

**Synchronization of œstrus in ewes
with Provera sponges/PMSG, prostaglandin $F_{2\alpha}$
or the prostaglandin analogue, ICI 80996,
and fertility following natural mating or artificial insemination**

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Summary. Following the synchronization of œstrus with different treatments during the breeding season, the fertility of naturally-mated ewes has been compared to that of ewes artificially inseminated with fresh semen. The following treatments were used :

- I. sponges impregnated with progestagen (50-60 mg of medroxyprogesterone acetate) left *in situ* for 14 days and an i.m. injection of 500 IU of PMSG at sponge withdrawal ;
- II. injections of prostaglandin $F_{2\alpha}$ (dose : 15 mg/injection) at intervals varying from 9 to 14 days (see tables) ;
- III. injections of the prostaglandin $F_{2\alpha}$ analogue, ICI 80996 (dose : 100 μ g/injection), with the same time intervals as in treatment II.

Lambing rate and prolificacy after natural service at the induced œstrus was 55 and 140 p. 100, 32.5 and 153 p. 100 and 60 and 133 p. 100 for ewes receiving treatments I, II or III, respectively, compared to 62.5 and 120 p. 100 for the untreated controls.

Double artificial insemination (AI) 48 and 58 h after the sponge/PMSG treatment resulted in a lambing rate of 25 p. 100 and a prolificacy of 133 p. 100. The lambing rate and prolificacy of ewes inseminated 58 and 68 h after the final injection of $PGF_{2\alpha}$ or ICI 80996 were 27.8 and 149 p. 100 and 45.5 and 165 p. 100, respectively.

Single AI 55 h after sponge withdrawal gave a lambing rate of 37.8 p. 100. The lambing rate of ewes inseminated 56 h after the final ICI 80996 injection was higher (54.8 p. 100) than that of ewes inseminated at 60 h (37.5 p. 100) or 66 h (30.8 p. 100). However, two inseminations 56 and 66 h after the final ICI 80996 injection gave an even more elevated lambing rate (61.9 p. 100).

These results demonstrate that ICI 80996 can successfully control œstrus in the ewe during the breeding season, thus offering an alternative to sponges/PMSG and that the fertility subsequent to both natural mating and AI is equivalent to that of the controls.

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Introduction.

The ability to successfully control oestrus and ovulation in sheep would provide a number of practical and economic advantages. First, it would allow batch-lambing, resulting in better flock management at lambing and thereby reducing feeding and labour costs. Second, if combined with AI it would allow the exploitation of genetically superior sires. However, the most important prerequisite of AI is the ability to synchronize ovulation with no subsequent impairment of fertility.

In the past, synthetic progestagens have been used with PMSG to control ovulation in sheep. Lowered fertility of the treated animals has been the major problem associated with this method of oestrous control (Robinson *et al.*, 1967; Holst and Moore, 1970). Treating ewes with progestagens also leads to the abnormal production of cervical mucus (Smith and Allison, 1971). Further, the timing of the preovulatory LH peak relative to the onset of progestagen-induced oestrus is significantly different from that of natural oestrus (Lintin *et al.*, 1973).

More recently, following the demonstration that $\text{PGF}_{2\alpha}$ is the uterine luteolysin in the ewe (Goding, 1974), this material and its analogue, ICI 80996, have been used to induce oestrus in cyclic ewes (Acritopoulou *et al.*, 1977; Baird and Scaramuzzi, 1977). The endocrine changes in progesterone and LH that occur during the ICI 80996-induced oestrous cycle closely resemble those found during a natural oestrous cycle (Acritopoulou *et al.*, 1977). Moreover, there is a high degree of synchrony in the return to oestrus and ovulation following treatment with the prostaglandin analogue (Acritopoulou *et al.*, 1978).

The experiments described in the present investigation were therefore designed to compare the effectiveness of the three different methods of controlling oestrus and ovulation in ewes during the breeding season. The fertility of ewes with induced oestrus and either AI or natural mating has been compared to that of untreated controls.

Material and methods.

Sheep of two native milking breeds, the Karagounico (K) and the Serres (S), showing reproductive activity from early summer through mid-autumn, were used in all the experiments. They varied in age from 18 months to 8 years. All four experiments were carried out during the breeding season in three consecutive years (1978-1980). To ensure that the sheep had regular oestrous cycle activity, teaser rams were introduced for 1 h twice daily (at 07.00 and 18.00 h) beginning one month before the start of the experiments. The ewes in all the experiments were treated without reference to the stage of the oestrous cycle.

