

CONTROL OF OVULATION IN CYCLING EWES WITH A  
PROSTAGLANDIN F<sub>2α</sub> ANALOGUE.

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## GENERAL INTRODUCTION

The secretion of progesterone by the corpus luteum controls the delicate hypothalamic - pituitary - ovarian interplay in the normal oestrus cycle of the female animal (Niswender, Nett & Akbar, 1975). In the cyclic ewe the functional activity of the corpus luteum is terminated rather abruptly on day 15 of the oestrus cycle as is indicated by the fall in the progesterone concentration in the peripheral blood (Plotka & Erb, 1967; Thorburn, Basset & Smith, 1969; Moore, Barrett, Brown, Schindler, Smith & Smyth, 1969; Van Niekerk, Morgenthal, Sanders & Malan, 1973; Yuthasastrakosol, Palmer & Howland, 1975) and the morphological regression of the corpus luteum (Stacy, Gemmell & Thorburn, 1975; McClellan, Abel & Niswender, 1977). As there is no evidence of a primary pituitary involvement, some other active mechanism was suspected to cause lysis of the corpus luteum (Robertson, 1977). The role of the normal uterus in luteal regression was soon proved (Kiracofe, Menzies, Gier & Spies, 1966; Bland & Donovan, 1969; Inskoop, 1973). Evidence is accumulating that this luteolytic substance which is produced by the endometrium is a prostaglandin since there is a local utero-ovarian cycle, whereby the corpus luteum stimulates the uterus to produce prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) which in turn destroys the corpus luteum (Hafez, 1975).

Thus the luteolytic property of these naturally occurring lipids (Nalbandov, 1976) has some exciting potential applications in practical animal breeding (Thompson & Witherspoon, 1974; Cooper & Furr, 1974). So for instance, it has been demonstrated by various workers that the administration of prostaglandin  $F_{2\alpha}$  causes luteal regression in horses (Lamond, Buell & Stevenson 1975; Miller, Lauderdale & Geng, 1976; Talksdorff, Jochle, Lamond, Klug & Merkt, 1976), cattle (Rowson, Tervit & Brand, 1972; Van Niekerk, Belonje & Morgenthal, 1974; Elving, Brand & De Bois, 1975; Motlik, Pavlok & Fulka, 1976; Roche, 1976; Stellflug, Louis, Gorewit, Oxender, Hafs, 1977) and sheep (Goding, Cain, Cerini, Cerini, Chamley & Cumming, 1972; McCracken, Carlson, Glew, Goding, Baird, Green & Samuelsson, 1972; Douglas & Ginther, 1973; Otake, Kikuma, Nomoto, Domeki & Nakahara, 1975; Land, Baird & Scaramuzzi, 1976; Hughes, Lucas & Notman, 1977).

In the ewe a single intramuscular injection of PGF<sub>2α</sub> or one of its analogues during the mid-luteal phase of the oestrus cycle results in rapid luteolysis followed by a return to oestrus (Douglas & Ginther, 1973; Acritopoulou, Haresign, Foster & Lamming, 1977; Van Zyl, 1977). The hormonal events following prostaglandin treatment do not differ significantly from those of a natural oestrus (Bindon, Blanc, Pellitier, Terqui & Thimonier, 1976; Acritopoulou, et al 1977). In addition, the morphological changes induced in the corpus luteum of the sheep by administered prostaglandins are similar to those occurring normally (Stacy, Gemmell & Thorburn, 1976; Stacy & Gemmell, 1976). However, the corpus luteum of the ewe is only responsive to prostaglandin during part of the mid-luteal phase. So for instance certain PGF<sub>2α</sub> analogues cause luteal regression only between days 5 and 10 of the oestrus cycle (Van Zyl, 1977), whereas the more potent analogues (e.g. Cloprostenol, ICI 8 0996) were found to be more effective in this respect (Fairnie, Cumming & Martin, 1976a). To overcome this refractory period when ewes are not responsive to prostaglandins it is necessary either to give two injections 8 or 9 days apart (Haresign, 1976), or to treat ewes with progestogens prior to a single injection of prostaglandin (Van Zyl, 1977). In view of the high degree of synchrony of ovulation and the absence of observed endocrine imbalances following the treatment of cyclic ewes with prostaglandins, the use of prostaglandins in the control of oestrus and ovulation in sheep warrants further investigation, especially in conjunction with A.I. The work reported in this thesis, set out to further investigate the use of a synthetic prostaglandin (Cloprostenol "Estrumate", ICI 80996) in the control of oestrus and ovulation in sheep. In addition a comparison is made between the use of this luteolytic agent and alternative methods of synchronisation of oestrus and ovulation for their possible use in fixed time insemination programmes.

CHAPTER ITHE DETERMINATION OF AN EFFECTIVE DOSAGE OF CLOPROSTENOL AND THE RESPONSIVE PERIOD IN THE OESTRUS CYCLE OF THE EWE.

The corpus luteum of the ewe is responsive to prostaglandins during a limited period of the mid-luteal phase only (Douglas & Ginther, 1973; Acritopoulou, Haresign, Foster & Lamming, 1977). Van Zyl (1977) found that a prostaglandin analogue (Prostalene-Syntex) terminates the oestrus cycle in sheep only between days 5 and 10. On the other hand, a single injection of 75µg to 100µg of the more potent PGF<sub>2α</sub> analogue, Cloprostenol ("Estrumate", ICI 80996) was found to be sufficient to induce luteal regression in sheep (Cooper & Furr, 1974; Trounson, Willadsen & Moor, 1976; Challis, Foster, Furr, Robinson & Thorburn, 1977) between days 4 and 14 of the oestrous cycle (Acritopoulou & Haresign, 1977 unpublished observations as quoted by Haresign, 1978).

This experiment was therefore designed to determine the lowest effective dosage of Cloprostenol and the responsive period of the cycle during which it causes luteolysis and subsequent oestrus.

MATERIAL & METHODS

## EXPERIMENT 1

During the breeding season (March, 1977) 16 mature South African Mutton Merino ewes were randomly allocated to four treatment groups each of which received the following intramuscular dosages of Cloprostenol (ICI 80996):

- Group 1 : 0,25 ml (62,5µg) Cloprostenol
- Group 2 : 0,5 ml (125µg) Cloprostenol
- Group 3 : 1,0 ml (250µg) Cloprostenol
- Group 4 : 2,0 ml (500µg) Cloprostenol

Commencing 24 hours after the injection of Cloprostenol, all the ewes were tested twice daily (07h00 and 17h00) with the aid of active vasectomised rams in order to determine the onset and occurrence of oestrus.

## EXPERIMENT II

Forty mature South African Mutton Merino ewes of which the oestrus periods had been accurately recorded were used. The day on which oestrus was recorded was taken as day 0 and day 1 as the day of ovulation (Boshoff, 1972). These ewes were then arranged into groups according to the stage of their cycle as follows:

Days 3,4,5 & 6 : Four ewes per day

Days 7,8,9 & 10 : Two ewes per day

Days 11,12,13 & 14: Four ewes per day

All these ewes received a single intramuscular injection of 125 µg Cloprostenol, whereafter they were tested at regular intervals (08h00 and 17h00) with vasectomised rams to detect the onset and occurrence of oestrus.

## RESULTS:

## EXPERIMENT 1

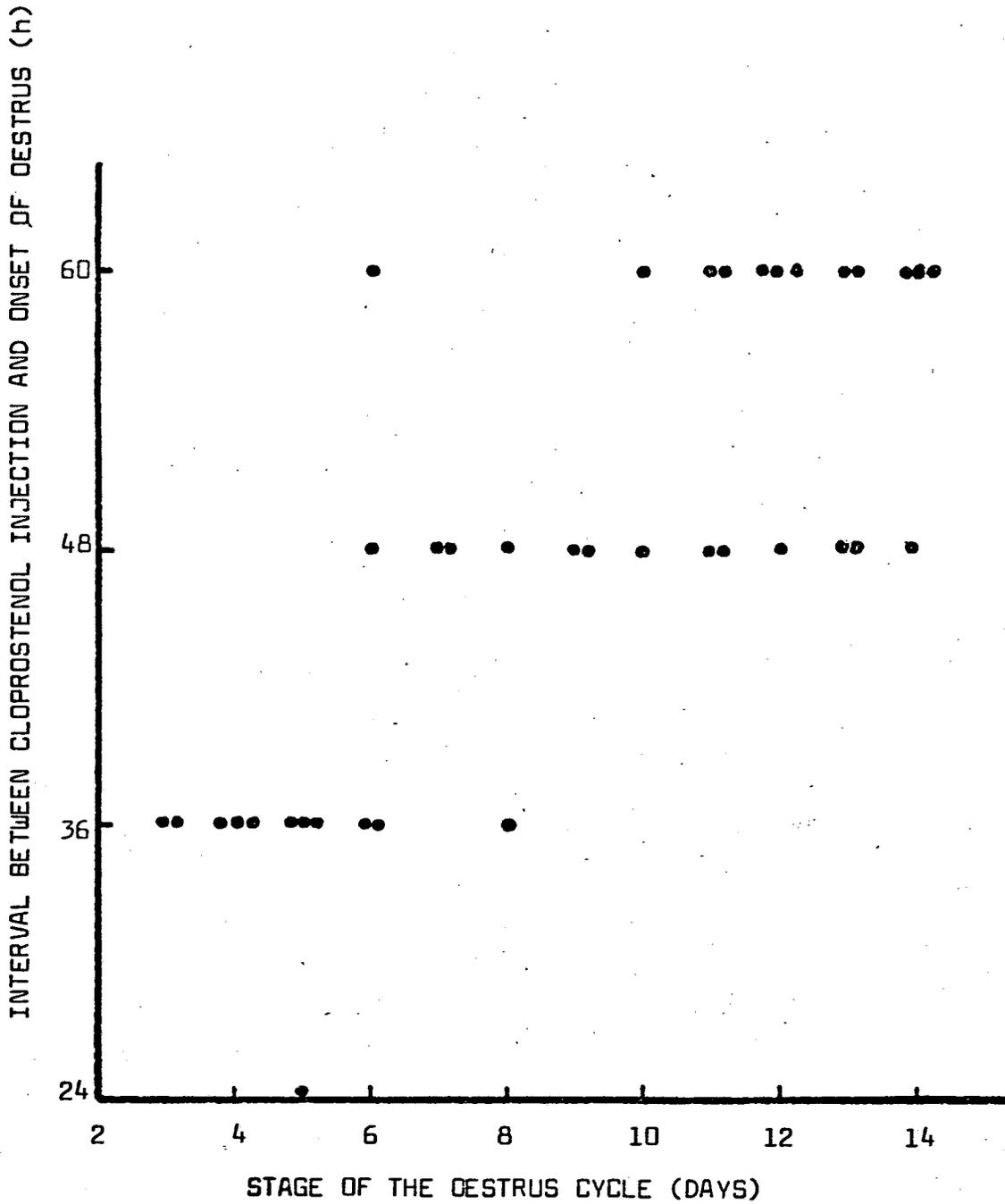
The effect of the different dosages of Cloprostenol on the oestrus response in sheep is presented in Table 1.1.

Table 1.1 The effect of the dosages of Cloprostenol on oestrus response and the time of onset of oestrus in sheep following treatment.

Group	Dosage	n	Number in Oestrus	Interval to onset of Oestrus (h)	
				Following injection	Range
1	62,5µg	4	4	110,5 ± 52,6	48 - 178
2	125 µg	4	3	102,7 ± 53,6	58 - 178
3	250 µg	4	4	60,0 ± 12,0	48 - 72
4	500 µg	4	4	65,0 ± 7,0	58 - 72

From Table 1.1 it is obvious that by increasing the dosage of Cloprostenol an improvement in the degree of synchronisation of oestrus is achieved. It should be noted that in Group 2 only the average time of three ewes were taken as the fourth ewe had a silent ovulation and showed oestrus again 17 days later.

Figure 1.1 The relationship between stage of the oestrus cycle when 125 $\mu$ g Cloprostenol was administered and the interval between treatment and the onset of oestrus.



## EXPERIMENT II

The effect of stage of the cycle when Cloprostenol was administered is presented in Figure 1.1. All ewes in this study showed oestrus except for two ewes from day 3 and one from day 4. It was therefore concluded that the corpora lutea at this stage of the cycle may not always be sensitive to Cloprostenol. From Figure 1.1 it is obvious that the stage of the cycle at which the prostaglandin is administered had a significant effect on the interval between the injection of Cloprostenol and the onset of oestrus.

## DISCUSSION

From the results of this experiment it is obvious that 62,5 µg Cloprostenol is sufficient to induce luteal regression and subsequent oestrus. At higher dosage levels (250µg and 500µg) the occurrence of oestrus was more closely synchronised and it is suspected that the higher dosages also caused rapid luteolysis of corpora lutea less susceptible to the lower dosages (62,5µg and 125µg). However the limited number of animals could affect the significance of this observation. Previous workers (Trounson, et al 1976) have shown that 100µg Cloprostenol is sufficient to induce luteal regression whereas others use 125µg (Fairnie, Cumming & Martin, 1976a). In the present study 125µg Cloprostenol proved to cause luteal regression between days 4 and 14 of the oestrus cycle, which corroborates the work of Acritopoulou & Haresign (1976). There was a clear effect of the stage of the cycle during which prostaglandin was injected and the interval between Cloprostenol treatment and the onset of oestrus, suggesting that luteolysis occurs more rapidly in fresh than in older corpora lutea. It is therefore concluded that the dosage of 125µg Cloprostenol is sufficient to cause luteolysis, although higher dosages may cause a higher degree of synchronisation.

CHAPTER 2

SYNCHRONISATION OF OESTRUS IN SHEEP WITH CLOPROSTENOL IN COMBINATION WITH PROGESTOGEN TREATMENT : (i) THE EFFECT OF DOSAGE CLOPROSTENOL (ICI 80996) ON THE REPRODUCTIVE EFFICIENCY AND SERUM PROGESTERONE AND LH CONCENTRATIONS. (ii) THE EFFECT OF TIME OF CLOPROSTENOL ADMINISTRATION RELATIVE TO SPONGE WITHDRAWAL ON THE REPRODUCTIVE EFFICIENCY OF THE EWE.

It has now been established that luteolysis can be induced with Cloprostenol between days 4 and 14 of the oestrus cycle of the ewe and therefore only about 65% of ewes will respond when a single injection is given to a flock of ewes without regard to the stage of the cycle. Therefore to ensure that all the ewes are in the responsive stage of the cycle, it is necessary either to give two injections of Cloprostenol 8 or 9 days apart (Fairnie, Wales & Gherardi, 1977; Haresign, 1976) or to treat ewes with progestogens prior to an injection of a prostaglandin  $F_{2\alpha}$  analogue (Van Zyl, 1977).

