

Luteal lifespan and fertility after estrus synchronization in goats

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The present experiment aims to examine the efficiency of estrus synchronization using progesterone and equine chorionic gonadotrophin (eCG) and to look at luteal function. During the non-breeding and breeding season, 5 adult female Korean native goats were injected intramuscularly with 2.5 ml of physiological saline as the control. A progesterone impregnated intravaginal sponge was then kept in the same goats for 10 days followed, after a week, by an intramuscular injection of 500 IU eCG. Five adult female Nubian goats were mated with a fertile buck during the non-breeding season. During the non-breeding season 2 of the 5 goats showed a normal estrous cycle (ranging from 18 to 21 days) and 3 a short estrous cycle (ranging from 3 to 6 days). During the breeding season the equivalent figures were 1 and 2. The major axes of the corpus luteum (CL) were measured by means of calipers built into the ultrasonography system, and the concentrations of plasma progesterone (P₄) were determined by double antibody radioimmunoassay. The mean major axes of the CL in goats showing the short cycle (6.1 ± 0.5 mm) was significantly smaller than in those showing the normal cycle (8.9 ± 0.5 mm; $p < 0.01$) and also the value of P₄ in goats showing the short cycle (4.2 ± 2.1 ng/ml) was significantly lower than for those showing the normal cycle (10.3 ± 4.3 ng/ml; $p < 0.05$) at day 3 following ovulation. Three out of 5 Nubian goats became pregnant but only one goat carried to full term. The present experiment indicated that a combination of progesterone and eCG was effective in inducing estrus, although it resulted in a high incidence of short luteal lifespan. The low kidding rate and high incidence of embryonic loss may be due to the instability of the luteal lifespan.

Keywords: eCG, goats, luteal lifespan, progesterone

Introduction

Estrus synchronization has been the main method used to manage reproduction in farm animals. The most widely used procedures for estrus synchronization and/or induction of estrus in small ruminants are a combination of an intravaginal progesterone impregnated sponge and an intramuscular injection of equine chorionic gonadotrophin (eCG) [8,15,18]. Corteel *et al.* [9] reported that the frequencies of estrus, ovulation, and pregnancy were greater in ewes with a combined treatment of progesterone and eCG than in ewes treated with only progesterone. Moreover, previous studies reported a high level of estrus synchronization using a combination of progesterone and eCG in ewes [20] and goats [26,32]. A combination of progesterone and eCG has been used in ewes and goats during the non-breeding as well as breeding seasons [2,8,15,18]. However, it has been suggested that long periods of progesterone treatment are associated with lower fertility [10,31]. Fertility increased when the period of progesterone treatment was reduced from 21 to 11 days [9]. Cardwell *et al.* [7] reported that 88% of ewes showed estrus within 108 h of removal of an implant that had been in place for 10 days.

Usually, eCG is given 2 days before removal of the progesterone sponge in cycling goats [6]. A dose of 500 IU eCG has been reported to synchronize estrus in goats [12,23]. However, the fertility rate of progesterone and eCG treated ewes has been variable, ranging from 33 to 77% [1,12,23,24]. This variation may be attributed to the luteal lifespan after insemination following estrus synchronization. Unlike ewes, goats require a functional corpus luteum (CL) during the whole gestation period [13]. In addition, there are few previous reports on luteal function and the cause of low fertility after ovulation following estrus synchronization in goats. Thus, the objectives of the present study were to examine the effect of using a combination of intravaginal progesterone impregnated sponge (PIIS) and intramuscular injection of

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eCG for estrus synchronization and to determine the luteal lifespan associated with the maintenance of conception and fertility following estrus synchronization in goats.

Materials and Methods

Animals

A total of ten cyclic adult female goats, five cyclic adult Korean native goats (#198, #183, #587, #913, #196) and five Nubian goats (#165, #178, #179, #181, #200) ranging in age from 3 to 7 years, weighing 23.0–33.5 kg were kept in a sheltered outdoor barn under natural lighting conditions. The animals were dewormed and their health was checked by blood analysis before starting the experiment. They were given alfalfa/grass hay and water *ad libitum*.

During the non-breeding season, (from May to August in Japan), Korean native goats were allocated to the pre-treatment term. After checking the estrus cycle and sampling the blood for the progesterone assay, the same Korean native goats were then allocated to the treatment phase of the experiment. In addition after estrus synchronization, fertility during the non-breeding season was determined using Nubian goats mated with a fertile buck at 12 h and 24 h following the onset of estrus.

The same procedure was then carried out during the breeding season, from September to December, with the same Korean native goats assigned to the pre-treatment phase and then to the treatment phase.

