

—Full Paper—

The Effects of Short-Term Nutritional Stimulus Before and After the Luteolysis on Metabolic Status, Reproductive Hormones and Ovarian Activity in Goats

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Abstract. The effect of short-term nutritional supplementation on hormonal and ovarian dynamics was studied in goats. Cycling Shiba goats were divided randomly into maintenance (group M, n=4) and high-energy (group H, n=4) groups. After the detection of the ovulation (Day 0, 1st ovulation), group H received a high-energy diet providing 2.5 times of the maintenance energy requirement for 7 days from Day 7 to Day 13 and were administered 2 mg of prostaglandin F_{2α} (PGF_{2α}) on Day 10 to induce luteal regression followed by the follicular phase. Follicular and luteal dynamics were monitored using ultrasonography daily or every other day, and blood samples were collected daily from Day 0 to the third ovulation (3rd ovulation) following the second ovulation (2nd ovulation) induced by PGF_{2α} administration. Blood samples were also collected at 10-min intervals for 6 h on Day 9 and Day 11 for analysis of pulsatile LH secretion. The mean concentrations of glucose and insulin were significantly (P<0.05) higher in group H than in group M on Days 8, 9, 12, 13 and Days 8, 9 and 10, respectively. For both the 2nd and 3rd ovulations, no significant difference was detected in ovulation rate between groups M and H. On the other hand, the interpeak interval for wave-like patterns of FSH in group H was significantly (P<0.05) shorter than in group M during the period between the 1st and 2nd ovulations (4.3 ± 0.3 vs. 6.5 ± 1.5 days). The mean LH pulse frequency in group H was significantly (P<0.05) greater than in group M on Day 11 (4.5 ± 0.6 vs. 3.3 ± 0.5 pulses/6 h). The present study clearly demonstrated that short-term (7 days) nutritional supplementation promoted pulsatile LH and wave-like FSH secretions in cycling goats. However, no significant increase in ovarian performance was found under such endocrine and metabolic conditions.

Key words: Follicle stimulating hormone (FSH), Goat, Luteinizing hormone (LH), Nutritional stimulus, Ovarian activity

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It is well known that nutritional manipulation modulates ovarian functions such as follicular development and ovulation rate in polyovular domestic animals including ewes [1], gilts [2], and goats [3]. There have been several reports of increases in ovulation rate in ewes treated with short-term dietary supplementation of lupin grain, such as for 4 [4], 7 [5], or 11 [6] days during the estrous cycle. Although the nutritional condition in domestic animals has been recognized as a key determinant of reproductive performance, the stimulatory effect of nutritional supplementation on reproductive function remains equivocal. In a recent report that contradicts the above studies, short-term nutritional stimulation of ewes with corn grain and soybean meal during the luteal phase did not increase their ovulation rate [7]. Previous studies have demonstrated that the pattern of daily follicle stimulating hormone (FSH) secretion is wave-like in ewes [8] and goats [9]. There are contradictory results regarding the relationship between ovulation rate and FSH secretion in ewes following nutritional stimulation [10, 11]; however, it is still unknown if an increase in ovulation rate is associated with a wave-like pattern of FSH secretion. In male sheep, pulsatile luteinizing hormone (LH) secretion is stimulated

by nutritional supplementation with lupin grain [12]. Rhind *et al.* [13] reported that high levels of food intake induce an increase in ovulation rate and that the LH pulse frequency increases 25–48 h before the preovulatory LH surge during the follicular phase in ewes. In contrast to these studies, Ritar and Adams [14] reported that supplementation of the diet of intact ewes with lupin grain does not induce a change in the LH pulse pattern.

Several studies have indicated that the timing of short-term nutritional supplementation during the estrous cycle is important for it to be effective in stimulating the ovulation rate in ewes [15]. More specifically, it has been reported that short-term nutritional supplementation stimulates the ovulation rate when the treatment starts from the mid- or late luteal phase of the estrous cycle [4, 6, 16]. Recent studies have suggested that insulin-mediated glucose uptake is an important factor regulating the nutritional effects on folliculogenesis [17] and gonadotropin secretion [18, 19] in sheep.

