg in body weight) maintained at the National Institute of Agrobiological Sciences were used. They were loosely held in an individual stanchion in a condition-controlled room and maintained on a standard pelleted diet and dry hay with free access to water and supplemental minerals. During the experiment, they were kept individually in cages, and each goat was fitted with an indwelling catheter (Medicut; Nippon Sherwood Medical Industries, Tokyo, Japan) in the jugular vein. Five μg/head of TAK-683 dissolved in 5 ml of 0.5% dimethyl sulfoxide in physiological saline was administered intravenously through the catheter. This dose of TAK-683 was chosen as a minimally effective dose for LH stimulation based on doses used in previous studies of gonadectomized goats [5]. Blood samples were collected through the catheter at 10 min intervals from –2 to 6 h after the TAK-683 treatment, and then at 2 h intervals from 6 h to 24 h after treatment. They were centrifuged at 3,000 rpm for 20 min at 4ºC, and plasma was separated and stored –20ºC until the LH assay.

Experiment 2

Animals and treatment: Five cycling Shiba goats (2–12 years of age and 18.5–39 kg in body weight) maintained at the Tokyo University of Agriculture and Technology were used. They were housed in an outside paddock with a shelter area and were fed maintenance diets of alfalfa hay cubes twice a day. Water and mineral salts were provided freely. They were kept individually in cages temporarily when they were subjected to treatment and frequent blood sampling.

Goats were assigned to the IV (n = 4), SC (n = 3), and Control (n = 4) groups. Three goats (#2, #21, and #27) were utilized first in the Control group, and then utilized in the IV and SC groups. The goats were kept untreated for at least one estrous cycle before they were allocated to another group. The other two goats were either assigned to the IV or control groups. Prior to the experiment, ovarian structures were monitored by transrectal ultrasonography every other day or daily to check the normality of estrous cycle and to determine the time of spontaneous ovulation. At 7–10 days after ovulation, all goats were injected with prostaglandin $F_{2\alpha}$ (2 mg dinoprost i.m., Pfizer, Tokyo, Japan) in order to induce the regression of corpus luteum, and controlled internal drug-releasing devices containing 0.3 g of progesterone (CIDR; EAZI-BREED^{TM} CIDR-G$^{®}$; Pfizer New Zealand, Auckland, New Zealand) were inserted for 10 days. On the day of CIDR removal, prostaglandin $F_{2\alpha}$ was injected again to all goats. Goats were fitted with an indwelling catheter and received 5 μg of TAK-683 intravenously (IV group) or subcutaneously (SC group) at 12 h after the CIDR removal. The Control group received 5 ml of vehicle intravenously.

Blood samples (2–6 ml) were collected at 10 min intervals for –2 to 6 h and at 2 h intervals for 6 to 24 h through the catheter and then at 6 h intervals for 24 to 48 h by jugular venipuncture after the administration of TAK-683 or vehicle. Thereafter, blood samples were collected by venipuncture daily until the ovulation was confirmed by ultrasonography. Obtained blood samples were centrifuged at 3,000 rpm for 20 min at 4ºC, and plasma was separated and stored at –20ºC until the assays.

Ovarian ultrasonography: Ultrasonography was performed daily from the first injection of prostaglandin $F_{2\alpha}$ to the confirmation of ovulation after TAK-683 or vehicle treatment. Ultrasound examination and blood sampling was performed every other day during the luteal phase, then daily during the follicular phase until the subsequent ovulation. Ovaries were monitored by transrectal ultrasonography (HS-1500V; Honda Elect, Aichi, Japan) equipped with a 7.5 MHz linear probe (HLS-375M), as previously described [12]. All follicles and corpus lutea that grew larger than 2 mm in diameter were monitored. Digital images were recorded, and the diameter of each follicle and corpus luteum was measured using image analysis software (GT Finder, TEAC, Tokyo, Japan). Ovulation was defined as the disappearance of a large follicle that had been observed the previous day and was re-confirmed by the development of corpus luteum at the same location.

