Nutritionally Induced Body Weight Loss and Ovarian Quiescence in Shiba Goats

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Abstract. Four female Shiba goats were used to determine the influence of body weight loss by dietary restriction on estrous cyclicity. The dietary restriction was started on the day following ovulation. The goats were fed hay cube and straw at an amount of 30% of energy requirement based on weekly body weight measurement. The ovaries were monitored daily by transrectal ultrasonography and blood samples were collected daily by jugular venipuncture for ovarian steroids analysis. After the start of food restriction, all animals lost body weight and entered ovarian quiescence. Intervals to the onset of ovarian quiescence tended to depend on the body weight of each animal at the start of food restriction. The mean concentration of progesterone during the mid-luteal phase (from 7 to 13 days after ovulation) in the last estrous cycle before ovarian quiescence was significantly lower than that in normal estrous cycle of the control period (19.7 ± 2.8 vs 12.3 ± 2.2 ng/ml, P<0.05), whereas there was no significant difference in the length of the luteal phase, determined as the period when corpora lutea existed and concentrations of progesterone were equal to or greater than 1 ng/ml (15.8 ± 1.5 vs 15.0 ± 2.8 days, P>0.1). A rise of estradiol concentration and follicular growth in the follicular phase following a decline of progesterone level after luteal regression tended to be suppressed at the onset of ovarian quiescence. It seems that the present results are consistent with previous findings that nutritionally induced body weight loss influences the secretion of ovarian steroids and eventually induces ovarian quiescence.

Key words: Nutrition, Body weight, Ovarian steroids, Ovarian quiescence, Goat

in gilts stopped after 46 day of food restriction, at which point they had lost 14.5% of their body weight [6]. These reports suggest that body weight changes are associated with nutritional cessation of the estrous cycle.

The Shiba goat has been proven to be a useful experimental model for scientific study of domestic ruminants. We previously demonstrated that pulsatile luteinizing hormone (LH) secretion in goats of low body weight was more susceptible to acute food deprivation than that in heavy animals [9], suggesting that there is a critical body weight influencing endocrine activity in response to metabolic insufficiency. Anestrus or amenorrhea has been demonstrated to be caused by nutritionally induced body weight loss in cows [4], rats [10], hamsters [11] and women [12]. In small ruminants, several studies have examined the effects of long-term restricted feeding on metabolic and hormonal environments in sheep [13–15]. However, ovarian and endocrine profiles preceding the onset of nutritionally induced anestrus have not been demonstrated because of the technical difficulty of successive monitoring of ovarian structures in these species. Recently, we have successfully adopted transrectal ultrasonography for monitoring the follicular and luteal dynamics during the estrous cycle in Shiba goats [16]. The objectives of this study were to monitor successive endocrine and ovarian changes until the onset of ovarian quiescence in food restricted Shiba goats.

**Materials and Methods**

**Animals**

Adult female Shiba goats maintained for experimental uses at Tokyo University of Agriculture and Technology were used. They are non-seasonal breeders under natural daylight and their normal body weight ranges from 15 to 30 kg [17]. They received hay cube daily according to dietary requirements established by the National Research Council (NRC, 1981). They were kept with stanchion in indoor cages where the daylight was maintained on a 12 h light and 12 h dark cycle.