In our previous studies, we have demonstrated that body nutritional status modulates gonadotropin secretion [20], ovarian cyclicity [21] and ovarian responses to a steroid treatment [22] in goats. Several studies concerning the stimulatory action of nutrition on endocrine and ovarian function have been reported in goats receiving nutritional manipulation for a relatively long period [23, 24]. However, there is little information available regarding ova-

rian and hormonal responses to a short-term nutritional stimulus in goats. The aim of the present study was to evaluate the effects of short-term (7 days) high levels of feeding from the luteal phase to the follicular phase on glucose, insulin, gonadotropin and progesterone secretion and their associations with follicular and luteal dynamics in cycling goats.

Materials and Methods

Animals

All experiments were carried out at the Tokyo University of Agriculture and Technology. Six Shiba goats with regular estrous cycles were used and two of these animals (#22, #27) were used twice (control and treatment). These goats are non-seasonal breeders under natural daylight in Japan; the length of a normal estrous cycle is approximately 20–21 days [25]. Some aspects of their reproductive characteristics have been described elsewhere [25]. We have reported previously that the mean (\pm S.D.) ovulation rate is 2.8 ± 1.0 (range 2–5) in Shiba goats [26]. The goats were kept outdoors with maintenance diets of 700 g of haycubes/head/day (approximately 6 MJ of digestible energy/day) given twice daily and were transferred to indoor cages temporarily when they were subject to ultrasonographic examination of their ovaries and frequent blood sampling. All procedures were approved by the University Committee for the Use and Care of Animals at the Tokyo University of Agriculture and Technology (#18–86).

Experimental design

After detection of ovulation (Day 0, 1st ovulation), the goats were allocated randomly to one of two groups, and either remained on the maintenance diet (group M; $n=4$, 24.0 ± 6.6 kg body weight) or were fed a high-energy diet (group H; $n=4$, 26.6 ± 5.0 kg body weight) from Day 7 to Day 13. The high-energy diet comprised 1000 g hay-cubes/head/day and 300 g of concentrated feed (Challenge 16; All In One; Nihon Nosan, Tokyo, Japan)/head/day (approximately 15 MJ of digestible energy/day), providing about 2.5 times the maintenance energy requirement of total digestible nutrients (TDN) established by the National Research Council of the United States of America. The feeding behavior of animals was observed for 1 h to confirm that the animals ate all of the feed or were satiated, and the remaining feed was then removed. On Day 10, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 2 mg/head of dinoprost (Panacelan Hi; Meiji Seika Kaisha, Tokyo, Japan) was administered intramuscularly to induce luteal regression followed by the follicular phase. For analysis of the pulsatile patterns of LH, blood samples (1 ml) were collected *via* indwelling jugular catheters every 10 min for 6 h from –24 to –18 h (Day 9; for the luteal phase) before $PGF_{2\alpha}$ injection and from 24 to 30 h (Day 11; for the follicular phase) after $PGF_{2\alpha}$ injection. For the determination of the concentrations of glucose, insulin, FSH and progesterone, daily blood sampling (5 ml) by jugular venipuncture was initiated on Day 0 and continued until the third ovulation (3rd ovulation) following the second ovulation (2nd ovulation) induced by $PGF_{2\alpha}$ injection. Plasma was separated by centrifugation immediately after blood collection and was stored frozen at –20 C until analysis. Transrectal ultrasound examinations were conducted every other day during the luteal

phase and were conducted daily during the follicular phase (from the onset of luteolysis until detection of the subsequent ovulation) to monitor follicular and luteal dynamics. Body weight was measured weekly from Day 0.

