S. Afr. J. Anim. Sci. 9, 185-192 (1979)

THE SYNCHRONISATION OF OESTRUS IN SHEEP. 1. DOSAGE AND TIME OF PROSTAGLANDIN ADMINISTRATION FOLLOWING PROGESTAGEN PRETREATMENT

Receipt of MS 20-04-1979

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(Key words: *Synchronisation, oestrus, sheep, progestagen, prostaglandin, progesterone, LH)*
(Sleutelwoorde: *Sinkronisasie, estrus, skape, progestogeen, prostaglandien, progesteroon, LH)*

OPSOMMING

SINKRONISASIE VAN ESTRUS BY SKAPE. 1. DIE INVLOED VAN DOSIS EN TYD VAN PROSTAGLANDIEN TOEDIENING RELATIEF TOT INTRAVAGINALE PROGESTEROON SPONSONTTREKKING

Ten einde die refraktoriese periode teen cloprostenol (ICI 80996) te oorbrug (voor dag 4 en na dag 14 van die estrus siklus), het 'n behandeling met intravaginale sponse bevattende medroksi -progesteroon asetaat vir agt dae die inspuiting van 31,25, 62,5 of 125 µg prostaglandien voorafgegaan. Die aanteeldoeltreffendheid van die verskillende behandelde groepe het geen patroon gevolg nie alhoewel die konsepsie tussen die eerste (63,7 persent) en tweede (81,9 persent) nabehandelings estrus periodes betekenisvol verskil het ($P < 0,05$). Die tempo van afname in progesteroon konsentrasie na beëindiging van behandeling is nie betekenisvol beïnvloed deur die verskillende dosisse prostaglandien nie. Heelwat variasie in die voorkoms van die LH piek ten opsigte van die aanvang van estrus is gevind en dit blyk dat die stadium van die estrus siklus 'n invloed uitoefen op die interval tussen die beëindiging van behandeling en die voorkoms van die LH piek. Die tyd van prostaglandien (cloprostenol) toediening relatief tot intravaginale progestogeen sponsonttrekking (-48, -24 of 0 uur) het geen betekenisvolle effek op beide die voorkoms van, of the lengte van die daaropvolgende estrus periode gehad nie. Namate die aanvang van progesteroon behandeling verander het van dag 2 tot dag 17 van die estrus siklus, het die ooie wat die prostaglandien inspuiting op die stadium van sponsonttrekking ontvang het (0 uur), 'n duidelike afname in die tydsduur tussen die beëindiging van hormoon behandeling en estrus getoon. Die konsepsiesyfers en fekunditeit vir die onderskeie behandelings groepe (-48, -24 of 0 uur) het nie betekenisvol van mekaar verskil nie en daar was ook geen betekenisvolle verskil in die konsepsiesyfer by die eerste en tweede (normale) nabehandelings estrusse nie.

SUMMARY

In order to overcome the refractory period of the ovary to cloprostenol (ICI 80996) treatment (prior to day 4 and subsequent to day 14 of the oestrous cycle), ewes were treated with intravaginal progestagen sponges for eight days and injected with either 31,25, 62,5 or 125 µg cloprostenol on the day of sponge withdrawal. A dosage of 31,25 µg proved adequate to induce luteolysis, but conception rates were significantly lower ($P < 0,05$) at the first (mean 63,7 per cent) than at the second (mean 81,9 per cent) post-treatment oestrus. Following the cessation of treatment the change in serum progesterone concentration was not affected by the dose of cloprostenol, but the commencement of the LH peak relative to the onset of oestrus varied markedly. The stage of the cycle at which intravaginal sponge treatment commenced tended to have an effect on the interval between the cessation of treatment and the onset of the LH peak. The time of prostaglandin (cloprostenol) administration relative to intravaginal progestagen sponge withdrawal (-48, -24 and 0 h) exerted no significant effect on either the ensuing incidence or the duration of oestrus. As the onset of the progestagen treatment progressed from day 2 to day 17 of the oestrous cycle for the group receiving the prostaglandin injection at sponge withdrawal (0 h), the interval between cessation of treatment and the onset of oestrus tended to decrease. The conception rates and fecundities of the three treatment groups (-48, -24 and 0 h) did not differ significantly from each other, nor was there a significant difference between the conception rate at the first and the second (normal) post-treatment periods of oestrus.

