Studies on Induction of Estrus and Ovulation, and the Subsequent Fertility in Anestrous Ewes during the Non-breeding Season

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Abstract

Induction of estrus and ovulation in 20 non-lactating anestrous ewes using 750 i. u. PMSG (Group A : 10 ewes) or 15 mg $PGF_{2\alpha}$ (Group B : 10 ewes) after a 9 days insertion of MAP (60 mg of 6-Methyl-17-Acetoxyprogesterone) intravaginal sponges was studied during the non-breeding season.

All of the 10 ewes and 4 out of the 10 ewes of Groups A and B, respectively, showed estrus within 5 days after treatment. Ovulation was observed in 4 out of 4 laparotomized ewes in Group A, whereas no ovulation was observed in the 4 laparotomized ewes of Group B. Non-return, pregnancy and lambing rates and number of lambs per ewe were : Group A ; 80% (8/10), 40% (4/10), 30% (3/10) and 2.33 (including 3 dead lambs after birth), Group B ; 75% (3/4), 25% (1/4), 25% (1/4) and 1.00. In Group A, a peak of plasma estradiol-17 β was observed on the day before the incidence of estrus and plasma progesterone levels increased after ovulation. A similar estradiol-17β peak was observed in all 4 ewes of Group B, but no ewe exhibited estrus. Underlying reasons were discussed.

It was concluded that treatment with MAP sponge and PMSG is more effective for induction of estrus and ovulation in ewes during the anestrous season, and that multiple births are also obtained by the use of PMSG after progestogen treatment.

Introduction

Induction of estrus and ovulation in the sheep during the non-breeding season is a significant method to produce more lambs per year. Many workers have been utilizing progestogen intravaginal sponge and Pregnant Mare Serum Gonadoropin (PMSG) for this purpose (GORDON, 1958 ; WISHART, 1966 ; LASTER and GLIMP, 1974 ; CHRISTENSON, 1976 ; FLETCHER et al., 1980 ; LUNSTRA and CHRISTENSON, 1981a). However, the treatment of ewes with progestogen and PMSG during anestrous season results in reduced lambing responses (GORDON, 1963 ; ROBERTS and EDGAR, 1966 ; BETTS et al., 1969 ; RANKIN et al., 1969).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been widely used for regression of corpus luteum to initiate a fertile estrus in cycling ewes (OHTAKE et al., 1975 ; HARESIGN, 1976 ; FUKUI and ROBERTS, 1977 ; HARESIGN and ACRITOPOULOU, 1978). A combined treatment with a short period administration (9-12 day) of progestogen and $PGF_{2\alpha}$ thereafter has also been studied to im-

prove fertility at the first regulated estrus in cattle (HEERSCHE et al., 1974; THIMONIER et al., 1975) and in cycling ewes (LOUBSER and Van NIEKERK, 1981). However, previous reports on the use of $PGF_{2\alpha}$ in anestrous ewes have not been noted.

In the present study, effect of progestogen intravaginal sponge (MAP: 60 mg of 6-methyl-17-acetoxyproggesterone) and subsequent $PGF_{2\alpha}$ treatment was compared with that of MAP sponge and subsequent PMSG treatment in anestrous ewes for induction of estrus and ovulation. Fertility following the treatments was also investigated.

Materials and Methods

The present study was conducted between May 25th and July 18th, 1981. Five Corridale, 6 Suffolk and 9 Corridale x Suffolk non lactating ewes (University Farm: 2, M Farm: 6 and H Farm: 12) were randomly divided into two groups (A and B) of 10 ewes each. Identification was made by colour-branding of each ewe.

All ewes were pretreated with MAP sponges for 9 days. The method of sponge insertion as described by FUKUI and ROBERTS (1979) was followed. Immediately after sponge removal, Group A ewes were treated with a single intramuscular injection of 750 i. u. PMSG in 1.0 m*l* of phosphate buffered saline, while Group B ewes were treated with a single intramuscular injection of 15 mg $PGF_{2\alpha}$ in 3 m*l* of the solution (Prostin $F_{2\alpha}$: Upjohn Co., U.S.A.).

