Effect of Low Dose of hCG on Induction of Fertile Estrus in Shiba Goats Pretreated Intravaginally with Progesterone during the Early Postpartum Nursing Period

Noritoshi KAWATE1), Mie YAMAZAKI1), Hiromichi TAMADA1), Toshio INABA2) and Tsutomu SAWADA1)

1) Laboratory of Theriogenology, 2) Laboratory of Cell Pathobiology, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, Sakai, Osaka 599–8531, Japan

Abstract. The present study was conducted to determine the effect of a low dose of human chorionic gonadotropin (hCG) on the induction of fertile estrus in Shiba goats pretreated intravaginally with progesterone during the early postpartum period. Nursing Shiba goats (n=13) around 3 weeks after the parturition were pretreated with a controlled internal drug release dispenser (CIDR) containing 0.3 g of progesterone for 7 day, and 60 µg of cloprostenol 24 hr before CIDR removal. Goats in Group I (n=6) received subcutaneous injections of a low dose of hCG (1 IU/kg) after the removal of CIDR at 12-h intervals until the occurrence of estrus. Goats in Group II (n=7, control) received subcutaneous injections of saline instead of hCG. The rate of estrus induction in Group I (83.3%) tended to be higher than that in Group II (42.9%), although the difference was not significant (P=0.14). Time from the removal of CIDR to the beginning of estrus in Group I was significantly shorter than that in Group II (P<0.05). A prominent peak of estradiol-17β concentration in plasma was observed with a maximum value at 30 h after the removal of CIDR in Group I, while two relatively small rises were detected with a maximum value at 48 h in Group II. The pregnancy rate in Group I (83.3%) was significantly higher (P<0.05) than in Group II (28.6%). These results suggest that the injections of low dose hCG after the treatment with CIDR promote follicular maturation and fertile estrus induction in the goats during the early postpartum nursing period.

Key words: Low dose hCG, Estrus induction, Goat, Postpartum nursing period, Estradiol-17β

dose of human chorionic gonadotropin (hCG) stimulates the maturation of antral follicles, while a high dose of the hormone triggers ovulation of the mature antral follicles [7]. However, to the best of our knowledge, there have been no reports on the use of low dose hCG successfully inducing estrus in ruminants with ovarian acyclicity during the early postpartum period.

It has been suggested that treatment with exogenous progesterone via an intravaginal device such as a controlled internal drug release dispenser (CIDR) may assist initiation of ovulation followed by a formation of CL with a normal life-span in postpartum suckled beef cows [8]. By extension, in the goat during the early postpartum nursing period, treatment with CIDR before injections of low dose hCG may help to induce fertile estrus. The main objective of the present study, therefore, was to examine the effect of a low dose of hCG after implantation of CIDR on induction of fertile estrus in the Shiba goat during the early postpartum nursing period. We also tried to clarify the endocrinological mechanism of the estrus induction by the low dose of hCG.

Materials and Methods

Animals

A total of 13 Shiba goats (2.7 ± 0.4 years; 35.8 ± 2.1 kg; mean ± SEM) nursing their kids during the postpartum period were used in this study. Weaning of the kids was performed at 3 months after parturition. They were housed under natural conditions of day length and temperature. They were fed concentrates and hay, with water always available.

To examine ovarian activity from parturition to hormone treatment, blood samples (10 ml) were collected thrice a week at 2- or 3-day intervals with heparinized syringes through a 22-g needle from the jugular vein. The collected blood was immediately centrifuged at 800 × g for 25 min at 4 C, and the supernatant was saved at −30 C until assayed for progesterone.