Hormonal treatment

The goats in the pre-treatment phase were injected intramuscularly with a single dose of 2.5 ml physiological saline two days before a progesterone-free sponge was removed after 10 days. The animals in the treatment term were injected intramuscularly with 500 IU of eCG (Denka, Japan) two days before removal of the intravaginal sponge impregnated with progesterone (PIIS; Wako Pure Chemical, Japan) that had been in place for 10 days. The sponge was cut to a size of 3 × 5 cm and a string was attached to aid its removal from the vagina. The sponge was placed in a syringe with its top cut off. One gram of Oronine ointment (Otsuka Seiyaku, Japan) and 40 mg progesterone were then placed in the end of the syringe in front of the sponge. The same protocol described above was carried out during both the non-breeding and breeding seasons.

Observation of ovarian dynamics and estrus

A real-time ultrasonography B-mode scanner equipped with 6.5 MHz transducer (EUB-405; Hitachi-Medical, Japan) was used to observe ovarian images transrectally. The daily ultrasonographical examination was carried out at 16:00 h. The major axes of the follicles and the CL on the ultrasonographic images were measured and the luteal lifespan was recorded at intervals from the day of CL

formation after estrus synchronization until the day luteal regression was complete. During this time the value of plasma progesterone (P₄) was maintained over 1 ng/ml. The major axes of the follicle and CL were measured by means of calipers built into the ultrasonography system. Estrus was checked by observing the goats' reactions to an adult male goat every 12 h after sponge removal. Female goats were determined to be in estrus when showing standing estrous behavior. Nubian goats in estrus were mated twice with a fertile male goat at 12 h and 24 h following the onset of estrus.

Blood sampling and hormone assay

Blood was collected into heparinized tubes from the jugular vein of each animal and centrifuged at 1,670 g for 20 min immediately after sampling. The plasma samples were stored at –20°C until hormonal analysis. Double antibody RIA was used to determine the concentrations of P₄ using antisera to progesterone (GDN 337) [30]. The intra- and interassay coefficients for progesterone were 4.2 and 8.0%, respectively.

Data analysis

Values were expressed as the mean ± SD. Differences between phases and between non-breeding and breeding seasons were analyzed by the student's t-test. A *p*-value of less than 0.05 was considered statistically significant.

Results

Table 1 shows the reproductive responses of goats to the estrus synchronization treatment during the non-breeding and breeding seasons. During the non-breeding season, no goat injected with physiological saline showed estrus. In contrast, during the non-breeding season all 5 goats given the estrus synchronization treatment showed estrus and ovulation. During the breeding season all 5 goats given physiological saline showed estrus. However, 1 (#587) of the 5 goats did not show estrus after the estrus synchronization treatment because the PIIS was inserted early in the luteal phase of the breeding season, but by day 9 after removal of the PIIS, this goat showed natural estrus and ovulation. Ovulation occurred normally in all goats showing estrus. However, the major axes of the dominant follicles were influenced by the season. Goats allocated to both the pre-treatment phase and the hormonal treatment phase during the breeding season had significantly larger dominant follicles than those allocated during the non-breeding season ($p < 0.01$; between pre-treatment phases $p < 0.01$; between treatment phases). Although the mean major axes of the dominant follicles in the hormonal treatment phase were larger than those of the pre-treatment phase, no significant difference was observed. Also, there was no difference in the number of CL between the pre-treatment and treatment phases.

Table 1. Estrous response, status of ovulation and numbers of corpus luteum (CL) following estrus synchronization during the non-breeding and breeding seasons

	Non-Breeding season		Breeding season	
	Pre-treatment term	Treatment term	Pre-treatment term	Treatment term
No. of goats	5	5	5	5*
No. of goats showed estrus (%)	—	5 (100)	5 (100)	4 (80)
No. of goats which ovulated (%)	—	5 (100)	5 (100)	4 (80)
Major axes of dominant follicles	6.3 ± 0.8 ^a	6.7 ± 1.0 ^c	8.4 ± 0.5 ^b	9.1 ± 2.3 ^d
No. of CL	—	2.8 ± 1.1	1.4 ± 0.6	2.0 ± 0.8

*One of the 5 goats did not respond to the treatment possibly because the progesterone impregnated intravaginal sponge (PIIS) was inserted too early in the luteal phase. †Data are expressed as mean ± SD. $p < 0.01$: a vs b, $p < 0.01$: c vs d.

