FERTILITY IN MERINO EWES IN ARTIFICIAL INSEMINATION PROGRAMMES FOLLOWING SYNCHRONIZATION OF OVULATION USING CLOPROSTENOL, A PROSTAGLANDIN ANALOGUE

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SUMMARY

Ten field and laboratory experiments were conducted in which Merino ewes were treated with one or two 125µg injections of Cloprostenol, an analogue of prostaglandin $F_{2\alpha}$ (PG). All treatments commenced in the luteal phase of the oestrous cycle. Sixty-six percent of ewes were detected in behavioural oestrus within 64 hr of completion of treatments although 95% of all treated ewes ovulated in apparent synchrony.

The time of onset of behavioural oestrus was earlier in ewes treated with two injections of PG eight days apart compared to treatments with two injections of PG 11 or 14 days apart.

Fertility, as assessed from the number of preimplantation embryos obtained by flushing oviducts and uteri of inseminated ewes, was lower in ewes treated with two injections of PG eight days apart when compared to unsynchronized control ewes.

The results indicate that the timing between injections of PG is critical for ewe fertility and this may reflect the need for adequate progesterone priming of the reproductive tract to achieve normal fertilization rates in artificially inseminated ewes.

INTRODUCTION

Cloprostenol, an analogue of prostaglandin $F_{2\alpha}$ (PG), causes luteolysis in ewes similar to that seen at the normal completion of the luteal phase in ewes (Stacy and Gemmel 1976) and its use for synchronization of ovulation in sheep in artificial insemination programmes has been described by Fairnie et al. (1976).

This paper presents the results of ten field and laboratory experiments which examined the fertility of ewes treated with PG.

MATERIALS AND METHODS

Parous Merino ewes in the luteal phase of their cycles (as determined from the use of harnessed vasectomized rams) were treated with two 125µg injections of PG spaced either eight (PG 8), eleven (PG 11) or fourteen days apart (PG 14). Harnessed, vasectomized rams were released with the ewes at the completion of PG treatment and the ewes were checked every 24 hr for mating marks.

Artificial insemination (AI) was carried out using the methods described by Martin and Fairnie (1976), on ewes either within 24 hr of being detected in behavioural oestrus or at 64 hr after completion of PG treatments, regardless of whether or not the ewes had been detected in oestrus. Untreated ewes which were detected in behavioural oestrus in the 24 hr period prior to AI, served as controls.

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The fertility of ewes was defined in terms of the time of onset of behavioural oestrus, the occurrence of ovulation as assessed by laparoscopy or laparotomy, and, either the presence of fertilized eggs or developing embryos as determined by flushing oviducts and uteri. Ewes which had not apparently ovulated in response to the PG treatments were not included in the data presented in Table 3. Chi-square analysis was used to assess the significance of differences in fertility.

RESULTS

TABLE 1 Incidence of oestrus detected within 64 hr of completion of Cloprostenol (PG) treatment, and incidence of ovulation in some of these ewes selected randomly regardless of whether or not they had been detected in oestrus (PG 8 = Two injections of PG eight days apart etc.)

Treatment group	Oestrous detection		Incidence of ovulation	
	Number examined	% detected	Number examined	% ovulated
PG 8	217	69%	87	95%
PG 11	173	82%	93	94%
PG 14	739	61%	100	97%
TOTAL	1129	66%	280	95%

The data in Table 1 indicate that while only two thirds of PG-treated ewes were detected in behavioural oestrus within 64 hr of completion of treatments, all but a very few ewes ovulated. *No* significant differences either in incidence of oestrous detection or incidence of ovulation were found between groups.

Table 2 shows that more ewes in the PG 8 treatment group were detected in behavioural oestrus within 40 hr of completion of PG treatments than in other groups (PG 8 vs PG 11, $\chi^2 = 34.02$, $P < 0.01$).

TABLE 2 Time of onset of oestrous behaviour in those ewes detected in behavioural oestrus, within 64 hr of completion of Cloprostenol (PG) treatments (PG 8 = Two injections of PG eight days apart etc.)

Treatment group	N	Up to 40 hours	40–64 hours
PG 8	149	54%	46%
PG 11	141	20%	80%
PG 14	257	10%	90%

Table 3 shows that when fertility is assessed soon after AI, the PG 8 treatment significantly depressed fertility in oestrous ewes compared to control ewes ($\chi^2 = 7.48$, $P < 0.01$). There was no significant difference between oestrous ewes in the PG 11 and PG 14 treatment groups and the control group. In addition, failure to be detected in behavioural oestrus by the time of AI was also associated with a significant depression in fertility in the PG treatment groups ($\chi^2 = 9.83$, $P < 0.01$).