Ultrasound examination

Transrectal ultrasound examinations were performed as described previously [26]. Ovulation was detected by collapse of a large follicle and was reconfirmed by development of corpus lutea at the same location. Follicles that grew to more than ≥ 4.5 mm in diameter were defined as dominant follicles. The day of emergence of a dominant follicle was identified when the growing dominant follicle reached at least 3 mm in diameter. The growth rate of a follicle was calculated by dividing the increase in diameter by the number of days between measurements.

Assays

Plasma glucose was quantified by the glucose oxidase method. Insulin concentrations were determined by an RIA kit as described previously [20]. Standards of insulin were evaluated by WHO International Standard 1st-IRP 66/304 (1 U=35.3 μ g). The assay sensitivity was 0.21 μ U/ml. The concentrations of LH and FSH were measured by a double-antibody radioimmunoassay with small modifications as described previously [27]. The sensitivity of the assays for LH and FSH were 0.062 and 1.74 ng/ml, respectively. The intra- and interassay coefficients of variation were 22.6 and 29.4% for LH and 31.8 and 25.3% for FSH, respectively. The concentrations of progesterone were measured by a double-antibody radioimmunoassay as described by Taya *et al.* [28]. The sensitivity of the progesterone assay was 48.5 pg/ml. The intra- and interassay coefficients of variation for progesterone were 15.1 and 5.3%, respectively.

Statistical analysis

Data are expressed as means \pm SD and the intergroup differences were determined by Student's *t*-test. $P < 0.05$ was considered to be statistically significant. For the identification of LH pulses in the frequently collected samples and of the peak of FSH fluctuations in the daily samples, a cluster analysis program was used [29]. The nadir and peak clusters for LH pulse detection were 2/2 points, and the *t* statistics for significant increase and decrease were 4.8/4.0. The nadir and peak clusters for detection of the peak of FSH were 1/1 points, and the *t* statistics for significant increase and decrease were 1.8/1.8.

Results

Body weight and metabolic profiles

The changes in body weight and the concentrations of glucose and insulin are shown in Fig. 1. The increases in the body weight as compared with that of Day 0 in group H were significantly greater than those in group M on Days 7 and 14. The glucose concentrations in group H started to increase immediately after the start of the high-energy diet, reached a peak level on Day 9 and then gradually decreased. The plasma concentrations of insulin in group H also started to increase in parallel with the glucose levels,

reached a peak level on Day 10 and then gradually decreased. In group M, the concentrations of glucose and insulin maintained constant values throughout the experiment, although the concentrations of insulin tended to be higher from day 11 to day 14. The mean concentrations of glucose and insulin were significantly higher in group H than in group M on days 8, 9, 12 and 13 and days 8, 9 and 10, respectively.

Ovarian and hormonal dynamics

The growth rates of dominant and ovulatory follicles from Day 7 to Day 14 in groups M and H were 0.7 ± 0.2 and 0.6 ± 0.1 mm/day, respectively, indicating there was no significant difference between them (Table 1). During both the 2nd and 3rd ovulations, no significant difference was detected in the maximum diameter of the ovulatory follicle, ovulation rate or the length of inter-ovulatory

intervals from the preceding ovulation between groups M and H. The mean times of emergence of the ovulatory follicles for the 2nd ovulation in groups M and H were 12.3 ± 4.9 and 10.0 ± 0.6 days after the 1st ovulation, respectively ($P > 0.1$).

A representative profile of the follicular dynamics and daily FSH concentrations are shown in Fig. 2, and the daily changes in the FSH concentrations during the period from the 1st to 3rd ovulation in all animals are shown in Fig. 3. The first peak of wave-like FSH secretion after the 1st ovulation occurred on Day 4 or Day 5 in all animals of both groups. All the animals in group H then showed subsequent peaks of wave-like FSH secretion within 3 days after the start of nutritional treatment (before Day 10), whereas all the animals in group M showed FSH peaks 4 days or later after the start of treatment (after Day 11). The mean interpeak interval for wave-like FSH fluctuations in group H was significantly shorter than in group M during the period from the 1st to 2nd ovulation (4.3 ± 0.3 vs. 6.2 ± 1.3 days, Table 2). On the other hand, there was no significant difference in the interpeak interval of FSH secretion between the two groups during the period from the 2nd to 3rd ovulation.