The corpus luteum of the ewe is responsive to prostaglandins only during a limited period of the mid-luteal phase of the oestrous cycle (Douglas & Ginther, 1973; Acritopoulou, Haresign, Foster & Lamming, 1977). To obviate this refractory period of the corpus luteum to prostaglandin, ewes can be treated with progestagens prior to the injection of a prostaglandin analogue (Van Zyl, 1977; Greyling, 1978). Administration of prostaglandin $F_{2\alpha}$ or its analogues has resulted in luteolysis associated with the cessation of progesterone secretion, but the fertility is variable (Fairnie, Wales & Gherardi, 1977; Fukui & Roberts, 1976; Fairnie, Martin & Rogers, 1978). Previous workers have shown that a dose of 100 µg (Trounson, Willadsen & Moor, 1976) or 125 µg (Fairnie, Cumming & Martin, 1976) cloprostenol (ICI 80996) is sufficient to induce luteal

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regression in the ewe, but no work has been cited in which a minimum effective dose of cloprostenol following a progestagen pretreatment has been determined.

The following experiments were therefore designed to determine the effect on reproductive efficiency of ewes, of different dosages and of different times of cloprostenol administration relative to intravaginal progestagen sponge withdrawal, when a short progestagen pretreatment was employed.

Procedure

Experiment 1

During the active breeding season (March, 1977) the oestrous cycles of 185 South African Mutton Merino ewes were monitored. From this flock 102 ewes were allotted to three groups of 34 ewes, in such a way that at the start of treatment each group was represented by two ewes on each day of the entire oestrous cycle (day of oestrus = day 0). All the ewes were treated with 60 mg medroxyprogesterone acetate (MAP) intravaginal sponges (Repromap : Upjohn) for eight days. Before insertion, these sponges were impregnated with 2,0 ml of an antibiotic preparation (Streptopen; Glaxo Allenburys). On the day of sponge withdrawal the three groups were treated (i.m.) with the following doses of cloprostenol:

Group 1 : 125 µg
Group 2 : 62,5 µg
Group 3 : 31,25 µg

Following the injection of the prostaglandin analogue all the ewes were tested for oestrus with the aid of vasectomised rams. From 30 of these ewes (10 from each group representing days 2, 4, 6, 8, 10, 11, 12, 13, 15 and 17 of the oestrous cycle, respectively), venous blood was collected at six-hour intervals. Sampling commenced at the time of cloprostenol injection and continued to the end of oestrus. Serum was recovered and stored at - 20 °C. Serum progesterone concentration was determined by the radioimmuno assay (R.I.A.) technique of Yousefnejadian, Florensa, Collins and Sommerville (1972) as modified by Faure (1975) and quality tested by Dierkse (1977). The R.I.A. method of Niswender, Reichert, Midgley and Nalbandov (1969) as modified by Millar and Aehnelt (1977) was utilized to measure serum LH concentrations.

Experiment 2

Intravaginal progestagen sponges (MAP 60 mg) were inserted (April 1977) for a period of 9 days in 78 S.A. Mutton Merino ewes of which the oestrous cycles had been monitored. The ewes were then allocated to three groups each of 26 individuals and the groups were balanced with respect to day of the oestrous cycle on which progestagen treatment commenced (day of oestrus = day 0). The groups were then treated as follows:

Group 1 : 125 µg i.m. cloprostenol injection ("Estrumate" ICI 80996) 48 hours prior to sponge withdrawal (-48h).

Group 2 : 125 µg i.m. cloprostenol injection 24 hours prior to sponge withdrawal (-24h).

Group 3 : 125 µg i.m. cloprostenol injection at sponge withdrawal (0h).

Following cloprostenol administration and sponge withdrawal, all the ewes were regularly tested for oestrus (08h00 and 16h00) with the aid of vasectomised rams. Ewes in oestrus were inseminated 12 hours after first detection and again at 12-hour intervals for as long as they remained in oestrus.

