Effects of GnRH and hCG Treatments for Enhancing Corpus Luteum Function to Increase Lambing Rate of Ewes Artificially Inseminated During the Non-Breeding Season

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Abstract. Two experiments were conducted to enhance the corpus luteum (CL) function for increasing lambing rate of ewes treated with GnRH or hCG after artificial insemination (AI) during the non-breeding season. Ewes (Experiment 1: $n=102$, Experiment 2: $n=37$) were pretreated with a controlled internal drug release dispenser (CIDR) for 12 days and 500 IU equine chorionic gonadotropin (eCG) 1 day before CIDR removal. In Experiment 1, the ewes were treated on Day 12 (Day 0=CIDR removal): 1) Group I; GnRH (100 μg), 2) Group II; hCG (500 IU), 3) Group III; 0.6% saline (2 ml) for control. Pregnancy and lambing rates and prolificacy were not significantly different among the groups. But, there was significant ($P<0.01$) difference in the lambing rate between parous (27%) and non-parous (72%) in Group I. On Day 17, plasma progesterone (P_4) levels of Group I were significantly ($P<0.01$) lower than those of Group III, but on the contrary, the plasma P_4 levels of Group II were significantly ($P<0.01$) higher than those of Group III. In Experiment 2, the ewes were treated: 1) Group I; hCG (500 IU) on Day 6, 2) Group II; hCG (500 IU) on Day 9, 3) Group III; no treatment for control. Pregnancy and lambing rates and prolificacy were not significantly different among the groups, but on Days 12 and 15, the plasma P_4 levels of Groups I and II were significantly ($P<0.01$) higher than those of Group III. The present results indicate that a single hCG treatment on Days 6, 9 and 12 after CIDR removal stimulates CL and increased P_4 concentration, but the increased P_4 levels did not reflect on the fertility of treated ewes.

Key words: Lambing, Corpus luteum, Non-breeding season, Ewe.

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The necessity of corpus luteum (CL) and progesterone (P_4)-secreting capacity of CL for the establishment of early pregnancy in domestic animals is well known [1–4]. Fertility of ewes induced estrus during the non-breeding season has varied independently with mating methods (40 to 70% in lambing rate) [5–10]. Wilmut *et al.* [4] suggested that if the CL function was inadequate, a substantial loss in fertility occurred. Additionally, several

researchers have reported a direct association between progesterone (P_4) concentrations following breeding and embryonic development and pregnancy maintenance [1, 11, 12].

The gonadotrophin releasing hormone (GnRH) modulates the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH), and the endogenous surge of LH during estrus is needed for ovulation and luteinizing of granulosa and theca cells to form luteal cells for subsequent progesterone secretion [13]. The human chorionic gonadotropin (hCG) treatment is known to increase

P₄ and total weight of CL [14, 15], and the responses are apparently due to a direct effect of this gonadotropin on hypertrophy and (or) to increase blood flow to the CL [16, 17].

Fukui *et al.* [18] attempted to improve the fertility in ewes during the non-breeding season by administering GnRH at estrus and administering hCG immediately after mating or Days 10 to 12 after mating, but all the treatments did not achieve further improvement of fertility. Kittok *et al.* [14] improved the fertility in lactating ewes by administering 250 IU hCG on Days 11, 12, and 13 after mating. Nephew *et al.* [15] reported that treatment with hCG on Day 11.5 after the onset of estrus stimulated uterine secretions and conceptus growth sufficiently to influence pregnancy rates.

Up-to-date, a single or multiple injection of hCG at the late luteal phase after mating has resulted in contravercial effects on conception rate in sheep. Also, there is little information on a single injection of GnRH at the late luteal phase in inseminated ewes during the non-breeding season. As GnRH modulates the secretion of LH, a single injection of GnRH or hCG at the late luteal phase needs to examine the effect on lambing rate in anestrus ewes.