Hormone treatment

Hormone treatment was started at 24–31 days after parturition. All of the goats used for the experiment received a CIDR containing 0.3 g of progesterone (InterAg, N.Z.) in the vagina for 7 days. In a previous report [9], the first estrus after parturition in Shiba goats was reported to be 50–80 days in 3 out of 4 goats when kids were weaned at 50–60 days, but in the other goat the first estrus occurred within 3 weeks after parturition. Therefore, in the present study, we decided to inject 60 µg of cloprostenol (Resipron S, Teikoku Hormone Mfg. Co., Ltd., Tokyo) intramuscularly to the goats 1 day before removal of CIDR. In order to examine the effects of low doses of hCG on expression of fertile estrus, the goats were randomly assigned into 2 groups without information of the change of progesterone concentration in blood after parturition. Goats in Group I (n=6) received a subcutaneous injection of hCG (1 IU/kg, 1 ml/animal, Gonatropin, Teikoku Hormone Mfg. Co., Ltd.) at the time of the removal of CIDR (0800 h). Thereafter, the injection was repeated every 12 h, at most for 60 h, until the animal showed estrous behavior. The dose of hCG used in this study was inferred from the dose for induction of ovulation in goats [10], based upon the ratio of the dose for follicular maturation to the dose for induction of ovulation in rats [7]. Goats in Group II (control, n=7) received subcutaneous injections of saline (1 ml) instead of hCG. Estrous behavior was checked with vasectomized male goats thrice daily (0800, 1400 and 2000 h) for 60 h after the removal of CIDR. Each goat showing estrus was naturally mated with intact male goats at the time of estrus detection. An identical male goat was used for mating to a certain female throughout the estrus. In total, 3 male goats that were known to be fertile were used for the natural mating. Each male goat was chosen for mating as evenly as possible in each experimental group to lessen the effects of the use of different males on the fertility. Pregnancy diagnosis was performed by transrectal ultrasonography using a real-time B-mode instrument with a 7.5 MHz linear array transducer (Aloka UST-660, Tokyo) 30–45 days after the mating. If at least one embryo with heartbeats was observed in the uterus, the goat was diagnosed as pregnant [11].

To clarify the effectiveness and mechanism of hormone treatment on estrus induction, blood samples (10 ml) were collected daily during the treatment of CIDR, thrice daily (0800, 1400 and 2000 h) for 72 h after the removal of CIDR, and thereafter daily for 21 days. Plasma was separated and saved, as described above, until assayed for estradiol-17β,
Hormone assays

The concentration of progesterone in plasma was measured by a radioimmunoassay as described previously [12] using the processed standard curve. Anti-progesterone-11-bovine serum albumin serum (GDN#337) was used for this assay. The sensitivity of the assay was determined to be 78 pg/ml. The intra- and inter-assay CV for progesterone were 8.2% and 15.7%, respectively.

The concentration of estradiol-17β in plasma was measured by a radioimmunoassay as described previously [13]. Before the assay of samples, the accuracy of the assay was estimated with the processed standard curve. Anti-estradiol-17β-6-bovine serum albumin serum (GDN#244) was used for this assay. The sensitivity of the assay was determined to be 0.36 pg/ml. The intra- and inter-assay CV for estradiol-17β were 11.8% and 17.8%, respectively.

The concentration of LH in plasma was measured by a radioimmunoassay as described previously [2]. In brief, ovine β LH antigen (AFP-35B, NIDDK, USA) was radiiodinated using the lactoperoxidase method. Ovine β LH antibody (NIDDK-anti-oLH Beta-1, NIDDK, USA) and a bovine LH standard (NIH-LH-B10, NIAMDD, USA) were used for the assay. The sensitivity of the assay was determined to be 122 pg/mL. The intra-assay CV for LH was 3.4% (n=6). All samples were quantified within an assay.

Statistical analysis

Differences in the time from the removal of CIDR to beginning and end of estrus, in the duration of estrus, and the concentrations of hormones between groups were evaluated using ANOVA, followed by Fisher’s protected least significant difference post-hoc analysis. Criterion used to apply the least-significant difference was 5%. The Stat View® computer program (Abacus Concepts, Inc., Berkeley, CA) was employed. A LH surge was defined as a LH peak over 20 ng/ml. Conception and pregnancy rate was defined as the proportion of the number of pregnant goats to that of mated and all goats, respectively. Birth rate was defined as proportion of the number of goats that kidded at least a live kid, to that of all goats. Differences in the conception, pregnancy and birth rate between groups were evaluated using the chi-square test.