TABLE 3 Fertility in those control and Cloprostenol (PG) treated ewes submitted to laparotomy examination as determined from flushing oviducts and uteri prior to implantation, according to whether or not the ewes were detected in behavioural oestrus prior to artificial insemination (PG 8 = Two injections of PG eight days apart etc.)

Treatment group	Detected in oestrus	Number examined	% fertile
PG 8	+	75	33%
PG 8	-	39	17%
PG 11	+	75	38%
PG 11	-	13	10%
PG 14	+	64	52%
PG 14	-	35	17%
Control	+	58	58%

DISCUSSION

All ewes commenced PG treatments during the luteal phase of their cycle and the following assumptions can be made. After the first PG injection, luteolysis occurred and this was followed two or three days later by ovulation with or without a preceeding oestrus. The length of the normal oestrous cycle in ewes is at least 16 days with ovulation occurring on day one and luteolysis from day 14 i.e. the corpus luteum is developing and present for at least 13 days of the cycle. The second PG treatment interrupts this sequence at a time of maximal production of progesterone. In the case of the PG 8 treatment group, the tract is exposed to progesterone for at least seven days less than would normally be the case. For the other groups there is a deficit of progesterone of at least four days (PG 11) and one day (PG 14) in most ewes. In the case of ewes treated with one PG injection in the "mid-luteal" phase of the cycle (e.g. days 10 to 12; Fairnie et al. 1976) there has probably been a progesterone deficit of at least five to seven days i.e. similar to the PG 8 treatment group.

Table 1 shows that 66% of ewes were detected in behavioural oestrus following completion of PG treatments. However ovulation occurred in 95% of ewes regardless of oestrus being detected. It is unlikely that this difference was due to poor detection of oestrus as the fertility in ewes that had ovulated but had not been detected in oestrus was lower than in detected ewes (Table 3). The earlier onset of oestrus in the PG 8 treatment group (Table 2) compared to other treatment groups has not been reported by other workers. Lightfoot et al. (1979) reported that 41% of ewes treated with two injections of PG, ten days apart were detected in oestrus within 48 hr of completion of treatments. Boland et al. (1978) state that the later in the cycle that PG is given the later the ewes will be coming into oestrus. It seems from Table 2 that the stage of the cycle being interrupted affects oestrous behaviour and needs to be more carefully defined in the literature to enable information on time of oestrous onset to be of use. Indeed, as one of the major benefits of a synchronization system is to allow fixed-time insemination, the time of oestrous onset and ovulation needs to be predictable.

The fertility of inseminated ewes (Table 3) is poorer in the PG 8 treatment group even when these are inseminated at oestrous detection rather than at a fixed time 24 hr later. Boland et al. (1978) reported a seven percent fertilization rate in ewes treated with two injections of PG, 11 days apart and

inseminated 56 hr after the second PG. Haresign (1978) and Lightfoot et al. (1979) used natural mating with ewes treated with two injections of PG 9 and 10 days apart and reported acceptable levels of fertility. Hawk and Conley (1975) have reported reduction of sperm transport in PG-treated ewes and more recently Hawk (personal communication 1978) stated that this may be due to high sperm mortality within the cervix of ewes treated with PG midway through the cycle. Challis et al. (1976) suggest that there may be reduced uterine motility during oestrus in PG-treated ewes. This may also be a factor in reducing sperm numbers recovered after mating from the oviducts of treated sheep.

In the light of these findings, failure of fertilization in the PG-treated ewes in AI programmes could be attributed to a failure of sperm transport. However Fairnie and Wales (unpublished observations) have not been able to demonstrate differences in sperm transport in PG-treated ewes after AI which would account for the observed differences in fertility. Variable numbers of sperm are recovered from the reproductive tract of PG-treated ewes after AI using low numbers of sperm, making it difficult to detect significant differences under these conditions. Perhaps the higher sperm numbers anticipated after natural mating may be sufficient to overcome the sperm transport problems recorded by Hawk and Conley (1975) and allow a reasonable fertilization rate in PG-treated ewes following natural mating.

Differences in fertility between PG-treated ewes appear to be related to the progesterone deficit and it seems that the reproductive tract cannot be deprived of more than five days of progesterone without the risk of reduced fertility in artificially-inseminated ewes.

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