**Experimental procedure**

Four cycling goats (1–5 years of age) with various body weights (heavy, moderate and light) were determined to have shown at least 2 times of normal estrus behavior, ovulation and subsequent luteal development before the start of dietary manipulation. Their body weights were 35 (#4, 4 years of age), 25 (#20, 5 years of age), 14 kg (#31, yearling) and 14 kg (#32, yearling) at the start of the experiment. Food restriction was started on the day following ovulation. All animals were fed hay cube (range: approximately 50–400 g/day) and straw (50 g/day) at an amount of 30% of total digestible nutrients as established by NRC in 1981, based on weekly body weight measurements. The limited diet level was chosen as the most severe level not to affect clinical general condition in consideration of previous reports [9, 18]. The follicular development, ovulation and development of corpora lutea were monitored daily by transrectal ultrasonography until the determination of ovarian quiescence as described previously [16]. The diameter of detectable follicles and the major axis of the corpus luteum were measured with the scanner's electronic calipers. Blood samples (12 ml) were collected by jugular venipuncture at the time of the ultrasonographic observation. Plasma was separated by centrifugation immediately after the collection and stored frozen at –20 C until the assays for concentrations of progesterone and estradiol. Because we have reported that the ovulation occurs within 7 days after the onset of luteal regression during the normal estrous cycle in Shiba goats [16], the animals that had no ovulation for 14 consecutive days (twice normal value) after the regression of corpus luteum from the ovarian image were diagnosed as having ovarian quiescence. The onset of ovarian quiescence was determined as the first day of successive daily bleeding dates when concentrations of progesterone were lower than 1 ng/ml.

**Assays**

Concentrations of estradiol-17β and progesterone were measured by the radioimmunoassay described by Taya et al. [19]. Intra-assay coefficient of variation for estradiol-17β was 11.2%, and intra- and inter-assay coefficients of variation for progesterone were 10.1% and 20.4%, respectively. The sensitivities for the estradiol-17β and progesterone assays were 0.08 pg/ml and 0.07 ng/ml, respectively.
Statistical analysis

Data for major axis of corpora lutea after polyovulation are represented as mean ± SD of individual corpora lutea, and values for follicular diameter in each day were designated as the diameter of the largest follicle in the ovaries on that day. In the present study, differences in progesterone levels during mid-luteal phase and in the length of luteal phase between normal maintenance and the food restriction period were analyzed. We previously reported that corpora lutea stopped growing around 6 days after ovulation and then the size remained constant until 14 days after ovulation in the normal estrous cycle of Shiba goats [16]. Therefore, the mean concentration of progesterone from 7 to 13 days after ovulations was defined as progesterone level in the mid-luteal phase in individual animals. The length of the luteal phase was determined as the number of days when corpora lutea were detected by ultrasonography and concentrations of progesterone were equal to or greater than 1 ng/ml.

Data are expressed as mean ± SD. Two-way ANOVA for repeated measures (between = treatment, within = day) or two sample t-test or the Cochran-Cox test, which is used for analysis of differences between two mean values with heterogeneity of variance [20], was used to evaluate the effect of food restriction. P<0.05 was considered to be statistically significant.

Results

After the start of food restriction, all four animals lost body weight and entered ovarian quiescence. The relationship between body weight changes and the estrous cycle is shown in Fig. 1 and Table 1. Intervals to the onset of ovarian quiescence tended to depend on the body weight of each animal at the start of food restriction. The heaviest animal (#4) had five ovulations after the start of food restriction and entered ovarian quiescence at 19.5 kg of body weight. The moderate animal (#20) had one ovulation and entered ovarian quiescence at 20.5 kg of body weight. The intervals to the onset of ovarian quiescence for #4 and #20 were 136 and 34 days, respectively (Table 1). The two light-weight animals (#31, #32) had no ovulation following the first luteal regression after the start of food restriction.

The profiles of progesterone and corpora lutea in the estrous cycles before and after the start of food restriction are shown in Fig. 2. The mean progesterone levels during mid-luteal phase in the last estrous cycle before ovarian quiescence was significantly lower than that in the normal estrous cycle before the start of food restriction (19.7 ± 2.8 vs 12.3 ± 2.2 ng/ml, P<0.05 with two sample t-test), whereas there was no significant difference in the length of the luteal phase (15.8 ± 1.5 vs 15.0 ± 2.8 days, P>0.1 with two sample t-test).

The profiles of estradiol concentration and follicular growth following luteal regression just before the start of food restriction and at the onset of ovarian quiescence are shown in Fig. 3. At the
onset of ovarian quiescence, mean estradiol concentration on the 1st day and mean follicular diameter on the 3rd day after the day when progesterone concentrations fell below 1 ng/ml after the onset of luteal regression was significantly (P<0.01 with two sample t-test for follicular diameter and P<0.05 with Cochran-Cox test for estradiol) lower than those just before the start of food restriction. The follicular diameter and estradiol concentration for 4 days (Day 0 to Day 3) from the day when progesterone concentrations fell below 1 ng/ml after the onset of luteal regression was suppressed at the onset of ovarian quiescence (P<0.01 with two-way ANOVA for repeated measures).