Hormone assays: Plasma LH [13] and estradiol (E_2) [14] were measured in duplicate by a specific RIA method. The sensitivity of the LH and E_2 assays was 0.29 ng/ml and 1.46 pg/ml, respectively. Intra- and inter-assay coefficients of variation were 14.0 and 23.7% for LH and 8.7 and 11.9% for E_2, respectively.

Plasma progesterone was measured in duplicate by competitive ELISA [15]. The sensitivity of the assay was 0.17 ng/ml, and intra- and inter-assay coefficients of variation were 7.9 and 14.8%, respectively.

Plasma TAK-683 concentrations of goats in the IV and SC groups

were determined by sensitive sandwich ELISA [16]. The sensitivity of the assay was 20 pg/ml. For goat plasma pool containing TAK-683 at 500 pg/ml, intra-assay coefficient of variation was 5.1%.

Statistical analyses

Data are expressed as mean and standard deviation (SD). Mean values of measured parameters that were not normally distributed were compared between groups by using the Kruskal-Wallis test or Friedman test. If overall significance was detected, pair wise comparisons were conducted by using the Mann-Whitney *U* test. P values less than 0.05 were evaluated as statistically significant.

A surge-like release of LH was defined according to the previous study that determined spontaneous LH surge in cycling goats [13]: an increase in LH concentrations over the level of 10 ng/ml in plasma. Further, duration of the surge was calculated as the period when plasma LH concentration was higher than 10 ng/ml in the samples collected at 2-h intervals.

Results

Experiment 1

Changes in LH concentration after TAK-683 treatment in all four ovariectomized goats are shown in Fig. 1. One goat (OVX107) showed an immediate increase in plasma LH concentration after TAK-683 treatment, and the mean LH concentration for 6 h after treatment was about 1.9 times higher than that of 2 h pre-treatment. The remaining three goats did not show obvious increases in LH concentration after TAK-683 treatment. Mean LH concentration for 6 h after TAK-683 treatment in the four goats examined were not significantly different compared with that of 2 h pre-treatment (3.8 ± 1.6 *vs*. 2.9 ± 1.3 ng/ml). In samples collected during the period from 8 to 24 h after TAK-683 administration, LH concentrations fluctuated within the range of 1.8 and 5.9 ng/ml (mean, 3.4 ± 1.1 ng/ml), and no surge-like release of LH (> 10 ng/ml) was detected in any goats.

Experiment 2

Profiles of LH and E_2 for 48 h after TAK-683 treatment: Changes in LH concentration in samples collected at 10-min intervals for –2 to 6 h after the treatment of TAK-683 or vehicle are shown in Fig. 2. LH concentration in all goats in the IV and SC groups showed a sustained increase after the administration of TAK-683 and reached over the level of 10 ng/ml (surge-like release of LH). The area under the curve for LH concentration during the period from 0 to 6 h after TAK-683 administration in the IV and SC groups were significantly ($P < 0.05$) greater than that in the Control group (IV: 26.5 ± 16.4 ng/ml•6 h, SC: 32.1 ± 10.4 ng/ml•6 h, and Control: 0.9 ± 0.4 ng/ml•6 h, respectively). In the profiles of LH and E_2 during the period from –12 to 48 h after administration (Fig. 3), LH concentrations at 4 and 6 h in the IV and SC groups were significantly higher when compared with that in the Control group. LH concentrations in the IV and SC groups reached a peak at 5.8 ± 2.5 and 4.6 ± 0.4 h after administration, respectively ($P > 0.1$). In contrast, no surge-like release of LH was detected in the Control group throughout the frequent sampling period from –2 to 48 h after administration (Fig. 3).

Profiles of plasma E_2 level were similar among the three groups before TAK-683 treatment. After the administration of TAK-683, E_2 concentration increased and reached its peak at 6 or 12 h in all goats in the IV and SC groups (mean: 9.0 ± 3.5 and 6.0 ± 0.0 h, respectively). A significant difference was detected in the E_2 concentration at 6 h after administration between the SC and Control groups. Plasma E_2 concentration in both the IV and SC groups decreased gradually from 6 h after TAK-683 treatment in parallel with the decrease in LH concentration and were significantly lower than that in the Control group at 24 and 30 h after administration. Further analyses of the surge-like release of LH in terms of peak concentration, peak time, and its duration found no difference between the IV and SC groups (Table 1).

