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Intravaginal administration of progesterone using a new technique for sustained drug release in goats

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Abstract. The objective of the present study was to develop and evaluate a sustained release vaginal progesterone (P_4) capsule containing a mixture of mucoadhesive polymer and silicone fluid. Goats were administered a gelatin capsule containing 0.4 g of P_4 mixed in silicone fluid and either a hydroxypropylmethylcellulose (HM) or polyaclil starch (PA) base. The mean plasma P_4 concentrations at 2 and 12 h after administration were significantly higher in goats treated with PA capsules than in those with HM capsules. The plasma P_4 concentrations in goats treated with HM capsules increased and remained above 1.0 ng/ml for 96 h after administration, whereas the plasma P_4 concentrations in goats treated with PA capsules remained above 1.0 ng/ml for only 24 h after administration. In the next experiment, an HM capsule was attached to a silicone device and inserted in the vagina for 10 days. The plasma P_4 concentration remained similar to that of the natural luteal phase for 9 days. These results suggest that a mixture of mucoadhesive polymer and silicone fluid has the potential to be applied clinically as a sustained release base for estrus synchronization or hormonal therapy.
Key words: Intravaginal administration, Mucoadhesive polymers, Progesterone

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The vaginal mucosa is a feasible, and safe route for drug delivery due the large surface area of the vaginal mucosal cavity itself, ample blood supply, and permeability to a wide range of compounds [1] including peptides [2, 3] and steroid hormones [4, 5]. Vaginal suppositories, gels or tablets have been used to administer drugs including steroid hormones, prostaglandins, and contraceptive and antibacterial agents in human medicine [6, 7]. In contrast, the use of vaginal suppositories or gels in farm animals is not common, although a P_4-immersed polyurethane sponge and silicone elastomer device for controlled internal drug release (CIDR) is widely used to synchronize estrus in ewes [4, 8], cows [9–11], and goats [12, 13]. The ability of exogenous P_4 to block ovulation [12] and to induce preovulatory luteinizing hormone secretion after the cessation of treatment [13] makes it applicable for estrus synchronization [14] and the treatment of ovarian disorders. However, long term insertion of these devices (i.e., 14 days) resulted in lower plasma P_4 concentrations than the natural luteal phase at the end of treatment [15], and therefore, CIDR should be removed at an appropriate time.

Recent studies have focused on the use of mucoadhesive polymers such as polyacrylic-acid bases and cellulose derivates to improve the efficacy of drug release and absorption in the vagina [16, 17]. In order to apply these drug release techniques to the hormonal treatment of farm animals, we developed P_4 capsules based on a mixture of silicone fluid and mucoadhesive polymers for vaginal drug release. In the present study, goats were used as an experimental animal model and we evaluated the P_4 profiles after administration of P_4 mixed with silicone fluid and a hydroxypropylmethylcellulose (HM) or polyaclil starch (PA) base.

In *Experiment 1*, three out of six goats treated with HM capsules and two out of five goats treated with PA capsules showed a transient increase of plasma P_4 concentration to 0.8–2.7 ng/ml at 2 to 12 h after capsule administration, which then decreased to less than 1.0 ng/ml. We speculated that the capsules might have dropped from the vagina some hours after administration in these goats, and they were excluded from further analysis. The plasma P_4 concentrations in the remaining three goats treated with HM capsules increased and remained above 1.0 ng/ml until 96 h after administration, with a range from 1.2 ± 1.2 ng/ml to 3.4 ± 1.0 ng/ml (Fig. 1). In contrast, the plasma P_4 concentrations in the remaining three goats treated with PA capsules remained > 1.0 ng/ml from 2 to 24 h after administration. The mean plasma P_4 concentrations at 2 and 12 h after administration were higher in goats treated with PA capsules than in those treated with HM capsules ($P < 0.05$, Fig. 1). Thereafter, the plasma P_4 concentrations at 48, 72, and 96 h after insertion were higher in goats treated with HM capsules than in those treated with PA capsules ($P < 0.05$). The plasma P_4 concentrations at 144 h were lower than 1.0 ng/ml in all goats, and did not differ significantly between the groups.