The treated ewes were run with 5 mature rams (University Farm: 1, M Farm: 2 and H Farm: 2) fitted with harness and crayons and were examined for estrus twice daily (08: 00 and 17: 00 hrs) for a period of 5 days after treatment. Ovulation was examined by laparotomy 8 day after treatment in 4 ewes of each group. The ewes were observed for non-return to service by the harnessed rams between 16 and 21 days after the first estrus. Pregnancy diagnosis was carried out by an ultrasonic Doppler method between 50 and 70 days after treatment and lambing rates were also recorded.

Blood was collected from 4 ewes of each group (at the University and M Farms) beginning from 17 days before the progestogen sponge insertion until 20 days after treatment. The frequency of blood collection was variable with 1-3 days interval and the time of sampling was between 10 a. m. and noon. Blood plasma was isolated and stored at -20°C until measurement of estradiol-17β (E_2) and progesterone (P) concentrations by radioimmunoassay (RIA) following the method of MAKINO (1973).

Results

Results are summarized in Table 1. All of the 10

Table 1. Results of induction of estrus and ovulation, and the subsequent fertility in ewes treated with MAP sponges and either PMSG or $PGF_{2\alpha}$ during the non-breeding season

Treatment (Groups)	No. of ewes	No. of ewes in estrus[a]	No. of ewes ovulated/ laparotomized (No. ovulation)	N. R. (16 – 21 days)	Pregnant[b] (50 – 70 days)	Lambing	No. lambs born/ lambing ewes
MAP + PMSG[c] (A)	10	10**	4/4 (11)	8	4	3 (30.0%)	2.33[e]
MAP + $PGF_{2\alpha}$[d] (B)	10	4	0/4 (0)	3	1	1 (25.0%)	1.00

a : The period within 5 days after treatment

b : Examined by an ultrasonic method

c : MAP sponge for 9 days and 750 i. u. PMSG injection (I. M.)

d : MAP sponge for 9 days and 15 mg $PGF_{2\alpha}$ injection (I. M.)

e : 3 lambs died after birth

** : $P<0.005$ to the figure in the same column

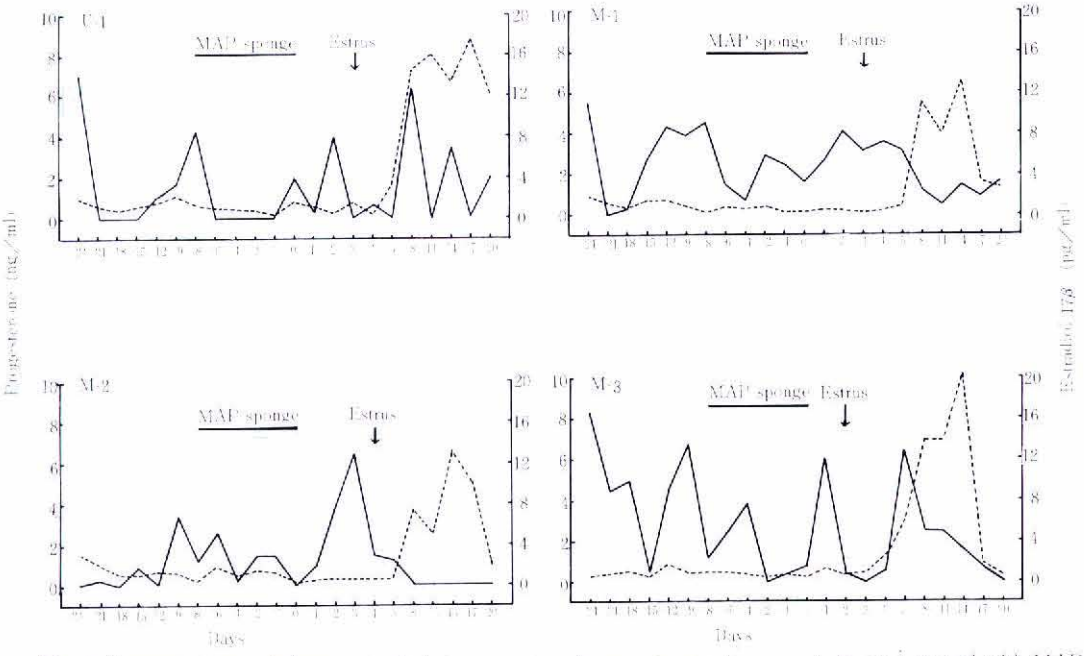

Fig. 1. Concentrations of plasma estradiol (————) and progesterone (·····) in ewes treated with MAP sponge and PMSG thereafter during the non-breeding season

