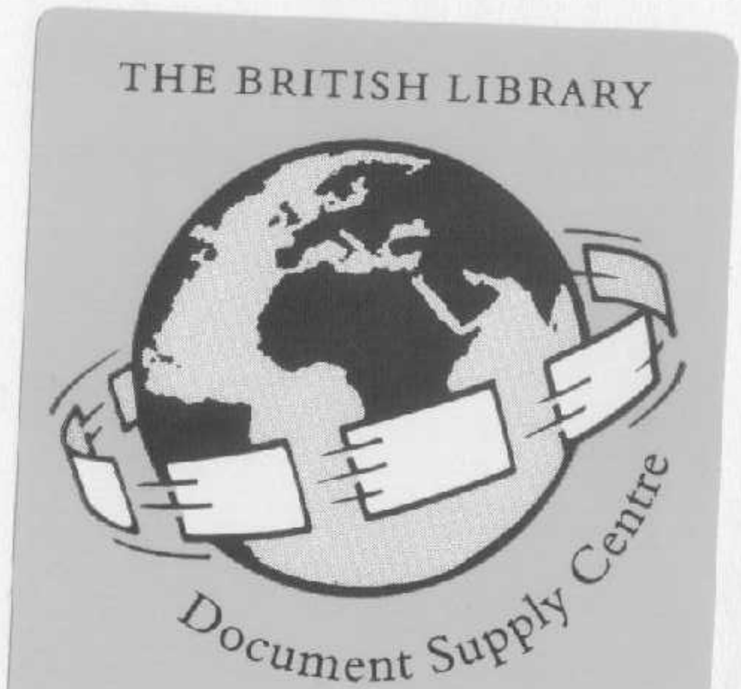

要　　約

体外で成熟させた牛卵胞卵子を媒精後，さらに体外で培養することにより脱出胚盤胞までの発育が観察された。

屠殺された牛卵巣の小卵胞（1～7 mm）から採取した供試卵子は卵丘細胞が卵子の表面に1/3以上付着しており，卵子細胞質が均一で良好なものを用いた。25 mM HEPES 緩衝 Earle 型 TCM 199 に5％仔牛血清を添加した培地内で20～24時間の成熟培養後，媒精に供した。

精子は凍結精巣上体精液を用いた。カフェイン 5 mM 含 BSA 不含の B.O. 液で洗浄後，精子濃度 30～36×10^6個/ml になるよう調整した。さらに BSA を 10 mg/ml 含む B.O. 液で等量希釈し2～3時間の前培養後に媒精に供した。

媒精6時間後に卵子を発生培地に移し，継続培養した。発生培地としては仔牛血清（0～10％）を含む 25 mM HEPES 緩衝 Earle 型 TCM 199 を用いた。媒精48あるいは72～77時間後に卵丘細胞層より卵子を遊離させたが，遊離後も卵子は卵丘細胞層上で継続培養した。

その結果，40.5～63.2％ が2細胞期以上へ，33.2～56.1％ が4細胞期以上へ，20.0～33.8％ が8細胞期以上へ 18.5～30.4％ が桑実期以上へ，4.1～25.4％ が胚盤胞期以上へ発育した。また低率（2.1～3.7％）ながらも脱出胚盤胞も観察された。

Effects of Types of Vaginal Sponge Impregnated with Progestogen on Estrus Induction and Lambing Rate in Seasonally Anestrous Ewes

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Summary. For out-of-seasonal breeding, types of vaginal sponges and progestogens were compared in two field trials. The materials used were as follows; I) 60 mg medroxyprogesterone acetate (MAP)-impregnated sponge (supplied by the Upjohn International, Australia), II) 500 mg progesterone-impregnated empty-MAP sponge, and III) 500 mg progesterone-impregnated self-made sponge. The sponge was inserted in the vagina for 12 (Trial 1) and 9 days (Trial 2). A 600 IU PMSG was injected intramuscularly in ewes at the time of sponge removal, and a synthetic LH-RH (Conceral, 100 μg) was also given in ewes at the time of estrus detection. In trial 1, the proportion of estrous ewes within 5 days after treatment was satisfactory (94–100%) with no significant differences among the groups. The mean time of induced estrus was significantly ($P<0.05$) earlier in the ewes treated with 500 mg progesterone-impregnated sponges than that with MAP sponges. The lambing rates were 85, 60 and 57% for groups I, II and III, respectively. However, they were not significantly different. In trial 2, the proportion of estrous ewes was generally low (68–88%) with no significant differences among the treatments. The lambing rates of the ewes mated following the sponge removal were also low (31–33%). However, there were no significant differences among the types of sponges and progestogens used. The present results indicate that the self-made vaginal sponge impregnated with 500 mg progesterone has similar effects to the MAP sponge for estrus induction and lambing rate in ewes treated during the non-breeding season.