All ewes were tested for oestrus 14-17 days later, in order to inseminate ewes returning to service at their second post-treatment cycle.

Results

Experiment 1

The oestrous response, conception rates and lambing performance following the different levels of cloprostenol are set out in Table 1.

Dose of cloprostenol did not significantly affect the interval between the cessation of treatment and the onset of oestrus, the duration of oestrus or the oestrous response, nor was any significant trend apparent in the conception, lambing rate and fecundity of these groups (Table 1). Therefore, the data was pooled for further analysis. No significant trend was found in the reproductive performance of the ewes during day 2 to day 5 following the cessation of treatment (Table 2). However, conception rate was significantly ($P < 0,05$) lower at the first than at the second post-treatment oestrus (Table 3).

From Figure 1 it is obvious that dose of cloprostenol dit not affect the rate of decrease and the mean serum progesterone concentrations of the respective groups following the prostaglandin injection. The differences in the mean concentration of serum progesterone of the three treatment groups at, and around, oestrus were also not significant (Fig. 2). The peak level measured during the LH surge after the cessation of treatment did not differ between the treatment groups (Table 4), although there was marked variation within groups. The time relationship between the LH surge and the onset of oestrus did not differ between treatment groups (Fig. 3). However, the day of the oestrus cycle on which the intravaginal sponge treatment commenced exerted an effect on the interval between the cloprostenol injection and the maximum LH level of the LH surge (Table 4). Accordingly, this interval tended to decrease markedly from day 2 to day 17 of the oestrous cycle.

Table 1

The effect of an 8-day intravaginal progestagen treatment followed by different dosages of prostaglandin $F_{2\alpha}$ (cloprostenol) on the oestrous and reproductive performance of South African Mutton Merino ewes (percentages in brackets)

	Group 1 (125 µg)	Group 2 (62,5 µg)	Group 3 (31,2 µg)
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
FIRST POST-TREATMENT OESTRUS:			
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
SECOND POST-TREATMENT OESTRUS:			
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

The oestrous response, conception and fecundity of ewes exibiting oestrus from day 2 to day 5 following cloprostenol treatment (percentages in brackets)

		Day 2 (48h)	Day 3 (72h)	Day 4 (96h)	Day 5 (120h)
Group 1 (125 µg)	No. Ewes in Oestrus	11 (32,4)	11 (32,4)	8 (23,5)	3 (8,8)
	No. Ewes Lambing	7 (63,6)	7 (63,4)	3 (37,5)	1 (33,3)
	No. Lambs Born	12 (171,4)	10 (142,9)	4 (133,3)	1 (100,0)
Group 2 (62,5 µg)	No. Ewes in Oestrus	10 (29,4)	16 (47,1)	7 (20,6)	1 (2,9)
	No. Ewes Lambing	9 (90,0)	12 (75,0)	3 (42,9)	1 (100,0)
	No. Lambs Born	13 (144,4)	16 (133,3)	4 (133,3)	2 (200,0)
Group 3 (31,25 µg)	No. Ewes in Oestrus	12 (35,3)	8 (23,5)	8 (23,5)	4 (11,8)
	No. Ewes Lambing	7 (85,3)	6 (75,0)	5 (62,5)	3 (75,0)
	No. Lambs Born	10 (142,9)	7 (116,7)	7 (140,0)	6 (200,0)
Total	No. Ewes in Oestrus	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 Born	35 (152,2)	35 (152,2)	33 (132,0)	9 (180,0)

Table 3

The overall conception, lambing rate and fecundity of all the ewes treated with prostaglandin $F_{2\alpha}$ (cloprostenol) following an 8-day interavaginal progestagen pretreatment for the first and second post-treatment oestrous periods

	1st Oestrus		2nd Oestrus	
	Number	Percentage	Number	Percentage
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