The purpose of this study was to enhance the CL function for increasing lambing rate by a single injection of GnRH or hCG after artificial insemination (AI) in ewes induced estrus during the non-breeding season. In Experiment 1, effect of a single injection of GnRH or hCG at the late luteal phase (Day 12 after controlled internal drug release dispenser (CIDR) removal) was examined. In Experiment 2, effect of a single injection of hCG at the early luteal phase (Day 6 or 9 after CIDR removal) was examined. Pregnancy and lambing rates, P₄ concentrations, and prolificacy were investigated in both experiments.

Materials and Methods

The present experiments were conducted at the Shibetsu Sheep Farm (Experiment 1; 1996) during May to June, and at a private sheep farm, "Japan Lamb" (Experiment 2; 1997) during June to July, in Hokkaido, Japan. All ewes were pretreated with a CIDR for 12 days and 500 IU equine chorionic gonadotrophin (eCG) one day before CIDR removal.

Experiment 1

Forty-five parous and fifty-seven non-parous Suffolk ewes were divided into 3 groups after AI. The ewes were treated by an intramuscular injection of 100 µg GnRH (Conceral: Takeda Chemical Industries Co., Ltd. Japan) (Group I: n=34) or 500 IU hCG (Gonatropin: Teikoku-zouki Co., Japan) (Group II: n=34) or 2 ml of 0.6% saline (Group III: n=34) as a control on Day 12 (Day 0=the day of CIDR removal) after CIDR removal. AI was carried out at 44 to 54 h after CIDR removal without estrus detection, by an intrauterine AI with frozen-thawed semen using a laparoscope. Semen was collected by an artificial vagina from two mature Suffolk rams and was frozen in 0.2 ml pellets, as previously described [19], and thawed at 37 °C in a water bath immediately before insemination. Post-thawing motility ranged from 40 to 50%. The insemination dose was 0.2–0.4 ml containing 70×10^6 total sperm per uterine horn. Jugular venous blood (5 ml) was collected for determination of P₄ on Days 2 (at the time of AI), 12, 17 and 22 from the half number of ewes (n=17) in each group. Plasma was immediately separated and stored at -20 °C until measurement of P₄ with an enzyme-immunoassay (EIA) [20]. On Day 22, ewes with more than 1 ng/ml of P₄ levels were considered to be pregnant [21] and thereafter pregnancy was confirmed by a real-time ultrasonic scanning at Day 60 [22].

Experiment 2

Thirty-seven parous Suffolk ewes were divided into 3 groups after AI. The ewes were treated by an intramuscular injection of 500 IU hCG on Day 6 (Group I: n=13), or on Day 9 (Group II: n=12) and no treatment for control (Group III: n=12). AI was carried out at 35 to 38 h after CIDR removal without estrus detection, by a cervical AI with fresh-undiluted semen. Semen was collected by an artificial vagina from a mature Suffolk ram, and motility ranged 80 to 90%. The insemination dose was 0.2–0.3 ml containing 600×10^6 total sperm per ewe. Jugular venous blood was collected for determination of P₄ on Days 2 (at the time of AI), 6, 9, 15, 18, and 21 in all treated ewes of each group. Plasma was separated and stored at -20 °C for P₄ measurement, as in Experiment 1.

Statistical analysis

Data of pregnancy and lambing rates in both experiments were analyzed by chi-square test. The plasma P₄ concentrations and prolificacy in both experiments were analyzed to compare between each treatment and control groups by the Student's *t*-test.

Results

Experiment 1

Four ewes (Group I-one ewe, Group II-two ewes, Group III-one ewe) were excluded from the data due to death before lambing.

Results of pregnancy and lambing rates and prolificacy are shown in Table 1. The pregnancy and lambing rates in Groups I (55.9%, 51.5%) and II (61.8%, 59.4%) tended to be lower than those of the control (Group III: 67.8%, 63.6%), but there were no significant difference. Prolificacy was also not significantly different among the groups. There was a significant difference ($P<0.01$) in the lambing rates between parous (26.7%) and non-parous (72.2%) ewes in Group I (Table 2).