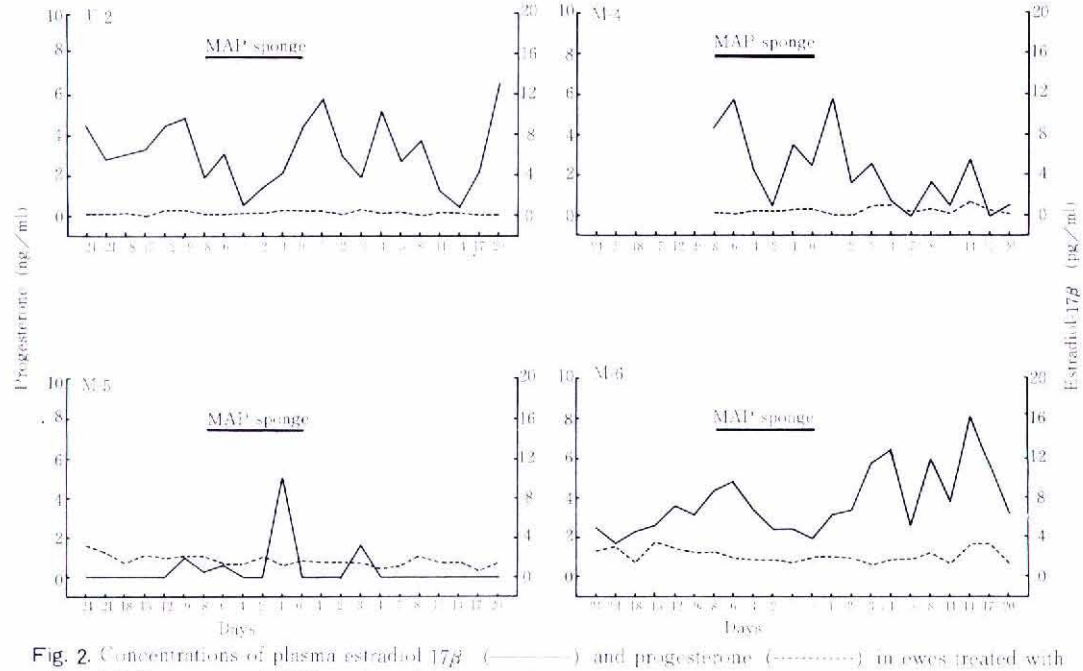

Fig. 2. Concentrations of plasma estradiol 17β (————) and progesterone (·····) in ewes treated with MAP sponge and PGF 2α thereafter during the non-breeding season

ewes in Group A and 4 out of the 10 ewes in Group B showed estrus within 5 days after treatment. There was a significant difference on the proportion of estrous ewes between the groups ($P<0.005$). Ovulation was observed in all of the 4 laparotomized ewes of Group A with a total number of 11 ovulations (i. e. 2.75/ewe) including 2 ewes with 5 and 4 ovulation sites, respectively. On the other hand, ovulation was not observed in the 4 laparotomized ewes of Group B. The ovaries of those ewes contained a number of small follicles sized 1–3 mm in diameter.

Non-return rates of Group A (80% : 8/10) and Group B (75% : 3/4) were similar with no significant difference. The proportions of pregnant ewes were 4 out of 10 and 1 out of 4 for Groups A and B, respectively. Three ewes of Group A and one ewe of Group B lambed with a gestation period range of 147 to 151 days. The number of lambs per ewe were 2.33 and 1.00 for Groups A and B, respectively. In Group A, one ewe lambed to quadruplets while another lambed to twins. However, 2 of the quadruplets and one of the twins died within a day after birth.