*Figures differ significantly $P < 0,05$

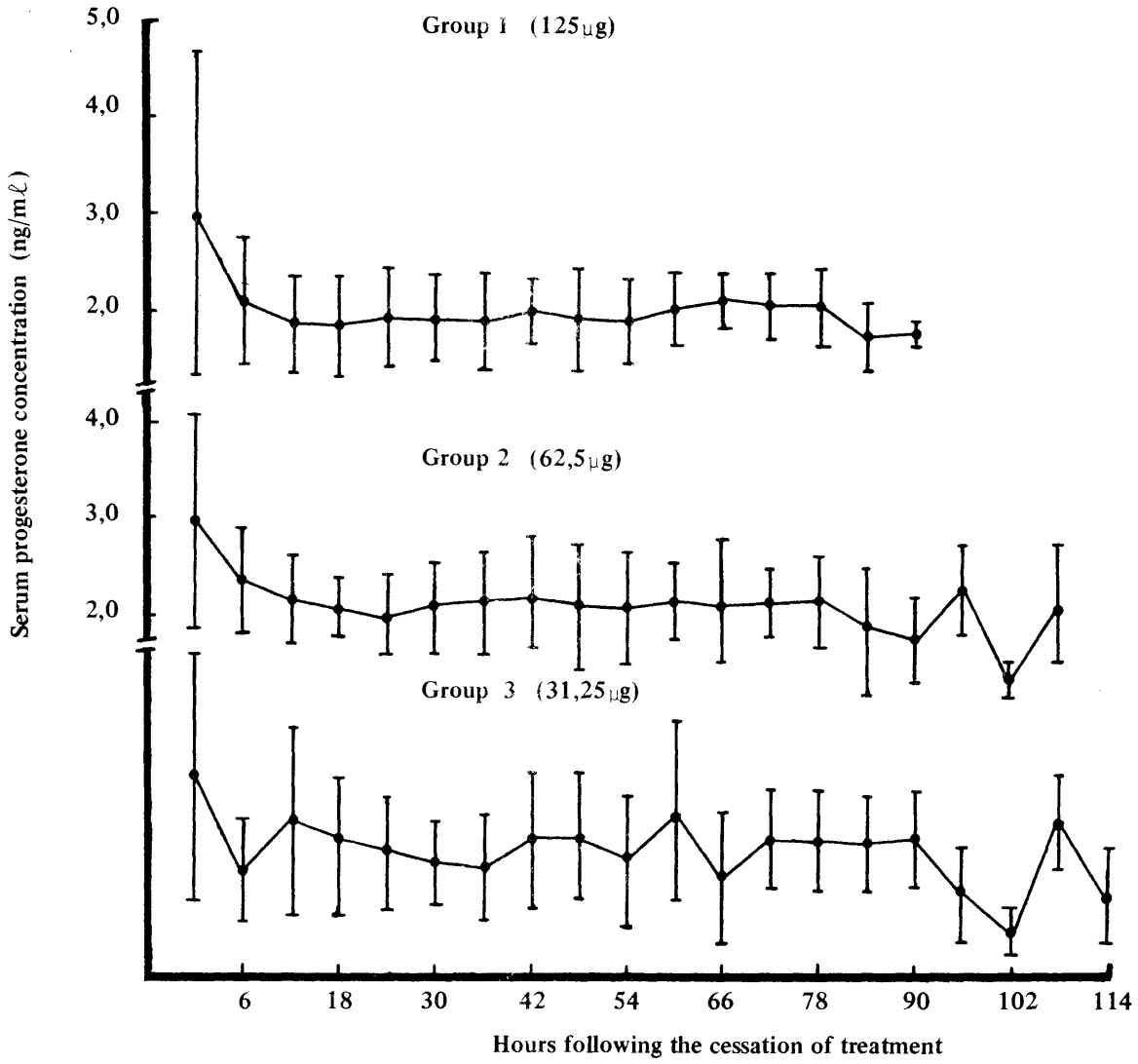

Fig. 1 The effect of different dosages of cloprostenol on the mean serum progesterone concentration