The profiles of pulsatile LH secretion on Day 9 (1 day before PGF_{2α} injection) and on Day 11 (1 day after PGF_{2α} injection) of representative animals are shown in Fig. 4. There was no significant difference in the mean pulse frequency on Day 9 or the mean concentrations of LH on Day 9 and Day 11 between groups H and M (Table 2). However, LH pulses on Day 11 in group H were stimulated by the treatment (Fig 4, bottom). The mean LH pulse frequency in group H was significantly greater than that in group M on Day 11 (4.5 ± 0.6 vs. 3.3 ± 0.5 pulses/6 h, Table 2).

For 16 days after the 2nd ovulation, no significant differences were detected in the progesterone concentration or mean diameter of the corpus luteum between groups H and M (Fig. 5).

Discussion

The present study showed that short-term (7 days) feeding of high level of nutrition stimulated the frequency of pulsatile LH secretion during a follicular phase induced by PGF_{2α} in cycling goats. Recent studies have revealed the clear wave-like secretion of FSH in daily blood samples in goats [9, 27]. The present study also demonstrated that nutritional supplementation promoted the occurrence of a new FSH wave and shortened the interpeak interval of wave-like FSH secretion. However, under such endocrine conditions, high-energy feeding had no effect on ovarian functions such as follicular growth, ovulation rate or development of the corpus luteum. From the present results, short-term nutritional

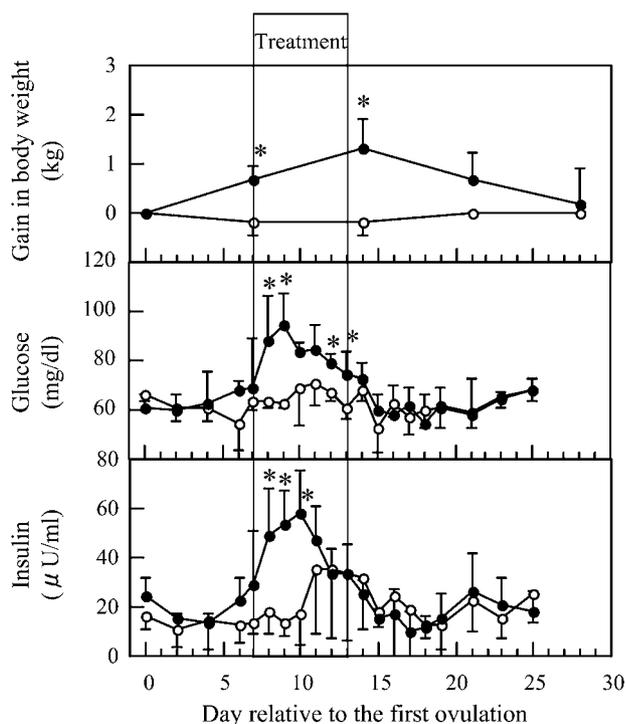


Fig. 1. Changes in body weight from Day 0 (upper) and the concentrations of glucose (middle) and insulin (bottom) in groups H (solid circles) and M (open circles). * $P < 0.05$ vs. group M. Gain in body weight indicates the values of increases in body weight compared with Day 0.

Table 1. The effects of nutritional supplementation on ovarian follicles, ovulation rate and length of the inter-ovulatory interval

	The growth rate of dominant follicles from Day 7 to Day 14 (mm/day)	2 nd ovulation		Length of the inter-ovulatory interval from 1 st to 2 nd ovulation	3 rd ovulation		
		Maximum diameter of the ovulatory follicle (mm)	Ovulation rate		Maximum diameter of the ovulatory follicle (mm)	Ovulation rate	Length of the inter-ovulatory interval from 2 nd to 3 rd ovulation
Group M (n=4)	0.7 ± 0.2	5.8 ± 0.8	2.8 ± 1.0	18.0 ± 5.7	5.6 ± 0.8	3.0 ± 1.2	23.8 ± 4.6
Group H (n=4)	0.6 ± 0.1	5.7 ± 0.3	2.5 ± 1.0	14.8 ± 0.5	5.6 ± 0.7	3.5 ± 1.7	19.3 ± 2.1

Mean \pm S.D.

supplementation before and after luteolysis stimulates the secretion of LH and FSH but does not have any stimulatory effects on ovarian activity.