The results from *Experiment 1* showed that HM capsules had a

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longer release time of P_4 than PA capsules, but dropped out shortly after administration in half of the animals. To address this issue, a Y-shaped intravaginal silicone device was prepared, and the capsule was attached to the device (Fig. 2). *Experiment 2* was then conducted to examine the time course of P_4 release from the HM capsule in the vagina. In all goats, the silicone device kept in vagina, and also no abnormal discharge from the vagina was observed for 10 days inserted. The mean plasma P_4 concentration at 1–9 days after administration of HM capsules with the device was 7.6 ± 2.3 ng/ml, and was not significantly different from that of the natural luteal phase level (i.e., prior to prostaglandin $F_{2\alpha}$ injection, 7.5 ± 1.9 ng/ml, Fig. 3). At 1 day after removal of the device, the plasma P_4 concentrations decreased to lower than 1.0 ng/ml in all goats.

Controlled drug delivery is one of the most attractive and challenging areas in medical science. Recent studies in related biological science disciplines have contributed to the design of novel methods to make drug delivery more effective. The present study confirmed that administration of P_4 by mixing with sustained-release base materials consisting of mucoadhesive polymer and silicone fluid could maintain the circulating P_4 concentrations over a long period. Mucoadhesive drug delivery systems can be formulated as tablets, solid inserts, pessaries, films, gels, micro- and nano-particles and sprays [16]. Mucoadhesive polymers enhance both the retention time of the pharmaceutical formulation and the drug absorption through

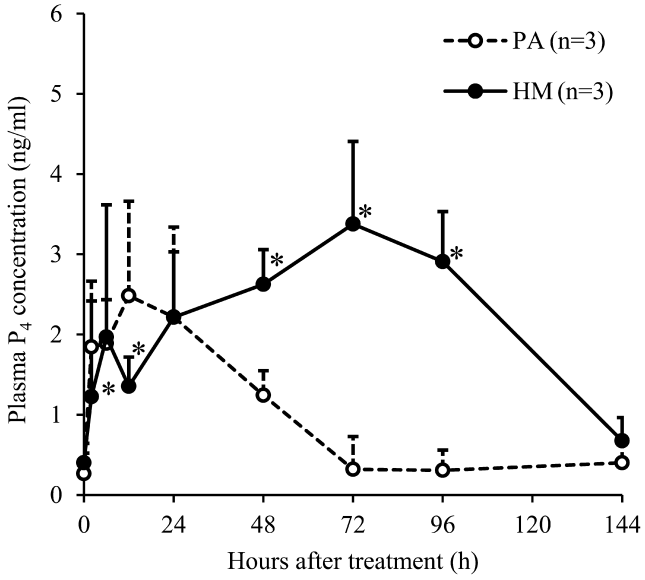

Fig. 1. Changes in plasma progesterone (P_4) concentrations after administration of polyaclil starch (PA) (open circle) and hydroxypropylmethylcellulose (HM) (closed circle) capsules. The Hour 0 sample was collected just before capsule insertion. Asterisks indicate significant difference between groups ($P < 0.05$).

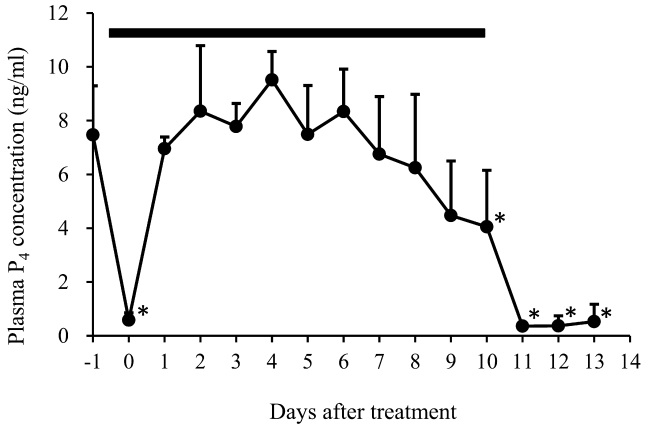

Fig. 3. Changes in plasma progesterone (P_4) concentration after administration of hydroxypropylmethylcellulose (HM) capsule with the silicone intravaginal device. Prostaglandin $F_{2\alpha}$ was injected 1 day before HM capsule administration to induce luteolysis. The Day 0 sample was collected just before capsule insertion. Black bar indicates the insertion period of the HM capsule and silicone device. Asterisks indicate significant difference compared to 1 day before HM capsule administration ($P < 0.05$).