In order to study these alternatives, this chapter reports on the first of a series of experiments in which Cloprostenol was used to synchronise oestrus in sheep in combination with an intravaginal progestogen sponge pretreatment. These experiments were designed to determine the effects of different dosages Cloprostenol and the time of Cloprostenol administration following a short progestogen pretreatment on the reproductive efficiency of ewes. In addition the serum progesterone and LH concentrations of these ewes were studied.

MATERIAL AND METHODS

## EXPERIMENT 1

During the active breeding season (March, 1977) the oestrus cycles of a flock of 185 South African Mutton Merino ewes were monitored. From this flock 102 ewes were selected and allotted to three groups in such a way that each group contained 34 ewes with 2 ewes at each day of the oestrus cycle. All the ewes were treated with 60 mg medroxy progesterone acetate (MAP) intravaginal sponges (Repromap : Upjohn) for 8 days. Before insertion, these sponges were also

impregnated with 2,0ml of an antibiotic preparation (Streptopen; Glaxo Allenburys). On the day of sponge withdrawal the three groups were treated with the following intramuscular dosages of prostaglandin (Cloprostenol, ICI 80996):

- Group 1 : 0,5ml (125 $\mu$ g)
- Group 2 : 0,25ml (62,5 $\mu$ g)
- Group 3 : 0,125ml (31,3 $\mu$ g)

Following the injection of Cloprostenol all the ewes were tested for oestrus with vasectomised rams. From 30 of these ewes (10 from each group representing the days 2,4,6,8,10,11,12,13,15 and 17 of the oestrus cycle respectively) venous blood was collected at six-hourly intervals starting at the time of Cloprostenol injection up to the end of oestrus. Serum was recovered and stored at - 20°C until it was analysed for progesterone and LH concentrations.

Ewes in oestrus were inseminated 12 hours after identification and again at 12 hour intervals for as long as they remained in oestrus. All ewes were again tested after 14 days so that ewes returning to service could be inseminated at their second cycle.

Serum progesterone concentration was determined by the radio-immuno assay (R.I.A.) technique of Yousefnejadian, Florensa, Collins & Sommerville (1972) as modified by Faure (1975) and serum LH concentration by the RIA method of Niswender, Reichert, Midgley & Nalbandov (1969) as modified by Millar & Aehnelt (1977).

## EXPERIMENT II

Seventy-eight S.A. Mutton Merino ewes of which the oestrus cycle had been monitored, were used in this trial (April, 1977). Intravaginal sponges (MAP 60mg) were inserted for a period of 9 days in all the ewes. The ewes were then allocated to three groups of 26 ewes each, balanced with respect to stage of the oestrus cycle. These groups were then treated as follows:

- Group 1 : Received a 125 $\mu$ g intramuscular injection Cloprostenol 48 hours prior to sponge withdrawal.
- Group 2 : Received a 125 $\mu$ g intramuscular injection Cloprostenol 24 hours prior to sponge withdrawal.
- Group 3 : Received a 125 $\mu$ g intramuscular injection Cloprostenol at sponge withdrawal.

After treatment and removal of the sponges, the ewes were tested twice daily (08h00 and 16h00) with the aid of vasectomised rams and artificially inseminated 12 hours later.

## RESULTS:

### EXPERIMENT 1

#### Reproductive performance:

The oestrus response, duration of oestrus and the reproductive efficiency following the different levels of Cloprostenol administration are set out in Table 2.1.

The different dosages of Cloprostenol did not affect the interval between the cessation of treatment and the onset of oestrus, the duration of oestrus or the oestrus response significantly, nor was any significant pattern apparent in the reproductive efficiencies of these groups (Table 2.1). Therefore data was pooled for further analysis. No significant pattern was found in the reproductive performance of the ewes from day 2 to day 5 following the cessation of treatment as can be seen in Table 2.2.

However fertility was found to be significantly ( $P < 0,05$ ) lower at the first post treatment oestrus as compared to the second post treatment oestrus (Table. 2.3), which was considered to be normal.

#### Changes in serum progesterone and LH concentrations:

From Figure 2.1 it is obvious that the different dosages of Cloprostenol did not affect the rate of decrease of and the mean serum progesterone concentrations of the respective groups following the prostaglandin injection. The differences in the mean concentrations of serum progesterone levels of the three treatment groups at and around oestrus were also insignificant (Figure 2.2). The release of the LH surge (LH peak value) relative to the cessation of treatment did not differ between the treatment groups (Table 2.4) although there was marked variation within groups. The position of the LH peak relative to the onset of oestrus did not differ between treatment groups (Figure 2.3). However, the day of the oestrus cycle on which the intravaginal sponge treatment started had a significant effect on the interval between the cessation of treatment and the onset and occurrence of the LH surge (Figure 2.4).

**Table 2.1** The effect of an 8 day intravaginal progestogen treatment followed by different dosages of prostaglandin F<sub>2α</sub> (Cloprostenol) on the oestrus and reproductive performance of South African Mutton Merino ewes (percentage in brackets)

	Group 1 (125µg)	Group 2 (62,5µg)	Group 3 (31,2µg)
No. Ewes	34	34	34
No. Ewes showing oestrus	34	34	34
Interval from cessation of treatment to the onset of oestrus (h)	70,09 ± 26,03	64,18 ± 18,7	73,94 ± 29,74
Range (h)	40 - 144	32 - 120	40 - 144
Duration of oestrus (h)	38,4 + 7,43	39,03 ± 7,06	38,06 ± 6,94
<b><u>FIRST OESTRUS:</u></b>			
Ewes conceiving	19(55,9)	25(73,5)	21(61,8)
Lambs born/Ewe treated	28(82,4)	35(102,9)	30(88,2)
Lambs born/Ewe lambing	1,47	1,40	1,43
<b><u>SECOND OESTRUS:</u></b>			
Ewes returning to service	15	9	13
Ewes conceiving	12(80,0)	8(88,9)	10(76,9)
Lambs born/Ewe mated	16(106,7)	14(155,6)	12(92,3)
Lambs born/Ewe lambing	1,33	1,75	1,20

Table 2.2 The oestrus response, conception rate and fecundity of ewes showing oestrus from day 2 to day 5 following Cloprostenol treatment (percentages in brackets)

	Day 2		Day 3		Day 4		Day 5	
	48h		72h		96h		120h	
Group 1 No. Ewes in Oestrus	11( 32,4)	11( 32,4)	11( 32,4)	8( 23,5)	3( 8,8)			
(125µg) No. Ewes Lambing	7( 63,6)	7( 63,6)	7( 63,6)	3( 37,5)	1( 33,3)			
No. Lambs	12(171,4)	10(142,9)	4(133,3)		1(100,0)			
Group 2 No. Ewes in Oestrus	10( 29,4)	16( 47,1)	7( 20,6)		1( 2,9)			
(62,5µg) No. Ewes Lambing	9( 90,0)	12( 75,0)	3( 42,9)		1(100,0)			
No. Lambs	13(144,4)	16(133,3)	4(133,3)		2(200,0)			
Group 3 No. Ewes in Oestrus	12( 35,3)	8( 23,5)	8( 23,5)		4( 11,8)			
(31,25µg) No. Ewes Lambing	7( 58,3)	6( 75,0)	5( 62,5)		3( 75,0)			
No. Lambs	10(142,9)	7(116,7)	7(140,0)		6(200,0)			
Total	33( 32,4)	35( 34,3)	23( 22,5)		8( 7,8)			
No. Ewes Lambing	23( 69,7)	25( 71,4)	11( 47,8)		5( 62,5)			
No. Lambs	35(152,2)	33(132,0)	15(136,4)		9(180,0)			

Table 2.3 The overall conception, lambing rate and fecundity of all the ewes treated with prostaglandin F<sub>2α</sub> (Cloprostenol) following an 8 day intravaginal progestogen pretreatment for then first and second post treatment oestrous period.

	1st Oestrus		2nd Oestrus	
	<u>Number</u>	<u>Percentage</u>	<u>Number</u>	<u>Percentage</u>
Total No. Ewes treated	102			
Total No. Ewes inseminated	102	100,0	37	100,0
Ewes lambing/Ewes treated	65	63,8*	30	81,1*
Lambs born/Ewes treated	93	91,2	42	113,5
Lambs born/Ewes lambing	93	143,1	42	140,0

\*P < 0,05

Figure 2.1 The effect of different dosages Cloprostenol on the mean serum progesterone concentration

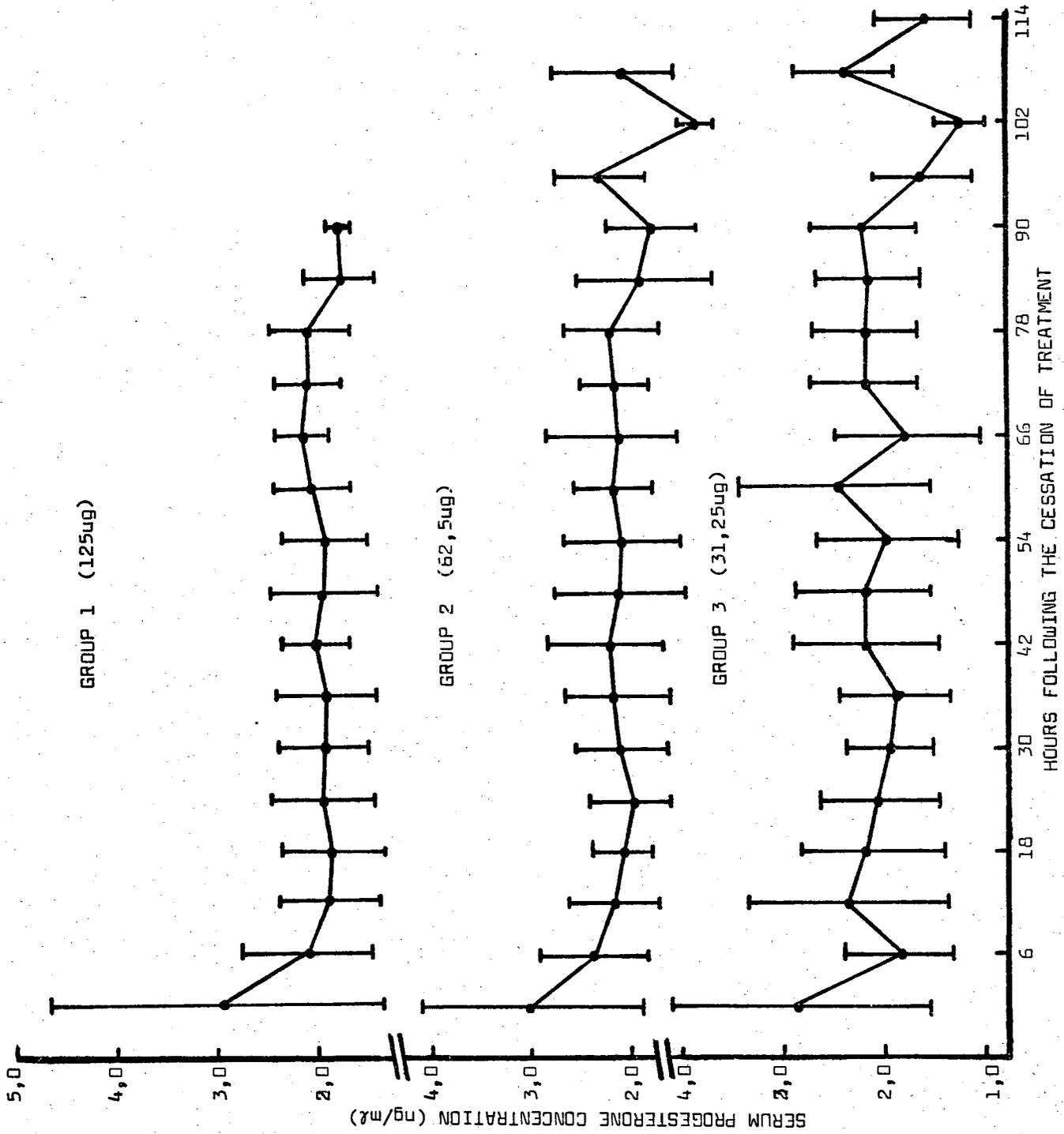


Figure 2.2

Mean serum progesterone concentrations relative to oestrus for ewes receiving Cloprostenol; 125µg (Group 1), 62,5µg (Group 2) and 31,25µg (Group 3) following an 8 day intravaginal sponge treatment.

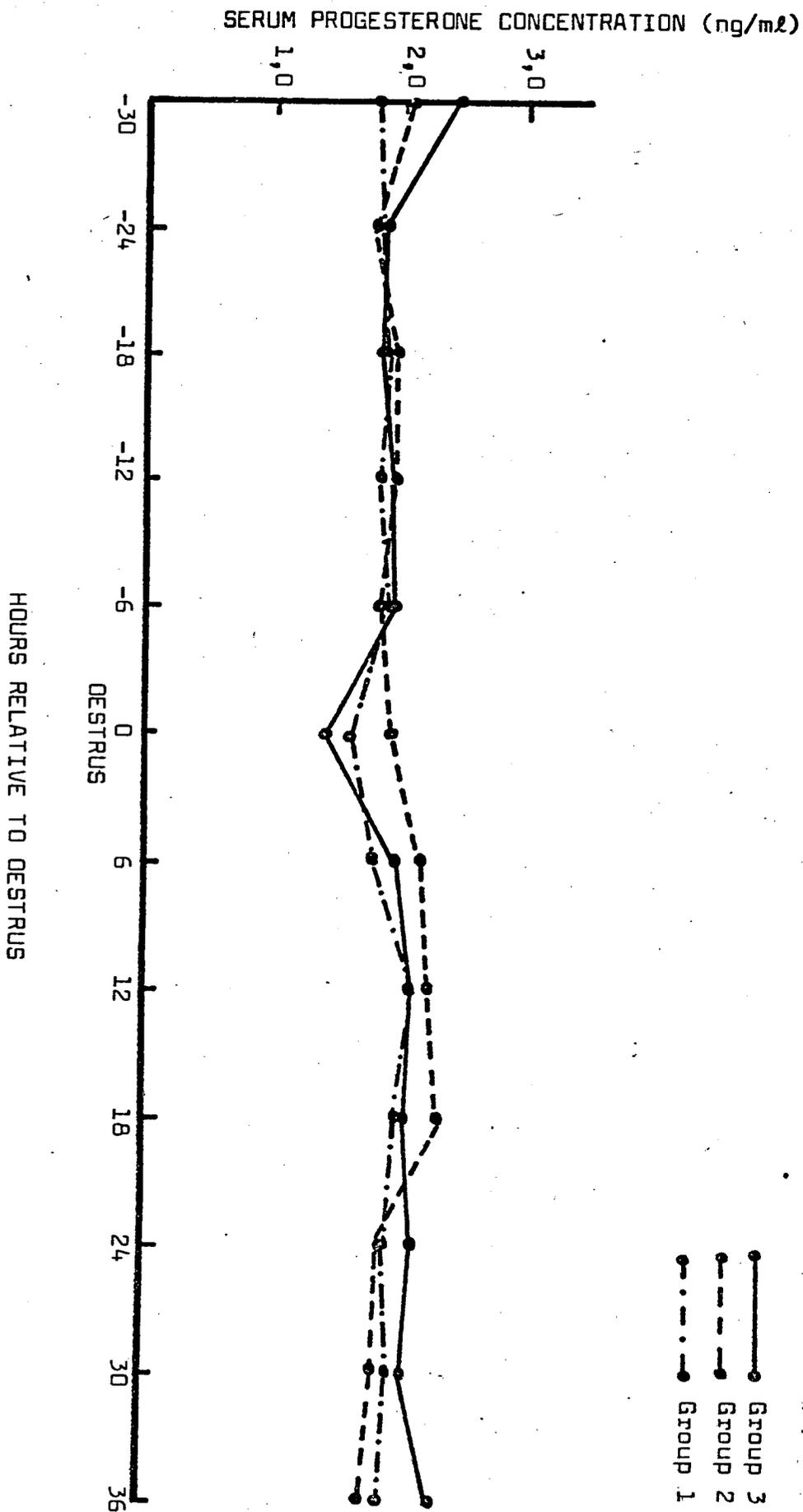


Table 2.4 The time interval (in hours) between the injection of 125 $\mu$ g (Group 1), 62,5 $\mu$ g (Group 2) and 31,3 $\mu$ g (Group 3) Cloprostenol, following an 8 day progesterone treatment, and the LH peak