The treatments used were as follows :

- I. Provera intravaginal sponges impregnated with 50-60 mg of medroxyprogesterone acetate were left *in situ* for 14 days. Upon sponge removal, an intramuscular injection of 500 IU of PMSG was given ;
- II. 15 mg of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) (Upjohn, Kalamazoo) were administered intramuscularly in a sterile, aqueous solution (5 mg/ml) ;

III. 100 µg of prostaglandin analogue, ICI 80996 (ICI Ltd, England), were given intramuscularly in 2 ml of a sterile saline solution (9 p. 1 000 NaCl).

The number of PGF_{2α} or ICI 80996 injections, the interval between them and the insemination time varied in the four experiments (see tables 1, 2, 3, 4). The choice was based on the data of preliminary studies (Acritopoulou, unpublished data). From 24 to 84 h after the injection of PMSG or the final injection of PGF_{2α} or ICI 80996, the treated ewes were checked for oestrus, using teaser rams at 4-hour (exp. 1) or 12-hour (exps. 2, 3, 4) intervals.

In experiment 1, the treated ewes were hand-mated to rams of proven fertility at the onset of oestrus and again 10 h later. Control ewes were checked for oestrus (at 07.00 h and 18.00 h) during the experimental period and were hand-mated at the detected oestrus and again 10 h later. In experiments 2, 3 and 4, AI was carried out using 0.2 ml of fresh diluted (1 : 1) semen (> 160 million motile sperm/0.2 ml of semen).

Following natural mating or AI after induced oestrus, the ewes of the different groups ran together with colour-marked fertile rams to cover returns to service. All data on the lambing and prolificacy of each ewe were recorded at lambing in all experiments. The χ^2 analysis and the t-test were used to statistically analyze the data.

Results.

In this investigation, we considered the return to oestrus up to 72 h after treatment as the « response to treatment ». « Treatment » refers to either the time of the PMSG injection or the final injection of PGF_{2α} or ICI 80996.

Experiment 1. — As shown in figure 1, by 48 h after PMSG injection, 90 p. 100

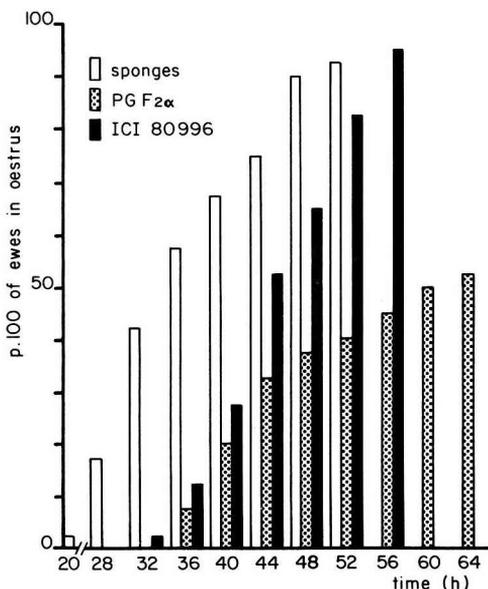


FIG. 1. — Time (h) from treatment to onset of oestrus.

TABLE 1
Timing of oestrus, lambing rate and prolificacy of hormonally-treated and control ewes

Group Treatment	I	II	III	IV
	Sponges/PMSG	PGF _{2α} (1)	ICI 80996 (1)	Controls
Time of natural mating	At induced oestrus and 10 h later			
Number of treated ewes	40	40	40	40
Number of ewes responding within 72 h of treatment	37	24	38	—
Percentage of ewes in oestrus after treatment (p. 100)	92.5 (a)	60.0 (b)	95.0 (a)	—
Time (h) from treatment to onset of oestrus.				
M ± SE	36.7 ± 1.3	48.1 ± 2.1	45.8 ± 1.1	—
Lambing rate (p. 100)	55.0 (c)	32.5 (d)	60.0 (c)	62.5 (c)
Prolificacy (p. 100)	140	153	133	120

(1) Two injections at a 9-day interval.
P < 0.05 in comparing a to b.
P < 0.025 in comparing c to d.