Table 2. Luteal lifespan following estrus synchronization during non-breeding and breeding seasons

	Non-Breeding season				Breeding season			
	Pre-treatment term		Treatment term		Pre-treatment term		Treatment term	
	N (%)	Luteal lifespan	N (%)	Luteal lifespan	N (%)	Luteal lifespan	N (%)	Luteal lifespan
No. of goats	5	—	5	—	5	—	5*	—
Normal cycle	—	—	2 (40)	17.4 ± 3.8	5 (100)	17.3 ± 1.0	1 (20)	19.0
Short cycle	—	—	3 (60)	4.7 ± 1.0 ^a	0	0	2 (40)	3.3 ± 0.6 ^b
Anoestrus	5 (100)	—	—	—	—	—	1 (20)**	—

†Data are expressed as mean ± SD. $p < 0.05$: a vs b. *One of the 5 goats showed the shorter cycle between the short and normal cycle. **One of the 5 goats did not respond to the treatment possibly because the PIIS was inserted too early in the luteal phase.

Table 2 shows the luteal lifespan of goats given the estrus synchronization treatment during the non-breeding and breeding seasons. The goats given the estrus synchronization treatment showed two kinds of responses with regard to luteal lifespan both during the non-breeding and breeding seasons. During the non-breeding season 2 of the 5 goats showed a normal estrous cycle ranging from 18 to 21 days and 3 showed a short estrous cycle ranging from 3 to 6 days. During the breeding season equivalent numbers for the normal and short estrous cycles were 1 and 2 goats, respectively. During the breeding season 1 goat showed an intermediate cycle, ranging from 10 to 12 days, after the estrus synchronization treatment. This was named the “shorter” estrous cycle. The mean luteal lifespan of the three goats showing the short cycle during the non-breeding season was significantly longer ($p < 0.05$) than that of those showing the short cycle during the breeding season. All goats in the pre-treatment phase showed a normal cycle during the breeding season.

The major axes of the CL and the plasma concentration of P₄ of the goats showing a normal or short estrous cycle during the non-breeding season are illustrated in Fig. 1 (day 0 = the day when ovulation occurred). The major axes of the CL of two goats (#198 and #183) started to increase

after ovulation and remained over 9 mm, then showed a sharp decline from day 14. The P₄ of these two goats rose from 3.7 ng/ml following ovulation to over 12 ng/ml at day 15. This is, classified as the normal cycle. The other three goats, classified as the short cycle, had premature regression of the CL which showed a rapid decline after ovulation, and the mean value of the P₄ was 1.7 ng/ml at ovulation. From day 3 this started to fall to below 1 ng/ml.

As shown in Fig. 1, the major axes of the CL had a significant positive correlation with the value of the P₄. The mean major axes of the CL in goats showing the short cycle (6.1 ± 0.5 mm) was significantly lower than in those showing the normal cycle (8.9 ± 0.5 mm; $p < 0.01$) and also the value of the P₄ of goats showing the short cycle (4.2 ± 2.1 ng/ml) was significantly lower than in those showing the normal cycle (10.3 ± 4.3 ng/ml; $p < 0.05$) at day 3 after ovulation.

Three out of 5 of the Nubian goats showed estrus by the estrus synchronization treatment and then were mated. Three pregnancies were confirmed by ultrasonography, but only one goat carried to full term. Two out of 3 goats aborted at day 46 and 54, respectively. The value of the P₄ in 3 pregnant goats is shown in Fig. 2 (day 0 = the day when estrus showed). Goat #178 kept a high P₄ level throughout

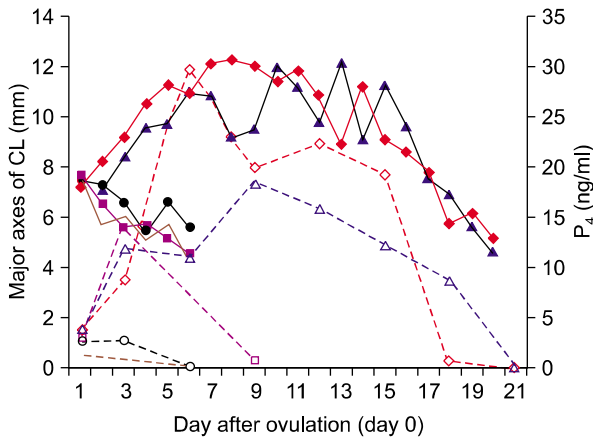


Fig. 1. Major axes of CL and plasma progesterone concentrations (P_4) in goats showing normal or short estrous cycles following estrus synchronization during the non-breeding season (day 0 = the day when ovulation occurred). *Solid lines: major axes of CL; Dotted lines: P_4 ; ◆: #198, ▲: #183, ■: #587, ●: #913, ▽: #196.

gestation which declined to 2.99 ng/ml at parturition, while the P_4 of goats #179 and #200 showed rapid decreases after reaching their peaks to decrease to concentrations of less than 1 ng/ml (0.035 ng/ml and 0.589 ng/ml, respectively) in the seventh or eighth week of gestation.