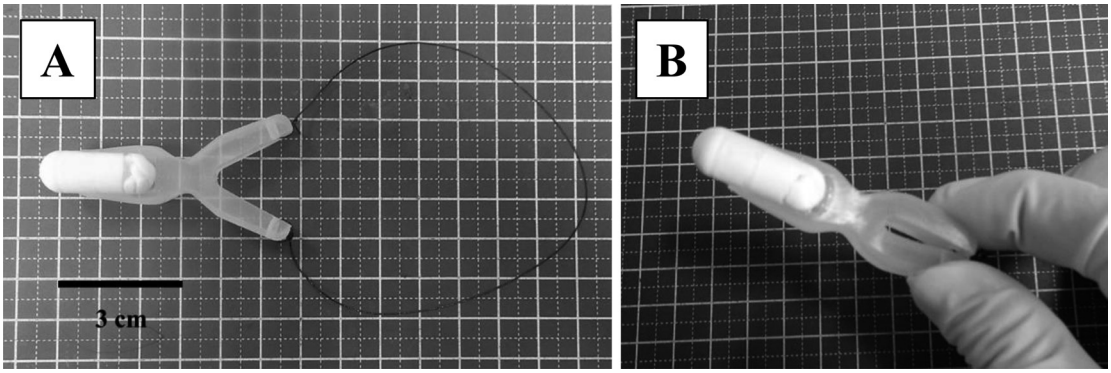

Fig. 2. Silicone intravaginal device for goats. The progesterone (P_4) capsule (length 26.1 mm, outside diameter 9.6 m, volume 1.37 ml) was attached to the upper surface of the device, and a sterilized suture was tied to the two wings of the device to allow withdrawal of the device from the vagina (A). Close the wings of the device by fingers and insert it into the vagina (B).

the mucosal surface [17]. In the present study, goats treated with PA capsules showed higher plasma P_4 concentrations at 2 and 12 h after administration than those treated with HM capsules. This indicates that the PA capsules collapsed shortly after administration and released the P_4 more rapidly than HM capsules. Polyacrylic-acid based gels have been used for sustained drug delivery *via* nasal and vaginal routes [18–20]. HM capsules had a longer action, maintaining P_4 release for at least 96 h after administration. HM is a water-soluble derivative of cellulose ether, and recent studies have examined its properties such as gelling, controlled release and mucoadhesion [21]. The high viscosity and retention property of the mixture of HM and silicon fluid formulated in this study resulted in slow release and absorption of P_4 in the vagina. In human medicine, vaginal P_4 suppositories or tablets are used for the treatment of infertility, and must be administered once or twice a day [22, 23] due to their short half-life. Although more detailed studies *in vitro* and *in vivo* are necessary for clinical application, HM-based vaginal suppositories or tablets may allow a reduced frequency of administration in human as well as veterinary medicine. However, it should be noted that the gelatin capsules used in the present study were not ideal for encapsulating the HM or PA paste; they did not adhere to the vaginal mucosa, and many appeared to drop out shortly after administration. Alternative capsule materials may overcome this issue.

When the P_4 capsule was attached to a silicone device in order to prevent loss from the vagina, plasma P_4 concentrations were maintained for 9 days at a level similar to that in the natural luteal phase of the goat's estrous cycle. In addition, the plasma P_4 concentrations after the administration of P_4 capsule with the device were more than two-fold higher than those in experiment 1. The device can keep the P_4 capsule in the deep portion of the device, which may increase the contact area and time of the P_4 capsule with the vaginal mucosa and resulted in the increase of plasma P_4 concentration. The silicone device we prepared appeared to cause no vaginal inflammation, since no abnormal discharge from the vagina was observed during the insertion period. The typical method for estrus synchronization in cows and small ruminants consists of 12 to 14 days of P_4 treatment [24, 25]. When using CIDR for estrus synchronization of goats, plasma P_4 levels increase rapidly after insertion, reach a maximum on Day 3, and then gradually decrease [12]. The subluteal serum P_4 concentrations induced by prolonged P_4 treatment generally result in extended growth of the ovulatory follicle [26]. For this reason, it was recently suggested that short-term protocols using P_4 treatment for 5–7 days are preferable for synchronizing estrus and ovulation [15, 27]. Although the HM capsules contained 0.4 g of P_4, which is more than the commercially available intravaginal devices for goats (e.g., CIDR-G, 0.33 g of P_4), the relatively constant release of P_4 for 9 days after insertion of the HM capsule is potentially sufficient for clinical use and may improve the results of estrus synchronization. Further studies are warranted to evaluate the efficacy of this P_4 capsule for estrus synchronization, including the effects on follicular growth, expression of estrus, and ovulation.

