Journal of Reproduction and Development, Vol. 70, No 6, 2024

Technology Report

Application of bovine progesterone intravaginal controlled-release formulation for estrus synchronization treatment in goats

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Abstract. Progesterone (P_4)-impregnated controlled internal drug-releasing device for goats and sheep (CIDR-G) is used in the estrus synchronization protocol; however, it is not commercially available in Japan. In this study, we investigated whether a modification of CIDR-B, designed for cattle, could be used as a P_4 release device for synchronizing estrus in goats. An *in vitro* analysis showed that the dissolution rate was not significantly different between CIDR-G and modified CIDR-B (CIDR-M). The administration of CIDR-G or CIDR-M to dairy goats resulted in plasma P_4 concentrations being maintained at the functional luteal phase levels (>2 ng/ml) in both groups for 12 days after administration. However, P_4 concentrations at 2, 4, and 8 days after the administration of CIDR-G were significantly higher than those after the administration of CIDR-M. These results suggest that the CIDR-M examined in this study can maintain P_4 levels for 12 days after its administration as a P_4 release device.

Key words: Breeding season, Dissolution test, Estrus synchronization, Goats, Intravaginal progesterone device

(**J. Reprod. Dev. 70:** 423–426, 2024)

Reproduction is among the most critical factors in livestock production, and estrus synchronization contributes to management stabilization by saving labor and improving the efficiency of breeding operations. Progesterone (P_4) is used for estrus synchronization and the treatment of reproductive disorders. A common method of administering P_4 is to insert hormone-containing drugs into the vagina for sustained release using a controlled intravaginal drug release (CIDR) device or a sponge. A CIDR contains an injection-molded silicone rubber skin mixed with P_4 on a "T"-shaped nylon skeleton [1, 2]. The wings of CIDR can be folded for insertion and, once inserted, return to their original "T"-shaped position and remain in the vagina of the animal by applying pressure to the vaginal wall. A CIDR has several advantages over a vaginal sponge, including the ability to contain higher concentrations of P_4 than that in a sponge, to protect vaginas from foul-smelling mucus expelled because of mild vaginal inflammation, and to incorporate synthetic P_4, structurally similar to natural hormones [3].

Globally, goats are raised as livestock to produce milk, dairy products, and meat. The main goat breeds raised in Japan are Saanen, a dairy breed, and Boer, a meat breed, raised mainly in Okinawa Prefecture. The number of goats raised in Japan in 2022 was 30,882, a 52.8% increase over the last decade [4], although this number is small compared to that of cattle and other livestock animals. Accordingly, livestock farmers and veterinarians increasingly demand relatively more efficient breeding programs and treatments for reproductive disorders. However, the CIDR designed for goats (CIDR-G) is not commercially available in Japan, probably because of its low sales prospects, whereas the CIDR designed for cattle (CIDR-B) is widely used on livestock farms in Japan. In an attempt to use CIDR-B in goats, CIDR-B was cut to the size of CIDR-G and administered to goats for estrus synchronization; however, the effectiveness of this approach in terms of P_4 release from the modified device has not been confirmed experimentally. Therefore, in this study, we examined whether a modified CIDR-B could be used for estrus synchronization in goats in terms of the dissolution rate of P_4 *in vitro* and blood P_4 profiles in adult dairy goats after administration.

An image of the modified CIDR-B (CIDR-M) and its applicator is shown in Fig. 1. *In vitro* P_4 dissolution profiles are shown in Fig. 2. No significant difference was observed in the P_4 concentrations 360 min after the start of the dissolution test between CIDR-G and CIDR-M.

The plasma P_4 concentrations in goats treated with CIDR-G and CIDR-M are shown in Fig. 3. Plasma P_4 concentrations were significantly higher in the CIDR-G group than in the CIDR-M group from day 2 to 12 after CIDR administration ($P < 0.01$). Plasma P_4 concentrations were maintained at the level of the functional luteal phase in goats (> 2 ng/ml) [5] both in the CIDR-G and CIDR-M groups throughout the CIDR insertion period. Although plasma P_4 concentrations in blood samples collected at 2, 4, and 8 days after CIDR administration in the CIDR-G group were significantly different from those in the CIDR-M group, no further differences were observed between the groups. No significant differences were observed in the profiles of P_4 concentrations between the breeding and non-breeding seasons, either independently or upon interaction with the CIDR (CIDR-G *vs*. CIDR-M).