As shown in Fig. 1, the plasma P₄ levels of Group II were significantly ($P<0.05$) higher than those of control ewes (Group III). On Day 22, the plasma P₄ levels of Group I were significantly ($P<0.05$) lower than those of control ewes (Group III). The plasma P₄ levels of pregnant ewes on Days 12, 17 and 22 were significantly ($P<0.01$) higher when compared with those of non-pregnant ewes (Fig. 2).

Experiment 2

Three ewes in Group III were dead before pregnancy diagnosis, and were excluded from pregnancy and lambing data.

Results of pregnancy, lambing and prolificacy are shown in Table 3. The pregnancy and lambing rates in Group II (25.0%, 25.0%) tended to be lower than those in Group III (44.4%, 44.4%), but there were no significant differences among the groups.

As shown in Fig. 3, the plasma P₄ levels of Groups I and II on Days 12 and 15 were significantly ($P<0.01$) higher than those in Group III. The plasma P₄ levels in ewes treated with hCG on Day 9 (Group II) increased rapidly after treatment with the highest P₄ value on Day 12, but the plasma P₄ levels beyond Day 15 declined sharply to the sig-

Table 1. Pregnancy and lambing rates of ewes treated with GnRH, hCG or saline on Day 12 after CIDR removal (Experiment 1)

Group	No. of ewes treated	No. of ewes pregnant (%) ^{*1}	No. of ewes lambed (%) ^{*2}	Prolificacy
I (GnRH)	34	19 (55.9%)	17 (51.5%)	1.35
II (hCG)	34	21 (61.8%)	19 (59.4%)	1.32
III (Saline)	34	23 (67.6%)	21 (63.6%)	1.52
Total	102	63 (61.8%)	57 (58.2%)	1.40

^{*1} A pregnancy test was performed by a real-time ultrasonic scanning at 60 days after artificial insemination.

^{*2} Four treated ewes (Group I-1 ewe, Group II-2 ewes, Group III-1 ewe) were dead before lambing, and they were excluded from lambing data.

Table 2. Lambing rates of parous and non-parous ewes (Experiment 1)

Ewe	No. of ewes lambed/treated* (%)			
	I (GnRH)	II (hCG)	III (saline)	Total
Parous	4/15 (26.7%) ^a	10/15 (66.7%)	9/15 (60.0%)	23/45 (51.1%)
Non-parous	13/18 (72.2%) ^b	9/17 (52.9%)	12/18 (66.7%)	34/53 (64.2%)

* Four treated ewes (Group I-1 ewe, Group II-2 ewes, Group III-1 ewe) were dead before lambing.

^{a-b}: $P<0.01$.

nificantly lower ($P<0.01$) levels than that of the control ewes (Group III).

Fig. 1. The mean concentrations of plasma progesterone (P_4) of ewes treated with GnRH (Group I), hCG (Group II) or saline (Group III) in Experiment 1. All values are mean $\pm$ SEM.

Fig. 2. The mean concentrations of plasma progesterone (P_4) from pregnant and non-pregnant ewes in Experiment 1. Pregnant ewes had more than 2 ng/ml P_4 levels on Day 22. All values are mean $\pm$ SEM.

Discussion

In Experiment 1, the effect of a single injection of GnRH or hCG on fertility of ewes at Day 12 after CIDR removal was investigated. However, the fertility of ewes was not improved. In the GnRH-treated group (Group I), the lambing rate of parous ewes was significantly lower than that of non-parous ewes. In the GnRH-treated ewes, the plasma P_4 levels on Day 17 tended to be low and on Day 22 were significantly lower than the control ewes. Especially, the plasma P_4 levels in parous ewes treated with GnRH declined immediately after treatment to Day 22 (data not shown). Many authors reported that GnRH treatment reduced plasma P_4 concentration [23–26], and the present result has supported this phenomenon (Fig. 1). On the contrary, there are reports that GnRH

Fig. 3. The mean concentrations of plasma progesterone (P_4) in ewes treated with hCG on Day 6 (Group I), Day 9 (Group II) and no treatment (Group III) in Experiment 2. All values are mean $\pm$ SEM.