TABLE 2
Lambing rate and prolificacy of ewes after synchronisation of ovulation

Group Treatment	I	II	III
	Sponges/PMSG	PGF _{2α}	ICI 80996
Time (h) from treatment to AI	At induced oestrus and 10 h later		
Number of treated ewes	48 & 58	58 & 68	58 & 68
Number of ewes responding within 72 h of treatment	60	25	34
Lambing rate of treated ewes (p. 100)	28.6	24.0	45.5
Prolificacy (p. 100)	133	133	146

(1) Two injections given on Days 1 and 14 no matter at what stage of the cycle treatment commenced.
(2) Three injections given on Days 1, 10 and 24 no matter at what stage of the cycle treatment commenced.
P < 0.10 in comparing a to b.

of the ewes of this group had returned to oestrus. Only 45 p. 100 of the PGF_{2 α} -treated ones were in oestrus by 56 h after the second PGF_{2 α} injection, while 95 p. 100 of the ewes treated with ICI 80996 came into oestrus within the same time interval. Treatment with ICI 80996 or PGF_{2 α} did not appear to affect the length of the oestrous cycle of the treated ewes since those not conceiving returned to service 15 to 19 days later. Provera sponges/PMSG and ICI 80996 were equally effective in synchronizing oestrus and subsequent fertility (table 1). These treatments, significantly better than PGF_{2 α} , were indistinguishable from the controls.

Experiment 2. — Table 2 shows that treating the ewes with ICI 80996 resulted in higher lambing rates after AI than treatment with PGF_{2 α} or sponges/PMSG. The χ^2 analysis of the data indicated that there was no significant difference in the lambing rate with 2 or 3 injections of PGF_{2 α} or ICI 80996. AI with the latter treatment, as compared to sponges/PMSG, resulted in a significantly higher lambing rate ($P < 0.10$).

Experiment 3. — The results in the two breeds were about the same ; out of 129 ewes, 83 (64.3 p. 100) did not return to service and 71 (55.0 p. 100) lambed, producing 82 lambs (116 p. 100). Following AI, the lambing peak (60 p. 100) of group S occurred on Day 146 and that of group K on Day 149.

TABLE 3

Fertility of ewes treated with 3 injections of ICI 80996

Group Treatment	Serres 3 injections of ICI 80996 ⁽¹⁾	Karagounico
Time (h) from treatment to AI	56 & 66	56 & 66
Number of treated ewes	62	67
Number of ewes responding within 72 h of treatment	58	62
Lambing rate of treated ewes (p. 100)	57.4	51.5
Prolificacy (p. 100)	126	106

⁽¹⁾ Injections given on Days 1, 10 and 24 without reference to the day of the oestrous cycle on which treatment commenced.

Experiment 4. — Fixed-time double AI of ewes 56 and 66 h after the second ICI 80996 injection resulted on a higher lambing rate than single AI at 56, 60 or 66 h after the second ICI 80996 injection (table 4). AI of sponge-treated ewes 55 h after PMSG injection gave a lambing rate of 37.8 p. 100 ; this percentage was similar to that (37.5 p. 100) of ewes treated with ICI 80996 and inseminated at 60 h. χ^2 analysis showed that double AI at 56 and 66 h or single AI at 56 h after treatment with ICI 80996 resulted in a significantly higher lambing rate than single AI at 60 or 66 h ($P < 0.05$). The treatment with sponges/PMSG resulted in a significantly

lower lambing rate at induced oestrus than double or single AI 56 h after ICI 80996 treatment ($P < 0.025$).

TABLE 4

Lambing rate and prolificacy of ewes treated with sponges/PMSG or ICI 80996 and artificially inseminated

Treatment	Sponges/PMSG		ICI 80996 (1)		
	55	56 & 66	56	60	66
Time (h) from treatment to AI					
Number of treated ewes	45	42	42	32	39
Number of ewes responding within 72 h of treatment	42	40	41	31	39
Lambing rate of treated ewes (p. 100)	37.8 (b)	61.9 (a)	54.8 (a)	37.5 (b)	30.8 (b)
Prolificacy (p. 100)	188	142	130	125	141

(1) Two injections at a 12-day interval.
 $P < 0.05$ in comparing a to b.

Discussion.

The present results clearly demonstrate the high potential of the $\text{PGF}_{2\alpha}$ analogue, ICI 80996, in the control of oestrus and ovulation in ewes during the breeding season under field conditions, thus confirming previous results (Acritopoulou, 1977).

