— Research Note —

Single or Double Inseminations at Fixed-Time Basis on Lambing Rate of Ewes Treated with Progestogen-Impregnated Intravaginal Sponges during the Non-Breeding Season

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Summary. Effect of a single or double inseminations at fixed-time basis on lambing rate of ewes treated with 2 different progestogen-impregnated intravaginal sponges during the non-breeding season was investigated in 3 trials (Farms T, H and S). A total of 236 mature Suffolk ewes were treated with either 60 mg medroxyprogesterone acetate (MAP) - or 500 mg progesterone (P) impregnated intravaginal sponge for 9 days and an intramuscular injection of 600 IU PMSG one day before sponge removal. The MAP- and P-treated ewes were inseminated once (36 hr) or twice (30 and 42 hr) with fresh-undiluted semen after sponge removal. The lambing rates in 4 groups were 44.6, 50.9, 46.6 and 50.0% for MAP-single, MAP-double, P-single and P-double inseminations, respectively. There were no significant differences in the lambing rates between the two types of sponges (46.5 and 48.3% for MAP and P sponges, respectively) and between the numbers of insemination (45.6 and 50.4% for a single and double insemination, respectively). The lambing rate in Farm H (33.7%) was significantly lower than those in Farms T (50.0%: $p < 0.05$) and S (61.7%: $p < 0.01$).

KEY WORDS: ARTIFICIAL INSEMINATION, INTRAVAGINAL SPONGE, NON-BREEDING SEASON, LAMBING RATE, SHEEP.

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Induced estrus in ewes treated with progestogen-impregnated intravaginal sponge and pregnant mare's serum gonadotropin (PMSG) during the non-breeding season is usually synchronized within 3 days after treatment (Fukui *et al.*, 1985, 1987, 1989; Kobayashi *et al.*, 1986). The subsequent fertility of ewes by natural mating is affected by many factors, such as the ratio of ram *versus*, ewe, libido and semen quality of rams used, and ages of ewes and rams (Fowler, 1984; Fukui *et al.*, 1986). In this respect, artificial insemination (AI) is probably the only alternative tool to be able to eliminate the factors affecting fertility of ewes. Espe-

cially, if a method of AI by a fixed-time basis is established (Dziuk *et al.*, 1972; Evans *et al.* 1986; Maxwell, 1986; Akaike *et al.*, 1989; Fukui *et al.*, 1989) without lowering lambing rate (60 to 80%), estrus detection by teaser rams becomes unnecessary and the mating system could be simplified.

In the present study, effect of a single or double fixed-time AI using fresh-undiluted semen was compared in seasonally anestrous ewes treated with 2 different progestogen-impregnated intravaginal sponges of 60 mg medroxyprogesterone acetate (MAP) and 500 mg progesterone (P).

Materials and Methods

Three trials were conducted at 3 sheep farms in Hokkaido, Japan: The Tawa Field Station (Farm T), Shibechya-cho during May, 1989, The Happy Farm (Farm H), Noboribetsu-shi during May, 1989 and the Shibetsu Sheep Farm (Farm S), Shibetsu-shi during July, 1989. A total of 236 mature Suffolk ewes used at the 3 farms (Farms T, H and S: 64, 88 and 84 ewes, respectively). The mean ages (years $\pm$ S.D.) of ewes were 4.4 ± 1.5 , 5.8 ± 1.7 and 3.8 ± 1.4 in the Farms T, H and S, respectively ($P < 0.05$).

At each farm, ewes were treated with either 60 mg MAP-impregnated (The Upjohn International Ltd., Australia) or self-made, 500 mg P-impregnated (Fukui *et al.*, 1987) intravaginal sponge. The sponges were inserted into the vagina for 9 days and an intramuscular injection of 600 IU PMSG (Serotropin, Teikoku-zoki Co., Japan) was administered to the ewes one day before sponge removal. At the time of sponge removal, it was found that 3 (2 and 1 ewes in Farms H and S, respectively) and 2 (Farms S) ewes out of 116 and 120 ewes treated with MAP and P sponges respectively, had lost their sponges during the insertion period, and those 5 ewes were eliminated from experiment.