The major axes of the CL in goats showing the normal, short estrous cycle during the breeding season are depicted in Fig. 3 (day 0 = the day when the PIIS was inserted). The PIIS was inserted into the vagina at the luteal or late luteal regression phase in goats #196, #198 and #913. Goat #913 ovulated at day 12 and goats #198 and #196 ovulated at day 13. Goat #198 had a normal sized CL. The major axes of the CL began to increase from 9.3 mm after ovulation and the size remained at over 10 mm for 16 days and then decreased. Goats #196 and #913 showed the short cycle with the CL regressing after ovulation. However, goat #183 had the “shorter” cycle. Goat #587 was excluded from the data because this animal did not respond to the treatment.

Discussion

The present study was designed to evaluate the efficiency of estrus synchronization by using a combination of progesterone and eCG and to record luteal function and fertility following estrus synchronization in goats during the non-breeding season. Schiewe *et al.* [28] and Saharrea *et al.* [27] reported that eCG treatments produced both normal and abnormal CL. Abnormal CL results in early luteal regression and short or shorter estrous cycles as shown in the present experiment. The life span of the CL and the P_4 concentration were used to classify the cycle as short (or shorter) and normal. There have been some

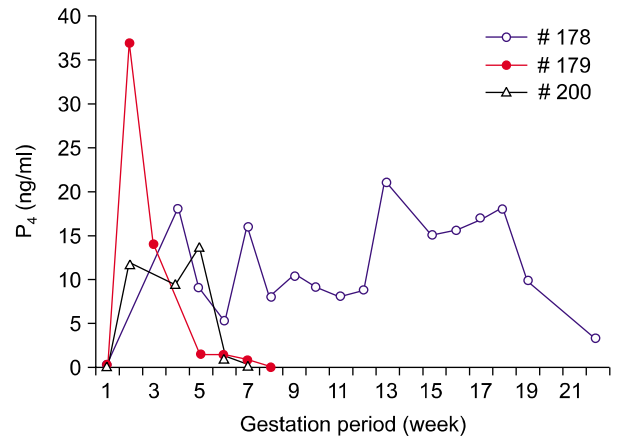


Fig. 2. Plasma progesterone concentrations (P_4) during gestation in 3 pregnant goats (day 0 = the day when estrus showed). *Goat #178 carried to full term, goat #179 aborted at day 46, and goat #200 aborted at day 54.

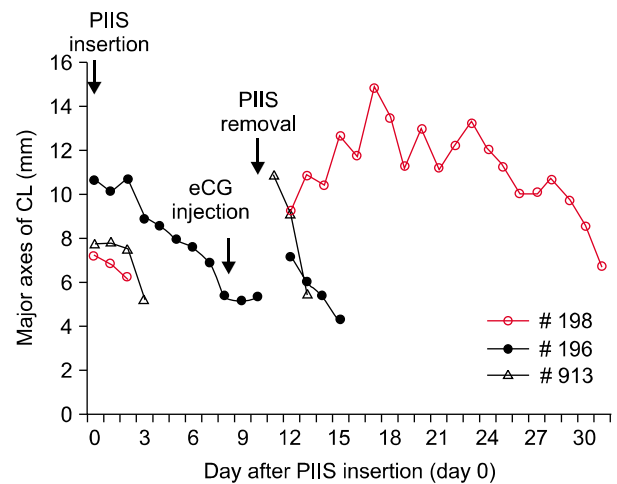


Fig. 3. The major axes of CL in goats showing normal or short estrous cycles following estrus synchronization during PIIS insertion and PIIS removal during the breeding seasons (day 0 = the day when PIIS was inserted).

reports of a short estrous cycle and/or an abnormal CL after eCG treatment for ovulation induction and/or estrus synchronization [3]. Therefore, it was assumed that it might be difficult to obtain a satisfactory result for the normal luteal lifespan in goats with a single eCG injection. More work is necessary to clarify this. Furthermore, since goats are seasonal breeders, it is assumed that their reaction to exogenous hormonal treatment varies during the non-breeding and breeding seasons. Pintado *et al.* [22] indicated that during the fall the seasonal influence on luteal regression is greater leading to a short estrous cycle. The proportion of estrous cycles with a abnormal duration was higher at the beginning and at the end of the breeding

season in cyclic Creole goats [25]. These authors suggested that a lack of progesterone priming before ovulation would cause an abnormal CL and a short estrous cycle.