KEY WORDS; VAGINAL SPONGE, NON-SEASONAL BREEDING, SHEEP.

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Vaginal sponges impregnated with synthetic progestogen have been widely utilized for synchronization and induction of estrus and ovulation in ewes. At the present time, mainly medroxyprogesterone acetate (MAP) and fluorogestone acetate (FGA) impregnated vaginal sponges in sheep have been internationally commercialized. On the other hand, in Japan, MAP-impregnated vaginal sponges for sheep are available for only research purposes (Fukui *et al.*, 1983, 1985; Kobayashi *et al.*, 1986). Recently, the demand for progestogen-impregnated vaginal sponges has been increasing especially for out-of-seasonal breeding in Japan. Therefore, it is necessary to develop a self-made vaginal sponge, as the progesterone-impregnated vaginal device, called "controlled internal drug release" (CIDR: Barnes and Welch, 1984/85; Davis *et al.*, 1985) and silastic vaginal ring (Watanabe *et al.*, 1986).

In the present study, two field trials were conducted to examine the effect of three different vaginal sponges impregnated with progestogens

on estrus induction and lambing rate in seasonally anestrous Suffolk ewes.

Materials and Methods

Trials 1 and 2 were performed during April, 1986 at the Tawa Field Station, Shibechya-cho, Hokkaido. Japan and May–June, 1986 at the Happy Farm, Noboribetsu-shi, Hokkaido, Japan, respectively. The animals used in Trial 1 were 50 maiden (2 years old) Suffolk ewes and for Trial 2, 74 maiden Suffolk × Finnish-Landrace ewes were used. Before both trials, the rams had been kept in a stud completely isolated for at least 3 months from the ewe flock.

Trial 1. The 50 ewes were divided into three groups by types of vaginal sponges and progestogens as follows; I) 60 mg MAP sponge (The Upjohn International Ltd., Australia: 16 ewes), II) 500 mg progesterone (Teikoku-zoki Co., Japan)-impregnated empty-MAP sponge (supplied by the Upjohn International Ltd., Australia: 17 ewes), and III) 500 mg progesterone-impregnated self-made sponge (17 ewes). The self-made sponges were prepared from polyurethane foam and were offered by the Teikoku-zoki Co., Japan. The cylindrical size was same (length 5.0 cm, diameter 2.3 cm) as the MAP sponge. A fishing string (no. 8), about 30 cm long, was passed through the sponge twice and knotted near the sponge and then again near the end of the double thread. This procedure was followed by that of the MAP sponge. The density of the vaginal sponges were 0.0245 g/cm^3 and 0.0204 g/cm^3 for groups I and II, III, respectively. For the groups II and III, impregnation of progesterone into the sponges was achieved by pipetting 6 ml ethanol containing 500 mg progesterone which were allowed to dry at room temperature for 24 hr.

The vaginal sponges were inserted into the vagina for 12 days from April 11th, 1986. On April 23rd, 1986, all sponges were removed and 600 IU pregnant mare's serum gonadotropin (PMSG: Serotropin, Teikoku-zoki Co., Japan) was injected (i.m.) to each ewes. One ewe in group II lost the sponge during the insertion period. All ewes were then introduced to 5 mature rams fitted with marking-hardnesses and crayons, and estrus was observed 6 hr intervals for 5 days after treatment. Estrous ewes were injected (i.m.) with 100 μg synthetic luteinizing hormone-releasing hormone, Fertirelin acetate (Conceral; Takeda Industrial Co., Japan), and received an ejaculation (hand service) 6 hr later by one of three mature Suffolk rams.