<u>Stage of cycle (Days)</u>	<u>Group 1</u> <u>125<math>\mu</math>g</u>	<u>Group 2</u> <u>62,5<math>\mu</math>g</u>	<u>Group 3</u> <u>31,3<math>\mu</math>g</u>
17	60	66	96
15	72	60	60
13	54	54	60
12	60	48	78
11	72	84	54
10	72	66	54
8	54	78	78
6	84	78	72
4	90	84	60
2	126	132	126
	<u>74,4 <math>\pm</math> 20,6</u>	<u>75,0 <math>\pm</math> 22,3</u>	<u>73,8 <math>\pm</math> 21,5</u>

## EXPERIMENT II

The time interval (in hours) between the cessation of sponge treatment and the onset of oestrus as well as the duration of oestrus for the respective groups, are presented in the (Table 2.5 and Figure 2.5) respective table and figure.

Figure 2.3 The position of the LH peak relative to the onset of oestrus for the three respective treatment groups each receiving 125 $\mu$ g, 62,5 $\mu$ g and 31,25 $\mu$ g Cloprostenol following an 8 day intravaginal progestogen sponge treatment

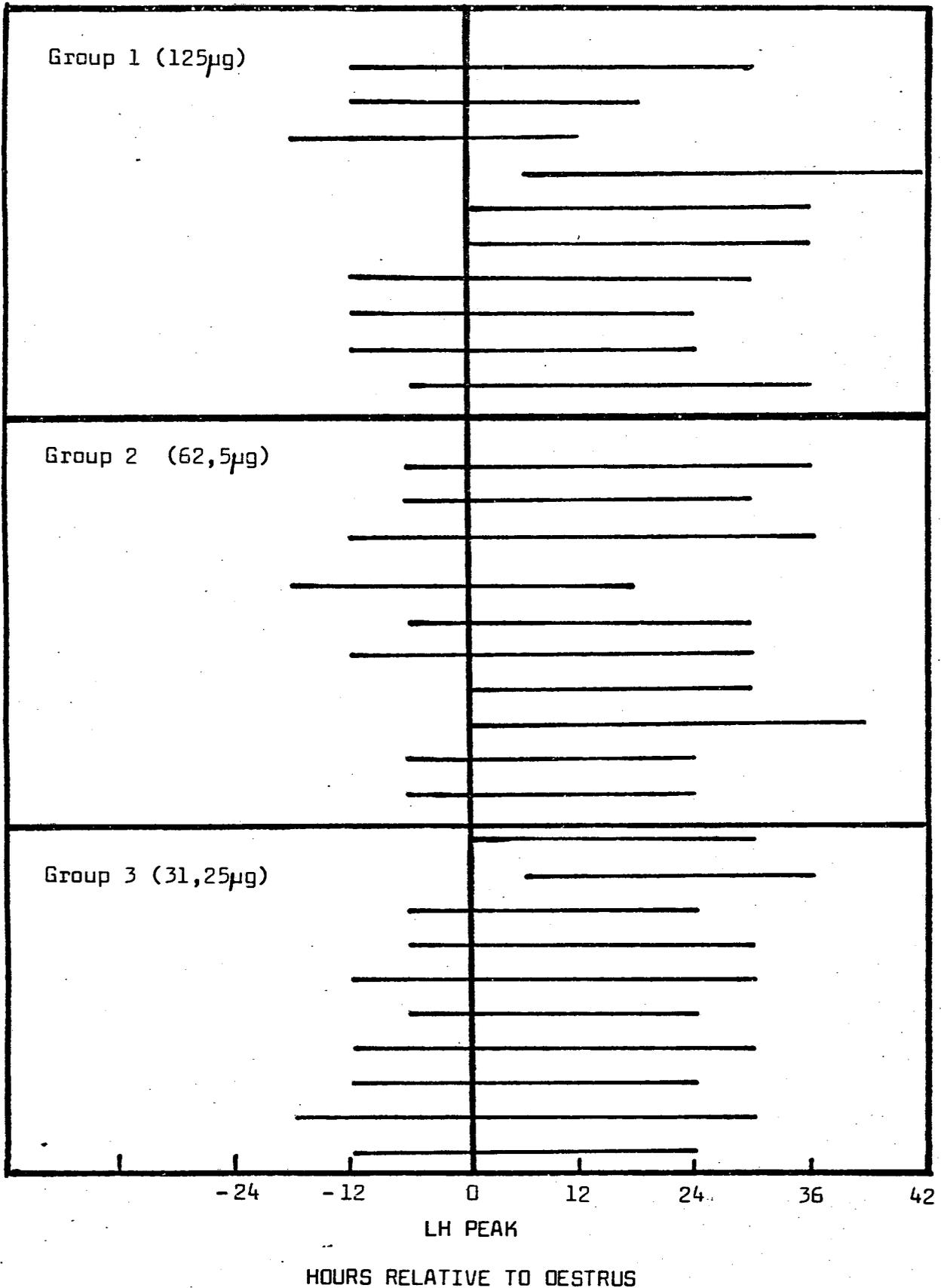
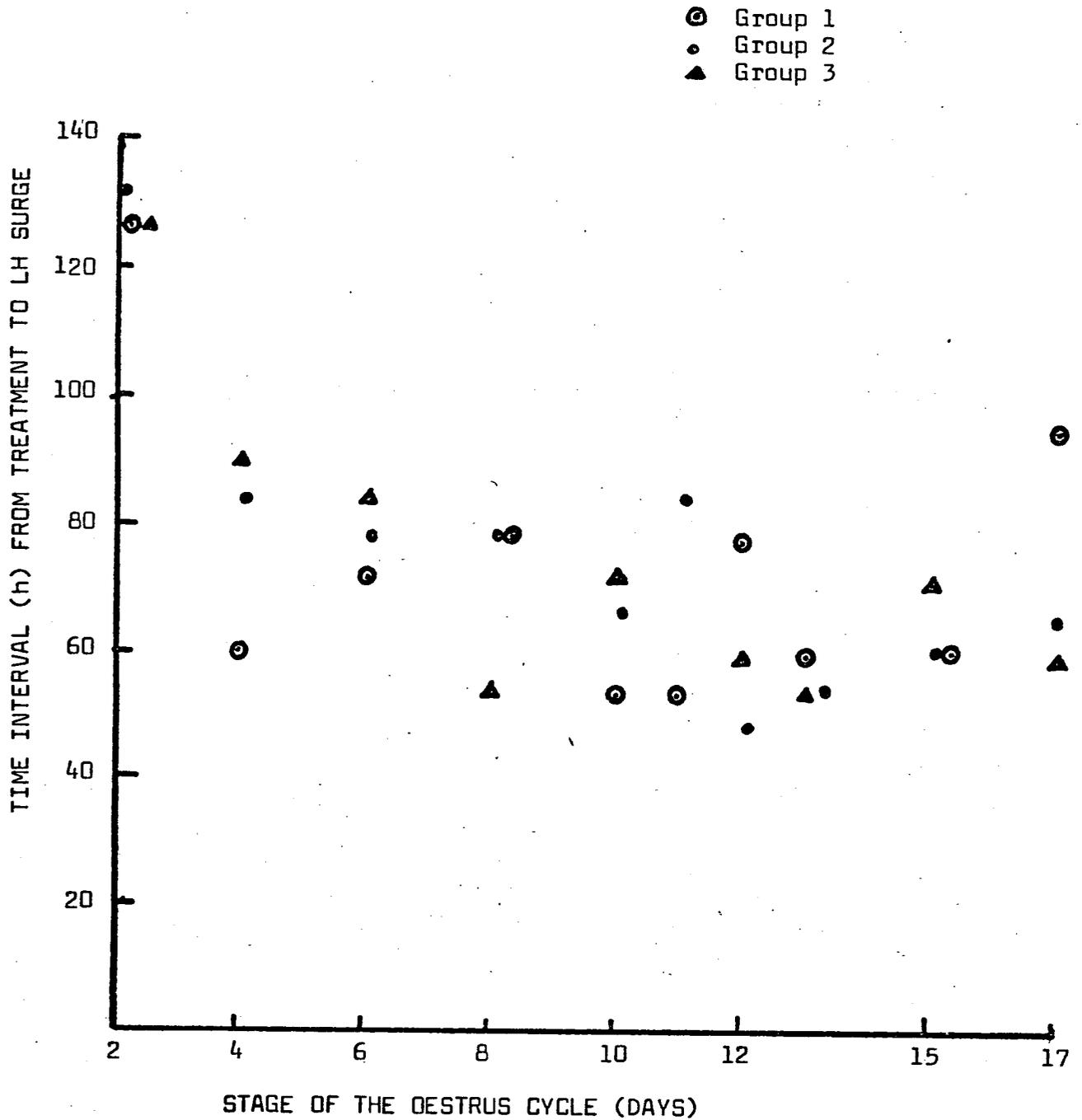


Figure 2.4 The time interval between the cessation of 125 $\mu$ g, 62,5 $\mu$ g and 31,25 $\mu$ g Cloprostenol administration, following an 8 day intravaginal sponge treatment and the LH surge in ewes



**Table 2.5** The onset of oestrus and duration of oestrus (in hours) following the treatment of ewes with intravaginal progestogens and Cloprostenol for the respective treatment groups

	Group 1 48h Prior	Group 2 24h Prior	Group 3 At sponge withdrawal
Oestrus response	58,04 ± 13,9	55,04 ± 12,7	61,9 ± 17,3
Duration of oestrus	36,4 ± 8,64	34,3 ± 7,89	38,6 ± 7,12

From these results it can be seen that the time of Cloprostenol administration had no significant effect on the interval between sponge withdrawal and the onset of oestrus, or the duration of oestrus. Although the stage of the cycle at which the progestogen treatment started did not have any affect on the interval between sponge withdrawal and the onset of oestrus in Groups 1 and 2 - in Group 3 this interval showed a marked decrease as the onset of the sponge treatment moved from day 2 to day 17 of the oestrus cycle (Figure 2.5).

Table 2.6 The conception rate, lambing rate and fecundity following different times of Cloprostenol treatment relative to sponge withdrawal.

	Group 1 - 48h		Group 2 - 24h		Group 3 0h	
		%		%		%
No. Ewes	26		26		26	
No. Ewes showing oestrus	26		26		26	
<u>FIRST OESTRUS:</u>						
Ewes lambing/ Ewe treated	20	76,92	19	73,08	20	76,92
Lambs born/ Ewe treated	33	126,92	30	115,38	27	103,85
Lambs born/ Ewe lambing	1,65		1,58		1,35	
<u>SECOND OESTRUS:</u>						
Ewes lambing/ Ewe treated	5	83,3	6	85,7	5	83,3
Lambs born/ Ewe treated	10	166,7	9	128,6	9	150,0
Lambs born Ewe lambing	2,0		1,5		1,8	

Figure 2.5 Time to the onset of oestrus (hours) following intravaginal progestogen sponge treatment and different times of Cloprostenol administration

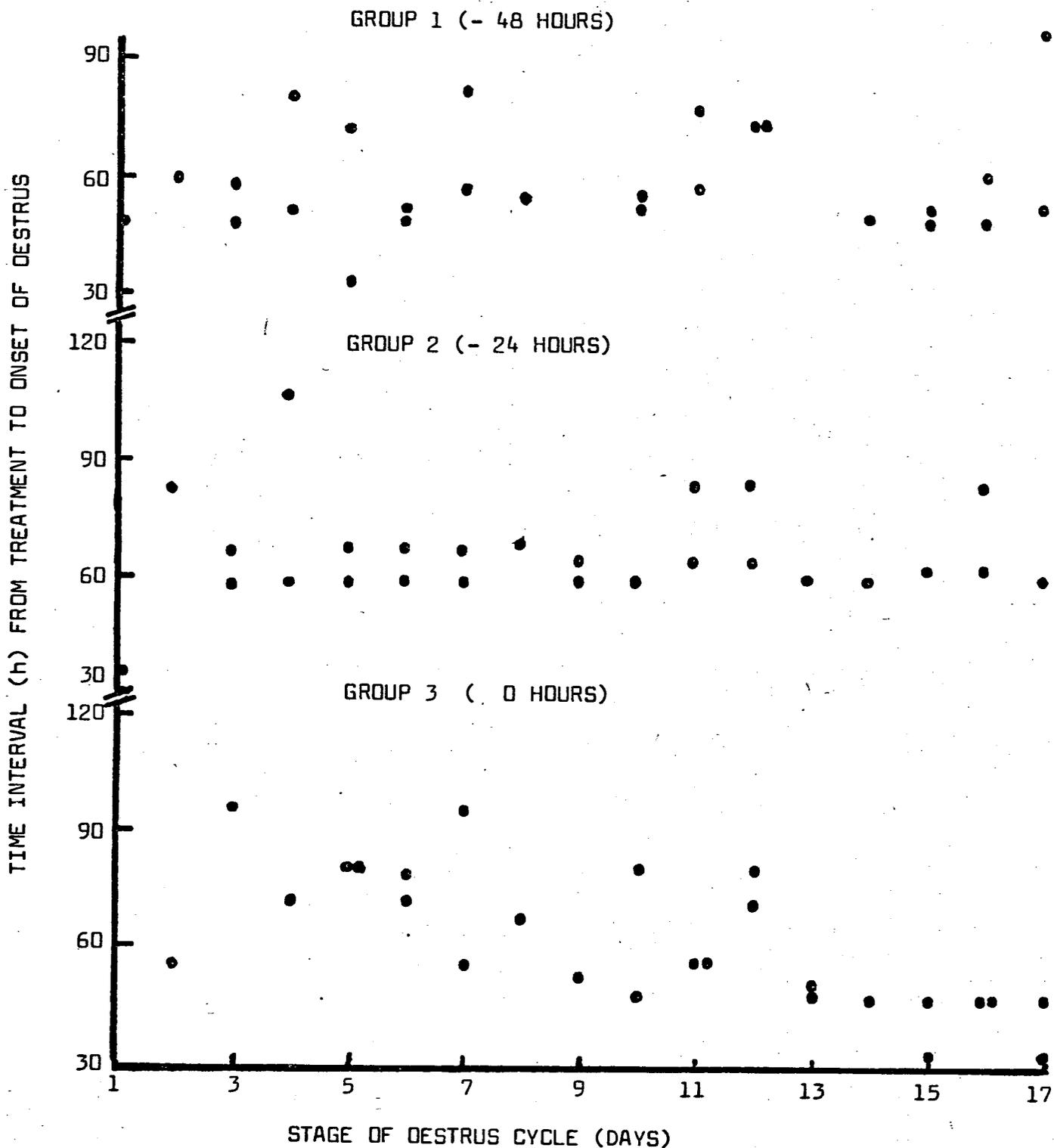


Table 2.7 The overall conception, lambing rate and fecundity of ewes following different times of Cloprostenol administration relative to sponge withdrawal for the first and second post treatment oestrus.