Ovarian response: The interval from the administration of TAK-

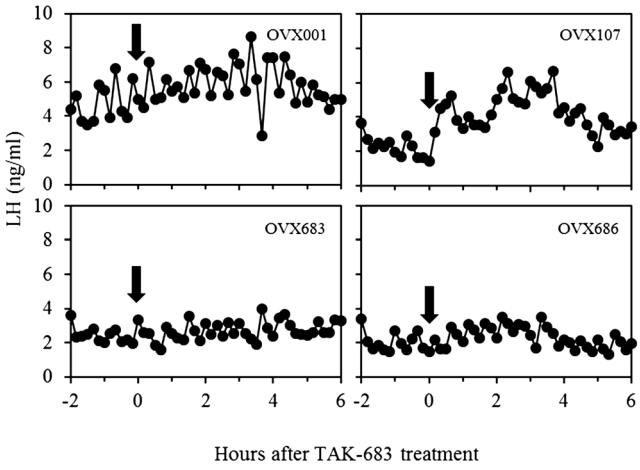

Fig. 1. Changes in LH concentration after the administration of 5 μg TAK-683 intravenously in four ovariectomized goats. Arrow indicates the time of TAK-683 injection.

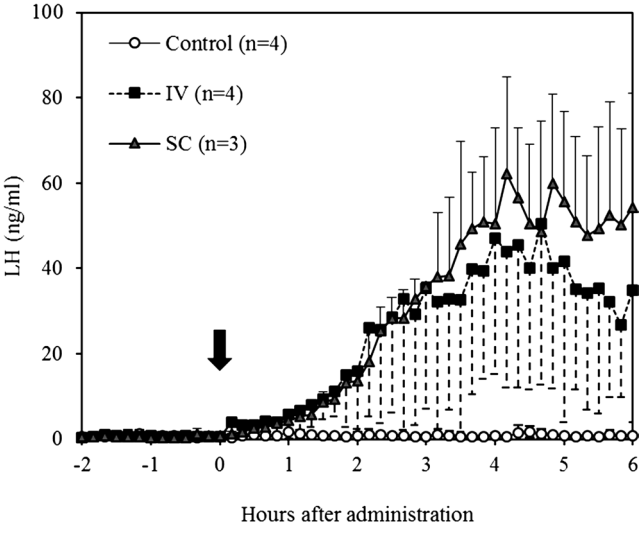

Fig. 2. Changes in LH concentration after the administration of 5 μg TAK-683 intravenously (IV) or subcutaneously (SC) or of vehicle (Control) in ovary-intact goats. Arrow indicates the time of TAK-683 injection.