Four goats tested during the non-breeding season did not reach estrus after CIDR removal in either the CIDR-G or CIDR-M treatments. In contrast, all three goats tested during the breeding season entered estrus between 1 and 3 days after CIDR removal in both the CIDR-G and CIDR-M treatments.

The results of the *in vitro* analysis revealed no differences in the dissolution rates of CIDR-G and CIDR-M. A previous study examining the pharmaceutical properties of commercially available CIDR showed that the *in vitro* rate of P_4 release from CIDR is impacted by many factors, including the surface area, drug load, addition of pore-forming materials, silicone shore hardness, and drug particle size; however, the only variables that impact the *in vivo* plasma P_4 levels are surface area and drug load [6]. In this

Received: September 12, 2024
Accepted: October 18, 2024
Advanced Epub: November 10, 2024

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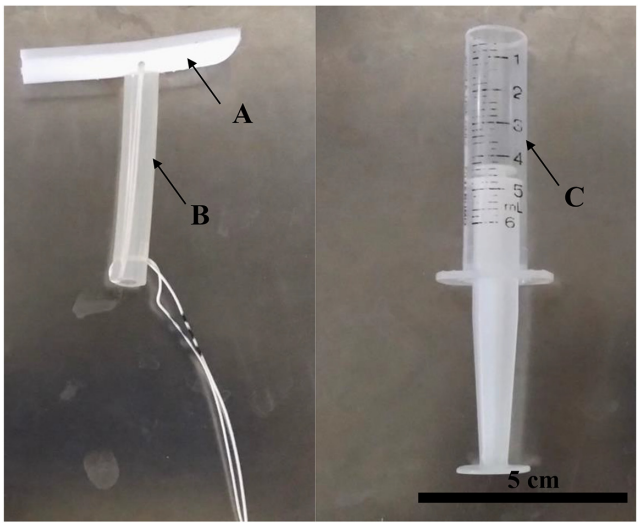

Fig. 1. Images of modified CIDR-B (CIDR-M) consisting of a P_4-impregnated silicone rubber piece (A) and central silicone tube (B), and its applicator (C). Scale bar = 5 cm.

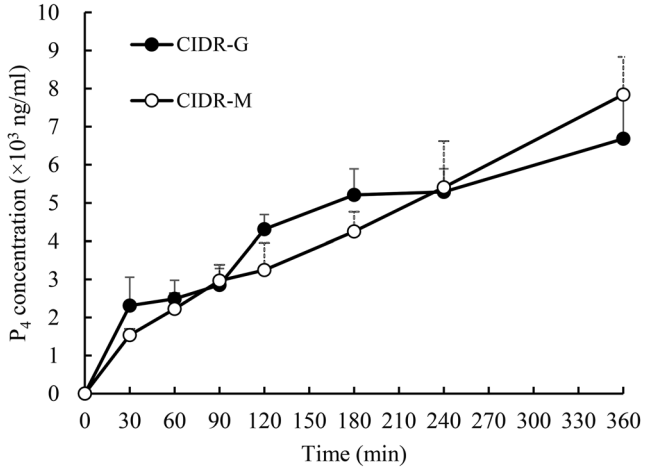

Fig. 2. Graph showing *in vitro* P_4 dissolution profiles of modified CIDR-B (CIDR-M) and CIDR-G (n = 3).