	<u>1st Oestrus</u>		<u>2nd Oestrus</u>	
	No:	Percentage	No:	Percentage
Total No. Ewes treated	78		19	
Total No. Ewes inseminated	78	100,0	19	100,0
Ewes lambing/Ewes treated	59	75,6	16	84,2
Lambs born/Ewe treated	90	115,4	28	147,4
Lambs born/Ewes lambing	1,52		1,75	

Although there was a tendency for fecundity to improve as the time of Cloprostenol administration prior to sponge withdrawal increased, the reproductive efficiencies of the three respective treatment groups did not differ significantly (Table 2.6). The overall conception, lambing rate and fecundity for the first post treatment oestrus was not significantly different from that of the second post treatment oestrus (Tables 2.6 and 2.7).

### DISCUSSION

The results of these experiments prove that the oestrus periods of sheep can be efficiently synchronised by means of a short progestogen pretreatment (8 - 9 days) followed by a single injection of prostaglandin from 48 hours prior to sponge withdrawal, to sponge withdrawal. By this treatment the insensitive period of the ovary to prostaglandins (around oestrus) can be overcome. According to the evidence in these experiments the luteolytic activity of a dosage of 31,25 $\mu$ g Cloprostenol proved to be sufficient to result in a synchronised oestrus following the progestogen pretreatment. Although fecundity tended to increase as the time of Cloprostenol treatment was given before sponge withdrawal (48 hours prior), this increase was not significant. It is therefore concluded that the time of Cloprostenol injection (between - 48 hours and 0 hours) does not affect reproductive efficiency.

However, fertility was slightly depressed at the first post treatment oestrus and it is suspected that in part this lowered fertility rests with the effects of intravaginal progestogen treatment on the reproductive efficiency of sheep (Robinson, Moore, Holst & Smith, 1967; Deweese, Glimp & Dutt, 1970; Van der Westhuysen, Van Niekerk & Hunter, 1970a; Hunter, Belonje & Van Niekerk, 1971). Various workers have found that progesterone or progestogens disturbs the time relationship between the release of LH and oestrus (Cumming, Blockey, Brown, Catt, Goding & Kaltenbach, 1970; Lintin & Lamming, 1973; Lishman, Botha & Louw, 1974; Van der Westhuysen, Malan & Dierkse, 1977). In this experiment a similar inconsistency in the occurrence of the LH peak relative to oestrus was found, but in agreement with Van der Westhuysen, et al (1977) and Dierkse (1977) this release in LH could not be related to the serum progesterone concentrations. It is of interest however, that the day of the oestrus cycle on which Cloprostenol was administered, often effected the interval between the cessation of treatment and the LH peak. This suggests that the hormonal events of the period prior to oestrus has a significant influence on the subsequent oestrus.

It is therefore concluded that oestrus can be synchronised by the use of an 8 - 9 day progestogen treatment, followed by a low dosage of Cloprostenol injected between 48 hours prior to sponge withdrawal and at sponge withdrawal. However, it is still not clear whether the addition of prostaglandins following the progestogen treatment did in fact have any additional effect beneficial to progestogens on the reproductive performance of the ewe. The effect of residual progestogens following the intravaginal treatment could have had a super-imposed effect on the rapid drop in progesterone normally following prostaglandin  $F_{2\alpha}$  treatment and on the subsequent onset of oestrus and fertility. For this reason, further studies of this and other techniques of overcoming the insensitive period of the corpus luteum to prostaglandin in the synchronisation of oestrus in the ewe is necessary.

CHAPTER 3SYNCHRONISATION OF OESTRUS IN SHEEP WITH A  
DOUBLE INJECTION CLOPROSTENOL:

THE EFFECT OF DOSAGE CLOPROSTENOL AND THE STAGE OF THE OESTRUS CYCLE ON OESTRUS RESPONSE AND SERUM PROGESTERONE CONCENTRATIONS.

It is a known fact that the corpus luteum of the ewe is only responsive to Cloprostenol treatment between days 4 and 14 of the oestrus cycle (Acritopoulou & Haresign unpublished observations as quoted by Haresign, 1978). Thus to overcome this insensitive period of the ovary to prostaglandin, a double injection of Cloprostenol was given 8 to 14 days apart (Fairnie, et al 1976a; Haresign, 1976). The minimum effective dosage Cloprostenol sufficient to induce luteolysis and the corresponding drop in serum progesterone concentration of this double injection regime was thus investigated in this experiment.

MATERIAL AND METHODS

This experiment was performed during February (1978) on 64 mature S.A. Mutton Merino ewes of which the stage of their oestrus cycles were known. The ewes were divided into four groups of 16 ewes, each group consisting of two sheep on days 2,4,6,8,10,12,14 and 16 of the oestrus cycle. The four groups each received the following levels of Cloprostenol:

- Group 1 : Two intramuscular injections of 0,03125mg (0,125ml) Cloprostenol with a 10 day interval.
- Group 2 : Two intramuscular injections of 0,0625mg (0,25ml) Cloprostenol with a 10 day interval.
- Group 3 : Two intramuscular injections of 0,125mg (0,5ml) Cloprostenol with a 10 day interval.
- Group 4 : Two intramuscular injections of 0,25mg (1,0ml) Cloprostenol with a 10 day interval.

Following the second injection of Cloprostenol the ewes were tested and blood samples (10ml) were collected at eight hour intervals for a period of 96 hours or until they showed oestrus. The serum was recovered and stored at - 20°C until it was used to determine the serum progesterone concentration by the RIA technique of Yousef=

nejadian, Florensa, Collins & Sommerville (1972) as modified by Faure (1975).

## RESULTS:

The response of the ewes to the different dosages of Cloprostenol is presented in Table 3.1.

From this table it is obvious that an increase in the dosage of Cloprostenol was accompanied by a significant increase in the oestrus response. It is also clear that the higher dosage (250 $\mu$ g) caused the occurrence of oestrus for the group as a whole to be more closely synchronised. The time to the onset of oestrus did not differ significantly between these treatment groups, but there was a great variation in response from Group 1 (31,25 $\mu$ g) where only 50% responded within the 96 hour observation period as compared to Group 4 (250 $\mu$ g) where all the ewes responded within 56 hours ( $P < 0,05$ ).

Changes in the serum progesterone concentrations of all the treated ewes are summarized in Figures 3.1 to 3.4. From these figures it is obvious that 250 $\mu$ g of Cloprostenol caused complete and rapid luteolysis in all the ewes (Figure 3.4) while lower dosages often failed (Figures 3.1 - 3.3). For the sake of comparison, the rates of decrease in serum progesterone concentration of all the ewes which responded are presented in Figure 3.5. Although the injection of 250 $\mu$ g Cloprostenol caused the most rapid decrease in serum progesterone concentration, these differences between groups were not significant. The mean serum progesterone concentrations at oestrus for the different groups were also found to be not significantly different.

## DISCUSSION

Data from this experiment prove that oestrus can be synchronised very efficiently by two injections of 250 $\mu$ g Cloprostenol given at a 10 day interval. Dosages of 31,25 $\mu$ g, 62,5 $\mu$ g and 125 $\mu$ g were often insufficient to induce complete luteolysis and in those ewes not responding fully to the luteolytic effect of Cloprostenol, an initial decline in progesterone following Cloprostenol injection was followed by a gradual increase in serum progesterone concentration and a suggested recovery of luteal function. Similar results have been reported

Table 3.1

The oestrus response, the interval to onset of oestrus (hours) and serum progesterone concentrations of ewes receiving two injections of 31,25µg, 62,5µg, 125µg and 250µg Cloprostenol respectively at a 10 day interval.

	Group 1 (31,25µg)	Group 2 (62,5µg)	Group 3 (125µg)	Group 4 (250µg)
Number of Ewes	16	16	16	16
Number of Ewes showing oestrus(%)	8(50,0) <sup>a</sup>	9(56,25) <sup>a</sup>	13(81,25) <sup>b</sup>	16(100,0) <sup>b</sup>
Interval between Second Injection and onset of Oestrus (h)	39,6 ± 21,17 <sup>a</sup>	41,56 ± 4,71 <sup>a</sup>	45,38 ± 9,91 <sup>a</sup>	44,31 ± 7,59 <sup>a</sup>
Range (h)	8 - 72	35 - 48	32 - 72	32 - 56
Mean Serum Progesterone Concentration at Oestrus	1,44 ± 0,57 <sup>a</sup>	1,45 ± 0,53 <sup>a</sup>	1,31 ± 0,33 <sup>a</sup>	0,96 ± 0,49 <sup>a</sup>

<sup>a</sup> <sup>b</sup> Within the body of the table, figures having the same superscript are not significantly different from each other.

Figure 3.1 The mean serum progesterone concentrations of those ewes failing to show oestrus and showing oestrus respectively, for the double Cloprostenol injection treatment

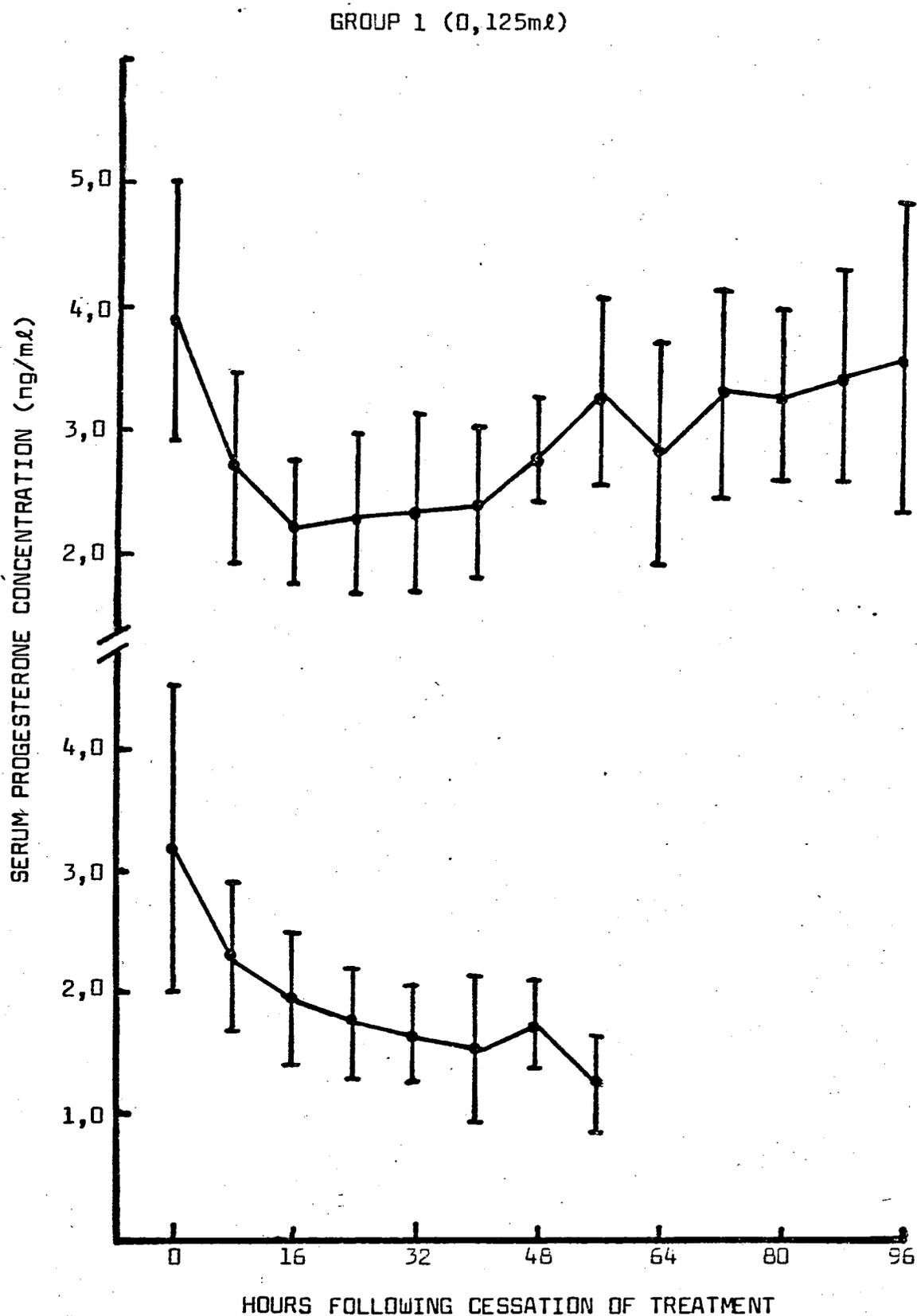


Figure 3.3 The mean serum progesterone concentrations of those ewes failing to show oestrus and showing oestrus respectively, for the double Cloprostenol injection treatment