In the present study, no difference was observed between the non-breeding and breeding seasons in the combined effect of the progesterone impregnated sponge and eCG on the responses of luteal function after estrus synchronization and following transition of the major axes of the CL. The present authors observed that, irrespective of season, the same individual goats showed a short or normal cycle. It was considered that the incidence of an abnormal CL might be related to the endogenous progesterone capacity of the goats to show a short or shorter cycle. To treat goats showing a short cycle after estrus synchronization on the farm, it may be necessary to prevent premature luteal regression by administration of exogenous progesterone.

Gilbert *et al.* [14] reported that an abnormal CL of less than 2 mm in diameter and pale in color began to regress about day 3 after ovulation. In the present study, we observed a small CL (about 7 mm in diameter) beginning to regress on day 1 after estrus. The P₄ of goats with the normal, shorter and short cycles showed a similar relationship to the mean major axes of the CL. The mean P₄ in goats with the normal cycle was higher than in those with the short estrous cycle. This result is consistent with the report that the P₄ was significantly higher in goats which had a normal-looking CL (12.6 ± 1.63 ng/ml) than that of goats that had both a normal-looking and regressing CL (5.33 ± 1.44 ng/ml) during the breeding season [27]. We observed abnormal CL and a short cycle in the treatment phase during both seasons. This result is similar to that reported by Okada *et al.* [21]. The present study has shown that the P₄ in two goats showing the short cycle decreased to basal levels (0.28 ng/ml) on day 4. A previous study has shown that the P₄ of goats suffering premature luteal regression fell to basal levels between day 4 and day 5.5 [27].

Three mated goats were identified as pregnant by transrectal ultrasonography on Day 20 after mating. However, only one goat carried to full term. In this study, the pregnancy rate was higher than that of previous reports [5,19]. This means that the estrus synchronization treatment of combined progesterone with eCG worked satisfactorily. However, the kidding rate was only 33% (1 out of 3). A high loss of embryos was also reported by Ahmed *et al.* [1] and Romano [26]. Johnson *et al.* [17] observed that two ewes that received 5 mg of progesterone had live fetuses 25 days after mating but returned to estrus before 40 days of gestation. These researchers indicated that treatment with a low concentration of progesterone increased the size of the largest and second largest follicles, and also increased the concentration of estradiol and that the low pregnancy rate was attributed to the high concentration of estradiol. It was suggested that the high incidence of embryonic loss in the

present study could be attributed to the high concentration of estradiol caused by the low concentration of progesterone, although the present study had no estradiol assay.

Bearing in mind that a lack of priming progesterone causes a short estrous cycle, the amount of progesterone used in the present study could not meet the requirement for keeping a normal luteal lifespan. During the time the PIIS was inserted, the mean P₄ was 5.4 ng/ml and 2.4 ng/ml on the 1st and 2nd day, respectively but after that, it decreased quickly to values of less than 1 ng/ml and maintained this low value until PIIS removal. The pregnancy that was supposed to be carried to term was impeded by functionally poor luteinization. Battye *et al.* [4] reported that premature release of PGF_{2 α} from the endometrium might be the cause of premature regression of the CL in superovulated goats. Flint and Sheldrick [11] and Hooper *et al.* [16] reported that uterine release of PGF_{2 α} was associated with the secretion by the ovary of oxytocin which controlled luteal regression.

In the present study the goats did not show any estrus and ovulation during the general non-breeding season (from May to August) and showed estrus and ovulation during the breeding season (from September to December). This result may indicate that the Korean native goat is a short day seasonal breeder, while previous researchers reported that it is a year-round breeder [29]. Contradictory findings like these were also reported by Rivera *et al.* [25] in Creole goats. According to these reports, it was considered that discrepancies between the results of our experiment and previous studies could be attributed to the change in the photoperiod and the latitudes where the animals were kept.

In conclusion, hormonal treatment by the intravaginal insertion of a progesterone sponge was thought to play a role in progesterone priming. A combination of a small dose (40 mg) of progesterone and 500 IU eCG was effective for estrus synchronization in Korean native goats during both the non-breeding and breeding seasons. We could not find any significant seasonal influence on estrus synchronization, including rates of estrus, ovulation and luteal lifespan. However, it was suggested that the high embryo loss after synchronization treatment and mating was caused by a functionally poor CL bringing low doses of progesterone. Further study should be conducted using other methods such as high doses of progesterone to improve the reproductive performance of goats.

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