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—Original—

A Comparative Study of Induction of Estrus and Ovulation by Three Different Intravaginal Devices in Ewes during the Non-Breeding Season

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Abstract. The aim of the present study was to compare three methods of estrus synchronization in ewes during the non-breeding season. Forty-two ewes were randomly grouped for three treatments with different intravaginal devices for 12 days: Group A) CIDR, Group B) Self-made P sponge, Group C) MAP (medroxyprogesterone acetate) cream sponge. Furthermore, all groups were divided into two treatments with (R) or without ram presence to examine the "ram effect". Blood was collected from all treated ewes, and progesterone (P_4), estradiol 17-β (E_2) and luteinizing hormone (LH) concentrations were measured by enzyme-immnoassay. All ewes showed estrus behavior between Day 0 to 3 after device removal, and the mean onset times of their estrus were 23.0, 33.0 and 21.0 h for Groups AR, BR and CR, respectively. On Day 5 as examined by laparoscopy, the ovulation rates (and number of ovulated ewes) were 1.45 (11/11), 1.25 (12/14) and 1.21 (14/14) for Groups A, B and C, respectively. In Group C, the time to LH surge was significantly ($P<0.05$) later (32.4 h) than those in Groups A (27.0 h) and B (25.5 h). Ram presence did not affect the number of ovulated ewes, ovulation rate or time to LH surge. The ram introduction group had significantly ($P<0.05$) lower E_2 concentrations during the period from 0 h to 36 h than the groups without ram presence. These results suggest that the self-made P sponge or MAP cream sponge was effective as well as CIDR, and ram introduction was not necessary, for induction of estrus and ovulation during the non-breeding season.
Key words: Induction of estrus, Intravaginal device, Non-breeding season, Sheep

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Estrus synchronization is generally applied for reproductive management of sheep flocks worldwide [1], and several methods have been performed with varying degrees of success [2]. The attempted methods are control of daily length and/or hormonal treatments such as natural progesterone, synthetic progestogens, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or gonadotrophin releasing hormone (GnRH), and isolated ram introduction [3]. Recently, a method using $PGF_{2\alpha}$ combined with GnRH was reported in ewes with a successful lambing rate after natural mating or artificial insemination [1, 4]. Treatment with GnRH and $PGF_{2\alpha}$ is a practical method for controlling ovarian follicular and luteal functions and for increasing the precision of estrus synchronization in cyclic and acyclic postpartum cows and heifers [5–8]. However, this method is very costly for ewes and it is not a practical method in Japan. The main hormonal method for estrus synchronization in ewes is intravaginal devices impregnated with

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progesterone or synthetic progestogen such as medroxyprogesterone acetate (MAP) or fluorogestone acetate (FGA). The controlled internal drug release (CIDR) device has been developed in New Zealand and it has been proven to be effective [5, 9, 10]. The intravaginal devices impregnated with progesterone, MAP or FGA are generally inserted in the vagina for a period of 10–14 days and combined with an injection of equine chorionic gonadotrophin (eCG) or follicle stimulating hormone (FSH). They have shown success inducing estrus and ovulation during the non-breeding season [11, 12]. There have been many other studies which have used different intravaginal devices, but these devices are difficult to import and use for out-of-season breeding in Japan. To solve this problem, a new intravaginal device impregnated with progesterone or synthetic progestogens needs to be developed [9, 10, 13, 14]. Recently, a new method using MAP dissolved in a cream (cream method) has been developed in Japan [15]. In this study, we compared three methods of estrus induction and synchronization in ewes during the non-breeding season: 1) CIDR, 2) self-made progesterone impregnated intravaginal sponge (self-made P sponge), and 3) MAP cream method. Ram introduction effects on ovarian response, the onset of estrus times and hormonal (P_4, E_2, LH) profiles were also examined.

Materials and Methods

Animals

The present study was conducted during the non-breeding season from May to June 2002. Thirty Suffolk (Sf), 10 South-Down (SD) and 2 Dorset-crossed (D) ewes were used in this study (Total, n=42). The ewes were fed 1 kg of hay per head (once daily) and a concentration pellet containing 15% crude protein (about 200 g per head per day), and were allowed free access to water, salt and mineral blocks. The ewes were not mated or inseminated during the study.