Results

The concentration of progesterone in the plasma in 10 out of 13 goats used for this experiment was low (<1.0 ng/ml) from parturition to the beginning of hormone treatments for 24–31 days (Fig. 1A). Four and six animals of the 10 goats with low concentrations of progesterone belonged to Groups I and II, respectively. In 2 out of 13 goats, the concentration of progesterone was low for 1–2 weeks after the parturition and increased to over 1.0 ng/ml 12 or 18 days after parturition, but declined to a low level within 2 days before the hormone treatments (Fig. 1B). Both goats were revealed to belong to Group I. The concentration of progesterone, in 1 out of 13 goats, increased to over 1.0 ng/ml 21 days after parturition and remained high at least for 13 days. The treatment with CIDR was started in the luteal phase (Fig. 1C) for this goat which belonged to Group II. Thus, percentages of goats without recovery of normal ovarian cyclic activity before the hormone treatments in Groups I and II were 100.0% (6/6) and 85.7% (6/7), respectively.

The induction rate of estrus after the removal of CIDR in Group I tended to be higher, but not significantly (P=0.14), than that in Group II (Table 1). The time from the removal of CIDR to the beginning of estrus in Group I was significantly shorter than that in Group II (P<0.05). In Group I, estrus was induced in 5 out of 6 goats. Four of the 5 goats received 3 hCG injections, while the other goat received injections of hCG four times. The ranges of the time from the removal of CIDR to the beginning of estrus in Groups I and II were 30–36 and 48-54 h, respectively. The ranges of the time from the removal of CIDR to the end of estrus in Groups I and II were 48–60 and 60–78 h, respectively. The duration of estrus in Group I tended to be longer, but not significantly (P=0.09), than that in Group II.

The concentration of progesterone in plasma in both of Groups I and II significantly decreased from 0 to 6 h after the removal of CIDR (Fig. 2A, P<0.05). No significant differences of the progesterone level were observed between both groups at any time point. The concentration of estradiol-17β in plasma in Group I increased after the removal of CIDR and
reached a maximum level at 30 h then decreased rapidly to a basal level at 48 h (Fig. 2B). The differences between the maximum (30 h) and the basal (0 h and 48 h) values of the estradiol-17β in Group I were significant (P<0.05). The time of maximum level of estradiol-17β in Group I corresponded to the beginning of estrus in the 5 goats of this group which showed estrus. The concentration of estradiol-17β in plasma in Group II slightly increased from 0 to 12 h after the removal of CIDR and returned to a basal level at 24 h. The concentration of estradiol-17β at 24 h in Group I was significantly higher than that at 24 h in Group II (P<0.05). The concentration of estradiol-17β in Group II slightly increased again from 24 h and reached a maximum level at 48 h then decreased gradually to a basal level at 72 h. The differences between the maximum (48 h) and the basal (0, 24 and 72 h) values of estradiol-17β in Group II were not significant. The time of the maximum level of estradiol-17β in Group II corresponded to the beginning of estrus in the 3 goats of this group which showed estrus. There was no significant difference between the maximum values of estradiol-17β in Groups I and II. The concentration of LH in plasma in Group I significantly increased from 30 to 36 h and decreased from 36 to 48 h after the removal of CIDR (Fig. 2C, P<0.05). The concentration of LH in plasma in Group II increased slightly from 36 to 48 h and significantly from 54 to 60 h, then decreased significantly from 60 to 72 h after the removal of CIDR (P<0.05). Four and three goats showed the LH surge out of 5 and 3 goats that showed estrus in Groups I and II, respectively.