Trial 2. The 74 ewes were divided into three groups and were treated same as in Trial 1 except the 9-days insertion period of the vaginal sponges. In this trial, 5 and 3 ewes in groups II (20.8%) and III (12.0%) respectively, lost their sponges during the insertion period. The injections of PMSG and Fertirelin acetate, and the methods of estrus detection and hand service were also the same as in Trial 1. Five and 4 mature Suffolk rams were used for the estrus detection and the hand service, respectively. In Trial 2, the ewes which did not show estrus for 5 days after treatment including those lost the sponges during the insertion period were injected (i.m.) again with 600 IU PMSG 16 days after the first injection. The ewes were joined with 3 rams for 7 days, and estrus induction and lambing rate were examined.

In both trials 1 and 2, number of estrous ewes and time of estrus detection in each group were recorded. Fertility was expressed as lambing rate, and prolificacy (no. of lambs born/no. of ewes lambed) were also examined. However, in Trial 1, 8 ewes had lost their ear-tags before lambing, and therefore they had to be excluded from the lambing data.

Data on the number of estrous ewes and the lambing rate were compared by chi-square analysis. Data on the time of estrus detection and prolificacy were subjected to analysis of variance, and differences were analyzed by Student's *t*-test (Steel and Torrie, 1960).

Results

Trial 1. Incidence and time of induced estrus in the three groups are shown in Table 1. The proportions of estrous ewes within 5 days after treatment were 100%, 100% and 94.1% for groups I, II and III, respectively with no significant differences. The ewes treated with the progesterone-impregnated sponges (Groups II and III) came into estrus significantly ($P<0.05$) earlier than those in group I with MAP sponges. Fig. 1 shows the pattern of estrus incidence in ewes of

Table 1. Incidence and time of estrus in ewes treated with different vaginal sponges (Trial 1)

Group*1	No. ewes treated	No. ewes held sponges	No. ewes in estrus*2	Mean time of estrus onset (h)*3 (Mean±SD)
I	16	16	16 (100)[a]	32.1± 9.7[a]
II	17	16	16 (100)[a]	23.7±10.4[b]
III	17	17	16 (94.1)[a]	24.0± 8.7[b]

*1: Group I; 60 mg MAP sponge, Group II; 500 mg progesterone in the empty MAP sponge, Group III; 500 mg progesterone in the self-made sponge.
*2: Within 5 days after treatment.
*3: Times from the sponge removal.
a, b: The figures with different superscripts in each column are significantly different ($P>0.05$)