The dose of 15 mg of $\text{PGF}_{2\alpha}$ appeared to be insufficient to cause luteolysis in ewes of the Karagounico and Serres breed since only 60 p. 100 of the treated animals returned to oestrus. Hackett and Robertson (1980) found that when the dose was increased from 15 to 20 mg, the oestrous response increased from 70 to 100 p. 100. However, Rommel *et al.* (1979) obtained an 85 p. 100 synchronisation of oestrus in ewes treated with only 10 mg of $\text{PGF}_{2\alpha}$ while Hackett *et al.* (1980) obtained identical results with 15 mg of $\text{PGF}_{2\alpha}$, both groups of workers using the double-injection regime.

The mean time interval (in this study : 36.7 ± 1.3 h) from the end of the sponge/PMSG treatment to the onset of oestrus is similar to that reported by Evans and Robinson (1980) in Border Leicester \times Merino ewes. However, this value is higher than that of 31.4 ± 0.6 h found by Cognié *et al.* (1970). Similarly, the mean time interval from the second injection of ICI 80996 to the onset of oestrus, recorded in the present study, is longer than that reported in previous experiments (Acritopoulou *et al.*, 1978). It must be emphasized here that when oestrus was checked at 4-hour intervals, as in experiment 1, it could only be detected with an accuracy of ± 4 h. Inaccurate and/or false detection of the onset of oestrus by the teaser rams must also be taken into account.

Of the three methods of ovulation control tested in the present study, the ICI 80996 treatment resulted in higher fertility when combined with either natural

mating or AI. Lambing rate and prolificacy of naturally-mated ewes synchronized with ICI 80996 were equivalent to those of control ewes (table 1), indicating that treatment with this analogue does not depress fertility. Similarly, Bielanski (1978) and Haresign and Acritopoulou (1978) reported no difference in lambing rate between ICI 80996-treated and control ewes. Fertility in naturally-mated, $\text{PGF}_{2\alpha}$ -treated ewes was unsatisfactory due to poor response to the treatment since only 56.5 p. 100 of the ewes lambed in response to this method. This percentage is close to the one reported by Hackett *et al.* (1980).

The effectiveness of the ICI 80996 treatment in controlling breeding was more clearly demonstrated when AI was used; the lambing rate with fixed-time AI and ICI 80996-induced oestrus reached acceptable levels (experiment 2, table 2), agreeing with the findings of Fairnie *et al.* (1976) and Greyling and van der Westhuysen (1980 a). In contrast, fertility with fixed-time AI and synchronization with either sponges/PMSG or $\text{PGF}_{2\alpha}$ was far from satisfactory (table 2). Contrary to our results, fixed-time double AI after ovulatory induction with the sponges gave a conception rate of more than 55 p. 100 for Le Roux (1976), Langford *et al.* (1979) and Greyling and van der Westhuysen (1980 a). The results of experiment 2 show that increasing the number of $\text{PGF}_{2\alpha}$ or ICI 80996 injections from 2 to 3 does not improve the fertility of the treated ewes. This suggests that the double-injection regime (on Days 1 and 14) may be employed as it has the great advantage over the three-injection regime of being time, labour and drug-saving as well as disturbing the animals less.

The results of experiment 3 were very encouraging, demonstrating that when the ICI 80996 treatment is used for oestrous control in sheep, fertility after AI is equivalent to that obtained with natural mating (tables 1, 3).

In experiment 4, the lambing rate was lower after AI and sponge/PMSG-induced oestrus. The lower fertility of animals synchronized by this method of ovulatory control has been a major problem; the treatment of ewes with progestagens has been shown to increase or decrease the production of cervical mucus during the ensuing oestrus (Moore *et al.*, 1967; Smith and Allison, 1971). Indeed, evidence has accumulated suggesting poor sperm transport and survival within the female reproductive tract after progestagen treatment (Quinlivan and Robinson, 1967; Robinson, 1973; Hawk and Cooper, 1977).