Table 1. Effects of low dose hCG injections on the induction of estrus during the early postpartum nursing period and the fertility in goats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of treated goats</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Induction rate of estrus (%)</td>
<td>5/6 (83.3)</td>
<td>3/7 (42.9)</td>
</tr>
<tr>
<td>Time from removal of CIDR to beginning of estrus (h)</td>
<td>31.2 ± 1.2 *</td>
<td>50.0 ± 2.0</td>
</tr>
<tr>
<td>Time from removal of CIDR to end of estrus (h)</td>
<td>54.0 ± 1.9</td>
<td>66.0 ± 6.0</td>
</tr>
<tr>
<td>Duration of estrus (h)</td>
<td>22.8 ± 1.2</td>
<td>16.0 ± 4.0</td>
</tr>
<tr>
<td>Conception rate (%)</td>
<td>5/5 (100.0)</td>
<td>2/3 (66.7)</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>5/6 (83.3 *)</td>
<td>2/7 (28.6)</td>
</tr>
<tr>
<td>Birth rate (%)</td>
<td>4/6 (66.7)</td>
<td>1/7 (14.3)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM for time and duration.

a Goats in Group I were treated with low doses of hCG after the removal of CIDR.
b Goats in Group II were treated with saline after the removal of CIDR.
c Conception rate was defined as proportion of the number of pregnant to that of mated goats.
d Pregnancy rate was defined as proportion of the number of pregnant to that of treated goats.
e Birth rate was defined as proportion of the number of goats that kidded at least a live kid, to that of treated goats.

The asterisks indicate significant differences compared with Group II (*, P<0.05).
Changes of the concentration of progesterone in plasma in goats for 21 days after the induced estrus or the end of injections of hCG or saline are shown in Fig. 3. The data of both groups were further divided into 2 categories, with or without the induced estrus, since the change of the progesterone level was quite different between the 2 categories. The concentrations of progesterone in the estrous goats of Groups I and II increased after the onset of estrus and reached maximum levels around 8 days and maintained this high level until day 21. On the contrary, the concentration of progesterone was low throughout the 21-day period in the Group II goats without estrus. The concentration of progesterone in the anestrous goat of Group I slowly rose but the level was relatively low compared with the goats with estrus and declined to a basal level on day 18.

The conception rate in Group I tended to be higher, but not significantly (P=0.17), than that in Group II (Table 1). The pregnancy rate in Group I was significantly higher than that in Group II respectively.

Changes of the concentration of progesterone in plasma for 21 days after the induced estrus or the end of injections of hCG or saline are shown in Fig. 3. The data of both groups were further divided into 2 categories, with or without the induced estrus, since the change of the progesterone level was quite different between the 2 categories. The concentrations of progesterone in the estrous goats of Groups I and II increased after the onset of estrus and reached maximum levels around 8 days and maintained this high level until day 21. On the contrary, the concentration of progesterone was low throughout the 21-day period in the Group II goats without estrus. The concentration of progesterone in the anestrous goat of Group I slowly rose but the level was relatively low compared with the goats with estrus and declined to a basal level on day 18.

The conception rate in Group I tended to be higher, but not significantly (P=0.17), than that in Group II (Table 1). The pregnancy rate in Group I was significantly higher than that in Group II respectively.
(P<0.05). In Group I, the number of live kids per pregnant goat was 1.5 ± 0.3 (mean ± SEM, n=4). One of the pregnant goats in Group I delivered 4 kids at almost full term (137 days after mating) but all of the kids were dead. In Group II, only one of 2 pregnant goats delivered 4 kids and 2 of them were alive. Another goat diagnosed as pregnant 32 days after mating lost the fetus before the next pregnancy check performed on day 75. The birth rate in Group I tended to be higher, but not significantly (P=0.05), than that in Group II (Table 1).