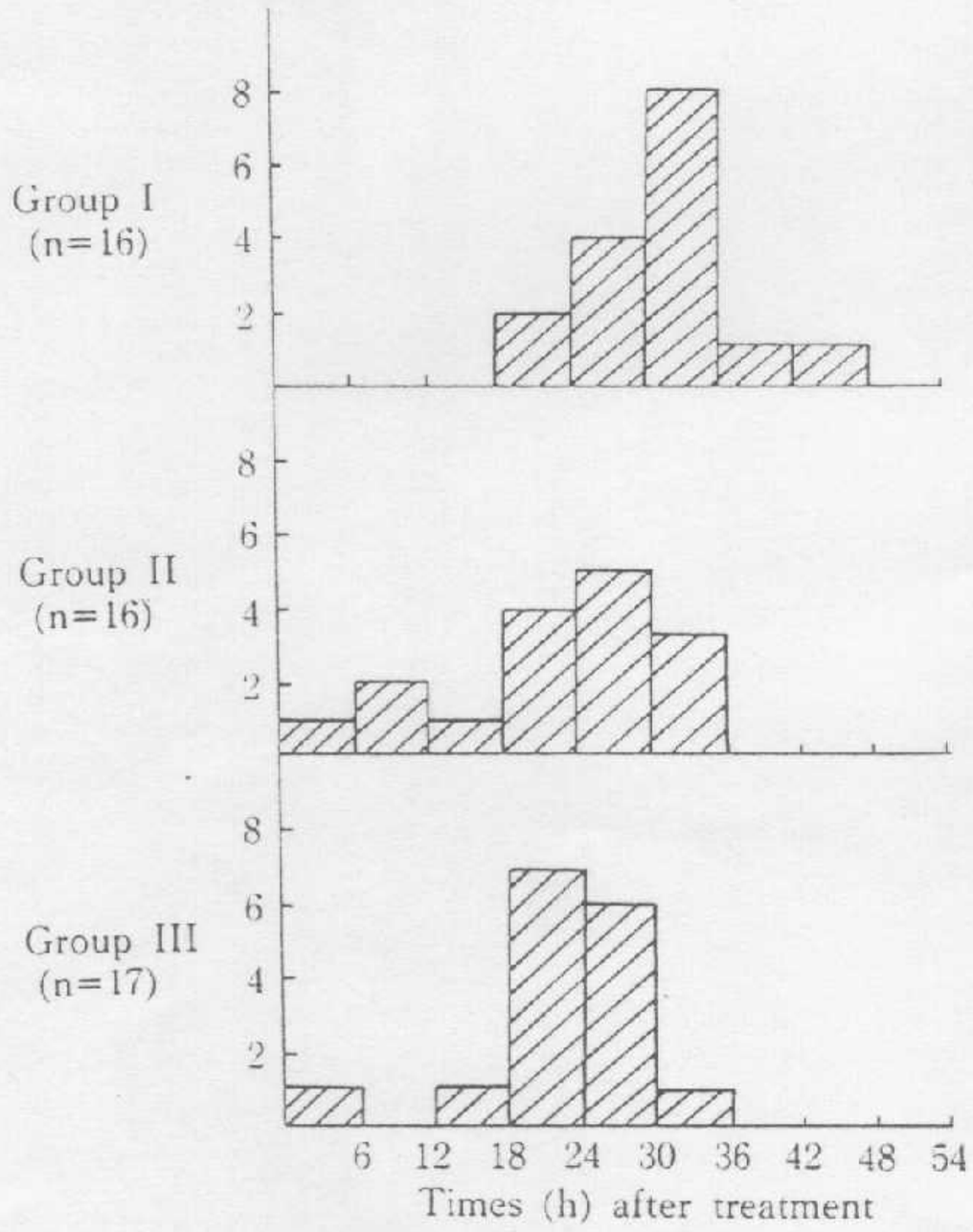

Fig. 1. The patterns of estrus incidence in ewes treated with different vaginal sponges.

each group. The types of vaginal sponge and progestogen did not affect on the proportion of estrous ewes, but the types of progestogen affected on the time of estrus incidence.

As described in the section of the Materials and Methods, 8 ewes (3, 2 and 3 ewes for groups I, II and III, respectively) lost their ear-tags and were not able to be identified to which group they belonged. Therefore, they were excluded from the lambing data. The lambing rates in each group were 84.6%, 60.0% and 57.1% for groups I, II and III, respectively (Table 2). The lambing rates of ewes treated with progesterone-impregnated sponges tended to be lower as compared with that of MAP sponges. However, there were no significant differences in the lambing rates among the groups. The prolificacy was also not significantly different among the groups.

Table 2. Lambing results of ewes treated with different vaginal sponges (Trial 1)

Group*1	No. ewes available*2	No. ewes lambed (%)	Prolificancy
I	13	11 (84.6)[a]	1.81[a]
II	15	9 (60.0)[a]	1.44[a]
III	14	8 (57.1)[a]	1.75[a]

*1: The same as in Table 1.
*2: The number of the ewes available for lambing results decreased due to the loss of the ear-tags (see the Table 1).
a: The figures in each column are not significantly diffenent ($P>0.05$).

Trial 2. The results of Trial 2 are summarized in Table 3. The rate of sponge loss was high in groups II (20.8%) and III (12.0%) as compared with that in group I (2.0%). The proportions of estrous ewes were lower in groups II (68.4%) and III (68.2%) than that of group I (88.0%), although they were not significantly different. The lambing rates by the first treatment were 31.8%, 30.8% and 33.3% for groups I, II and III, respectively with no significant differences. Of 24 ewes (3, 11 and 10 ewes for groups I, II and III, respectively) treated with the second PMSG, 1, 5 and 7 ewes were marked by rams, and 1, 5 and 5 out of those ewes

Table 3. Estrus incidence and lambing results of ewes treated with different vaginal sponges (Trial 2)