Treatment

Ewes were randomly grouped for three treatments with different intravaginal devices inserted for 12 days: Group A) CIDR (type G containing 0.3 g progesterone: InterAg, Hamilton, New Zealand) (Sf=10, SD=4), Group B) Self-made P sponge (containing 0.5 g progesterone) [9, 12, 14] (Sf=10, SD=4), Group C) intravaginal MAP cream sponge (containing 0.06 g medroxyprogesterone acetate with inunction) (Sf=10, SD=2, D=2). Furthermore, all groups were divided for two additional treatments, with (R) or without ram introduction. The intravaginal cream was blended 0.06 g medroxyprogesterone acetate with inunction (Oronain-H-Nankou: Otsuka Seiyaku Co., Japan), that is sold commercially, which was used to coat the sponges. All ewes received an intramuscular injection of 500 i.u. eCG (Serotropin: Teikoku-zoki Co., Japan) 24 h before removal of the intravaginal devices (the day of device removal: Day 0).

On Day 0, three mature rams fitted with a marking-harness and crayons were introduced to Groups AR, BR and CR for detection of estrus onset. Estrus detection was performed at 6 h intervals until Day 3.

On Day 5 after removal of intravaginal devices, ovulation was examined by laparoscope in all ewes. The ewes with newly formed corpus luteum were considered to have ovulated.

Collecting blood samples and Hormonal measurement

Blood (5 to 10 ml) from the jugular vein was collected from all ewes from Day –12 to Day 4 once a day for measurement of progesterone (P_4) concentrations, from Day 0 to Day 4 twice a day for measurement of estrogen (E_2) concentrations, and from 9 h to 37 h at 2 hour intervals for measurment of LH concentrations (Day 0 and 0 h: the day and time of intravaginal devices removal). The collected blood was centrifuged (3000 × g, 15 min) at 4 C, immediately, and plasma was separated and stored at –30 C, until measurement.

Plasma P_4 and E_2 concentrations were measured by double-antibody enzyme immuno-assay (EIA) using 96-well ELISA plates according to the methods of Miyamoto *et al.* [16], and Acosta *et al.* [17, 18]. The study of Fukui *et al.* [19] showed that the antibody of P_4 does not cross-react with plasma MAP. The standard curves of P_4 and E_2 EIA ranged from 2 to 20 ng/ml and 2 to 2000 pg/ml, respectively. The average intra- and inter-assay coefficients of variation (CVs) of P_4 were 5.8% and 8.2%, respectively, and of E_2 were 6.1% and 8.9%, respectively.

Plasma LH concentration was determined by EIA according to the biotin-streptavidin amplification technique [18, 20]. Intra- and inter-assay

Table 1. Estrus incidence, LH surge and ovulation rate of ewes treated with three different intravaginal devices and ram introduction (+) or not (–)

Ram presence	Treatment (Group)	No. ewes treated	Onset time of estrus*1 (No.ewes)	Time to LH surge*1 (No.ewes)	Ovulation rate*2 (No.ewes ovulated)
+	CIDR (A)	7	23.0 ± 1.84 (7)	27.0 ± 3.43^{abc} (6)	1.57 ± 0.30 (7)
	Self-made P (B)	7	33.0 ± 5.74 (7)	24.6 ± 1.60^{c} (5)	1.00 ± 0.00 (5)
	MAP cream (C)	7	21.0 ± 3.38 (7)	33.4 ± 1.60^{b} (5)	1.23 ± 0.18 (7)
	sub-total	21	25.1 ± 2.18 (21)	28.3 ± 1.66 (16)	1.32 ± 0.13 (19)
–	CIDR (A)	4*3	–	27.0 ± 1.41^{abc} (4)	1.25 ± 0.25 (4)
	Self-made P (B)	7	–	26.1 ± 1.94^{ac} (7)	1.25 ± 0.13 (7)
	MAP cream (C)	7	–	31.4 ± 2.14^{ab} (5)	1.21 ± 0.11 (7)
	sub-total	18	–	28.0 ± 1.12 (16)	1.28 ± 0.11 (18)
Total	CIDR (A)	11	–	27.0 ± 1.09^{d} (10)	1.45 ± 0.21 (11)
	Self-made P (B)	14	–	25.5 ± 1.28^{d} (12)	1.25 ± 0.13 (12)
	MAP cream (C)	14	–	32.4 ± 1.30^{e} (10)	1.21 ± 0.11 (14)

*1 Time from intravaginal device removal.
*2 No.of corpus luteum / No. of ovulated ewes.
*3 Three ewes lost CIDRs during the insertion period and were excluded from data.
a-c, d, e $P<0.05$

coefficients of correlation were within 13 %.