Previous studies have demonstrated that a high level of feeding influences ovarian dynamics by increasing the concentrations of metabolic substances and/or by stimulation of gonadotropin secretion in ewes [6, 13] and heifers [30]. The present results contradict these reports as no changes in ovarian dynamics, including ovulation rate, were observed despite increases in the levels of both metabolic substances and gonadotropin secretion. Although there are a few reports demonstrating nutritional effects on ovarian dynamics in goats [23, 24], ovulation rate was not increased in Spanish goats that received 1.5 times their basic energy requirement for 20 months [24]. The previous and present studies in goats suggest that the nutritional effect in terms of inducing increases in ovulation rate is relatively weaker in goats than in ewes. Another

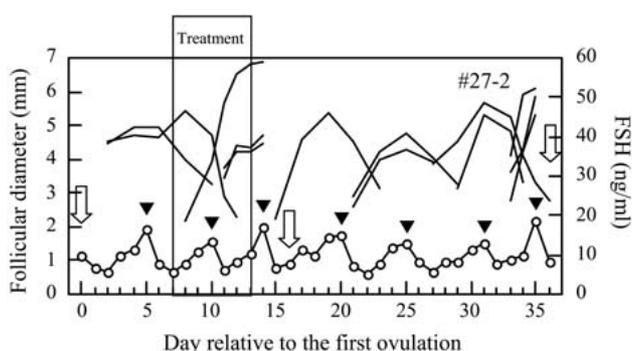


Fig. 2. Representative profiles of follicular dynamics (solid line) and daily FSH (open circles) from the 1st ovulation to the 3rd ovulation in goat #27-2 of group H. Arrows and solid triangles represent ovulation and the peak of wave-like FSH secretion identified by cluster analysis, respectively.

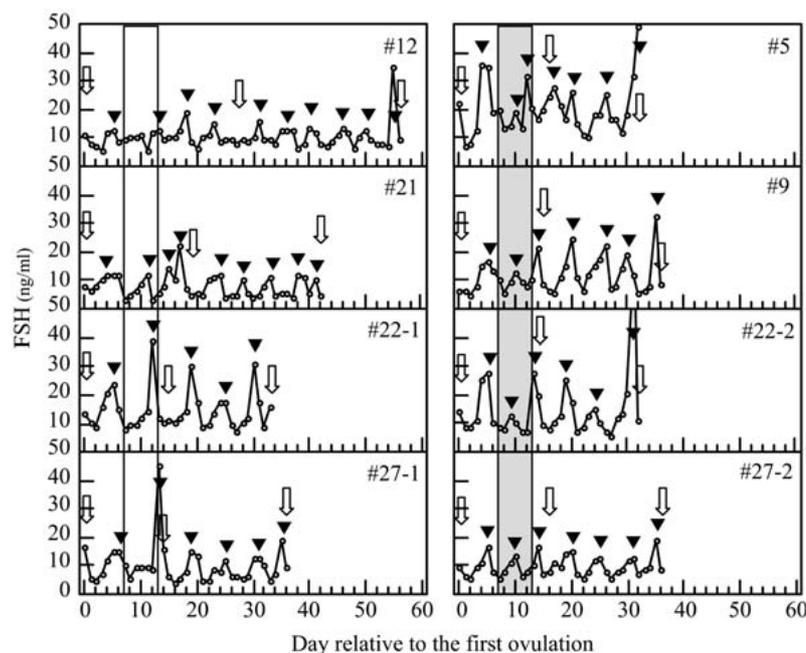


Fig. 3. Secretory profiles of daily FSH from the 1st ovulation to the 3rd ovulation in all animals of groups M (left panels) and H (right panels). Arrows and solid triangles represent ovulation and the peak of wave-like FSH secretion identified by cluster analysis, respectively. The gray area of group H indicates the period of the high-energy diet treatment.