Group*1	No. ewes treated	No. ewes held sponge	No. ewes in estrus (%) *2	No. ewes secondly treated*3	No. ewes secondly mated (%)	No. ewes lambed (%)			Prolificacy
						1st	2nd	Total	
I	25	25	22 (88.0) [a]	3	1 (33.3) [a]	7 (31.8) [a]	1 (100)	8 (32.0) [a]	1.62[a]
II	24	19	13 (68.4) [a]	11	5 (45.5) [a]	4 (30.8) [a]	5 (100)	9 (37.5) [a]	1.11[a]
III	25	22	15 (68.2) [a]	10	7 (70.0) [a]	5 (33.3) [a]	5 (71.4)	10 (40.0) [a]	1.60[a]

*1: The same as in Table 1.
*2: Within 5 days after treatment.
*3: The ewes which had lost the sponges and did not come to estrus after the first treatment were again injected with 600 IU PMSG 16 days after the first PMSG.
a: The figures in each column are not significantly different ($P>0.05$).

lambed in each group. When combined the results following the first and second treatments, the lambing rates for the ewes treated initially were 32.0%, 37.5% and 40.0% for groups I, II and III, respectively. There were no significant differences on estrus incidence, lambing rate, and prolificacy among the three groups in both the first and second treatments.

As compared Trial 1 with Trial 2, the proportions of estrous ewes and lambing rates by the first treatment were lower in Trial 2 than those in Trial 1 as follows: estrus incidence for Trial 1 and 2 were 100% and 88.0% (Group I: not significant), 100% and 68.4% (Group II: $P<0.025$), and 94.1% and 68.2% (Group III: $P<0.05$). Lambing rates for Trial 1 and 2 were 84.6% and 31.8% (Group I: $P<0.005$), 60.0% and 30.8% (Group II: not significant), and 57.1% and 33.3% (Group III: not significant). The MAP sponges did not show significant differences on estrus incidence between Trials 1 and 2, but significantly affected on lambing rates. Meanwhile, the sponges impregnated with progesterone showed significant differences in estrus incidence between Trials 1 and 2 in both Groups II and III, but not in the lambing rates.

Discussion

Robinson (1965) first reported a synchronization of estrus in cyclic ewes using vaginal sponges impregnated with FGA or progesterone. Since then, vaginal sponges impregnated with synthetic progestogen (FGA, MAP and others) and progesterone have been widely used for not only synchronization of estrus in cyclic ewes, but also for out-of-seasonal breeding with different breeds of ewes (Moore and Holst, 1967; Dave *et al.*, 1969; Gordon, 1971; Boland *et al.*, 1981; Steffan *et al.*, 1982/83; Fukui *et al.*, 1985). In the present study using MAP sponges, estrus induction and lambing rates were satisfactory except the lambing rate in Trial 2.

The self-made vaginal sponges impregnated with 500 mg progesterone (Group III) showed the same effects on estrus induction and lambing rate as the empty-MAP sponges impregnated with the same dose of progesterone (Group II). On the dose of progesterone impregnated onto sponges, Robinson (1965), and Moore and Holst (1967) used 800 mg. However, Robinson (1965), and Holst and Moore (1965) showed in the cyclic ewes that even when a high dose of progesterone (720–800 mg) was impregnated onto vaginal sponges, estrus and ovulation were not completely controlled. A lower dose of progesterone (400–500 mg) has been also tested (Gordon, 1971; Crosby *et al.*, 1983; Hamra *et al.*, 1984). Crosby *et al.* (1983) compared vaginal sponges impregnated with 500 mg progesterone and MAP on the induction of estrus and lambing rate during the late anestrous season, and showed that estrus induction (92% and 98% for progesterone and MAP sponges, respectively) and lambing rates by a hand service (70% and 68%, respectively) were similar in both treatments. The present results also showed that the self-made vaginal sponges impregnated with 500 mg progesterone resulted in

similar effects on estrus induction and lambing rates in both trials as compared with MAP treatment.

