Effects of CIDR withdrawal - insemination interval on conception rate in sheep AI

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ABSTRACT

This study was undertaken to investigate the effects of time from CIDR withdrawal and ewe age on conception rates in a sheep artificial insemination programme. Four groups each of 50 Romney ewes (balanced for ewe age, two-tooths vs mixed age) were synchronised by intravaginal insertion of CIDRs for 11 days. Ewes in each group were inseminated at 36h, 42h, 48h or 54h respectively from CIDR withdrawal by cervical insemination using 200 million sperm per insemination of fresh semen. Conception was determined by measuring non-return rates using harnessed rams.

Conception rates increased ($P<0.10$) with the interval between CIDR withdrawal and fixed time insemination thus: 36h, 37%; 42h, 49%; 48h, 59%; and 54h, 60%. Conception rates were greater in mixed age ewes (58%) than in two tooth ewes (43%)($P<0.05$). There was no interaction between the effects of age and time of insemination relative to CIDR withdrawal. It is concluded that there may be advantages in extending the interval between CIDR withdrawal and insemination to 48 to 54 h as opposed to the current industry recommendations of 45 to 48 hours.

Keywords: Sheep, fixed time insemination, CIDRs, synchronisation.

INTRODUCTION

Sire referencing and commercial fixed time cervical sheep AI were introduced into the New Zealand ram breeding industry in 1984 through the MAFTechnology national sire referencing programme (Clarke *et al.*, 1984). Sire referencing is being used within the sheep industry and has the ability to increase the rate of genetic gain especially for medium and low heritability traits such as wool bulk, parasite resistance, fly strike resistance and wool colour (Baker and Parratt, 1988).

The results from cervical insemination programmes in sheep have been encouraging, but conception rates have been variable between and within flocks (Shackell *et al.*, 1990), limiting the uptake of the technology. The major factor limiting the uptake of sheep cervical AI has been the inconsistency of results between location and day of insemination.

The purpose of this study was to investigate the effect of fixed time insemination on conception rate in synchronised Romney ewes with the aim of developing a low cost method of "Do it Yourself" (DIY) insemination for widespread use by ram breeders. Several factors were investigated including the relationships between the time of onset of oestrus post CIDR withdrawal, onset of oestrus pre AI, the timing of AI post CIDR withdrawal and conception rate. Research concentrated on the effect of different fixed AI times on conception rate in two tooths and mixed age Romney ewes.

MATERIALS AND METHODS

The experimental group consisted of 200 Romney ewes from the Massey University MPT progeny test flock. There were 85 two tooths, and 115 mixed age ewes ranging in age from 3-5 years.

The trial was a 2 x 4 factorial design (2 levels of age x 4 levels of time from CIDR withdrawal to AI). For ease of management the 200 ewes were separated into two treatment blocks to be inseminated on consecutive days (henceforth referred to as days 1 and 2). Each block was further divided randomly into four sub-groups, balanced for ewe age (two-tooths vs mixed age) and liveweight, to be inseminated at 36h, 42h, 48h or 54h post CIDR withdrawal. Ewes were synchronised via intravaginal insertion of 'eazi-breed' CIDRs (9% progesterone), lubricated with an antiseptic gel, for eleven days. Vasectomised rams (mixed age and breed) fitted with sire-sine harnesses were run with the ewes at a ratio of 6:100. CIDRs were removed at varying times to ensure the appropriate interval between CIDR withdrawal and AI for each group. Six-hourly monitoring was conducted to determine onset of oestrus and at each monitoring time the oestrous ewes were drafted off and held separately.

Five mature Border Leicester x Dorset rams were used for the AI programme. They had been trained to serve the artificial vagina in 1991 and were retrained in 1992 over a period of one week using restrained cycling ewes from another group.

Semen collecting and processing techniques followed the guidelines outlined by Salamon (1987). All glass collection tubes were preheated to 32°C and wrapped in cotton wool to prevent light denaturing the sperm. Semen was collected via an artificial vagina (AV) which had been lubricated with vaseline and filled with water at 49°C. Between each collection the AV was washed with 5% hibitane, rinsed twice with distilled water and then sprayed with 70% ethanol. Semen was pooled and the motility scored from 1-5 (1 = poor, 5 = excellent) using the scoring system described by Salamon (1987). Any sample scoring under 3 was rejected. Concentration of sperm was measured using a haemocytometer (Salamon,

1987) and the sample stored in a water bath at 32°C until it was diluted to 10^9 sperm per ml. The diluent (Colas and Courot, 1976) consisted of 10g skim milk powder made up to 100 ml in distilled water (0.1g milkfat per 100 ml diluent). Penicillin was added at a rate of 0.03ml per 100ml of diluent. Diluted semen was allowed to cool to 15°C over a twenty minute period and then, using a 500ml beaker of water acting as a water bath, was held at 15°C until insemination (one to five hours later).