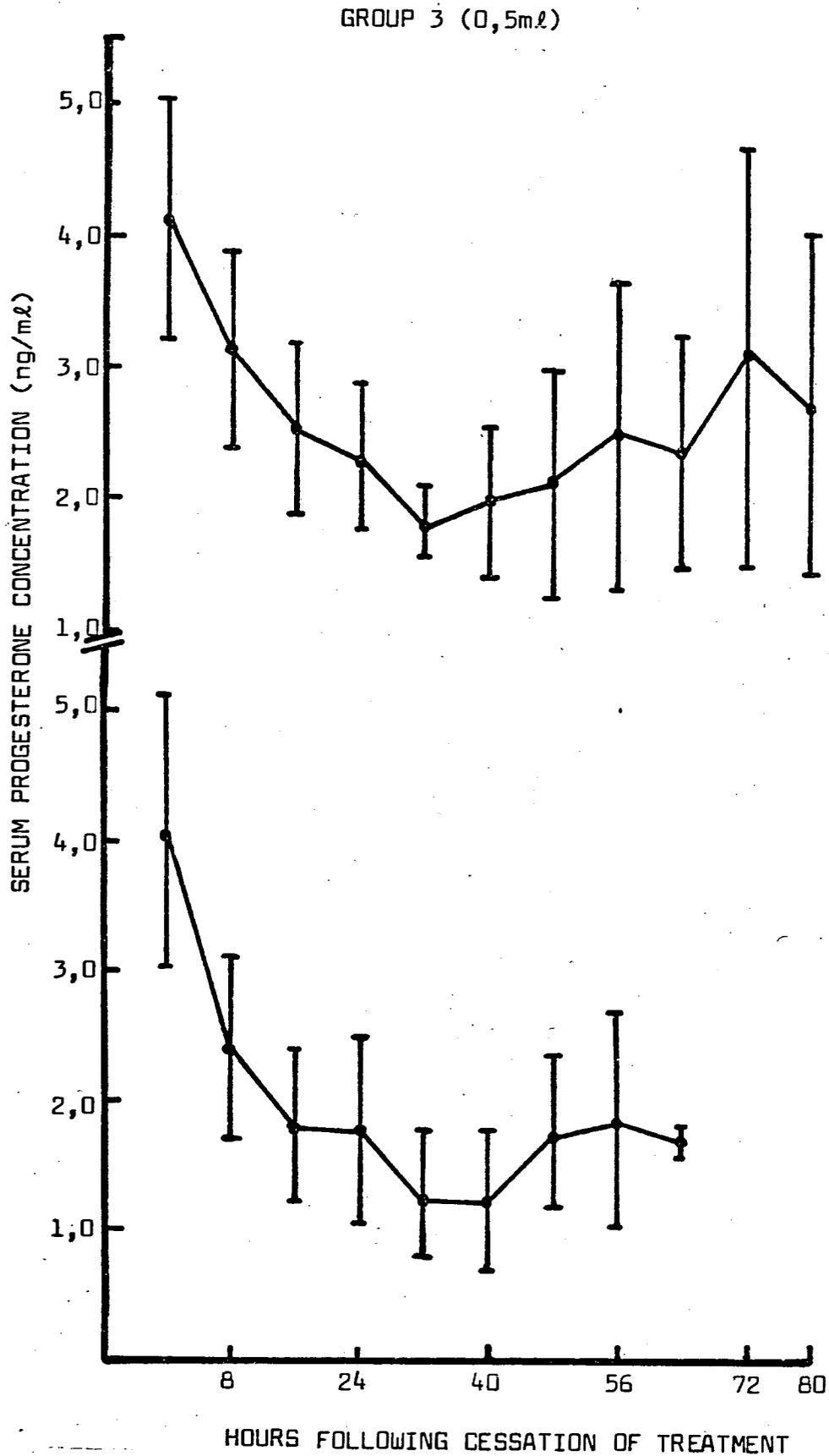


Figure 3.4 The mean serum progesterone concentrations of ewes showing oestrus for the double Cloprostenol injection treatment

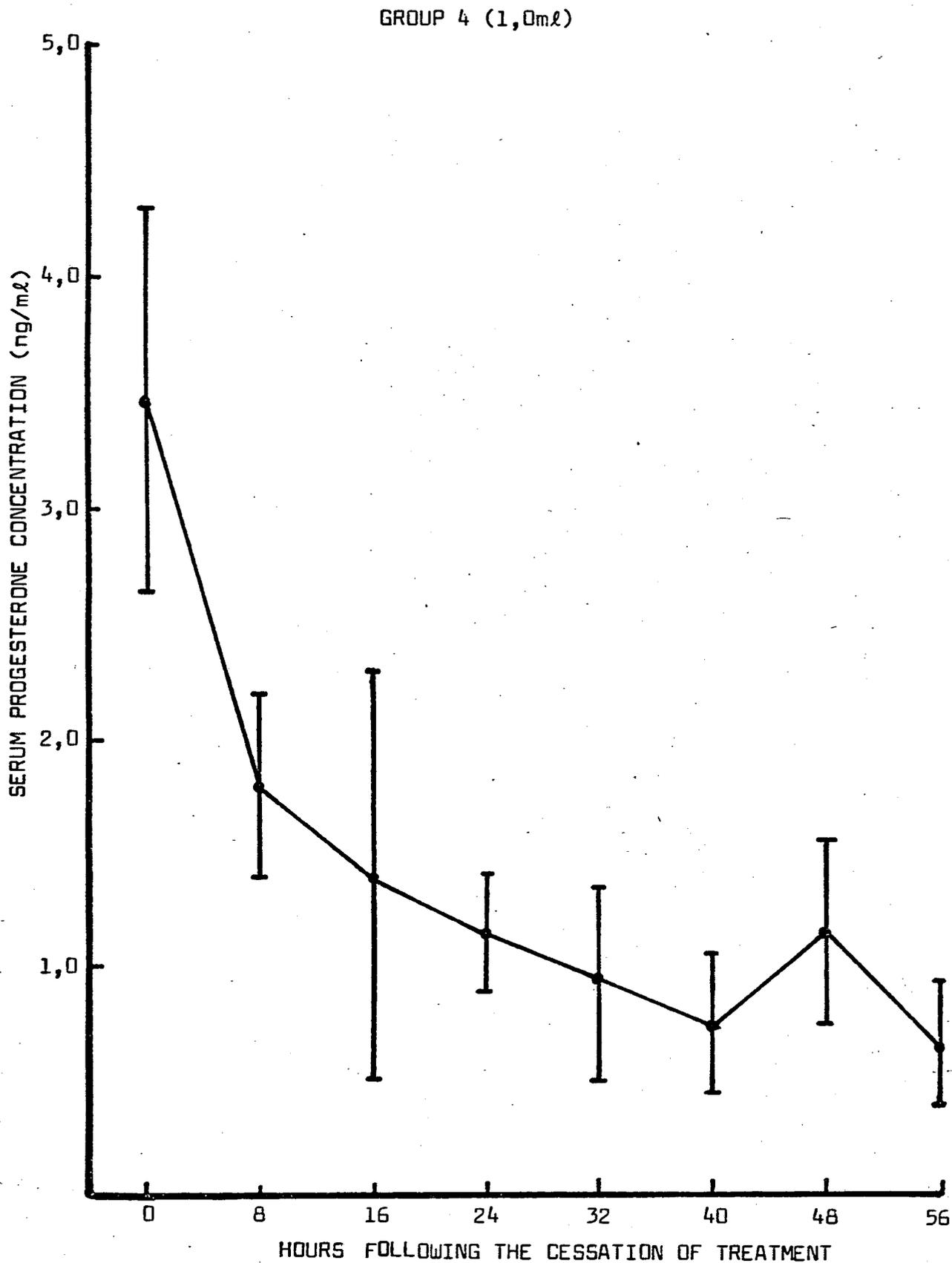
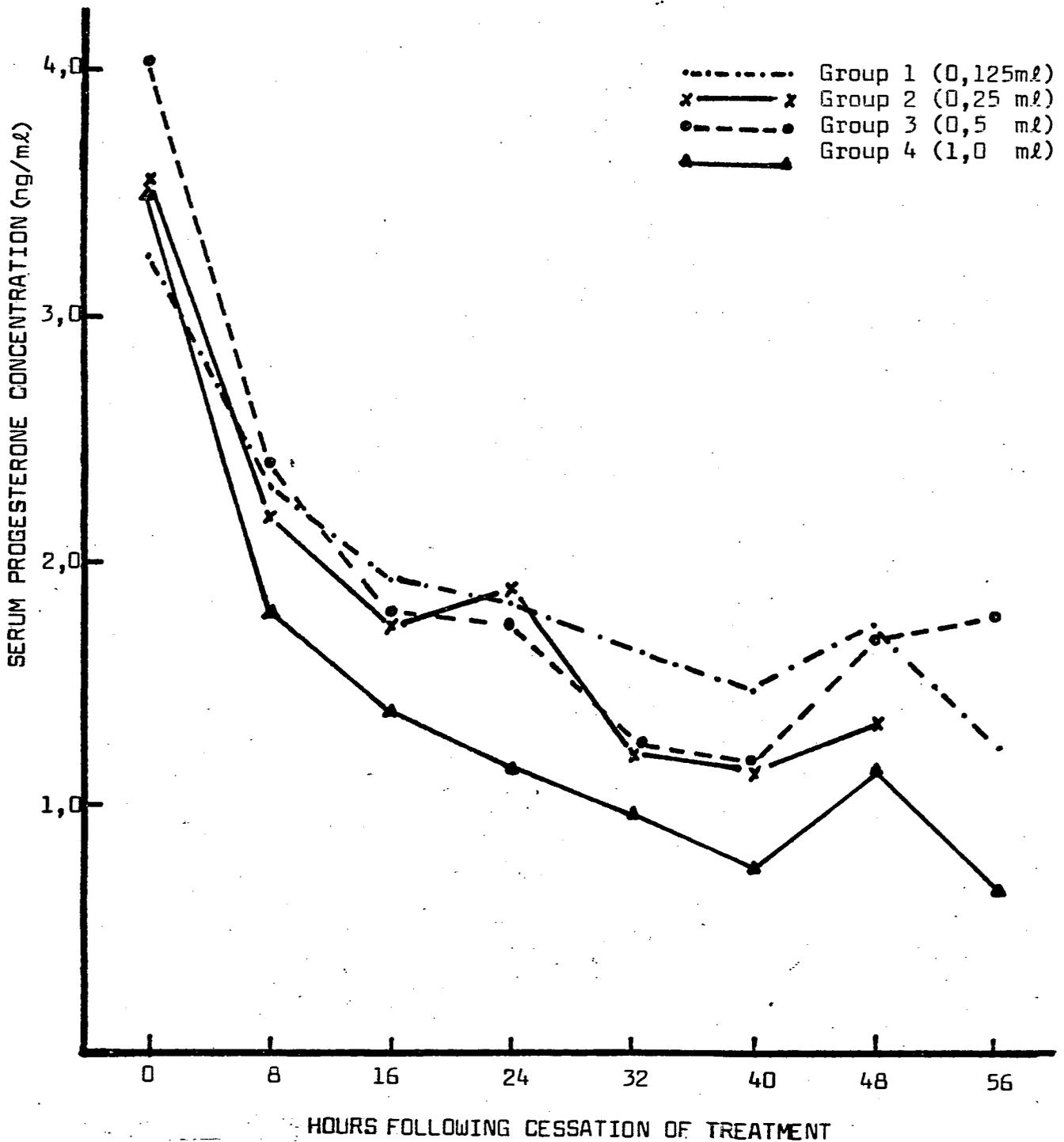


Figure 3.5 Serum progesterone concentrations of all ewes responding to the respective double injection treatments.



earlier (Thorburn & Nicol, 1971). The occurrence of this phenomenon increased in frequency as the dosage Cloprostenol decreased. The ewes not responding were not found to come from a specific stage of the cycle, but were randomly distributed throughout the cycle.

It is therefore concluded that i.m. injections of 250 $\mu$ g Cloprostenol cause luteolysis in all ewes and result in efficient synchronisation of oestrus when given at a 10 day interval whereas dosages of 125 $\mu$ g and lower are not always successful in this respect.

CHAPTER 4THE SYNCHRONISATION OF OESTRUS IN SHEEP : COMPARISON OF THE REPRODUCTIVE EFFICIENCIES OF CONTROL, INTRAVAGINAL SPONGE, INTRAVAGINAL SPONGE PLUS A PROSTAGLANDIN F<sub>2α</sub> ANALOGUE (CLOPROSTENOL) AND DOUBLE INJECTION CLOPROSTENOL TREATED EWES.

It has now been proved that the oestrus periods of sheep can be efficiently synchronised with progestogens (Deweese, Glimp & Dutt, 1970; Van der Westhuysen & Van Niekerk, 1971), progestogen - prostaglandin combination (Chapter II) or with the injection of sufficient prostaglandin twice with a 10 day interval. Fertility following the intravaginal progestogen treatment (Hawk & Conley, 1973; Gordon, 1976) and the intravaginal progestogen/Cloprostenol method (Chapter II) is slightly depressed. Fairnie, Cumming & Martin (1976a) found that the synchronised oestrus following a double treatment of Cloprostenol (125µg) resulted in acceptable fertility.

In order to evaluate the practical advantages and the fertility of ewes following these different techniques of synchronisation of oestrus, this experiment was designed to compare the reproductive efficiency of ewes following intravaginal progestogen, intravaginal progestogen/prostaglandin analogue and the double prostaglandin analogue treatments.

MATERIALS AND METHODS

During the normal breeding season (February, 1978) 140 mature and 60 maiden S.A. Mutton Merino ewes of which the oestrus cycles had been previously monitored, were allotted to four groups. Each group consisted of 50 ewes, balanced with regard to age and the stage of their oestrus cycle, so that days 1 to 17 of the oestrus cycle were represented in each group. These four groups each received the following treatment:

- Group 1 : Control group
- Group 2 : Intravaginal sponges (MAP - 60mg) for a period of 14 days.
- Group 3 : Intravaginal sponges (MAP - 60mg) for a period of 8 days and a 125 $\mu$ g intramuscular injection of Cloprostenol at sponge withdrawal.
- Group 4 : Two intramuscular injections of 250 $\mu$ g (1,0ml) Cloprostenol with a 9 day interval between the two injections.

For the sake of convenience the treatments were arranged so that the termination of sponge treatment (groups 2 and 3) and the last Cloprostenol injection (group 4) coincided. The ewes were then regularly (06h00, 12h00 and 16h00) tested for oestrus with the aid of vasectomised rams. Ewes in oestrus were inseminated 12 hours after identification and again at 12 hour intervals for as long as they remained in oestrus. The control group were tested and inseminated in the same way for the duration of an entire cycle.