Discussion

It has been reported that treatments with CIDR for 6–18 days and pregnant mare serum gonadotropin (PMSG) 1 day before or at the time of the removal of CIDR were performed to induce fertile estrus in seasonally anestrous ewes [14] and does [15]. However, estrus induction in anestrous goats during the early postpartum nursing period has not been reported as far as we know. It has been suggested that ovarian acyclicity in suckled beef cows is due to suppressed pulsatile LH release, but is not due to inhibited FSH release [6]. Furthermore, the use of FSH or PMSG to induce estrus in ruminants may lead to an increase in the number of ovulations and fetuses [16, 17]. Therefore, we used a low dose of hCG, but not PMSG, in the present study, to induce estrus in Shiba goats with ovarian acyclicity in the early postpartum nursing period. The results show that estrus is induced with high efficiency in the Shiba goat by a few injections of low dose hCG after the implantation of CIDR for 7 days. Injections of low dose hCG have been reported to stimulate the maturation of antral follicles in immature rats [7]. There have been, however, no reports on the use of low dose hCG in ruminants to stimulate the development of antral follicles and induce estrus. Thus, the present study is probably the first to show the successful use of low dose hCG after pretreatment with CIDR to induce estrus in ruminants with ovarian acyclicity. In the present study, we did not examine the efficacy of PMSG or FSH to induce fertile estrus of the Shiba goat in the early postpartum nursing period. Clearly, further studies are necessary to compare the efficiency of estrus induction and subsequent reproductive performance of the goat pretreated with CIDR between the treatments with the low dose of hCG and PMSG or FSH.

The present study also shows that the estrus of the goat induced by treatment with CIDR and low dose hCG is followed by normal development of the luteal function and is fertile enough to produce kids. Therefore, hormone treatment of goats during the early postpartum nursing period may be useful in reducing the interval from parturition to conception. Also, this study shows that the estrus of the goat induced by this treatment was tightly synchronized, suggesting that the hormone treatment can be applied to natural mating or artificial insemination at a fixed time. It was observed in this study that 3 hCG injections at 12-hour intervals after the treatment with CIDR are required in most cases to induce estrus in the goat during the early postpartum period. Further studies are necessary to try to reduce the number of times of hCG injection, for example using an oil suspension form, for practical use of this hormone treatment. It has been suggested that the use of hCG may stimulate the production of antibody against the hormone and, therefore, repeated use of this hormone may result in loss of its effectiveness in cattle [18]. Therefore, whether treatment with low dose hCG in goats can be used repeatedly without loss of its effectiveness remains to be determined.

The data in the present study show that the injections of low dose hCG after the treatment of CIDR enhanced secretion of estradiol-17β which formed a prominent peak coincident with the onset of estrous behavior, followed by a LH surge. These results suggest that the treatment with low dose hCG can stimulate the maturation of follicular function leading to higher incidence and earlier expression of estrus. In prepubertal rats, injections of low dose hCG increased not only secretion of estradiol-17β, but also LH receptor content in both theca and granulosa cells in the antral follicles during the course of follicular maturation, and subsequently mature follicles gained the ability to respond to LH surge [7]. In the rat, in response to the LH surge, the mature follicles undergo diminishment of aromatization and enhancement of progesterone and prostaglandins syntheses, and these events consequently lead to ovulation [19]. It has been previously shown that, during the development of bovine antral follicles, both
secretion of estradiol-17β and LH receptor content concomitantly increase [20, 21]. Hence, in the present study, it is plausible that the treatment with low dose hCG may also increase the LH receptor content thus allowing the follicles to ovulate and luteinize in response to the LH surge induced by the increment of estradiol-17β in the goat.

In conclusion, the treatment with a low dose of hCG after the implantation of CIDR for 7 days in the Shiba goat during the early postpartum nursing period stimulated the maturation of follicular function and induced fertile estrus.

Acknowledgments

The authors thank Dr. G. D. Niswender, Colorado State University, for providing the estradiol-17β antiserum (#244) and the progesterone antiserum (#337). We thank NIDDK, NIH and Dr. A. F. Parlow, National Hormone & Pituitary Program, Harbor-UCLA Medical Center, for providing the ovine β LH antigen (AFP-35B) and ovine β LH antibody (NIDDK-anti-oLH Beta-1). We thank Surge Miyawaki Co., Ltd., Tokyo and InterAg for supplying CIDR. We also thank Teikoku Hormone Mfg. Co., Ltd. for supplying Resipron S. This study was supported, in part, by a Grant-in-Aid for Scientific Research (C), Number 11836009, from the Japanese Society for the Promotion of Science.

References