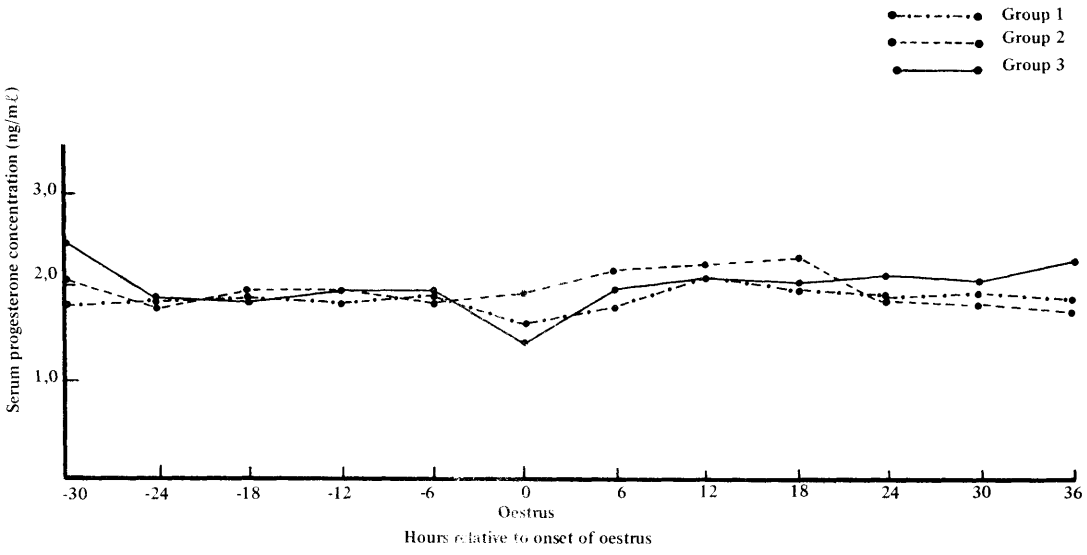

Fig. 2 Mean serum progesterone concentrations relative to oestrus for ewes receiving 125µg (Group 1), 62,5µg (Group 2) and 31,25µg (Group 3) cloprostenol following an 8-day intravaginal sponge treatment

Table 4

The time interval (hours) between cloprostenol injection and the maximum LH level

Stage of cycle (Days) when progestagen commenced	Group 1 125 µg	Group 2 62,5 µg	Group 3 31,3 µg
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
Mean	74,4 ± 20,6	75,0 ± 22,3	73,8 ± 21,5

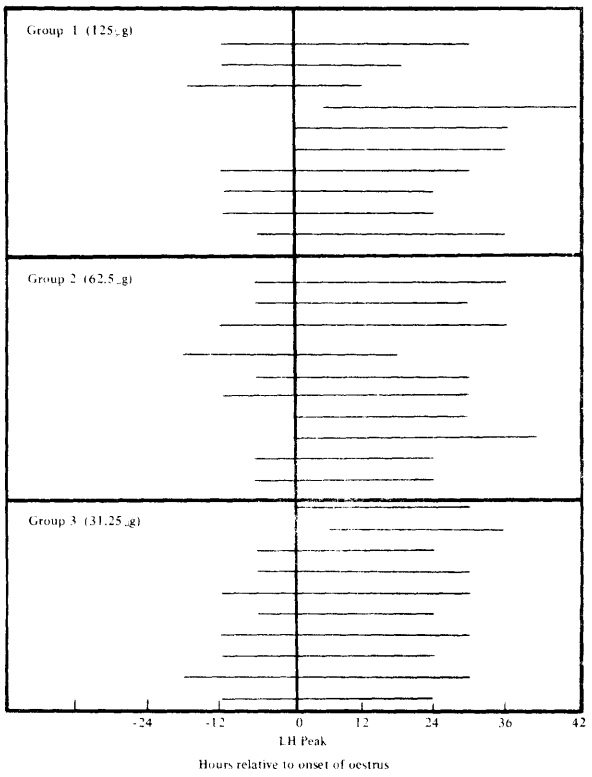

Fig. 3 The position of the LH surge relative to the onset of oestrus for the three treatment groups receiving different doses of cloprostenol

Experiment 2

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 (Table 5; Fig. 4). Although the stage of the cycle at which the progestagen treatment commenced did not exert an effect on the interval between sponge withdrawal and the onset of oestrus in Groups 1 and 2, this interval tended to decrease as the onset of the sponge treatment progressed from day 2 to day 17 of the oestrous cycle in Group 3 (Fig. 4).