#### RESULTS:

The oestrus response (Figure 4.1) and duration of oestrus following the different treatments are set out in Table 4.1

From Table 4.1 it can be seen that four ewes (one from Group 2 and three from Group 3) did not respond to the treatments, but the oestrous response of the groups did not differ significantly. Similarly, neither did the mean interval from the cessation of treatment to the onset of oestrus nor the duration of oestrus differ significantly between the treatment groups (Table 4.1). As regards the reproductive efficiencies (Table 4.2) it can be seen that the conception rates of the ewes that received a double injection of prostaglandin was significantly lower than the control group ( $P < 0,01$ ), the intravaginal sponge/prostaglandin group ( $P < 0,01$ ) and the group of ewes only receiving intravaginal sponges ( $P < 0,05$ ). In consequence, also the reproductive efficiencies (lambs born) per treatment group showed the same pattern. However, the fecundity for the four respective groups did not differ significantly from each other. The stage of the oestrus cycle when the treatments commenced, had no apparent effect on the oestrus response and conception rates of any of the groups.

**Table 4.1** The oestrus response, time interval to onset of oestrus and duration of oestrus for ewes treated with MAP sponges for 14 days, MAP sponges for 8 days followed by a Cloprostenol injection or two injections Cloprostenol at a 9 day interval.

	Group 1 Control	Group 2 MAP - 60mg	Group 3 MAP - 60mg + PGF	Group 4 PGF + PGF
Number of Ewes	50	50	50	50
Number of Ewes in Oestrus	50	49	47	50
Interval from cessation of treatment to onset of oestrus (h)	-	43,59 ± 16,93	58,02 ± 12,87	55,24 ± 27,36
Range (h)	4 - 360	24 - 102	33 - 96	24 - 170
Duration of Oestrus (h)	34,68 ± 5,75	30,18 ± 7,13	32,46 ± 7,39	34,96 ± 5,75

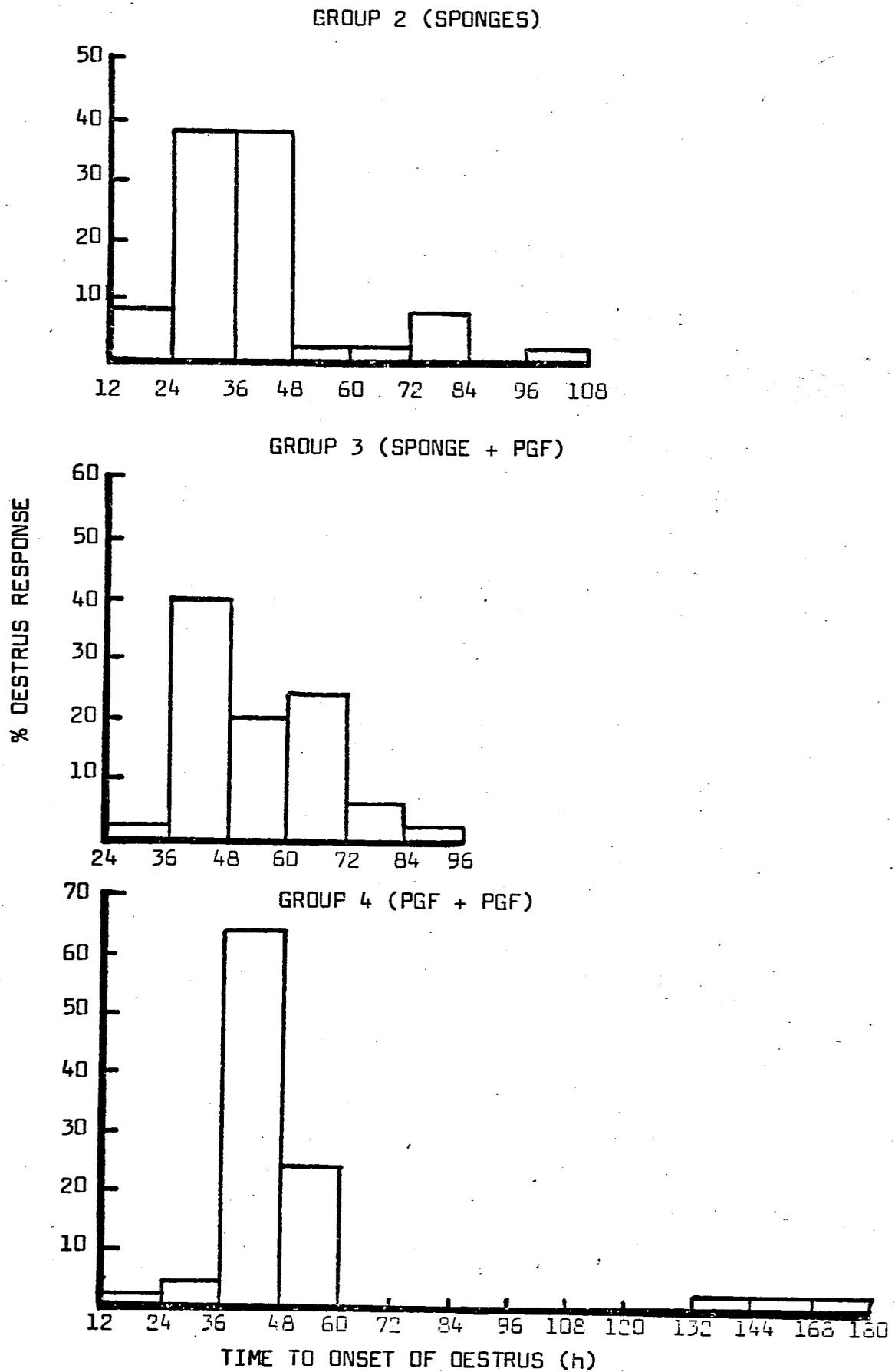
Table 4.2

The conception rate, lambing rate and fecundity of ewes treated with MAP sponges for 14 days, MAP sponges for 8 days followed by a Cloprostenol injection or two injections Cloprostenol at a 9 day interval.

	Group 1 Control	Group 2 MAP - 60mg	Group 3 MAP - 60mg+PGF	Group 4 PGF+PGF
	%	%	%	%
Number of Ewes	50	50	50	50
Number of Ewes conceiving/ Ewes showing oestrus	39 <sup>a</sup> 78,0	30 <sup>a</sup> 61,2	36 <sup>a</sup> 76,6	19 <sup>b</sup> 36,0
Ewes lambing/Ewes treated	39 <sup>a</sup> 78,0	30 <sup>a</sup> 60,0	36 <sup>a</sup> 72,0	18 <sup>b</sup> 36,0
Lambs born/Ewes treated	66 <sup>a</sup> 132,0	45 <sup>a</sup> 90,0	57 <sup>a</sup> 114,0	29 <sup>b</sup> 58,0
Lambs born/Ewe lambing	1,69 <sup>a</sup>	1,5 <sup>a</sup>	1,58 <sup>a</sup>	1,6 <sup>a</sup>

<sup>a</sup> <sup>b</sup> Within the body of the table, figures having the same superscript are not significantly different from each other.

Figure 4.1 The distribution of the occurrence of oestrus in ewes receiving intravaginal (MAP) sponges, intravaginal sponges/ Cloprostenol treatment and two injections of Cloprostenol at a 9 day interval



DISCUSSION

This experiment proves that the oestrus periods of sheep can be efficiently synchronised by the intravaginal progestogen sponge technique, the intravaginal progestogen sponge/prostaglandin combination or two injections of prostaglandin administered with a 9 day interval. Although in the present experiment the fertility of intravaginal progestogen sponge treated ewes and intravaginal progestogen sponge/prostaglandin treated ewes approached normality it was slightly depressed. These results corroborate previous findings on progestogen sponges (Robinson, 1967; Van der Westhuysen & Van Niekerk, 1971) and the progestogen/prostaglandin technique (Chapter 2) in sheep. The conception rate of the double prostaglandin treated group was highly significantly depressed, but the reason for this depressed fertility is obscure. Hughes, Lucas & Notman (1977) using a different synthetic analogue (ONO 453) of prostaglandin found conception rates of 70,8% with two injections given at a 7 day interval. Fairnie, Cummings & Martin (1976b) when comparing the use of sponges (Cronolone) with a double injection Cloprostenol, as a means of synchronising oestrus, found conception rates of 56% and 53% for the sponge and prostaglandin groups respectively. Recently, work has been published in which the effect on fertility of the time interval between the two consecutive injections were demonstrated (Fairnie, Wales & Gherardi, 1977; Fairnie, Martin & Rogers, 1978; Fairnie & Wales, 1978). According to these workers, the time interval between the two injections of prostaglandin (Cloprostenol) is critical for optimum fertility and should not be reduced to less than 13 or 14 days. From the results of this experiment it is therefore concluded that although the use of the double injection prostaglandin regime offers an efficient technique for synchronising oestrus, the disappointing fertility following injections at a 9 day interval renders it impractical. However, since injections at greater intervals tend to result in higher fertility (Fairnie, Wales & Gherardi, 1977), these possibilities need verification before this method for the control of ovulation can be accepted.

CHAPTER 5THE EFFECT OF SYNCHRONISATION OF OESTRUS IN SHEEP WITH A PROSTAGLANDIN ANALOGUE OR PROGESTOGEN SPONGES ON THEIR REPRODUCTIVE RESPONSE TO ARTIFICIAL INSEMINATION AT OESTRUS OR AT A FIXED TIME.

The ultimate aim of the synchronisation of the oestrus cycles of sheep is the practicability of a successful artificial insemination program. Such a program still requires twice daily use of vasectomised rams for the identification of ewes in oestrus. Thus the possibility of artificial insemination at a fixed time following progestogen intravaginal sponge withdrawal has been investigated (Robinson & Moore, 1967; Colas & Cognie, 1968; Van Niekerk & Belonje, 1970; Van der Westhuysen, Van Niekerk & Hunter, 1970b; Van Wyk, 1977) to eliminate this time consuming identification of ewes in oestrus. Not only is oestrus detecting the most time and labour consuming input in an AI programme, but is also the area where many problems occur due to poor detection and "silent heats". The provision of teaser animals and equipment is expensive and all these inputs can be reduced with a fixed time insemination (Eaton, 1976) and warrant the practical application thereof. Similarly, the efficiency of synchronisation of the oestrus periods in sheep following the double injection regime of prostaglandin led to the investigation of the application of fixed time AI following this technique of synchronisation. The success of fixed time AI will depend on the efficiency of synchronisation. So for instance it has been proved that the degree of synchronisation of the oestrus periods of sheep is much higher following the double injection of prostaglandin (Cloprostenol) than following the intravaginal progestogen treatment (Chapter 4). For this reason this experiment was planned to investigate the practicability of fixed time artificial insemination following synchronisation of oestrus with progestogens and prostaglandins respectively.

## MATERIAL AND METHODS

During the breeding season (May, 1978), 104 S.A. Mutton Merino ewes ranging from maiden to multiparous ewes were used. The ewes were randomly allotted to a 2 x 2 factorial designed experiment of equal group size with the following treatments:

- (1) Intravaginal progestogen sponges (Methyl acetoxy progesterone, MAP-60mg) for 14 days vs two injections of 250 $\mu$ g Cloprostenol with a 10 day interval between the injections.
- (2) Artificial insemination at oestrus vs AI at a fixed time following the cessation of treatments.

In the groups where oestrus ewes were identified with the aid of vasectomised rams, insemination with 0,1ml undiluted semen was performed 12 hours following the first positive test for oestrus and again 12 hours later. As regards the fixed time insemination groups, the times of insemination were based on the previous results (Chapter 4). The sponge treated group were inseminated 48 and 60 hours following sponge withdrawal, while the double prostaglandin treated group were inseminated 60 and 72 hours following the last injection.

### RESULTS:

All the ewes in the groups which were tested with vasectomised rams, showed oestrus within 96 hours, except for one ewe in the intravaginal sponge group. The oestrus response for these groups are presented in Figure 5.1. The times at which the fixed time groups were inseminated are also indicated in this figure.

From the distribution of oestrus (Figure 5.2) it can be seen that 80,7% of the sponge treated ewes had come into oestrus by the time of the first insemination (48 hours following the cessation of treatment) and 88,4% by the second insemination (60 hours). Similarly, in the prostaglandin treated ewes, 92,3% of the ewes had come into oestrus after 60 hours (first insemination) and 96,1% by the second insemination (72 hours).

The reproductive performances of these groups are presented in Table 5.1.

**Table 5.1** The conception rate, lambing rate and fecundity of ewes treated with MAP sponges for 14 days and two injections Cloprostenol at a 10 day interval for a fixed time insemination group respectively.

	Group 1		Group 2	
	MAP sponge control	MAP sponge fixed time %	PGF + PGF control	PGF + PGF fixed time %
No. Ewes in each group	26	26	26	26
Ewes conceiving with first oestrus	16 <sup>a</sup>	64,0	17 <sup>a</sup>	65,4
Lambs born/ Ewes treated	25 <sup>a</sup>	96,2	29 <sup>a</sup>	111,4
Lambs born/ Ewe lambing	1,56 <sup>a</sup>	1,54 <sup>a</sup>	1,71 <sup>a</sup>	1,43 <sup>a</sup>
				53,9
				76,9

<sup>a</sup> Within the body of the table, figures having the same superscript are not significantly different from each other.