Semen was collected with an artificial vagina from 5 to 10 Suffolk rams in each farm and only fresh ejaculates of good initial motility ($> 80\%$ in forward movement) and a concentration of at least 25×10^9 spermatozoa/ml (determined by haemocytometer) were used, within 30 min. The ewes treated with MAP or P sponge were inseminated once (36 hr after sponge removal) or twice (30 and 42 hr after sponge removal) at fixed-time basis without estrus detection. In each insemination, 0.2 ml of fresh-undiluted semen containing about 5×10^8 spermatozoa was deposited into the first fold of the cervix using a plastic pipette (curved tip) with the aid of a 'duck bill' speculum and a head light. At the time of insemination, score of body condition (1: very thin, 2: thin, 3: fair, 4: fat, 5: very fat) was measured in each ewe by body palpation. The mean ($\pm$ S.D.) scores were 3.4 ± 0.6 , 2.9 ± 0.5 and 3.3 ± 0.5 for Farms T, H and S, respectively. The score in Farm H was significantly ($P < 0.05$) lower than those in Farms T and S.

Fertility of inseminated ewes was expressed as lambing rate. Prolificacy (number of new-born lambs/number of lambed ewes) was also recorded. Data on lambing rate and prolificacy were analyzed by χ^2 test and Student's t-test, respectively.

Table 1. Lambing results of ewes treated with different intravaginal sponges and inseminated once or twice at fixed-time basis

Intravaginal sponges impregnated	AI group	No. ewes treated & inseminated	No. ewes lambed (%)	Prolificacy
60 mg medroxy-progesterone acetate	1	56	25 (44.6) ^a	1.56 ^a
	2	57	29 (50.9) ^a	1.59 ^a
	Total	113	54 (46.5) ^A	1.58 ^A
500 mg progesterone	1	58	27 (46.6) ^a	1.56 ^a
	2	60	30 (50.0) ^a	1.73 ^a
	Total	118	57 (48.3) ^A	1.65 ^A

a, A: The figures with the same superscript in each column are not significantly different ($P > 0.05$).

Results

The lambing results in a total of 3 farms are shown in Table 1. There was no significant differences in lambing rates between MAP (53/114: 46.5%) and P (57/118: 48.3%) sponges. The lambing rate by a single insemination was similar to a double insemination (45.6 and 50.4%, respectively), and they were not significantly different ($p>0.05$). Prolificacy was also not significantly different among the 4 groups. Table 2 shows results of lambing rate and prolificacy in 3 farms. The lambing rate in Farm H was significantly lower than those in Farms T (50.0%: $p<0.05$) and S (61.7%: $p<0.01$), but differences in prolificacy between groups were not significant.

Table 2. Lambing results of ewes treated and inseminated at three different farms

Farms	No. ewes treated & inseminated	No. ewes lambed (%)	Prolificacy
T	64	32 (50.0) ^a	1.84 ^d
H	86	29 (33.7) ^b	1.62 ^d
S	81	50 (61.7) ^c	1.46 ^d

a vs. b: $p<0.05$, b vs. c: $p<0.01$.

d: Not significant ($p>0.05$).

Discussion

The present study indicate that the type of progestogen-impregnated intravaginal sponges and a single or double insemination at fixed-time basis did not differ in lambing rate of ewes treated during the non-breeding season. The present study using the 2 types of intravaginal sponges has confirmed the previous results (Fukui *et al.*, 1987) showing that both MAP and P sponges were effective for induction of estrus and the subsequent lambing rate in seasonally anestrous ewes. Crosby *et al.*, (1983) compared intravaginal sponges impregnated

with MAP or 500 mg P on the induction of estrus and lambing rate during the late anestrous season, and showed that estrus incidence (98 and 92% for MAP and P sponges, respectively) and lambing rates by a hand service (68 and 70%, respectively) were similar in both treatments. More recently, a new intravaginal device called the controlled internal drug release dispenser (CIDR) impregnated with 300 to 400 mg progesterone has been tested in New Zealand (Barness and Welch, 1984/85) and found to be favorable compared with fluorogesterone acetate (FGA) sponge (Maxwell and Barness, 1986) and with MAP sponge (Rhodes and Nathanielsz, 1988).