Table 5

The onset of oestrus and duration of oestrus (hours) in ewes treated with 125 µg cloprostenol at different intervals in relation to the termination of intravaginal progestagen therapy

	Interval (hours) cloprostenol injection to pessary withdrawal:		
	Group 1 (-48h)	Group 2 (-24h)	Group 3 (0 h)
Interval between sponge withdrawal and onset of oestrus (h)	58,04 ± 13,9	55,04 ± 12,7	61,9 ± 17,3
Duration of oestrus (h)	36,4 ± 8,64	34,3 ± 7,89	38,6 ± 7,12

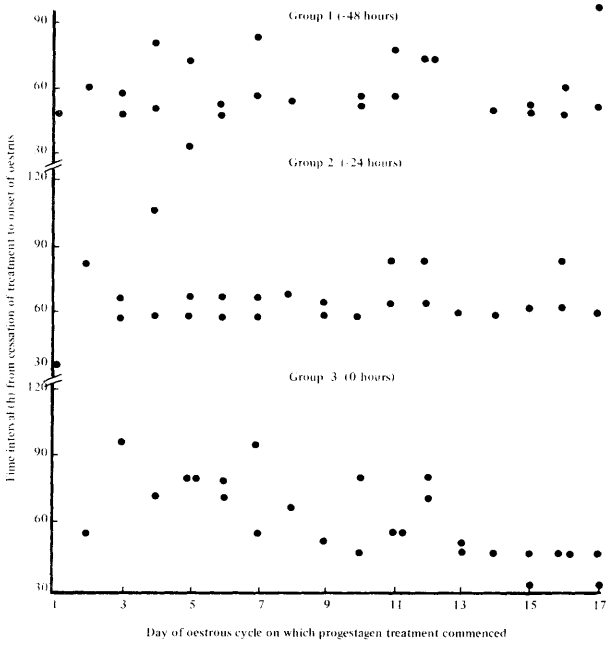

Fig. 4 Time to the onset of oestrus (hours) following intravaginal progestagen sponge treatment and different times of cloprostenol administration

Fecundity tended to improve as the time of prostaglandin administration prior to sponge withdrawal increased, but the conception rates and fecundities of the three treatment groups did not differ significantly

Table 6

The conception rate, lambing rate and fecundity following different times of cloprostenol treatment relative to sponge withdrawal (percentages in brackets)

	Group 1 -48h		Group 2 -24h		Group 3 0h	
No. Ewes	26		26		26	
No. Ewes showing oestrus	26		26		26	
FIRST OESTRUS:						
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	
SECOND OESTRUS:						
Ewes lambing/Ewe treated	5	(83,3)	6	(85,7)	5	(83,3)
Lambs born/Ewe treated	10	(166,7)	9	(126,6)	9	(150,0)
Lambs born/Ewe lambing	2,0		1,5		1,8	

Table 7

Cloprostenol administration and the overall conception, lambing rate and fecundity of ewes

	1st Oestrus		2nd Oestrus	
	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	

(Table 6). The overall conception, lambing rate and fecundity for the first post treatment oestrus was lower than for the second post treatment oestrus, but this difference was not significant (Table 7).