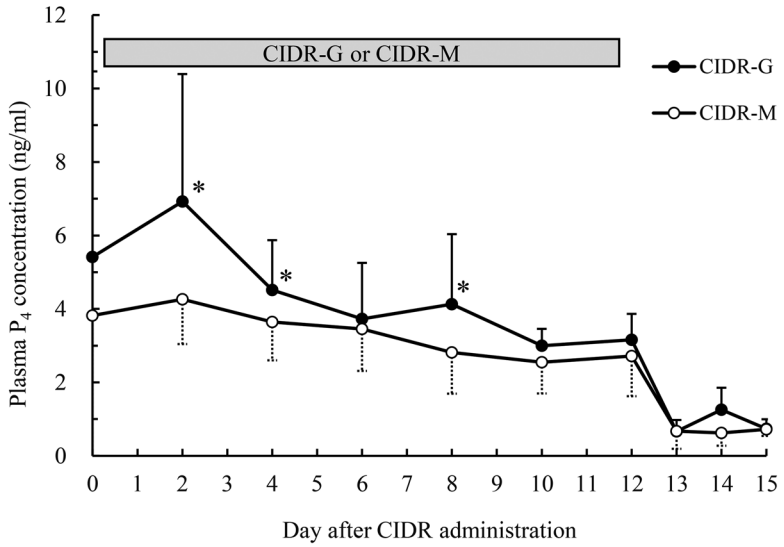

Fig. 3. Plasma P_4 concentrations in goats intravaginally treated for 12 days with modified CIDR-B (CIDR-M, n = 7) or CIDR-G (n = 7). Asterisks indicate a significant difference between the two groups ($P < 0.05$).

study, CIDR-B was cut into six pieces such that the amount of P_4 contained in a cut piece of silicone rubber was comparable to that in the silicone rubber of CIDR-G. This simple preparation method was sufficient for obtaining a dissolution rate of CIDR-M similar to that of CIDR-G *in vitro*.

When these two types of CIDR (CIDR-G and CIDR-M) were administered *in vivo*, the plasma P_4 concentrations of goats in the CIDR-G group on days 2, 4, and 8 after CIDR administration were significantly higher than those of goats in the CIDR-M group. However, no significant differences were observed on the remaining sampling days, i.e., days 6, 10, and 12 after CIDR administration. Based on the results of the *in vitro* study, factors other than the dissolution rate may affect the sustained release of the drug *in vivo*. A likely possibility is the pressure difference, as CIDR-G seemed to exhibit a stronger wing opening force than exhibited by CIDR-M, probably because CIDR-G has an internal plastic skeleton. In contrast, the CIDR-M has no skeleton in its wing section, and the P_4-impregnated silicone piece was attached to a silicone tube in the center to form a T-shape similar to that of the CIDR-G. The elastic force produced by the internal plastic skeleton of CIDR-G may have pushed the vaginal wall relatively wide and increased the contact surface area of CIDR-G, thereby increasing the absorption efficiency of P_4 through the vaginal mucosa. The possibility of these physical differences will be examined in the future.

Although plasma P_4 concentrations were not affected by the season of administration (breeding or non-breeding season), estrus was not observed during the non-breeding season in any goat, regardless of the CIDR type. Previous studies have shown that follicular growth is observed during both breeding and non-breeding seasons and is characterized by a wave-like pattern; however, the development

of larger follicles and greater follicular growth rates are observed in the breeding season than in the non-breeding season [7, 8]. To induce estrus and ovulation during the non-breeding season in goats, additional hormonal treatments using equine or human chorionic gonadotropin are effective in promoting the development of ovarian follicles [9–11]; however, these gonadotropins cannot be used repetitively because of the production of antibodies against these glycoprotein hormones [12] and animal welfare risks in horse breeding environments, as a large amount of blood is collected at a time or during repeated collection for the extraction of equine chorionic gonadotropin [13].

In conclusion, the CIDR-M prepared in this study maintained blood P_4 concentrations at the functional luteal phase levels for 12 days after its administration, although relatively low plasma P_4 concentrations were observed on days 2, 4, and 8 after CIDR administration. After CIDR-M removal, estrus was induced in all goats during the breeding season in the same manner as in the CIDR-G treatment. Based on the P_4 profiles obtained in this study, it is suggested that CIDR-M can maintain P_4 levels for 12 days after its administration and act as a P_4 release device for estrus synchronization. Attention must be paid to CIDR-M use in clinical practice because it is not prescribed. Future studies should examine how the observed differences in P_4 profiles affect the growth and ovulation of follicles and conception rate after mating.

Methods

Adult Saanen dairy goats maintained at the Tokyo University of Agriculture and Technology were used in this study. Goats were kept in a freely moving paddock and fed ort hay and a standard pelleted diet, with clean water and mineral salt available *ad libitum*. All procedures were approved by the University Committee for the Use and Care of Animals at the Tokyo University of Agriculture and Technology (approval no. R05-1).