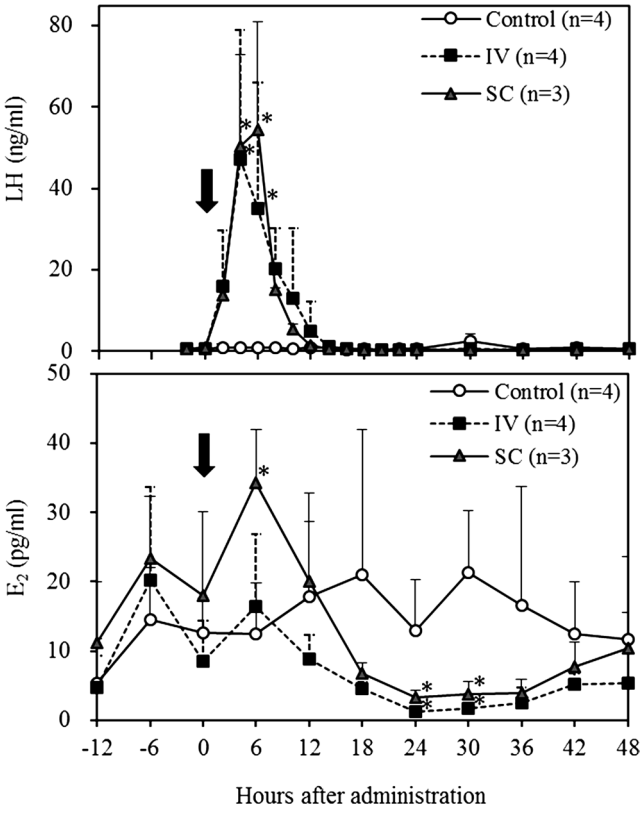

Fig. 3. LH and E_2 concentration during the period from –12 to 48 h after the administration of TAK-683 intravenously (IV) or subcutaneously (SC) or of vehicle (Control). Arrow indicates the time of TAK-683 injection. Asterisk indicates a significant difference ($P < 0.05$) as compared with the Control group by Kruskal-Wallis analyses of variance followed by Mann-Whitney-*U* test.

683 to ovulation was 5.5 ± 3.0, 2.2 ± 0.4, and 2.7 ± 0.6 days in the Control, IV, and SC groups, respectively ($P < 0.05$ between the IV and Control groups, Table 1). However, there was no significant difference between groups in the number and maximal diameter of ovulatory follicles.

TAK-683 did not affect luteal formation and progesterone profiles during the subsequent estrous cycle (Table 2). Most goats (3/4 in IV, 3/3 in SC, and 3/4 in Control groups) showed a normal cycle length (inter-ovulatory interval, 18–24 days) after the administration of TAK-683; thus, there was no difference in this parameter among the groups. Two goats (one in the Control and one in the IV groups) showed a longer cycle length (39 and 35 days, respectively), due to a prolonged follicular phase after luteal regression.

Plasma TAK-683 concentrations after TAK-683 administration: Plasma TAK-683 concentrations in the IV and SC groups are presented in Table 3. Plasma TAK-683 concentrations in the IV group increased acutely after administration, and TAK-683 concentrations at 10 and 30 min after administration in the IV group (8437.0 ± 2419.4 and 653.8 ± 168.5 pg/ml) were significantly higher than those in the SC group (290.2 ± 216.3 and 240.1 ± 68.1 pg/ml). However, the plasma TAK-683 concentration at 1 h after administration in the IV group decreased to a level similar to that in the SC group (191.4 ± 53.5 *vs.* 225.7 ± 43.1 pg/ml, respectively, $P > 0.1$), then continued to decrease to a value under the detectable limit at 3 or 4 h after administration in almost all goats (4/4 goats in IV and 2/3 goats in SC groups).

Discussion

Our previous study showed that intravenous administration of 50 μg/head of TAK-683 induced a surge-like release of LH during the follicular phase in ovary-intact goats [8], but not in ovariectomized goats, even though an episodic increase in the LH secretion was observed [7]. Similarly, in the present study, a smaller dose of

Table 1. Characteristics of the surge-like release of LH and subsequent ovulation after TAK-683 administration

Item		Control (n = 4)	IV (n = 4)	SC (n = 3)
LH	Peak concentration (ng/ml)	NA	62.4 ± 27.0	62.6 ± 23.2
	Hours from administration to the peak of LH	NA	5.8 ± 2.5	4.6 ± 0.4
	Duration of the surge (h) [a]	NA	5.5 ± 2.2	6.0 ± 0
Ovulation	Number of ovulatory follicles	4.0 ± 0.8	4.8 ± 2.7	4.0 ± 2.0
	Days from administration to ovulation	5.5 ± 3.0	2.2 ± 0.4*	2.7 ± 0.6
	Maximal diameter of ovulatory follicles (mm)	4.8 ± 0.6	4.5 ± 0.5	4.7 ± 0.6

Data are expressed as mean and SD. [a] Period when plasma LH concentration was higher than 10 ng/ml. NA: Data was not analyzed because LH surge was not observed during the 48 h of frequent blood sampling in any goats in the Control group. Asterisk indicates a significant difference ($P < 0.05$) as compared with the Control group by Mann-Whitney-*U* test.