The hormonal patterns before and after treatment are shown in Fig. 1 and 2 for PMSG and $PGF_2\alpha$ injections after MAP sponge treatment, respectively. The P level has been maintained at the basal line before and during treatment in both groups. In 4 ewes of Group A, a peak of E_2 (7.7–12.9 ng/m*l*) appeared on the day before the incidence of estrus. The second E_2 peaks were also observed 5 or 6 days later following the first peak in the ewes of U-1 (12.6 pg/m*l*) and M-3 (12.9 Pg/m*l*). The P levels increased to 4.0–10.0 ng/m*l* after ovulation in the 4 ewes which was confirmed by laparotomy. However, P levels in 3 ewes (M-1, M-2 and M-3) declined at the 20th day after treatment indicating non conception. The P level of U-1 was maintained at a high level (6.0 ng/m*l*) 20 days after treatment, and the ewe lambed guadruplets.

In Group B, a peak of plasma E_2 (10.0–12.9 pg/m*l*) was also observed in the 4 ewes immediately before (M-5) or after treatment, although no ewe exhibited estrus. Plasma P levels have been maintained at 0.04–1.58 ng/m*l* throughout the present study.

Discussion

The insertion period of vaginal sponges is usually for 13 to 16 days as an almost same length as the luteal phase of the ewe (ROBINSON, 1967; CUNNINGHAM et al., 1980). However, lowered fertility has been pointed out by the long period of progestogen tratment. (ROBINSON, 1968). The short period (7–12 days) of progestogen treatment has been investigated in the sheep (ROBERTS and BINDON, 1966; FUKUI and ROBERTS, 1979).

In the present study, the treatment with a 9 days insertion of MAP sponge followed by PMSG injection was successful for the induction of estrus and ovulation in anestrous ewes during the non-breeding season. This corraborates with other reports (DAWE et al., 1969; COOPER et al., 1971; GORDON, 1971; LUNSTRA and CHRISTENSON, 1981b). However, lambing was observed only in 3 out of the 10 estrous ewes which demonstrates that high embryonic mortality occurred during the early pregnancy period. The number of pregnant ewes decreased from 8 non-returned ewes at Day 16–21 to 4 ewes at Day 50–70 after treatment. Lowered fertility of estrous ewes treated with progestogen and PMSG during anestrous season is due to imparied fertilization (DAWE and FLETCHER, 1976; LUNSTRA and CHRISTENSON, 1981b) and embryonic mortality (COGNIE et al., 1975; LUNSTRA and CHRISTENSON, 1981b). A reduction in semen quality of rams as a possible major factor affecting the conception rate of ewes treated and mated during anestrous season (LUNSTRA and CHRISTENSON, 1981a), may explain the lowered lambing performance in the present study. The two rams used at the M Farm did not show a high libido due likely to their younger age than the other rams used at University and H Farms, and probably to seasonal influences. Only rams with good quality of semen as well as a high libido must be used to prevent lowered fertility especially during anestrous season (FUKUI and ROBERTS, 1981).

$PGF_2\alpha$ and it's synthetic analogues are very potent luteolytic factors in cycling ewes. However, in anestrous ewes, induction of estrus and ovulation is not feasible by injection of $PGF_2\alpha$ since $PGF_2\alpha$ cannot trigger LH release from the anterior pituitary (CARLSON et al., 1973). In the present study, estrus was induced in 4 out of the 10 ewes treated with MAP sponge and $PGF_2\alpha$, but no ovulation was observed in the 4 laparotomized ewes. The fact that one of the 4 estrous ewes lambed, is difficult to explain. Assumedly, one of 6 non-laparotomized ewes ovulated and conceived.