possible interpretation of the disagreement between the present and previous studies of ewes is that the present nutritional treatment method was inappropriate for induction of stimulatory effects on ovarian function. Viñoles *et al.* [11] reported that the timing of emergence of two ovulatory follicles was different in 3/4 of cases of twin ovulation in ewes with high body conditions; a large follicle emerged at an early stage and another small follicle emerged later, but ovulated at the same time. Bartlewski *et al.* [31] reported that in a prolific species of ewes, ovulatory follicles were supplied from not only the last follicular wave but also the penultimate wave at a higher rate than in non-prolific ewes. The time-relation between the stimulatory effect of gonadotropin by nutritional stimuli and emergence of the follicular wave for ovulatory follicles might be related to the reason for the failure to induce an increase in the ovulation rate. In the present study, the concentrations of glucose and insulin increased rapidly, whereas they subsequently decreased 2 or 3 days after the start of the treatment while the nutritional supplementation continued. Other methods for inducing more long-term stimulation of metabolic status might be appropriate for induction of an increase in the ovulation rate.

Short-term high-energy level feeding in the present experiment increased pulsatile LH secretion on Day 11 (4 days after the start of treatment), but did not affect pulsatile LH secretion on Day 9 (2 days after the start of treatment). It is possible that different effects on LH secretion between Day 9 and Day 11 were associated with the period of exposure to nutritional supplementation. However, this is not supported by a previous study in which the LH pulse frequency increased within one day after an acute change in nutrition in male sheep [32]. Furthermore, the LH pulse frequency was greater in gilts fed a high-energy diet than in gilts fed a maintenance diet during both the luteal and follicular phases [33]. On the other hand, the present results are consistent with the previous results in ewes showing that high levels of nutritional intake increase the frequency of LH pulses in the follicular phase, but not

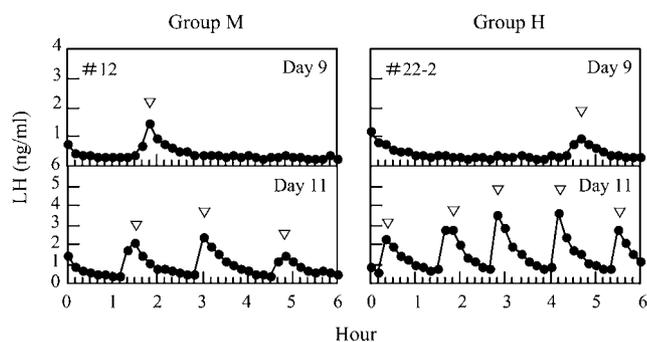


Fig. 4. Representative plasma LH profiles on Days 9 (upper) and 11 (bottom) in goat #12 of group M (left panels) and goat #22-2 of group H (right panels). Open triangles represent LH pulses identified by cluster analysis.

in the luteal phase [13, 34, 35]. The reason for the differences in the response of pulsatile LH secretion to short-term nutritional supplementation during the luteal phase is unclear, although it might be related to the difference in species and the timing of nutritional manipulation.

In the present study, short-term high-energy feeding stimulated secretion of gonadotropins during the follicular phase. A higher LH pulse frequency and FSH concentration in male sheep fed a high-energy diet are accompanied by increased concentrations of amino acids, glucose and insulin in plasma and cerebrospinal fluid [12]. Intracerebroventricular infusion of insulin stimulates the LH pulse frequency in diabetic [19] and diet-restricted [36] sheep models, suggesting that insulin and glucose are metabolic factors in regulation of pulsatile LH secretion through the gonadotropin-releasing hormone (GnRH) neurosecretory system. The increases in the concentrations of glucose and insulin for several days prior to the follicular phase in the present study supports the hypothesis that increased glucose and insulin in response to the acute change to a high-energy diet stimulates hypothalamic GnRH neurosecretion separately or synergistically as nutritional mediators.