Figure 5.1 The distribution of the occurrence of oestrus in ewes following treatment with intravaginal progestogen sponges and a double injection prostaglandin (Cloprostenol)

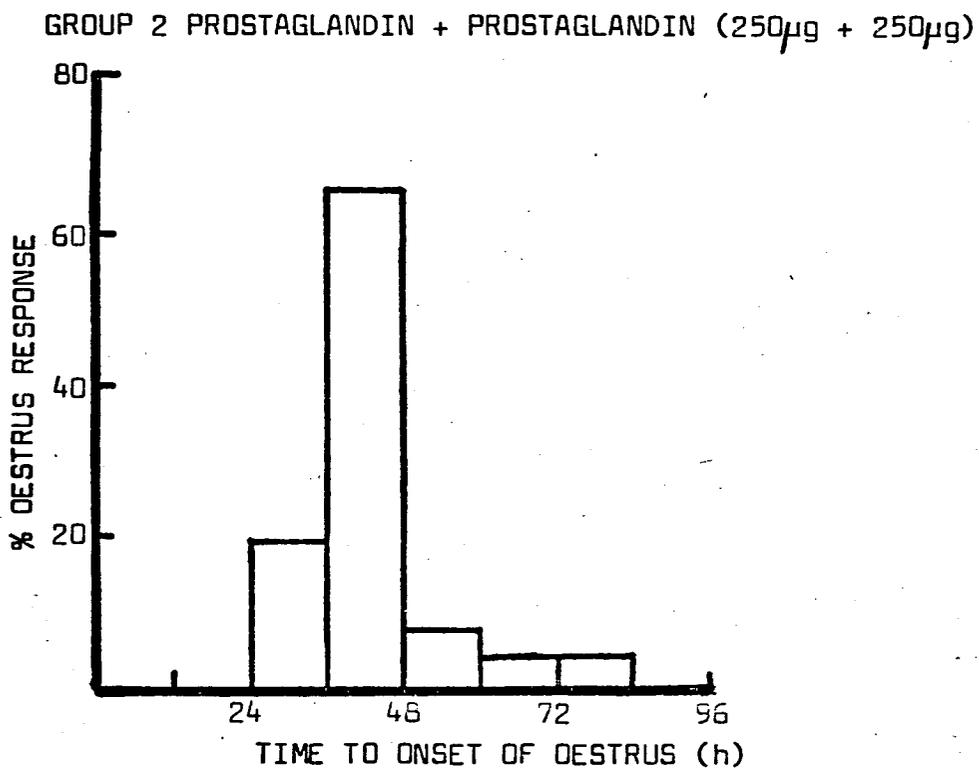
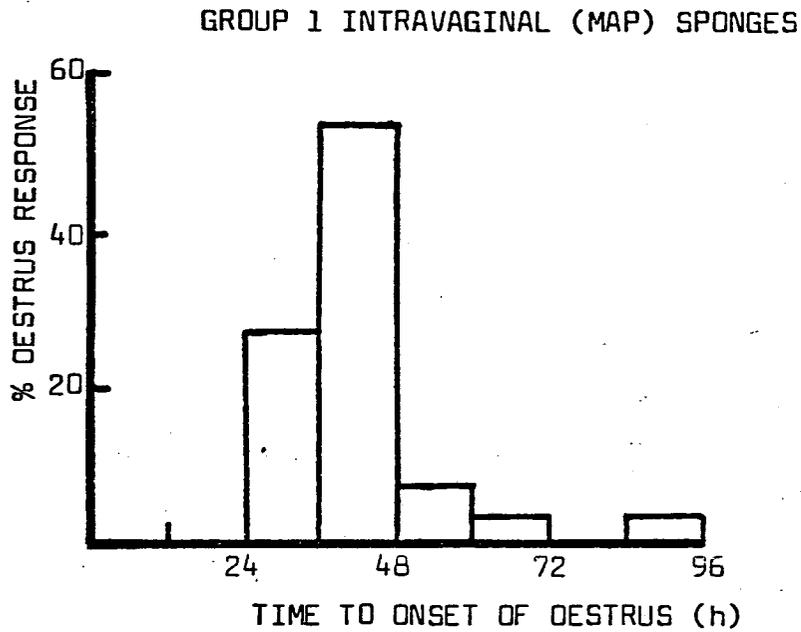
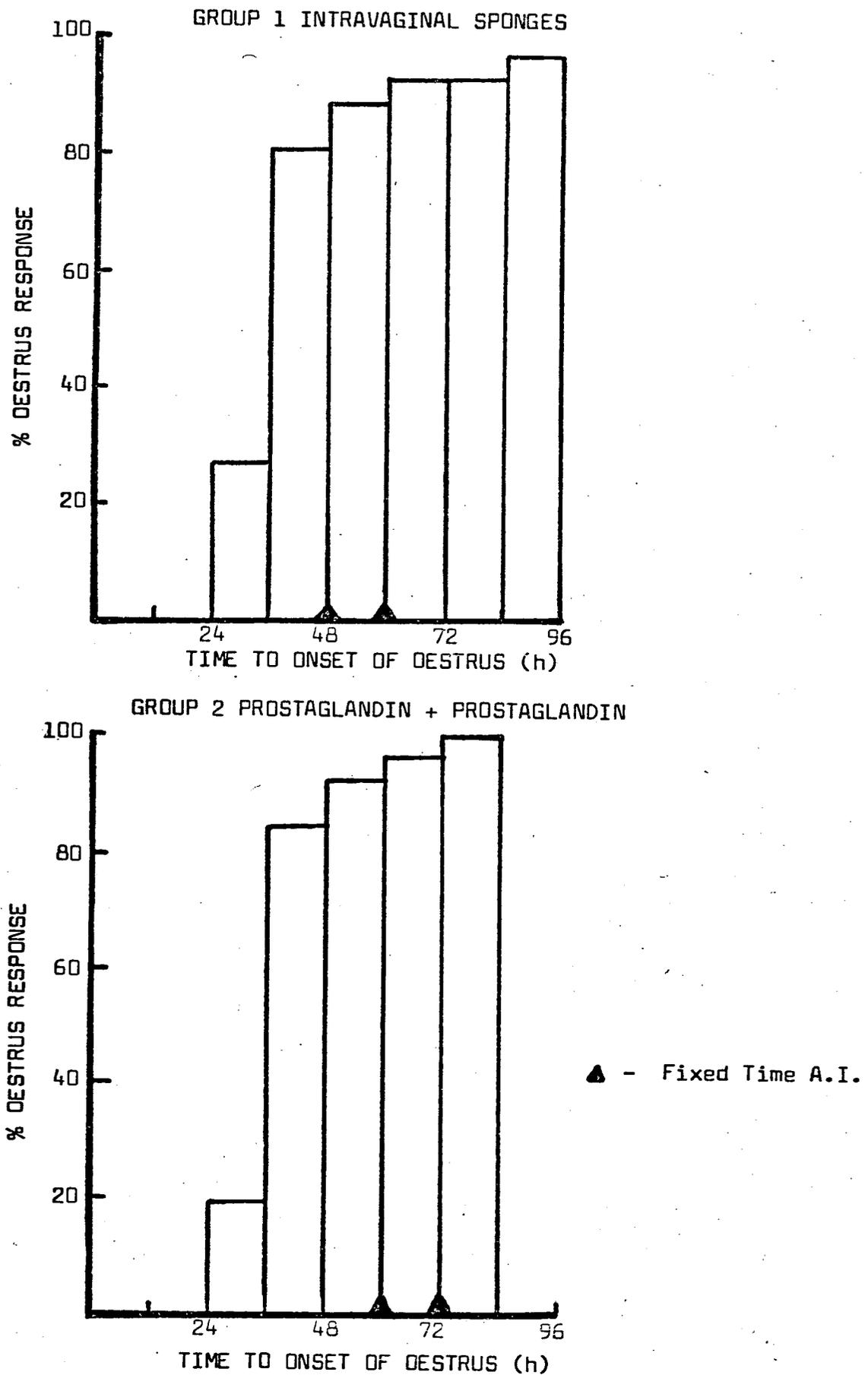


Figure 5.2 Oestrus response following treatment with intravaginal progestogen sponges and a double prostaglandin injection (Cloprostenol) (Accumulative)



The lambing rate and fecundity did not differ significantly between the respective groups.

### DISCUSSION

It has again been certified that both the intravaginal progesterone (MAP) sponge treatment and the double injection of prostaglandin is efficient in synchronising oestrus in randomly cycling sheep. In this experiment no differences were found between treatments in either the duration of time from the cessation of treatment or the distribution of oestrus periods following the respective treatments. Similarly, the reproductive performances of the Cloprostenol treated and sponge treated groups were not significantly different from each other, but the application of AI at a fixed time reduced conception rate consistently by approximately 10%. However, considering that ovulation takes place approximately 30 hours after the commencement of oestrus (Van der Westhuysen et al, 1970b), fixed time AI of the MAP-sponge treated ewes would probably be more efficiently spaced at 60 and 72 hours following sponge withdrawal (Figure 5.2). So for instance Petcu, Scheul & Barbu (1977) found the optimum time for insemination to be about 68 hours following the end of hormonal synchronisation. According to Colas, Brice & Guerin (1974) and Gordon (1975) (as quoted by Gordon, 1976) fixed time AI in sheep has progressed from two inseminations at 50 and 64 hours to a single insemination at 55 - 57 hours, without the conception rate necessarily being depressed. Similarly Dýrmundsson (1977) obtained conception rates of 65,4% following an insemination 48 - 56 hours following sponge withdrawal.

Likewise Fairnie, Wales & Gherardi (1977) inseminated ewes 64 hours following the cessation of a double Cloprostenol (125µg) treatment with a 14 day interval and found the fertility to be 60%. Other workers (Fukui & Roberts, 1978) found the fertility of ewes treated with a double injection prostaglandin  $F_{2\alpha}$  at a 12 day interval, to be much higher when inseminated at 70 hours (62%) following the cessation of treatment than insemination 46, 54 or 78 hours following the cessation of treatment. Although the lambing rates and fecundity following fixed time AI in this experiment did not differ significantly from those ewes which were inseminated at observed oestrus, the significant but consequently lower conception rates following fixed time

AI suggests that it should be subjected to further large scale field trials before its practical application could be justified.

CHAPTER 6THE EFFECT OF THE INTERVAL BETWEEN PROSTAGLANDIN (CLOPROSTENOL) INJECTIONS IN THE DOUBLE INJECTION REGIME, ON THE REPRODUCTIVE PERFORMANCE OF EWES.

To ensure that all ewes are at an appropriate stage of the oestrus cycle to respond to prostaglandin treatment, the double injection regime has been developed as a method of synchronisation of oestrus (Haresign, 1976). By this technique two injections of prostaglandin are given at a 8 or 9 day interval (Haresign 1978). At the time of this experiment, the effect of the interval between the two injections was unknown, but in two previous experiments (Chapter 4 and 5) in this series it was noticed that an increase in this interval from 9 to 10 days was accompanied by an increase in fertility. For this reason an experiment was planned to investigate the effect of this interval on the subsequent oestrus response and fertility.

MATERIAL AND METHODS

This experiment was carried out towards the end of the breeding season (July) on 50 mature Merino ewes. These ewes were randomly divided into four groups and each group received the following treatments:

- Group 1 : 20 control ewes.
- Group 2 : 10 ewes each receiving a double intramuscular injection Cloprostenol (125 $\mu$ g) at a 9 day interval.
- Group 3 : 10 ewes each receiving a double intramuscular injection Cloprostenol (125 $\mu$ g) at a 10 day interval.
- Group 4 : 10 ewes each receiving a double intramuscular injection Cloprostenol (125 $\mu$ g) at a 11 day interval.

For the sake of convenience, the oestrus cycles of the control ewes were synchronised two cycles previously with the aid of intravaginal progestogen (MAP) sponges and observations were made at the second (normal) oestrus period. All three treatment groups received the same treatment, except that the time interval between the two injections for the respective groups varied. The treatments were also

so timed that all three groups received their second injection on the same day.

Following the last injection, all the ewes from all four groups were regularly tested (07h00 and 16h00) for oestrus with the aid of vasectomised rams. Ewes that showed oestrus were inseminated 12 hours later and then again 12 hours later. Fourteen days after the first ewes showed oestrus, the ewes were again tested to determine the number of ewes returning to service. Ewes not returning to service were considered pregnant.

### RESULTS:

The time interval (in hours) between the last injection prostaglandin and the times at which the ewes showed oestrus, are set out in Table 6.1 and Figure 6.1.

It should be noted that of the 50 ewes, four did not show oestrus - three of which were in the control group and one in Group 3 (9 day interval). The time to the onset of oestrus was more closely synchronised for the 11 day interval group, but the differences between the respective treatment groups were not significant. Similarly there was no significant difference in the oestrus response for the different groups. The conception rates of the ewes are presented in Table 6.2.

From Table 6.2 it is apparent that as the interval between the two injections of Cloprostenoī was increased from 9 to 11 days, the conception rate increased correspondingly. So for instance, the conception rates of all the prostaglandin treated groups (days 9, 10 and 11) differed significantly from each other ( $P < 0,05$ ). The conception rate of ewes injected with a 11 day interval, did not differ significantly from that of the control ewes.

### DISCUSSION

The interval between the two injections of prostaglandin has no significant effect on the oestrus response of ewes, however it is apparent that fertility is significantly impaired as the interval between treatments is decreased below 11 days. The reason for the impaired fertility is still unclear, but it indicates that the hor=

**Table 6.1** The oestrus response and the time of onset of oestrus following a double injection of prostaglandin F<sub>2α</sub> (Cloprostenol) given at a 9,10 and 11 day interval respectively.

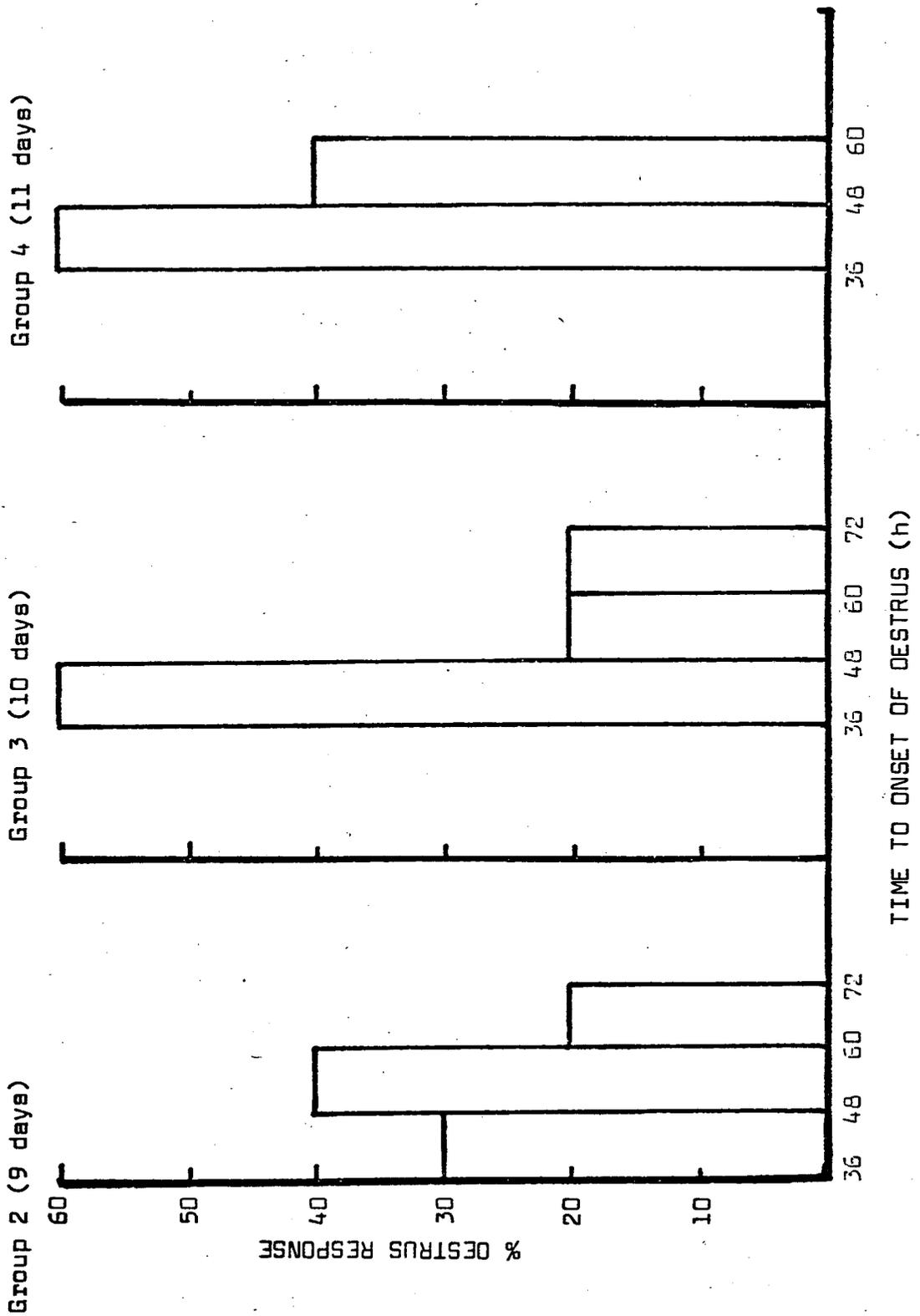
Group 1 (Control)		Group 2 (9 days)		Group 3 (10 days)		Group 4 (11 days)	
No.Ewes	% oestrus	No.Ewes	% oestrus	No.Ewes	% oestrus	No.Ewes	% oestrus
3	15	3	30	6	60	6	60
	72		48		48		48
3	15	4	40	2	20	4	40
	84		60		60		60
4	20	2	20	2	20		
	96		72		72		
2	10						
	108						
5	25						
	120						
17	85,0	9	90,0	10	100,0	10	100,0

Table 6.2 The conception rates following a double injection of prostaglandin F<sub>2α</sub> (Cloprostenol) given at 9,10 and 11 day intervals respectively.