The Plasma P was at basal level during the treatment of MAP sponge which seems to be due to a physiological process rather than to the availability of the hormone from the sponge (SYMONS et al., 1974). As shown in Fig. 2, a peak of E_2 (10.0-12.9 pg/m*l*) in plasma was observed within 5 days after $PGF_2\alpha$ treatment and had very similar concentrations and patterns to those of estrous ewes treated with PMSG following progestogen treatment (7.7-12.9 pg/m*l*). Regardless of season, serum estradiol concentration of 0.5-1.5 pg/m*l* fails to induce an LH surge, while estradiol concentrations greater than 4 pg/m*l* (GOODMAN and KARSCH, 1980) or with the range of 3-7 pg/m*l* (KARSCH et al., 1979) induce estrous behavior and produce an LH surge in ewes. Furthermore, GODING et al. (1969) reported that administration of E_2 induced LH release in anestrous ewes. As the E_2 concentration in the plasma of ewes treated with $PGF_2\alpha$ following the MAP sponge removal were higher than 4 pg/m*l* in the present study, it would be sufficient to induce estrous behavior and a LH surge. However, estrus was observed in only 4 out of the 10 $PGF_2\alpha$ treated ewes. This may be explained by the report of LAND et al. (1976) describing that estrogens may become less effective in inducing a LH release in ewes during anestrous season. From the fact that one ewe treated with MAP sponge and $PGF_2\alpha$ lambed, a LH surge may have been induced and ovulated.

For future works, LH levels in the plasma should be investigated.

Whether the 9 days progestogen pretreatment followed by $PGF_2\alpha$ injection is more effective than MAP sponge alone for induction of estrus and ovulation during anestrous season, could be elucidated by further detailed studies. It appears from the present study that treatment with MAP sponge and PMSG is more effective for induction of estrus and ovulation in anestrous ewes than treatment with MAP sponge and $PGF_2\alpha$ and that multiple births are obtained by the former treatment.

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非繁殖季節におけるめん羊の発情および排卵誘起と受胎性について

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摘　　要

めん羊の非繁殖季節(5月～7月)において，コリデール種，サフォーク種そして両種の雑種，計20頭の雌羊を用いて発情，排卵誘起を試みた。方法は，全頭に合成黄体ホルモン（6-Methyl-17-Acetoxyprogesterone: MAP) 60 mg を含む腟内スポンジを9日間腟深部に挿入した。腟内スポンジ除去後10頭づつの2群に別け，750 i.u. PMSG（A群）または15 mg $PGF_2\alpha$（B群）を各々筋肉内注射した。その後，マーキング・ハーネスを付着した正常雄羊を雌羊群に5日間同居させた。朝・夕（8時と5時）に発情観察を行なった。排卵は処置後8日目に各群の4頭を開腹し，確認した。受胎率として，Non-Return (N. R. 16-21日)，超音波ドップラー法による妊娠診断（50～70日）および分娩率を記録した。また，黄体ホルモン処置前17日から処置後20日までの期間，1～3日毎に各群4頭において頸静脈から採血し，血漿中のエストラジオール 17β (E_2) とプロゲステロン (P) 濃度をRIA法により測定した。

A群では10頭中全頭，B群では10頭中4頭が処置後5日以内に発情が出現した。排卵はA群の4頭すべてに観察された（排卵数/雌羊頭数：2.75)。一方，B群では排卵は認められず，1～3 mmの卵胞が多数存在していた。両群において，N.R.率，妊娠率，分娩率および出生子羊数/分娩母羊数は以下のとおりである。A群：80% (8/10)，40% (4/10)，30% (3/10) そして7/4 (2.33：3頭の死亡羊を含む)，B群：75% (3/4)，25% (1/4)，25% (1/4) そして1/1 (1.00)。発情を示したA群の4頭において，血漿中の E_2 レベルは発情前日に高いピーク(7.7～12.9 Pg/ml)が見られ，排卵後はPが上昇し処置後20日目のPレベルから受胎・不受胎が推察された。一方，B群の4頭においても，処置後 E_2 レベルのピークが見られたが発情，排卵は示さなかった。

以上の成績から，非繁殖季節中のめん羊の発情，排卵にはMAP処置後 $PGF_2\alpha$ よりもPMSG注射がより効果的であり，多胎分娩の可能性が示唆された。

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