In conclusion, intravaginal administration of P_4 capsules using a sustained release drug technique employing a mixture of mucoadhesive polymers and silicone fluid could effectively maintain the plasma P_4 concentrations in goats. While the polyacrylic starch-based P_4 capsule had a higher release rate of P_4, the hydroxypropyl methylcellulose-based P_4 capsule showed slower and more constant release. The use of an intravaginal silicone device ensured retention of the P_4 capsule in the vagina and maintained the plasma P_4 concentration for 9 days at a level similar to that of the natural luteal phase in goats. This novel method of P_4 administration is applicable for estrus synchronization and hormone therapy in veterinary medicine.

Methods

Adult Shiba goats were maintained at Tokyo University of Agriculture and Technology. Shiba goats are non-seasonal breeders under natural daylight. All goats were kept in a freely-moving paddock and fed alfalfa hay cubes (660 g of dry matter/day), with clean water and mineral salt blocks available *ad libitum*. All procedures were approved by the University Committee for the Use and Care of Animals of Tokyo University of Agriculture and Technology (no. 22-67).

Preparation of sustained-release P_4 capsule

P_4 paste was prepared by mixing P_4 powder (Wako, Osaka, Japan), silicone fluid (Q7-9120, Dow Corning, MI, USA) as a dispersing agent, and a mucoadhesive polymer; PA capsules contained polyacrylic starch (SANWET®, Sanyo Chemical, Kyoto, Japan), and HM capsules contained hydroxypropyl methylcellulose (METOLOSE®, Shin-Etsu Chemical, Tokyo Japan). The P_4 paste was filled into a gelatin capsule with a volume of 1.37 ml (Matsuya, Osaka, Japan) and stored at 4ºC until administration. The formulations of the PA and HM capsules are shown in Table 1.

Experimental design

In *Experiment 1*, eleven adult female goats with normal estrous cycles (30.4 ± 4.7 [mean ± SD] of body weights) were assigned to HM capsule (n = 6) and PA capsule (n = 5) groups. Prostaglandin $F_{2\alpha}$ (2 mg of dinoprost; Pronalgon F; Pfizer, Tokyo, Japan) was administered intramuscularly to the goats during the mid-luteal phase (Days 7–14 of the estrous cycle), one day prior to P_4 capsule administration, for the purpose of inducing luteolysis. One capsule was inserted into the deep portion of the vagina using an applicator (Fig. 2) on the day after Prostaglandin $F_{2\alpha}$ injection. Two milliliters of blood were collected at 0, 2, 6, 12, 24, 48, 72, 96, and 144 h after the administration. Blood samples were centrifuged at 4ºC, 1750 g, and separated plasma samples were kept at –20ºC until assay.

In *Experiment 2*, five adult female goats with normal estrous

Table 1. Formulation of sustained-release progesterone (P_4) capsules

Composition	Weight (g) per capsule	
	PA capsule	HM capsule
Silicone fluid	0.81	0.81
Silica	0.07	0.07
Stearyl alcohol	0.02	0.02
Polyethyleneglycol	0.08	0.08
Progesterone	0.40	0.40
Polyacrylic starch	0.30	–
Hydroxypropylmethylcellulose	–	0.30

cycles (27.3 ± 3.9 kg of body weights) were used. Prostaglandin $F_{2\alpha}$ was administered as in Experiment 1. A silicone device with one HM capsule attached was inserted into the vagina for 10 days. When inserting the device into the vagina, close the wings of the device by fingers and insert manually it into the vagina (Fig. 2). Once insertion was completed, the device moved into the deep portion of the vagina due to the elasticity of the two wings. Two milliliters of blood were collected once a day from –1 to 13 days after device insertion. Blood samples were centrifuged at 4ºC, 1750 g, and separated plasma samples were kept at –20ºC until assay.

Hormone assay and data analysis

Plasma P_4 concentrations were measured by enzyme immunoassay according to the method described previously [28]. The intra- and inter-assay coefficients of variation were 14.6% and 12.4%, with an assay sensitivity of 0.4 ng/ml.

Data are presented as means ± SD. Changes in plasma P_4 concentration were compared by repeated measures ANOVA, and *post hoc* multiple comparisons were made using Tukey's test or Dunnett's test. Differences with $P < 0.05$ were considered significant.

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