Compared to those of experiment 3 (table 3), the data of experiment 4 (table 4) show that fixed-time double AI at 56 and 66 h, after either the two or three-ICI 80996-injection regime, results in a similar lambing rate (about 60 p. 100); this percentage approximates the lambing rate usually obtained at first service in untreated control ewes. These data strengthen our earlier reasons for using the two-injection method (see above). Moreover, they indicate that an interval of 12 or 14 days between the two ICI 80996 injections gives comparable results; reducing this interval to 8 or 9 days is followed by a drop in the fertility level (Fairnie *et al.*, 1977; Greyling and van der Westhuysen (1980 b). Hawk and Cooper (1977) found that sperm numbers were reduced in the cervix of ewes treated with $\text{PGF}_{2\alpha}$ on Day 10, but not in those treated on Day 16. This means that prostaglandins do not interfere with the patterns of sperm transport in the reproductive tract of the ewe at induced oestrus. Hawk and Cooper's results

(1977) suggested that some factor associated with the shortening of the oestrous cycle inhibited sperm transport. There were no differences in the number of spermatozoa recovered from any parts of the genital tract of either PGF_{2α}-treated or control ewes (Fukui and Roberts, 1977).

When the number of inseminations in the present study was reduced from two to one 56 h after injection of ICI 80996, the lambing rate decreased by 7. p. 100 (table 4). It is not easy to judge here if this decrease would justify or not a second insemination. In this study, single AI at 60 or 66 h after ICI 80996 treatment gave rather poor fertility, suggesting that such AI timing was too late for good results.

Although from the present data it appears that the optimal insemination time is around 56 h after the second injection of ICI 80996, Fairnie *et al.* (1976) and Fukui and Roberts (1978) working on Merino ewes, obtained higher conception rates when insemination was carried out from 64 to 70 h after the second ICI 80996 injection.

Conclusions.

Our data show that :

- 1) two injections of 100 µg each of the prostaglandin analogue, ICI 80996, given to cyclic ewes from 9 to 14 days apart, controlled oestrus in all ewes irrespective of the stage of the cycle when the treatment began ;
- 2) Provera sponges/PMSG and ICI 80996 were equally effective in synchronizing oestrus, and the results of hand-mating could not be distinguished from the performance of control ewes. PGF_{2α} was less effective (experiment 1) ;
- 3) double insemination was better than single in all the synchronization experiments ;
- 4) a single insemination 55 h after Provera sponge/PMSG treatment gave results comparable to those obtained with insemination 60 or 66 h after ICI 80996 treatment but poorer than those obtained at 56 h ;
- 5) the choice of insemination time was important ; 56 h was better than 60 or 66 h with ICI 80996.

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Résumé. Au cours de la saison de reproduction, la fertilité de brebis dont les œstrus ont été synchronisés par différents traitements a été comparée après insémination naturelle ou artificielle. Les traitements de synchronisation ont été les suivants :

I. Eponges intravaginales imprégnées de 50-60 mg d'acétate de médroxyprogestérone pour une durée de 14 jours plus 500 U.I. de PMSG lors de l'arrêt du traitement.

II. Injection de prostaglandine $F_{2\alpha}$ (dose 15 mg) ou

III. de l'analogue ICI 80996 (dose 100 μ g)

Après insémination naturelle, les taux d'agnelage et de prolificité sont respectivement de 55 et 140 p. 100, 32,5 et 153 p. 100, 60 et 133 p. 100 pour les femelles recevant les traitements I, II et III. Ils sont de 62,5 et 120 p. 100 pour des brebis ne recevant aucun traitement. L'insémination artificielle avec du sperme frais 48 et 58 h après retrait des éponges vaginales donne un taux d'agnelage de 25 p. 100 et une prolificité de 133 p. 100.

Le taux d'agnelage et la prolificité des brebis inséminées 58 h et 68 h après la dernière injection de $PGF_{2\alpha}$ ou de l'analogue ICI 80996 sont respectivement de 27,8 et 149 p. 100 ; et 45,5 et 165 p. 100.

Après une seule insémination artificielle, 55 h après arrêt du traitement progestagène, les taux de conception, d'agnelage et la prolificité sont de 53,3, 37,8 et 188 p. 100.

Une seule insémination artificielle, effectuée 56 h après la dernière injection de l'analogue ICI 80996, résulte en un taux plus élevé d'agnelage (54,8 p. 100) que lorsqu'elle est faite à 60 h (37,5 p. 100) ou à 66 h (30,8 p. 100). Il est cependant encore plus élevé (61,9 p. 100) avec deux inséminations réalisées 56 h et 66 h après la dernière injection d'ICI 80996.

Ces résultats montrent que l'ICI 80996 peut contrôler avec succès l'œstrus chez la brebis au cours de la saison de reproduction, et que, la fertilité consécutive après insémination naturelle ou artificielle est équivalente à celle des contrôles.

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