Group 1 Control		Group 2 9 Days		Group 3 10 Days		Group 4 11 Days	
No. Ewes Inseminated	Con- ceived						
17	14	9	1	10	4	10	7
	3		8		6		3
85,0	82,4	90,0	11,1	100,0	40,0	100,0	70,0
	17,6		88,9		60,0		30,0

%

FIGURE 6.1 The distribution of the occurrence of oestrus in ewes receiving a double injection prostaglandin 9, 10 and 11 days apart.



monal events preceding oestrus play an important role in the fertility at that oestrus. So for instance the short progestational phase of an 8 day interval group may affect sperm transport (Hawk, 1973) and Fairnie et al (1977) found treatment of prostaglandin 6 days apart markedly reduces the life span of the corpus luteum and exposure of the reproductive tract to its progesterone prior to insemination. However, on the evidence of this experiment and previous results (Chapters 4 and 5) it has been proved beyond doubt that the interval between injections should not be less than 11 days and could possibly be extended to a maximum of 13 to 14 days (Fairnie, et al, 1978; Fairnie & Wales, 1978).

SUMMARY

1. Preliminary observations proved that an intramuscular injection of 62,5 $\mu$ g Cloprostenol terminates the oestrus cycle of ewes. Higher dosages (125 $\mu$ g, 250 $\mu$ g and 500 $\mu$ g) caused a more abrupt termination of the cycle and more synchronised occurrence of oestrus. However, 125 $\mu$ g was only effective in terminating the oestrus cycle when injected between days 4 and 14.
2. In order to overcome this refractory period to Cloprostenol treatment, (days 15 through oestrus to day 4 of the oestrus cycle) ewes were treated with intravaginal progestogen sponges for 8 - 9 days and injected with Cloprostenol on the day of sponge withdrawal. A dosage of 31,25 $\mu$ g proved adequate, but conception rates were significantly lower at the first post treatment oestrus (mean 63,7%) as compared to the second post treatment oestrus (mean 81,9%). The change in the serum progesterone concentration following the cessation of treatment was not affected by the dosage of Cloprostenol (31,25 $\mu$ g; 62,5 $\mu$ g and 125 $\mu$ g), but the position of the LH peak relative to the onset of oestrus varied markedly. The stage of the cycle when the intravaginal sponge treatment started had a significant affect on the interval between the cessation of treatment, the onset of oestrus and the LH peak.
3. The time of Cloprostenol administration relative to intravaginal sponge withdrawal (-48, -24 and 0h) showed no significant effect on either the oestrus response or the duration of oestrus. However, for the group receiving the prostaglandin injection at sponge withdrawal (0h) the interval between cessation of treatment and oestrus showed a marked decrease as the onset of the progestogen treatment progressed from day 2 to day 17 of the oestrus cycle. The reproductive efficiencies of the three respective treatment groups did not differ significantly from each other, neither was there a significant difference between the reproductive performances at the first and the second post treatment oestrus.
4. An alternative method of bypassing the refractory period of the corpus luteum to prostaglandin is by giving two injections of prostaglandin 8 to 14 days apart. In this experi-

ment different dosages (31,25 $\mu$ g; 62,5 $\mu$ g; 125 $\mu$ g and 250 $\mu$ g) of Cloprostenol were injected at a 10 day interval. An increase in the dosage was followed by a significant increase in the oestrus response (50,0%; 56,3%; 81,3% and 100,0% respectively). The higher dosages (250 $\mu$ g) of Cloprostenol cause more rapid and complete luteolysis as is reflected in the decrease in plasma progesterone concentration, while lower dosages (31,25 $\mu$ g and 62,5 $\mu$ g) often fail.

5. The reproductive efficiencies of ewes treated with the intravaginal progestogen sponge (MAP), an intravaginal progestogen sponge (MAP) followed by an injection of Cloprostenol (125 $\mu$ g), a double injection of 250 $\mu$ g Cloprostenol at a 9 day interval and a control group were compared. The oestrus response, the interval from cessation of treatment to the onset of oestrus and the duration of oestrus did not differ significantly for the respective groups. The mean conception rate of ewes treated with a double injection of Cloprostenol at a 9 day interval was significantly lower (36%) than that of the other groups (mean of 71,9%).
6. In a 2 x 2 factorial experiment the reproductive efficiency of ewes treated with a double injection of prostaglandin at a 10 day interval and of a group of progestogen sponge (MAP) treated ewes were compared following insemination at observed oestrus and insemination at a predetermined time. The prostaglandin treated group was inseminated at 60 and 72 hours following the last injection of Cloprostenol and the sponge treated group at 48 and 60 hours following sponge withdrawal. Although the conception rates of the ewes were about 10% lower following fixed time A.I. as compared to A.I. at observed oestrus, these differences were not significant.
7. The reproductive efficiencies of ewes treated with two injections of prostaglandin (Cloprostenol) administered at intervals of 9, 10 and 11 days, were compared. The conception rates of ewes in these treatment groups were 11,1%; 40,0% and 70,0% respectively and that of the control group 82,4%. These differences indicate the importance of injecting Cloprostenol at an interval of at least 11 days.

OPSOMMING

1. Voorlopige waarnemings bewys dat 'n intramuskulêre inspuiting van 62,5µg Cloprostenol die estrus siklus van ooie beëindig. Hoër dosisse (125µg, 250µg en 500µg) veroorsaak 'n meer effektiewe en meer gesinkroniseerde estrus reaksie. Die gebruik van 125µg Cloprostenol is gevind om voldoende te wees om effektiewe luteoliese te veroorsaak vanaf dag 4 tot 14 van die estrus siklus.
2. Die sinkronisasie van die estrus periode met Cloprostenol is gedoen met 'n voorafbehandeling van intravaginale sponse, bevattende medroksie-progesteron asetaat vir 8 - 9 dae, om die refraktoriese periode te oorbrug (dag 15 - 3). Die aanteeldoeltreffendheid van die verskillende behandelde groepe het geen noemenswaardige patroon gevolg nie, alhoewel die vrugbaarheid betekenisvol verskil het vir die eerste na-behandelings estrus (gemid. 63,7%) en die tweede (normale) na-behandelings estrus (gemid. 81,9%). Aangaande die serum progesteron konsentrasies is gevind dat die tempo van afname in progesteron konsentrasie na beëindiging van behandeling, nie betekenisvol beïnvloed is deur die verskillende dosisse (31,25µg; 62,5µg en 125µg) van prostaglandien nie. Heelwat variasie in die posisie van die LH piek t.o.v. die begin van estrus is gevind en dit was duidelik dat die stadium van die estrus siklus 'n betekenisvolle effek op die interval tussen die beëindiging van behandeling en die voorkoms van die LH piek het.
3. Dit is gevind dat die tyd (-48, -24 en 0h) van Cloprostenol toediening relatief tot intravaginale spons onttrekking geen betekenisvolle effek op beide die estrus reaksie of die lengte van die estrus periode het nie. Alhoewel, vir die groep wat 'n prostaglandien inspuiting by spons onttrekking ontvang het (0h), is 'n duidelike afname in die interval tussen die beëindiging van behandeling en estrus waargeneem soos die stadium van progesteron behandeling gewissel het van dag 2 tot dag 17 van die estrus siklus. Die aanteeldoeltreffendheid vir die onderskeie behandelings groepe het nie betekenisvol verskil van mekaar nie en ook was daar geen betekenis-

volle verskil in die aanteeldoeltreffendheid by die eerste en tweede (normale) na-behandelings estrus nie.

4. 'n Alternatiewe metode om die refraktoriese periode van die corpus luteum tot prostaglandien te oorbrug, is deur die toediening van twee inspuitings prostaglandien 8 tot 14 dae uitmekaar. Verskillende dosisse (31,25 $\mu$ g; 62,5 $\mu$ g; 125 $\mu$ g en 250 $\mu$ g) Cloprostenol is gegee met 'n 10 dae interval tussen die inspuitings. 'n Vermeerdering van die dosis is gevolg deur 'n betekenisvolle verhoging in die estrus reaksie (50,0%; 56,3%; 81,3% en 100,0% respektiewelik). Die hoër dosisse (250 $\mu$ g) Cloprostenol veroorsaak meer vinnige en doeltreffende luteoliese terwyl die laer dosisse (31,25 $\mu$ g en 62,5 $\mu$ g) dikwels ondoeltreffend is. Die 250 $\mu$ g Cloprostenol groep het die vinnigste afname in die gemiddelde serum progesteron konsentrasie getoon.
5. Die aanteeldoeltreffendheid tussen intravaginale progesteron sponse (MAP) gevolg deur 'n inspuiting van Cloprostenol (125 $\mu$ g), 'n dubbele inspuiting Cloprostenol met 'n 9 dae interval en 'n kontrole groep is vergelyk. Die estrus reaksie, die interval vanaf beëindiging van behandeling tot begin van estrus en die lengte van die estrus periode het nie betekenisvol verskil vir die verskillende groepe nie. Die gemiddelde konsepsie syfer van ooie behandel met 'n dubbele inspuiting Cloprostenol met 'n 9 dae interval, was betekenisvol laer (36%) as die ander groepe (gemid. 71,9%).
6. In 'n 2 x 2 faktoriale eksperiment is vrugbaarheid, na inseminasie op waargeneemde estrus en inseminasie wat op 'n tydsbasis uitgevoer is, tussen 'n dubbele inspuiting prostaglandien groep met 'n 10 dae interval en 'n intravaginale (MAP) spons groep vergelyk. Die dubbel inspuiting groep is geïnsemineer 60 en 72 uur na die laaste inspuiting en die spons groep is geïnsemineer 48 en 60 uur na spons onttrekking. Alhoewel die konsepsie syfers van ooie ongeveer 10% laer was na inseminasie op 'n vasgestelde tyd teenoor inseminasie na waargeneemde estrus, was die verskil nie betekenisvol nie.

7. Die aanteeldoeltreffendheid van ooie wat met twee inspuitings prostaglandien (Cloprostenol) 9, 10 en 11 dae uitmekaar behandel is, is vergelyk. Die konsepsie syfer (vrugbaarheid) van die ooie in hierdie behandelings groepe was 11,1% 40,0% en 70,0% respektiewelik terwyl die van die kontrole groep 82,4% was. Hierdie verskille in vrugbaarheid dui op die noodsaaklikheid om die twee inspuitings Cloprostenol ten minste 11 dae uitmekaar te gee.

### CONCLUSION

The prostaglandin F<sub>2α</sub> analogue Cloprostenol (Estrumate, ICI 80996) has been proved to be a highly efficient luteolytic agent. Complete luteolysis and a concomittent decrease in serum progesterone concentration was found to occur without exception when a dosage of 250µg Cloprostenol was administered between days 4 to 14 of the oestrus cycle. In contrast to the high degree of synchrony of the oestrous periods of a flock following the injection of 250µg, lower dosages (31,25µg and 62,5µg) of Cloprostenol were less efficient in this respect and often failed to cause complete luteolysis and a subsequent drop in serum progesterone concentration. Nevertheless, ewes between days 15 of the oestrus cycle through the oestrus period to day 3 of the next cycle (approximately 30% of a flock), do not respond to a single injection and thus methods to bypass this refractory period were investigated. Both an intravaginal progestogen sponge (MAP) pre-treatment for 8 or 9 days prior to the prostaglandin injection, or two injections of Cloprostenol spaced 9 to 11 days apart were successful in this respect. However when the two injections were given 9 days apart fertility was greatly depressed and when this interval was increased to 10 and 11 days, it was accompanied by a subsequent increase in conception rate. This is in agreement with various other workers who suggest a 14 day interval to be the ultimate (Fairnie et al, 1976a,b).

A comparison of the reproductive efficiencies of ewes following different methods of synchronisation proved that oestrus can be successfully synchronised by means of intravaginal progestogen sponges (MAP) for 14 days, MAP sponges for 8 or 9 days followed by Cloprostenol administration and the double injection regime of prostaglandin. Whatever method was used, fertility was slightly depressed.

The high degree of synchronisation following prostaglandin treatment makes fixed time insemination a practical consideration. From these experiments it is concluded that artificial insemination without oestrus detection can be applied with equal success following intravaginal progestogen sponge treatment (48 and 60 hours following sponge withdrawal) and the double prostaglandin regime (60 and 72

hours following the last injection). From the distribution of oestrus periods, it appears that AI should be performed at 60 and 72 hours following the use of MAP sponges. The conception rate of ewes following fixed time insemination is some 10% lower than when AI is performed following oestrus detection.

In conclusion, the factors in favour of the double injection Cloprostenol regime as a synchronisation technique are firstly the ease of administration of the two prostaglandin injections, the absolute precise control, the close synchrony of oestrus and ovulation and the satisfactory fertility. In addition the possibility of uterine infection (Quinlivan & Robinson, 1967; Van der Westhuysen & Van Niekerk, 1971) is eliminated. However, treatments including Cloprostenol are still relatively expensive for use in sheep and the advantages not that much greater than the use of sponges alone. Fairnie et al (1976a) found a large day to day variation in the response of ewes to the double injection Cloprostenol technique. Although this could be attributed to the lower dosage (125 $\mu$ g) used in those experiments, the practicality and economics of the use of Cloprostenol as a means of controlling ovarian function in cyclic ewes should be tested on a larger scale.

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