It has been considered that there is a limit to the amount of progesterone that can be absorbed through the vagina (Moore and Holst, 1967). Neff and Lauderdale (1984) reported that only 12.3% of FGA-impregnated (usually contained 20–30 mg) vaginal sponges was released from the inserted sponge through the vagina. The absorption rate of impregnated progestogen may be related to the density of sponges. Morgan *et al.* (1967) using FGA found that slower rate of absorption from dense sponge (0.046 g/cm^3) was more effective than higher rate of absorption from less dense sponge (0.037 g/cm^3). However, the density of MAP sponge (0.0245 g/cm^3) and self-made sponge (0.0204 g/cm^3) was similar in the present study, and it did not significantly affect on estrus incidence and lambing rate between the two sponges used.

The reasons for lowered estrus incidence and lambing rates in Trial 2 are unknown. The lack of nutritional condition before hand service and late pregnancy may have been involved. A considerable difference in Trials 1 and 2 was the insertion period of vaginal sponges (12 and 9 days, respectively). Usually, progestogen sponges were inserted into the vagina for 12–14 days (12 days: Moore and Holst, 1967; Gordon, 1971; Boland *et al.*, 1981; Crosby *et al.*, 1983, and 14 days: Alifakiotis *et al.*, 1982; Steffan *et al.*, 1982/83). However, a shorter insertion period (7 days: Fitzgard *et al.*, 1985, and 9 days: Fukui *et al.*, 1983, 1985) has been examined with successful results on synchronization of estrus in cyclic ewes and induction of estrus during the non-breeding season. Morgan *et al.* (1967) examined the absorption rate of progestogen sponges (FGA) from the vaginal wall in the different insertion periods, and found that 50% was absorbed in the first 4 days, 75% by 8 days and 94% by 16 days. Morgan *et al.* (1967) concluded that estrus control was effective at 8-day insertion period and much less effective at 16 days.

The sponge-holding rate of ewes during the insertion period was satisfactory in Trial 1 including the self-made vaginal sponges. However, the rate of sponge loss (18.6%) in Trial 2 was high although the insertion period was shorter in Trial 2 than in Trial 1 (9 and 12 days). All ewes lost their sponges were in Groups II and III with which progesterone had been impregnated. No clear reasons were able to be found. At the time of sponge insertion in Trial 2, more ewes were difficult to deposit the sponges into the bottom of the vagina, as compared with those in Trial 1.

In conclusion, the type of vaginal sponges and progestogen used in the present study did not show significant differences on estrus induction and lambing rate. The self-made vaginal sponge impregnated with 500 mg progesterone could be useful for out-of-seasonal breeding of ewes.

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季節性無発情雌羊の発情誘起および分娩率に対する膣内スポンジとプロジエストジエンの効果

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めん羊の季節外繁殖に使用するため，500 mg プロジエステロン含有の自家製膣内スポンジを作製した。この膣内スポンジの発情誘起および分娩率に対する効果を検討するために，2つの野外試験を実施した。方法は，I) 60 mg メドロキシプロジエステロン・アセテート (MAP) を含有した膣内スポンジ (MAPスポンジ・オーストラリア，アップジョン社製)，II) MAP不含の同上の膣内スポンジに，500 mg プロジエステロンを含有したもの，III) 自家製膣内スポンジに，500 mg プロジエステロンを含有したもの，の3種の膣内スポンジを使用した。膣内スポンジの挿入期間は，12日間（実験1）または9日間（実験2）とし，除去後 PMSG (妊馬血清性性腺刺激ホルモン) 600 IU を筋肉内注射した。発情発見時に，合成 LH-RH（コンセラール）100 μg を筋肉内注射し，その後6時間目に1回の自然交配を実施した。

実験1において処置後5日間の発情誘起率は，I)，II)，III) でそれぞれ，100%，100%，94%であり，有意差はなかったが，500 mg プロジエステロン含有の膣内スポンジでは (II および III)，処置後から発情発見までの時間が有意に ($P<0.05$) 短かかった。分娩率は，それぞれ，85%，60%，57%であったが，有意差は認められなかった。実験2の発情誘起率は，88%，68%，68%であり，使用した膣内スポンジ間に有意差はなかった。分娩率は，32%，31%，33%と各群とも低率であったが，処置間に有意差は見られなかった。

以上の成績から，500 mg プロジエステロン含有の自家製膣内スポンジは，従来の合成プロジエステロン (60 mg MAP) 含有の膣内スポンジと同程度の効果が認められ，めん羊の季節外繁殖に充分，使用できるものと思われた。