Discussion

Dietary treatment of 30% of energy requirement...
resulted in body weight loss and ovarian quiescence in all goats examined. These data support previous observations that body weight loss by restricted nutrient intake induces the cessation of estrous cycle in cows [4], rats [10], hamsters [11] and women [12].

Nutritionally induced anestrus was found at the 20% and 14.5% of body weight loss by food restriction in cows [8] and pigs [6], respectively. The present data showed that interval to ovarian quiescence tended to depend on the body weight of each animal at the start of food restriction. In the heaviest animal, approximately 20 weeks lapsed before ovarian quiescence, whereas the estrous cycle stopped immediately after the start of food restriction in two yearling, light goats. Although the number of animals involved was too small to be definitive on the relationship between body energy status (body weight, body size, adiposity etc.) and nutritionally induced ovarian quiescence, heavy animals might have more mobilizable reserves to maintain sufficient nutritional feedback for supporting ovarian activity than light animals. In connection with this, pulsatile LH secretion in light goats was more susceptible to acute food deprivation than in heavy animals [9].

In the present study, dietary restriction influenced the concentrations of progesterone during the mid-luteal phase just before the onset of ovarian quiescence, but not the length of luteal phase. Therefore it is possible that chronic dietary restriction may affect the activity of progesterone secretion from the luteal cells, but not the lifespan of the corpora lutea. However, the effect of dietary restriction on luteal function during the estrous cycle is still controversial. Various levels of dietary restriction increased [21], decreased [7, 22] or had no effect [23] on progesterone concentrations during the luteal phase in heifers. Food restricted cows had a decreased weight of corpora lutea during the estrous cycle [24]. Rhodes et al. [23] reported that the maximum diameter of corpora lutea was decreased, whereas no reduction of the duration of luteal phase was found in the last estrous cycle before anestrus in food restricted heifers. In this regard, more detailed endocrinological studies are awaited.

In the present study, mean estradiol concentrations and follicular growth at the onset of ovarian quiescence was lower than those in the follicular phase before the start of food restriction. This finding suggests that nutritionally induced ovarian quiescence (anestrus) begins with the deficiency of estradiol secretion inhibiting the preovulatory LH surge. Although metabolic control of estradiol secretion is not clear, some explanations for this finding may be considered. For example, it was clearly demonstrated that estradiol secretion from the follicle was regulated

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**Fig. 3.** The profiles of estradiol concentration in plasma (bottom panel) and follicular growth (upper panel) following luteal regression just before the start of food restriction (–●–, n=4) and at the onset of ovarian quiescence (–○–, n=4). No data is shown on day 4 for the pretreatment period because of ovulation. Day 0 is first day when progesterone concentrations fell below 1 ng/ml after the onset of luteal regression. Mean ± SD. * P<0.05, ** P<0.01 as compared with values before the start of food restriction.
by pulsatile LH secretion during the follicular phase in the ewe [25–27]. Imakawa et al. [28] reported that the suppressive effect of dietary restriction on the estrous cycle was partially attributable to a decrease in the frequency of LH pulses during the follicular phase of the estrous cycle in heifers. Insulin like growth factor-I is greatly influenced by nutritional status and has been identified to be involved in the regulation of steroidogenesis and the expression of LH receptor in the ovary [29]. The dysfunction of these hormonal and metabolic actions on the ovary might suppress estradiol secretion in the follicular phase under the condition of food restriction.

In conclusion, the present findings may indicate that nutritionally induced body weight loss suppresses progesterone secretion during the luteal phase, and further progress of body weight loss induces ovarian quiescence that starts in the follicular phase accompanying suppression of estradiol secretion in Shiba goats.

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References

15. Adam CL, Findlay PA, Kyle CE, Mercer JG. Effect