Table 2. Effect of TAK-683 on luteal formation, progesterone (P_4) profiles, and subsequent estrous cycle length

Item	Control (n = 4)	IV (n = 4)	SC (n = 3)
Maximal diameter of the CL (mm)	8.2 ± 0.6	7.4 ± 1.1	8.6 ± 0.8
Maximal P_4 concentration (ng/ml)	7.6 ± 0.8	6.2 ± 2.3	8.5 ± 1.6
Cycle length (interovulatory interval, days)	25.8 ± 9.0	25.3 ± 7.1	21.0 ± 1.0
AUC of P_4 during 14 days after ovulation	66.8 ± 10.3	57.1 ± 16.6	53.3 ± 27.0

Data are expressed as mean and SD.

Table 3. Plasma TAK-683 concentrations (pg/ml) in all goats administered with 5 μg TAK-683 via IV or SC

Group	Goat no.	Time (min or h) after treatment							
		10 min	30 min	1 h	2 h	3 h	4 h	6 h	12 h
IV	#2	9649.9	679.9	134.8	51.9	32.5	0.0	0.0	24.7
	#4	4816.0	432.2	157.1	35.0	0.0	0.0	0.0	0.0
	#21	9442.4	841.8	242.6	77.8	31.1	0.0	0.0	0.0
	#27	9839.5	661.4	231.1	58.3	0.0	0.0	0.0	0.0
	Mean	8437.0*	653.8*	191.4	55.8	15.9	0.0	0.0	6.2
	(SD)	(2419.4)	(168.5)	(53.5)	(17.7)	(18.4)	(0.0)	(0.0)	(12.4)
SC	#2	209.1	287.8	184.1	32.1	0.0	0.0	0.0	0.0
	#21	535.3	270.4	222.7	131.2	91.8	0.0	0.0	0.0
	#27	126.1	162.2	270.2	292.6	87.5	71.7	57.4	0.0
	Mean	290.2	240.1	225.7	152.0	59.8	23.9	19.1	0.0
	(SD)	(216.3)	(68.1)	(43.1)	(131.5)	(51.8)	(41.4)	(33.1)	(0.0)

The values under the detectable limit were indicated as 0.0 for statistical analysis. Asterisk indicates a significant difference ($P < 0.05$) as compared with the SC group by Mann-Whitney-*U* test.

TAK-683 (one tenth of the dose in the above study) induced a robust increase reaching peak values between 4 and 6 h post-injection in ovary-intact goats, whereas it did not have a significant stimulatory effect on LH secretion in ovariectomized goats. The peak concentration and duration of surge-like release of LH in both intravenous and subcutaneous trials are similar to those of spontaneous LH surge in cycling Shiba goats [13]. These results support our previous findings that acute administration of TAK-683 during the follicular phase results in a stimulatory effect capable of triggering an LH surge, which is a specific reaction under the presence of ovaries in goats.

A large number of studies have reported that kisspeptin action on LH secretion is transient. For example, a single treatment of the kisspeptin-10 or kisspeptin receptor agonist (FTM080) resulted in a short-term increase in plasma levels of LH in anestrous ewes [17]. The effects of intravenous administration of a single bolus of kisspeptin-10 elicited LH secretion with a relatively small amplitude and a short duration (for several hours), but failed to induce LH surge and ovulation in mares [18]. On the other hand, constant infusion of kisspeptin-10 produced a sustained increase in plasma levels of LH during the infusion period with the equivalent peak values of endogenous LH surge in ewes [19]. It has been demonstrated that TAK-683 is active after a single injection and capable of inducing a rapid and long-lasting increase of LH in men [4] and orchidectomized [5] and ovariectomized [7] goats. Biological stability of kisspeptin analogs for LH stimulation may be required for the induction of LH surge in ovary-intact females. Recently, Decourt *et al*. [20] has reported that a single administration of C6, another investigational kisspeptin analog, was successful in inducing LH surge followed by ovulation during breeding season in ewes, although the necessity of the presence of ovaries for the induction of LH surge was not evaluated.