Statistical analysis

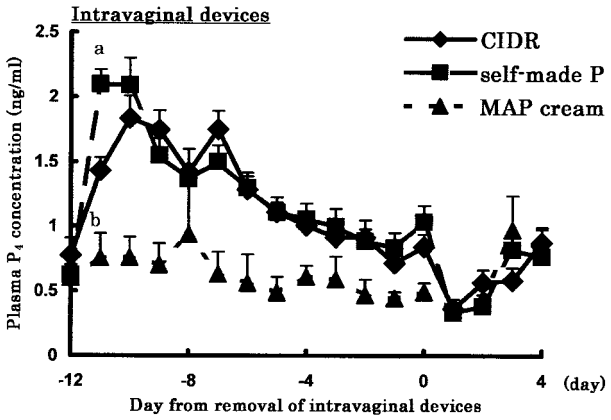
Data on the number of estrous and ovulated ewes, and ewes with recognized LH (> 10 ng/ml) surge were analyzed by Student's t-test. Mean onset times of estrus, ovulation rate and each hormonal concentration were analyzed by χ-square test. The results were considered significant for $P<0.05$.

Results

Three out of 7 CIDR-treated ewes in the group without ram introduction lost their CIDRs during the insertion period. Therefore, data from these 3 ewes were excluded from the experimental data (Table 1).

Irrespective of treatments, all ewes in the ram introduction group showed estrus. The mean onset times of estrus were 23.0, 33.0 and 21.0 h for Groups AR, BR and CR, respectively (Table 1). The treatment with the self-made P sponge induced estrus later than the CIDR and MAP cream treatments, but there were no significant differences. In the results of ovarian observation, all ewes in Group A (11/11) and Group C (14/14) ovulated, but 2 ewes had not ovulation in Group B (12/14) (Table 1). Ram presence resulted in a higher ovulation rate (1.32: 19/21) than without rams (1.28: 18/18), but there was no significant difference ($P<0.05$) (Table 1).

The mean plasma P_4 concentrations for each treatment are shown in Fig.1. There were no significant differences among the types of intravaginal devices and ram introduction. The MAP cream method did not affect P_4 levels during



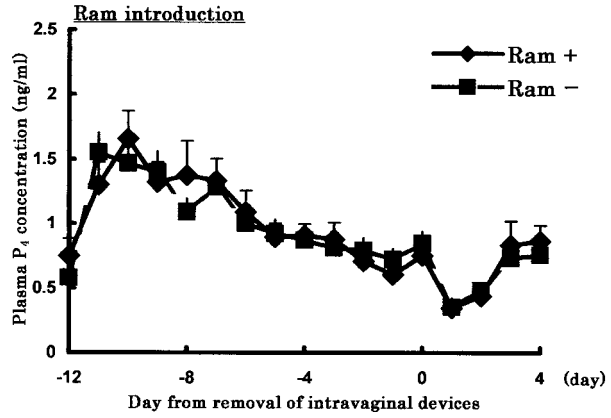
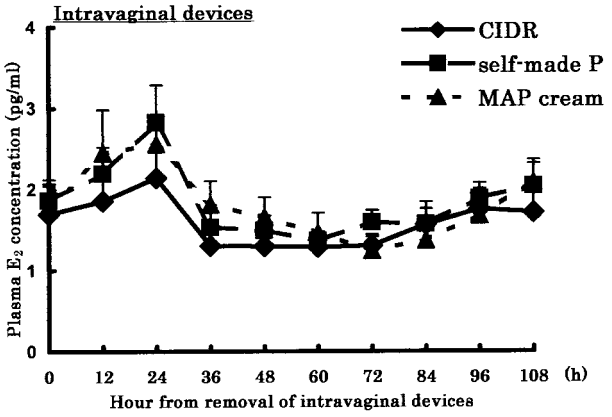


Fig. 1. The changes in plasma progesterone (P_4) concentrations after removal of intravaginal devices. [a-b] $P<0.05$.



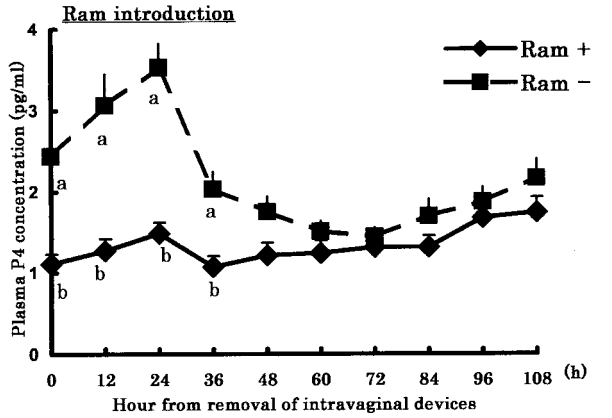


Fig. 2. The changes in plasma estradiol 17-β (E_2) concentrations after removal of intravaginal devices. [a-b] $P<0.05$.

the insertion period. The mean plasma E_2 concentrations are shown Fig. 2. The three intravaginal devices did not show any significant differences ($P<0.05$) among the plasma E_2 concentrations. However, the ram introduction group had significantly ($P<0.05$) lower E_2 concentrations during the period from 0 h to 36 h than the group without ram presence. The mean time of LH surge is shown in Table 1. The MAP cream method showed a significantly ($P<0.05$) later time of LH surge than the other two groups. There was no significant difference in the times of LH surge between the groups with or without ram introduction.

