
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

The timing of oestrus, LH surge and ovulation in ewes following synchronisation with MAP sponges FGA sponges or CIDR's

G. H. SHACKELL

Invermay Research Centre, Private Bag, Mosgiel, New Zealand.

ABSTRACT

Three groups of 10 ewes each were treated with Repromap sponges (MAP), Chronogest sponges (FGA) or Controlled Internal Drug Release devices (CIDR) for 11 days. At withdrawal the delay time until oestrus, LH surge and ovulation was measured.

There was no significant difference between the two sponge treatments in mean delay times although FGA treated ewes tended to be less variable in their activity.

CIDR treated ewes commenced standing oestrus sooner than MAP and FGA treated ewes (30h vs 42h and 40h respectively $P<0.001$). Similarly the onset of the LH surge (31h vs 46h and 46h $P<0.001$) and ovulation (59h vs 69h and 71h $P<0.001$) occurred sooner in the CIDR ewes compared to MAP and FGA sponges.

Keywords Oestrus, LH, ovulation, MAP, FGA, CIDR, oestrous synchronisation.

INTRODUCTION

Synchronisation of oestrus and ovulation is commonly used as an aid in breeding programmes designed to maximise sheep production. Where synchronisation of ewes is designed to ensure that a flock lambs during a concentrated period the synchrony of individuals may be imprecise, provided the mean day of oestrus occurs as planned. Embryo transfer programmes, however, require the onset of oestrus of individuals to be more predictable and synchronous for planning surgery schedules.

The least flexible synchrony of individuals is in "fixed-time" AI programmes where a technician is scheduled to inseminate the flock on a pre-determined date and time. Dziuk (1970) has suggested that conception rates are best when insemination occurs approximately 12 hours (h) before ovulation, which equates to about 18h after the onset of oestrus. However, Amir and Schindler (1972) inseminated ewes at various times between 4h to 8h and 32h to 36h after the onset of oestrus and noted no difference in conception rates. Experimentation to determine optimum conditions for artificial insemination (AI) requires large numbers of animals to confirm small percentage shifts in conception rate. Shackell *et al.* (1990) have shown that the time of AI after onset of oestrus can influence conception rates. When planning a fixed time AI programme it is desirable to know the timing of events following synchronisation treatment to ensure that ewes are inseminated at the most opportune time.

Following initial achievements of oestrus synchronisation in sheep using progesterone injections (Dutt and Cassida, 1948), technology has moved through the use of progestagen impregnated sponges (Robinson, 1965) to the more recent progesterone impregnated silicone elastomer devices (Welch, 1982). This development has tended to "tighten" the synchrony of the treated ewes and to decrease the variability of the onset of oestrus.

The study reported here was conducted to establish when oestrus, LH surge and ovulation occur following the treatment of ewes with MAP or FGA sponges or CIDRs. There was no augmentation with other hormones and fertility of the ewes was not tested. This information should assist in determining the optimum times for "fixed-time" AI and provide benchmarks for further, more intensive studies.

EXPERIMENTAL

Figure 1 illustrates the experimental design. Thirty mixed-age Coopworth ewes were randomly allocated to three treatment groups (n=10). On day 0 (9th May) commercial synchronisation devices were inserted in the anterior vagina of each ewe. The current ovarian status of the animals at the time of insertion of the devices was not considered. Ewes in group 1 (MAP) received sponges impregnated with 60mg Medroxyprogesterone acetate (Repromap; Upjohn). Group 2 (FGA) received sponges impregnated with 45 mg Fluorogestone acetate (Chronogest; Intervet) and group 3 (CIDR) received Controlled Internal Drug Release devices containing 9% progesterone (CIDR-G, Carter Holt Harvey, Hamilton, New Zealand). The devices were removed at 00h on day 11. From 00h on day 12 (24h after withdrawal) all ewes were blood sampled at four hourly intervals until 0800h on day 13 (56h after withdrawal). At each blood sampling ewes were teased with vasectomised rams to determine the onset of oestrus. From 0800h on day 13 ewes were laparoscoped at four hourly intervals to determine the time of ovulation. Once ovulation had been recorded ewes were no longer laparoscoped and the final observation was made at 0800h on day 14 (80h after withdrawal).

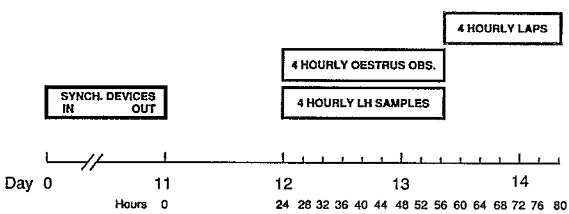

FIG 1 Experimental design.

L.H. Assays

Immediately following collection of blood into evacuated tubes each sample was centrifuged at 4°C and blood plasma decanted and stored at -20°C until assay. The determination of LH in the blood plasma was by the method described by Webster (1989).

Statistical Methods

All variables were analysed by least squares and tested for homogeneity of variance (Bartlett, 1937) over treatment groups.