To our knowledge, there are no reports regarding the effect of a high-energy diet on wave-like patterns of daily FSH secretion. A novel finding of the present study was that short-term nutritional supplementation shortened the interpeak interval of wave-like patterns of FSH. This may be due to increases in hypothalamic GnRH secretion induced by nutritional supplementation because the LH pulse frequency was stimulated transiently after the nutritional treatment in the present study. On the other hand, FSH secretion is

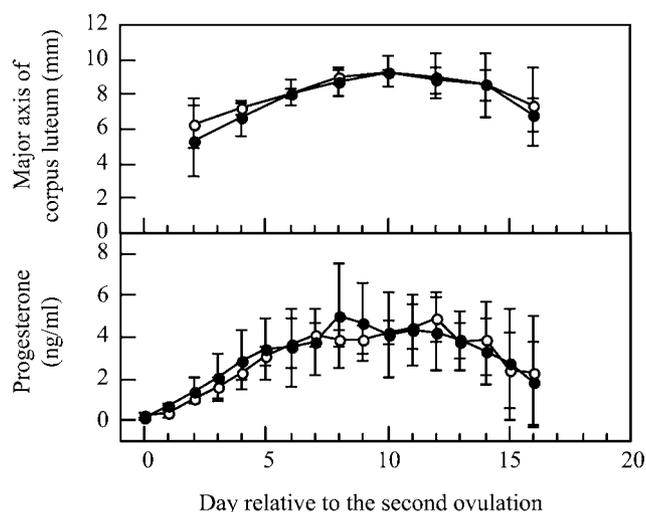


Fig. 5. Changes in the major axis of the corpus luteum (upper panel) and the concentrations of progesterone (bottom panel) after the 2nd ovulation in groups H (solid circles) and M (open circles). No significant difference was detected between the two groups.

considered to be mainly regulated by inhibin and oestradiol secretion from growing follicles [37]. There is a general consensus concerning the direct effect of nutritional supplementation on the local endocrine system of the ovary [38]. Another interpretation is that short-term nutritional supplementation had stimulatory effects on FSH secretion by suppressing inhibin and/or oestradiol secretion from the ovary, although a recent study has reported that secretion of inhibin into the follicular fluid is not stimulated in ewes following dietary supplementation with lupin grain [39].

In conclusion, the present study demonstrated that nutritional supplementation for 7 days during the period from the luteal phase to the follicular phase stimulated pulsatile LH secretion and a wave-like pattern of FSH secretion in parallel to increases in the glucose and insulin concentrations in cycling goats. However, increases in follicular growth, ovulation rate and formation of corpora lutea were not affected under such endocrine and metabolic conditions. The plasma concentrations of glucose and insulin were stimulated only for several days while the nutritional supplementation continued. Further studies regarding short-term nutritional treatment promoting ovarian performance by controlling gonadotropin secretion are needed.

Table 2. Characteristics of wave-like FSH secretion and pulsatile LH secretion

	The interpeak interval of wave-like FSH secretion (day)		LH pulse frequency (pulses/6 h)		Mean plasma concentration of LH (ng/ml)	
	From 1 st to 2 nd ovulation	From 2 nd to 3 rd ovulation	Day 9	Day 11	Day 9	Day 11
Group M (n=4)	6.2 ± 1.3	5.0 ± 0.6	1.0 ± 0	3.3 ± 0.5	0.3 ± 0.1	1.4 ± 0.4
Group H (n=4)	4.3 ± 0.3*	5.3 ± 0.5	1.0 ± 0	4.5 ± 0.6*	0.3 ± 0.1	1.2 ± 0.3

* P<0.05 compared with group M. Mean ± S.D.

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