Discussion

In this study, all ewes in the ram introduction group showed estrous behavior 1–2 days after removal of the intravaginal devices. Also, there was no significant difference in the ovulation rate and the number of ovulated ewes between the groups with and without ram introduction. These result are in agreement with previous reports [9, 14, 21] on synchronized estrus and ovulation using progesterone or synthetic progestogen impregnated devices in ewes. In Group BR, however, 2 out of 7 ewes showed estrous behavior more than 40 h after sponge removal, and this caused the prolonged mean time (33.0 h) of the estrous onset compared to the other 2 treatments (21–23 h). Furthermore, the same two ewes in Group B had no definitive LH surge and did not ovulate. It was considered that progesterone was not fully absorbed from the sponge and thus, the progesterone interfusion into the vagina was insufficient for induction of ovulation. In ewes

treated with progesterone or the synthetic progestogen impregnated intravaginal sponge, the successful control of the estrous cycle depends on the absorption of an effective dose, and the density of sponge [22, 23]. This suggests the possibility that the self-made intravaginal sponges impregnated with progesterone in this study had unequal absorption rates, and that this might have caused the insufficiency of estrous synchronization in 2 ewes. However, the other 5 ewes in Group BR ovulated, and if the absorption rate had been consistent, these 2 ewes might also have ovulated. All ewes in Group C ovulated, and this indicats that the absorption rate for MAP cream sponges was sufficient for induction of ovulation. There was no significant difference in the number of ewes with recognized LH surge for the three intravaginal devices. However, the time to LH surge was significantly prolonged in Group C (25–37 h) compared to those in Groups A (11–35 h) and B (19–35 h). This result was consistent with a previous study of Fukui *et al.* [9] in which performing estrus synchronization was performed by natural or synthetic progestogen impregnated intravaginal devices during the breeding season. However, the onset time of estrous behavior was earlier in this study than in previous studies [9, 13]. The MAP used in this study is an anticancer drug for human mammary or uterus cancer and the functional mechanism and absorption rate are likely different from the other progestogens used for estrus induction and synchronization in the previous studies.

In this study, ram introduction did not affect the number of ovulated ewes and the ovulation rate. This may have been caused by the intravaginal devices used in the present study with administration of eCG for induction of ovulation. Plasma progesterone concentrations were not significantly different in the groups with or without ram introduction and the type of intravaginal devices used. Ram effect is provided by "pheromone" which is secreted by the sudoriferous glands in the skin of the ram [24], and is considered to suppress negative feedback of estrogen during the non-breeding season. Thereafter, a rapid increase in the frequency of LH pulses occurs in ewes [25, 26], and subsequently ovulation is induced. In spite of these facts, the plasma E_2 concentration during the period from 0 to 36 h after treatment in the present study were significantly higher in the group without ram introduction than those in the ram introduction group. In this study, the ewes in the ram introduction group were completely isolated from rams in a different animal hut until Day 0, whereas the ewes without ram introduction were housed in different pens in the same field until Day 0. Pheromone effect is induced by olfaction or hearing [27], and there is the possibility that the presence of rams housed in the same animal hut affected the ewe group without ram introduction. The basal level of E_2 is generally 7–8 pg/ml and it increases to 10–15 pg/ml at the peak level in ewes during the breeding season [28]. In this study, however, the basal and peak levels of E_2 were low in accordance with a previous study [29] reporting that E_2 production was inhibited during the non-breeding season. These results suggest that the lower level of E_2 is still able to introduce estrus and ovulation.

MAP did not affect in the plasma progesterone levels, and the MAP cream method gare constantly low P_4 levels for Day –12 to Day 0. In Fig. 2, the plasma progesterone concentrations increased in both Groups A and B on Day –7, and for all groups after Day –1. Ovulation was confirmed in each treatment by laparoscopy on Day 5 and determination of progesterone levels.

In conclusion, the present results indicate that the self-made P_4 sponge or MAP impregnated cream sponge were efficient methods in ewes to induce estrus and ovulation during the non-breeding season; however, ram effect was not confirmed. A further study is needed to investigate the effects in ewes on pregnancy and lambing rates by natural mating or AI of treatments with self-made intravaginal devices of either sponge or cream form.

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