Discussion

The results of these experiments confirm that the oestrous periods of sheep can be efficiently synchronised by means of a short progestagen pretreatment (8 – 9 days) followed by a single injection of prostaglandin from 48 hours prior to 0h relative to sponge withdrawal. When using this treatment the insensitive period of the corpus luteum to prostaglandins (around oestrus) can be overcome (Van Zyl, 1977). According to the results obtained in this experiment the luteolytic activity of 31,25µg cloprostenol proved to be sufficient to result in a synchronised oestrus following the progestagen pretreatment. However, conception rate was slightly depressed at the first post-treatment oestrus. It is suspected that this lowered fertility in these experiments was partly due to intravaginal progestagen treatment (Robinson, Moore, Holst & Smith, 1967; Deweese, Glimp & Dutt, 1970; Van der Westhuysen, Van Niekerk & Hunter, 1970; Hunter, Belonje & Van Niekerk, 1971), since synchronisation of oestrus using progesterone or progestagens has been found to disturb 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), and sperm transport and survival (Quinlivan & Robinson, 1967, 1969; Hawk, 1971; Hawk & Conley, 1971). In this experiment a similar inconsistency in the occurrence of the LH peak relative to oestrus was noted. However, the results agreed with those of Van der Westhuysen, *et al.* (1977) and Dierkse (1977) who noted that the release of LH was unrelated to the serum progesterone concentrations. The finding that the day of the oestrous cycle on which progestagen was first administered often affected the interval between the cessation of treatment and the LH peak is of interest. This phenomenon could possibly result from the fact that the treatment lengthens the progestational phase when it starts in the second half of the oestrous cycle, but shortens it in ewes treated during the first half of the oestrous cycle. Thus in the former ewes oestrus is likely to be synchronised by the progestagen and in the latter ewes by the prostaglandin.

Although the procedure described here did synchronise oestrus, it is still not clear whether the repro-

ductive performance of the ewes benefitted by the addition of prostaglandins to the progestagen treatment. Following the intravaginal treatment, the residual effect of progestagen modified the effect of the rapid drop in progesterone (following prostaglandin $F_{2\alpha}$ treatment) on the subsequent onset of oestrus and fertility. Clearly, further studies on techniques of overcoming the insensitive period of the corpus luteum to prostaglandin when synchronising oestrus in the ewe, are necessary.

References

ACRITOPOULOU, S., HARESIGN, W., FOSTER, J.P. & LAMMING, G.E., 1977. Plasma progesterone and LH concentrations in ewes after injection of an analogue of prostaglandin $F_{2\alpha}$. *J. Reprod. & Fert.* 9, 337.

CUMMING, I.A., BLOCKLEY, M.A. DE B., BROWN, J.M., CATT, K.J., GODING, J.R. & KALTENBACH, C.C., 1970. The release of luteinizing hormone in ewes, following the withdrawal of intravaginal sponges containing progestogen. *Prod. Aust. Soc. Anim. Prod.* 8, 383.

DE WEESE, W.P., GLIMP, H.A. & DUTT, R.H., 1970. Comparison of medroxyprogesterone acetate orally and in vaginal sponges for synchronizing oestrus in ewes. *J. Anim. Sci.* 31, 394.

DIERKSE, E., 1977. *Verandering in serum progesteroon en luteiniserende hormoonpeile na sinkronisasie van estrus by skape.* M.Sc. Tesis (Landbou), Universiteit van Stellenbosch.

DOUGLAS, R.H. & GINTHER, O.J., 1973. Luteolysis following a single injection of prostaglandin $F_{2\alpha}$ in sheep. *J. Anim. Sci.* 37, 990.

FAIRNIE, I.J., CUMMING, I.A. & MARTIN, E.R., 1976. Use of prostaglandin analogue, ICI 80996, to synchronise ovulation in sheep in an artificial insemination programme. *Proc. Aust. Soc. Anim. Prod.* 11, 133.

FAIRNIE, I.J., WALES, R.G. & GHERARDI, P.G., 1977. Time of ovulation, fertilisation rate and blastocyst formation in ewes following treatment with prostaglandin analogue (ICI 80996) *Theriogenology* 8, 4.56.

FAIRNIE, I.J., MARTIN, E.R. & ROGERS, S.C., 1978. The lambing performance of Merino ewes following synchronisation of ovulation with cloprostenol a prostaglandin analogue (ICI 80996). *Proc. Aust. Soc. Anim. Prod.* 12 : 256.

FAURE, A.S., 1975. *Vroeë embrionale verlies weens wanvoeding by Merinoskape.* M.Sc. (Landbou) Tesis, Universiteit van Stellenbosch.

FUKUI, Y, & ROBERTS, E.M., 1976. In *Proceedings of the international sheep breeders congress, Muresk,* pp 483–494. ed. Tomes, G.J., Robertson, D.E. & Lightfoot, R.J., Western Australian Institute of Technology.