An arbitrary value of 3.5 ng/ml was chosen to determine when the LH surge had been detected. When two consecutive samples exceeded this value the first elevated value was taken to indicate the start of LH surge. In a small number of cases the elevated LH concentrations occurred at only one sampling. This value was invariably 10 - 20 times higher than the mean of all other readings for that animal and was deemed to be the LH surge.

Time of ovulation was regressed on time of onset of both oestrus and the LH surge.

RESULTS

Treatment means with SED's and significance levels are shown in Table 1. Ranges for variables and Bartlett's test for homogeneity are shown in Table 2.

Oestrus

All ewes showed oestrus following withdrawal of the synchronising devices. For ewes treated with CIDRs the mean time from withdrawal to oestrus was 30h. This was significantly earlier than for ewes treated with either MAP (42h) or FGA (40h) sponges ($P<0.001$). The difference between MAP and FGA treated ewes was not significant.

The pattern of onset of oestrus is shown in Figure 2. In the CIDR group the first ewe was observed in oestrus at 24h after withdrawal. At 32h 9 of the 10 ewes treated had shown oestrus and all ewes had shown oestrus by 36h. However only one of the MAP ewes showed oestrus at 36h, 9/10 by 48h and all ewes by 52h. For FGA treated ewes the first two animals were in oestrus at 36h, 9/10 by 44h and all ewes by 48h after withdrawal.

LH Surge and Maximum LH Levels

For ewes treated with CIDRs the LH surge started 31h after device withdrawal which was 15h sooner than for both MAP and FGA treated ewes (46h, $P<0.001$). In all

TABLE 1 Means of variables by treatment groups, with significance levels for CIDR vs sponge contrast.

Number of Hours		CIDR	MAP	FGA	SED	
to	Oestrus	29.6	42.4	40.4	1.75	***
	LH surge	30.8	46.0	46.4	2.83	***
	Maximum LH level	32.8	48.5	47.6	2.85	***
	Ovulation	58.8	69.5	71.2	2.36	***
from	Oestrus to LH surge	1.2	4.5	6.0	2.07	*
	LH surge to Ovulation	28.0	23.5	24.8	1.26	**
	Oestrus to Ovulation	29.2	28.0	30.8	1.83	NS

treatment groups the maximum LH levels were measured at a mean of 1.5h - 2.5h after the start of the surge with no significant difference between treatment groups. Two ewes in the MAP treatment group showed no change from basal LH levels throughout the sampling period.

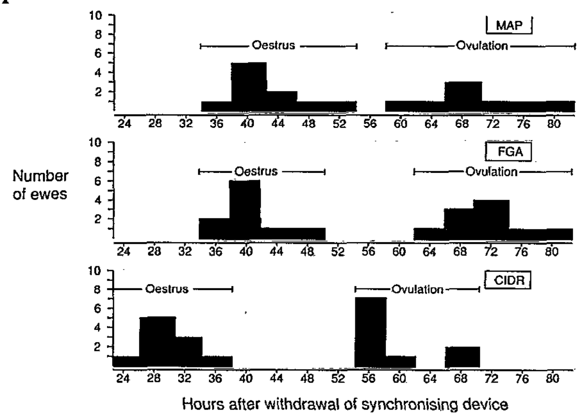

FIG 2 Pattern of oestrus and ovulation following synchronisation with intravaginal devices

Ovulation

The time of ovulation following withdrawal of synchronisation devices followed a similar pattern to the previous variables. The CIDR treated ewes ovulated approximately 59h after withdrawal, which was significantly earlier than MAP (70h) or FGA (71h) treated ewes (P<0.001). The two ewes in the MAP group which had no LH surge did not ovulate in the 80 h following sponge withdrawal. At the first observation at 56h, 7 of the 10 CIDR ewes had recently ovulated. As shown in Figure 2 there was a different spread of ovulation pattern. MAP treated ewes were the most variable with the eight ewes ovulating over a period of 20h whereas the 10 FGA ewes ovulated over 16h and the 10 CIDR ewes over 12h.

TABLE 2 Ranges of variables by treatment groups and Bartlett's test statistic for homogeneity of variance (χ^2_2 : critical value = 5.99)

Number of Hours to	CIDR	MAP	FGA	χ^2_2
LH surge	24.36	36.52	36.48	1.18
Oestrus	24.44	36.56	40.52	2.58
Ovulation	56.68	60.80	64.80	0.95

Oestrus to LH surge

In the CIDR treated ewes the LH surge occurred 1.2h after oestrus which was significantly earlier than the FGA ewes (6h, P<0.05) but not significantly different from the MAP ewes (4.5h, n.s.).

LH Surge to Ovulation

In CIDR treated ewes ovulation occurred 28h after the LH surge. This was significantly earlier than in either the MAP (23.5h) or FGA (24.8h) ewes ($P<0.01$).

Oestrus to Ovulation

There were no significant differences between treatment groups in the time from oestrus to ovulation.