Preparation of CIDR-M

The commercially available CIDR-B and CIDR-G (Pfizer New Zealand, Auckland, New Zealand), which contained 1.9 g (10% w/w) and 0.3 g (9% w/w) P_4, respectively, in silicone rubber, imported with permission from the Ministry of Agriculture, Forestry and Fisheries were used. Therefore, the silicone rubber of CIDR-B was cut into six pieces, such that the amount of P_4 contained in a cut piece of silicone rubber was comparable to that in the silicone rubber of CIDR-G. A piece of silicone rubber was attached to a silicone medical tube (outer diameter, 8 mm; inner diameter, 4 mm; Matsuyoshi & Co., Ltd., Tokyo, Japan) with a silk thread to form a T-shape (Fig. 1). An applicator was prepared for CIDR-M by cutting the tip of the outer cylinder of a 5 ml medical plastic syringe (Terumo Co., Ltd., Tokyo, Japan; Fig. 1).

In vitro P_4 dissolution test

CIDR-G (n = 3) and CIDR-M (n = 3) were put into 400 ml of 0.1% BSA-PBS solution (Sigma-Aldrich, St. Louis, MO, USA) in a 500 ml bottle individually and were shaken in a 38°C water bath (T-N225; Thomas Science Machine Ltd., Tokyo, Japan) at 60 round trips per min and 40 mm amplitude. Samples (100 µl of BSA-PBS solution) were collected at 0, 0.5, 1, 1.5, 2, 3, 4, and 6 h after the start of shaking. The same volume of the BSA-PBS solution was returned to the bottles. The samples were stored in a freezer at −20°C until assay.

In vivo P_4 measurement in goats

Seven Saanen dairy goats (1.7 ± 1.5 years old, 45.6 ± 11.3 kg of body weight) were used. All goats received both treatments (CIDR-G and CIDR-M) randomly, with a gap of at least 2 weeks before starting the next treatment. Four of the seven goats (#2, #5, #8, and #10) were used during the non-breeding season (March–September 2022), and three of these seven goats (#6, #7, and #9) were used during the breeding season (October 2022–February 2023). On the day of CIDR administration (day 0), 5 mg prostaglandin $F_{2\alpha}$ (dinoprost tromethamine, Pronargon F; Zoetis Japan Inc., Tokyo, Japan) was administered intramuscularly immediately before CIDR insertion to induce luteolysis. The vulva was cleaned and disinfected using 70% alcohol and cotton. CIDR-M was folded into the outer tube of the applicator (Fig. 1, C) and inserted deep into the vagina. CIDR-G was administered following the manufacturer's instructions. Each CIDR was removed on day 12 after its administration. Blood (5 ml) samples were collected from the jugular vein on day 0, every other day during CIDR insertion, and three consecutive days after CIDR removal (days 12–15). Blood samples were centrifuged at 1,750 × *g* for 30 min, and the separated plasma was stored at −20°C until assay. The observation of estrus was performed on five consecutive days (days 13–17) after CIDR removal by checking several signs of estrus (vocalization, tail wagging, vulvar swelling, mucus secretion, and immobility when mounted on by the buck).

P_4 assay and data analyses

P_4 concentrations were determined by enzyme immunoassay using the two-antibody method in 96-well plates, as previously described [14]. Rabbit anti-P_4 antibody (KZ-HS-P13; UCB Bioproducts, Brussels, Belgium) was used as the primary antibody, and affinity-purified anti-rabbit goat IgG (111-005-003; Jackson ImmunoResearch, West Grove, PA, USA) was used as the secondary antibody. The inter- and intra-assay coefficients of variation were 4.3% and 8.8%, respectively, with a sensitivity of 0.4 ng/ml.

All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria). The results of the *in vitro* dissolution test were compared using the Mann–Whitney U test to analyze the differences between the two types of CIDR. Plasma P_4 concentrations were analyzed using the repeated-measures analysis of variance, followed by Tukey's multiple comparison test. Differences were considered statistically significant at $P < 0.05$.

Conflict of interests: No conflict of interest.

Acknowledgments

This study was supported by the JSPS KAKENHI (grant number 23K05551). We thank Zoetis Japan, Inc. for providing the CIDR-G used in this study.

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