Based on a lot of literature, it is likely that one of the most plausible ovarian factors involving the TAK-683-induced LH surge is estradiol secreted from the follicle. There is a general consensus that estradiol at a certain level or more activates the hypothalamic GnRH surge-generating center regulating GnRH surge for induction of LH surge [21]. The present findings suggest the future studies are required to determine whether estradiol is an indispensable factor in the modulation of specific reaction of TAK-683 to GnRH surge-generating center. An *in vitro* study has showed that expression of G protein-coupled receptor 54 (GPR54), a receptor for kisspeptin, in the GnRH neurons is stimulated by the exposure to high levels of estrogen [22]. Administration of a high estradiol dose increased the mRNA expression of hypothalamic *Gnrh*, *Kiss1* and *Gpr54* in immature female rats [23]. Taken together, these findings and our current results suggest that estradiol partly mediates the surge generation influence at the hypothalamic level by modulating the expression pattern of GPR54. A possible mechanism for the ovary-dependent response of LH to TAK-683 treatment is that TAK-683 plays a role in the continuous activation of GnRH neurons for GnRH release with a synergetic action by peripheral estradiol, which appears to play a role to up-regulating GPR54 expression in the hypothalamic GnRH neurons.

For another ovarian factor, a recent study indicated that progesterone receptor signaling in kisspeptin neurons is required for normal kisspeptin neuronal activation and LH surge during the positive feedback in transgenic mice lacking progesterone receptors exclusively in the hypothalamic kisspeptin cells [24]. The duration and level of progesterone pre-exposure have highly significant effects on the estradiol-dependent mechanisms that drive the LH surge in ewes [25] and cows [26]. Progesterone from the corpus luteum in the preceding luteal phase may be involved in the modulation of TAK-683 action on LH secretion during the follicular phase of the estrous cycle.

It has been shown that the interval from the LH surge peak to ovulation ranged from 16–24 h in Shiba goats [13], suggesting that the surge-like release of LH induced by the TAK-683 treatment resulted in the advance of the occurrence of ovulation in the IV and SC groups as compared with the Control group. Once past the peak of the surge-like release of LH, plasma E_2 levels in both TAK-683 treated groups decreased gradually to a lower level than that in the Control group. The sustained rise in LH which occurs during the process for ovulation initially stimulates and then markedly inhibits aromatase activity and eventually all steroid secretion from the follicles [27]. However, profiles of luteal size and progesterone during the subsequent

estrous cycle after the first ovulation induced by TAK-683 were not different from those in the Control group. This indicates that there is no apparent deficiency in the development and function of the corpus luteum after the ovulation induced by TAK-683. It appears that the surge-like release of LH induced by TAK-683 treatment exerted a similar action on the ovaries as compared with that of endogenous LH surge during the periovulatory period of normal estrous cycle.

Despite plasma concentrations of TAK-683 for the initial 30 min after treatment in the SC group being much lower than those of the IV group, the different routes of administration did not influence the outcomes in terms of the peak concentration of LH surge and its peak time. Plasma TAK-683 concentrations decreased to undetectable level at the peak time of LH elevations in 6 of the 7 goats given TAK-683 intravenously or subcutaneously, indicating that the GnRH/LH surge was triggered and sustained by the initial action of TAK-683. Taking those into consideration, the minimum dose of TAK-683 via intravenous for the induction of LH surge is estimated to be less than 5 µg/head in the follicular phase in goats.

In conclusion, the present study reconfirmed our previous observation that TAK-683 can induce the surge-like release of LH capable for ovulation in the follicular phase, and it revealed that one or more ovarian factor(s) modulate the specific action of TAK-683 on LH secretion. This implies that the responses of LH secretion to the TAK-683 treatment are influenced by the steroidal milieu during this stage of the reproductive cycle in female animals.

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