GREYLING, J.P.C., 1978. *Control of ovulation in cycling ewes with a prostaglandin $F_{2\alpha}$ analogue.* M.Sc. (Agric) Thesis, University of Stellenbosch.

HARESIGN, W., 1976. Controlled breeding in sheep using the prostaglandin analogue ICI 80996. *Proc. Brit. Soc. Anim. Prod.* 22, 137.

HAWK, H.W., 1971. Sperm destruction in the sheep vagina. *J. Anim. Sci.* 33, 255.

HAWK, H.W. & CONLEY, H.H., 1971. Loss of spermatozoa from the reproductive tract of the ewe and intensification of sperm "breakage" by progestagen. *J. Reprod. Fert.* 27, 339.

HUNTER, G.L., BELONJE, P.C. & VAN NIEKERK, C.H., 1971. Synchronised mating and lambing in spring-bred Merino sheep: The use of progesterone-impregnated intra-vaginal sponges and teaser rams. *Agroanimalia* 3, 133.

LINTIN, K. & LAMMING, C.E., 1973. The effects of progestagens and PMSG on peripheral plasma LH levels in the Clun Forest ewe. *J. Reprod. Fert.* 35, 603.

LISHMAN, A.W., BOTHA, W.A. & LOUW, B.P., 1974. Release of LH in ewes treated with progestagen and oestrogen during the anoestrous season. *S. Afr. J. Anim. Sci.* 4, 143.

MILLAR, R.P. & AEHNELT, C., 1977. Application of ovine luteinizing hormone (LH) radioimmuno assay in the quantitation of LH in different mammalian species. *Endocrinology* 101, 760.

NISWENDER, G.D., REICHERT, L.E. JR., MIDGLEY, A.R. & NALBANDOV, A.V., 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. *Endocrinology* 84, 1166.

QUINLIVAN, T.D. & ROBINSON, T.J., 1967. The number of spermatozoa in the fallopian tubes of ewes at intervals after artificial insemination following withdrawal of SC-9880 impregnated intravaginal sponges. In *Control of the ovarian cycle in sheep.* ed. T.J. Robinson, Sydney: Sydney University Press.

QUINLIVAN, T.D. & ROBINSON, T.J., 1969. Numbers of spermatozoa in the genital tract after artificial insemination of progestagen-treated ewes. *J. Reprod. Fert.* 19, 73.

ROBINSON, T.J., MOORE, N.W., HOLST, P.J. & SMITH, J.F., 1967. The evaluation of several progestagens administered in intravaginal sponges for the synchronisation of oestrus in the entire cyclic Merino ewe. In *The control of the ovarian cycle in sheep,* ed. T.J. Robinson. Sydney: Sydney University Press.

TROUNSON, A.D., WILLADSEN, S.M. & MOOR, R.M., 1976. Effects of prostaglandin analogue cloprostenol on oestrus, ovulation and embryonic viability in sheep. *J. Agric. Sci. Camb.* 86, 609.

VAN DER WESTHUYSEN, J.M., VAN NIEKERK, C.H. & HUNTER, G.L., 1970. Time of ovulation and possible application of artificial insemination in sheep on a time basis after the use of progestagen sponges. *Proc. S. Afr. Soc. Anim. Prod.* 9, 183.

VAN DER WESTHUYSEN, J.M., MALAN, J.E. & DIERKSE, E., 1977. Changes in plasma progesterone and LH concentrations during the progesterone synchronised oestrus in sheep. *S. Afr. J. Anim. Sci.* 7, 133.

VAN ZYL, G.J., 1977. *Sinkronisasie van bronstigheid in die skaapooi met prostaglandien* $F_{2\alpha}$. M.Sc. (Landbou) Tesis, Universiteit van Stellenbosch.

YOUSEFNEJADIAN, E., FLORENSA, E., COLLINS, W.P. & SOMMERVILLE, I.F., 1972. Radioimmunoassay of plasma progesterone. *J. Steroid Biochem.* 3, 893.