It has been reported that an intravaginal sponge impregnated with progesterone or CIDR induced estrus earlier by 12 to 24 hr than MAP sponge (Fukui *et al.*, 1987; Rhodes and Nathanielsz, 1988). Therefore, the timing of fixed-time insemination following treatment is likely affected by the type of progestogen-impregnated intravaginal sponge used. The timing for a fixed-time insemination has been also affected by the time of PMSG injection (Fukui *et al.*, 1989). Fukui *et al.*, (1989) reported that estrus incidence occurred between 36 and 48 hr after treatment when ewes were treated with MAP sponge and a PMSG injection at the time of sponge removal. On the other hand, estrus was synchronized between 24 and 36 hr after sponge removal, when a PMSG injection was given two days before sponge removal. The time of AI in the present study was determined by the result of an unpublished study using MAP sponge and a PMSG injection one day before sponge removal that estrus was synchronized between 30 and 42 hr. The present study has failed to show an advantage of a double injection with fresh-undiluted semen within one estrus. The result has confirmed the report of Evans and Maxwell (1987) suggested that the effect of a double insemination varies depending on the time of the first

insemination in relation to ovulation and that the increase in lambing rate has been expected by a double insemination with frozen-thawed semen, but is not always with fresh semen. In the present study, the time of the first insemination (30 hr after sponge removal) was slightly early for ewes treated with MAP sponges, because the mean time of estrus incidence was 32 hr compared with 24 hr in ewes treated with P sponges (Fukui *et al.*, 1987). Therefore, the first insemination for double AI should have performed as that for a single insemination (36 hr after sponge removal) in all ewes treated with either MAP or P sponges. Evans *et al.* (1987) reported that the time of fixed insemination is less critical using fresh than frozen semen. Because of the greater longevity of fresh semen compared with frozen semen, earlier insemination with fresh semen may be acceptable. On the other hand, with frozen-thawed semen, AI especially, intrauterine insemination should take place shortly before or after ovulation (Maxwell *et al.*, 1986; Akaike *et al.*, 1989).

The lambing rate by the double insemination in the present study was lower than the report by Langford *et al.* (1982) using FGA sponge (76%) and the previous report (Kobayashi *et al.*, 1987) using natural mating (60 to 80%). The ewes at Farm H were older and had a lower score of body condition compared with the other two farms. This may be caused by the low rate of lambing in Farm H as shown in Table 2. A live weight of 50 kg at joining and the score (3) of body condition are advocated for Merino ewe flock in Australia (King *et al.*, 1990). For increasing the estrus incidence and lambing rate of seasonally anestrus ewes, not only the hormonal treatment and mating method, but also physiological status (Fukui *et al.*, 1985) and nutrition (Egan *et al.*, 1984; Smith and Stewart, 1990) are important factors.

In conclusion, a single, fixed-time insemination (36 hr after sponge removal) with

fresh-undiluted semen was as effective as a double insemination in seasonally anestrus ewes. The self-made P sponge was also equally effective as was MAP sponge.

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非繁殖季節においてプロジェストジェン含有腔内スポンジで処置された雌羊の分娩率に 及ぼす1回または2回定時人工授精の効果

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非繁殖季節において2種のプロジェストジェン含有腔内スポンジとPMSG（妊馬血清性腺刺激ホルモン）で発情誘起された雌羊の発情発見をしないで処置後定時的に1回または2回人工授精を行いその分娩率を比較検討した。本研究は、1989年北海道内の3ヶ所の牧場（T, H, S牧場）において、計236頭のサフォーク種雌羊を用いて行った。各牧場の雌羊に、60mgメドロキシプロジェステロン・アセテート（MAP）含有腔内スポンジまたは500mgプロジェステロン（P）含有の自家製腔内スポンジを9日間腔内に挿入した。そして、スポンジ除去1日前に600IUのPMSGを筋肉内注射した。両群の雌羊に、処置後36時間目に1回または30と42時間目に2回、新鮮原精液（0.2ml, 5×10^8 精子）を子宮頸管外口部に

注入した。

3牧場の総分娩率は、44.6%（MAPスポンジ, 1回授精）, 50.9%（MAPスポンジ, 2回授精）, 46.6%（Pスポンジ, 1回授精）, 50.0%（Pスポンジ, 2回授精）であった。両スポンジの種類（MAP: 46.5%, P: 48.3%）および授精回数（1回: 45.6%, 2回: 50.4%）による分娩率および産子率には有意差は認められなかった。しかし、H牧場の分娩率（33.7%）は他の2牧場よりも有意に低かった。（T牧場: 50.0%, $P < 0.05$, S牧場: 61.7%, $P < 0.01$ ）。以上の結果、非繁殖季節において2種のプロジェストジェン含有腔内スポンジで発情誘起された雌羊の定時人工授精は処置後36時間目の1回授精で十分有効であると思われる。