Regression of Ovulation on Oestrus and LH Surge

The regressions of ovulation on oestrus and LH surge are given by:

1. Ovulation = 30.5 + 0.97 (SE = 0.121) *oestrus*, RMS (Residual Mean Square) = 17.46

2. Ovulation = 35.6 + 0.75 (SE = 0.047) *LH Surge*, RMS = 5.59

with no significant differences in slope or intercept with treatment group. The slope for the ovulation-oestrus regression was not significantly different from 1 ($P>0.05$).

DISCUSSION

All three devices used induced oestrus and ovulation following withdrawal. The variation in synchrony was less using CIDRs than either of the sponge treatments.

The major use of oestrus synchronisation in New Zealand is likely to be in artificial insemination (AI) programmes. In a large scale AI programme in New Zealand Shackell *et al.* (1990) have shown that in ewes treated with CIDRs the time of oestrus in relation to insemination has a significant effect on non-return rate. By inference time of ovulation is also likely to have an effect. It is therefore important that the timing of ovulation prior to "fixed time" A.I. is predictable and of low variability following synchronising treatment. Data reported here suggest that if MAP or FGA sponges are used to synchronise ewes insemination should be timed for about 10h later, after withdrawal, than if CIDRs are used. Using Dziuk's (1970) claim that insemination is most effective at 12h before ovulation timing of AI should be approximately 46h after the CIDR removal 57h after MAP sponge removal and 59h after FGA sponge removal (current protocols in NZ commonly recommend AI at 48h after CIDR withdrawal and 60h after sponge withdrawal).

The data presented here were collected during the breeding season at Invermay. Smith *et al.* (1991) have shown that the time to onset of oestrus following synchronisation with CIDRs shows considerable variation both within and between breeding seasons. The present data may therefore be used as a guideline when planning experiments to study breed, age of ewes or seasonal effects on synchronisation, or when planning "fixed time" AI programmes in the breeding season. As synchronisation treatment moves earlier or later than the breeding season the delay times from removal of devices until oestrus, LH surge and ovulation will be expected to increase. (Smith *et al.*, 1991).

Using the regression analysis from the present study the time of ovulation following sponge or CIDR removal had a $\pm$ 5 hr SD. The time from onset of oestrus to ovulation was constant with a $\pm$ 4h SD. Once the LH surge had occurred the time to ovulation could be predicted with a $\pm$ 2h SD with the delay to ovulation being shorter the later the surge occurred. Thus while knowing which treatment is being used allows prediction of the time of ovulation following withdrawal within a range of 20h, with 95% confidence limits, knowing when the onset of oestrus occurs reduces the range to 16 hours and knowing when the onset of the LH surge occurs reduced the range of predicted time of oestrus even further to an 8h span. Adding treatment group does not reduce the variability further. In the absence of a means of identifying the LH surge as it occurs, time of oestrus is the best predictor of time of ovulation following removal of synchronisation devices.

There may be a need to further reduce the variability of time to ovulation following synchronisation to achieve maximum fertility; even the CIDR treated animals ovulated over a 12h period. Fixed-time AI programmes are designed to inseminate at the most opportune time related to the average of animals in the flocks. Where the time of ovulation shows the least variability the chances of success of the AI programme should be enhanced if the AI is timed correctly.

ACKNOWLEDGEMENTS

Staff of the Animal Production Unit, Invermay, for assistance with sampling, T.R. Manley for the LH analyses and Dr R. Littlejohn for statistical advice.

REFERENCES

Amir, D.; Schindler, H. 1972. The conception rate of ewes after artificial insemination of ewes at different times during oestrus. *Journal of Reproduction and Fertility* **28**: 261-264.

Bartlett, M.S. 1937. Properties of sufficiency and statistical tests. *Proceedings of the Royal Society* **160**: 262-282.

Dutt, R.H.; Cassida, L.E. 1948. Alteration of the oestral cycle in sheep by use of progesterone and its effect upon subsequent ovulation and fertility. *Endocrinology* **43**: 208-217.

Dziuk, P. 1970. Estimation of optimum time for insemination of gilts and ewes by double mating at certain times relative to ovulation. *Journal of Reproduction and Fertility* **22**: 277-282.

Robinson, T.J. 1965. Use of progesterone impregnated sponges inserted intravaginally or subcutaneously for the control of the oestrus cycle in sheep. *Nature* **206**: 39-41.

Shackell, G.H.; Kyle, B.; Littlejohn, R.P. 1990. Factors influencing the success of a large scale Artificial Insemination programme in sheep. *Proceedings of the New Zealand Society of Animal Production* **50**: 427-430.

Smith, J.F.; Konlechner, J.A.; Parr, J. 1991. Factors influencing the time to onset of oestrus after synchronisation treatment in ewes. *Proceedings of the New Zealand Society of Animal Production* **51**: 15-23.

Webster, J.R. 1989. Exogenous metatonin treatment and reproductive seasonality in male red deer (*Cervus elaphus*) and sheep (*Ovis aries*) *PhD thesis*, University of Otago Dunedin, New Zealand.

Welch, R.A.S. 1982. Development of progesterone releasing intravaginal devices (CIDR dispensers). Annual Report of Agricultural Research Division, New Zealand Ministry of Agriculture and Fisheries, 1981/2. p 49.