Table 3. Pregnancy and lambing rates of ewes treated with hCG on Days 6, or 9 after CIDR removal (Experiment 2)

Group	No. of ewes treated	N. of ewes pregnant (%) ^{*1*2}	No. of ewes lambbed (%) ^{*2}	Prolificacy
I (Day 6)	13	6 (46.2%)	6 (46.2%)	1.50
II (Day 9)	12	3 (25.0%)	3 (25.0%)	1.00
III (Control)	12	4 (44.4%)	4 (44.4%)	1.25
Total	37	13 (38.2%)	13 (38.2%)	1.31

^{*1} A pregnancy test was performed by a real-time ultrasonic scanning at 60 days after artificial insemination.

^{*2} Three ewes in Group III were dead before a pregnancy test, and they were excluded from the pregnancy and lambing data.

treatment increased P_4 concentrations [27, 28]. Macmilan *et al.* [29] found that the effect of GnRH on P_4 production varied with the stages of the estrous cycle in cows. Although a clear reason for the low lambing rate in parous ewes treated with GnRH is unknown in the present study, GnRH treatment also induces a high level of plasma estradiol-17 β (E_2) in cows [24] and the following luteolysis induced by the high E_2 levels may be one reason for the decrease of P_4 levels and the low fertility in GnRH-treated ewes. However, the difference in the lambing rates between parous and non-parous ewes could not be explained from the present study.

In Experiment 1, the plasma P_4 levels on Day 17 in ewes treated with hCG were significantly higher than those of control ewes (Fig. 1). It has been reported that hCG treatment increased plasma P_4 concentration in cows and ewes, and enhanced lambing rate [14, 15, 30–32]. In the present study, although the plasma P_4 levels increased, lambing rates were not improved. Additionally, the decrease of the proportions between pregnancy and lambing was smallest (2.4%) in ewes treated with hCG compared with those in other groups, although the difference was not significant. The fertility may not be improved by the hCG administration at the late luteal phase (Day 12), because the plasma P_4 levels on Day 12 was already significantly lower in non-pregnant ewes as compared with that of pregnant ewes (Fig. 2). Therefore, we extended to investigate the effects of hCG treatment at the early luteal stage (Day 6 or 9) in Experiment 2.

In Experiment 2, fertility was also not improved, and the lambing rate of ewes treated with hCG on Day 9 tended to be low. The plasma P_4 levels treated with hCG on Day 9 increased on Day 12 with the highest levels, and declined to Days 18 and 21, immediately (Fig. 3). An increased of plas-

ma P_4 concentration observed in the ewes treated with hCG on Day 9 might be detrimental effect on CL function and/or embryonic development. On the other hand, the plasma P_4 levels in ewes treated with hCG on Day 6 gradually increased to Day 15 after treatment. Lucy *et al.* [24] reported in cattle that improved fertility may be associated with delayed or slow rising of concentrations of serum progesterone after ovulation. However, the present study failed to improve the fertility of inseminated ewes even with the slow increase of the plasma P_4 levels after hCG treatment on Day 6. The discrepancy should be clarified in the future study. For a future study, it would also be worthy to investigate the effect of hCG at Day 6 or earlier stage after CIDR removal using a large number of ewes. In the present study, a single 500 IU hCG was employed. Therefore, administration methods of hCG (dose and injection number) as well as the injection timing at the induced luteal phase should be involved in a future study.

In conclusion, a single GnRH or hCG treatment on Day 12 did not improve the fertility of ewes treated during the non-breeding season, although hCG administration stimulated CL to increase plasma P_4 concentration. Also, hCG treatment on Days 6 and 9 after CIDR removal did not improve the fertility.

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