Insemination was carried out on a raised herringbone platform holding 6-7 sheep standing at an angle with their backs to the inseminator. Cervical insemination involved placing the semen in, or as close as possible to, the cervical entrance. A vaginal speculum fitted with an illuminating light source was used. Each dose contained 200 x 10^6 sperm consisting of 0.2ml of air followed by 0.2ml diluted semen drawn into a 2ml glass pipette with a curved tip attached to a 1ml tuberculin syringe graduated in 0.05ml stages.

The AI rams were fitted with sire sine harnesses and crayon then released into the flock three days post insemination. Crayon marks were recorded every three days for two weeks to monitor returns and then the crayon colour was changed. Monitoring continued every three days for a further two weeks when crayon colour was again changed. A final recording of crayon marks was made 18 days after the last crayon change and the rams were removed.

Conception rate to AI was treated as a binomial trait and measured in terms of non-return rates. Data were analyzed using the SAS Catmod Model (SAS, 1985), involving logit transformation, ANOVA and calculation of means (%) on the retransformed values. All interactions were nonsignificant and therefore only main effects (treatment, age, day) are reported.

Three ewes were removed from the trial for the following reasons: one CIDR was left in a mixed age ewe; one CIDR was lost from a mixed age ewe; and one two tooth ewe became ill and later died.

RESULTS AND DISCUSSION

Table 1 shows the effects of day of insemination, ewe age and the time from CIDR withdrawal to AI on conception rates. There was a significant ($P<0.01$) effect of day of insemination, conception rates on day 1 (41%) being substantially lower than those on day 2 (61%). Since ewes were randomly assigned to the "day of insemination" groups (balanced for age and treatment), it is assumed that this was a chance effect. Nevertheless, a difference of this magnitude is of concern in terms of the viability of commercial AI programmes given that the insemination technique used on each day was identical. There was also a significant ($P<0.05$) effect of ewe age on conception rate, the mixed age ewes having higher conception rates (58%) than the two-tooth ewes (43%). Conversely, Harvey *et al.*, (1986) found that conception rates were higher in two tooths than in 3- or 4-year old ewes when results were averaged across a number of subtrials involving different AI protocols. Shackell *et al.*, (1990), in a much larger study, also found that conception rates declined as ewe age increased.

The effect of interval from CIDR withdrawal to AI on conception rate was significant only at the $P<0.10$ level (Table 1). This reflected the fact that conception rates at 48-54 hours post-CIDR withdrawal (59-60%) were higher than those at 36 hours (37%) and 42 hours (49%). Findlater *et al.*, (1988) reported that lambing rates to AI were higher when ewes were inseminated 48 h or 60 h after sponge withdrawal than at 72 h but, because they used sponges and frozen-thawed semen delivered via the intrauterine route, results may not be directly comparable with those of the present study. The use of vasectomised rams to determine onset of oestrus may have an effect on the interval between CIDR withdrawal and onset of oestrus. No other studies have examined conception rates following AI with fresh semen over the range of post-CIDR withdrawal times reported here.

TABLE 1: Effect of day of insemination, ewe age and time from CIDR withdrawal to AI on conception rates

Parameter	No. of ewes	Conception Rate	Significance (P)
Day			
1	97	-0.35 ± 0.21[a] (41%)[b]	
2	100	0.45 ± 0.21 (61%)	0.006
Age			
Two tooth	84	-0.29 ± 0.22 (43%)	
Mixed Age	113	0.30 ± 0.19 (58%)	0.04
Interval[c]			
36 h	50	-0.54 ± 0.30 (37%)	
42 h	49	-0.04 ± 0.29 (49%)	
48 h	49	0.37 ± 0.29 (59%)	
54 h	49	0.41 ± 0.29 (60%)	0.08

[a] Logit-transformed mean ± s.e.
[b] Retransformed mean (% conceiving).
[c] Interval from CIDR withdrawal to AI.

Figure 1 shows the number of ewes cycling at varying intervals post-CIDR withdrawal based on the 6-hourly observations of oestrous behaviour. The greatest proportion (36%) of the ewes cycled 30-36 hours after CIDR withdrawal. This is within the expected range of 34.7 to 36.5 hours from CIDR withdrawal to onset of oestrus when using G-type CIDRs (Welch, 1984). However, 34 of the ewes (17% of the mob) did not exhibit oestrous behaviour (ie did not show crayon marks from mating with the ram). The expected range is 10-15% (Harvey, 1989) and some trials achieved up to 98% synchronisation (Harvey *et al.*, 1984). The increased stress associated with regular monitoring, mustering and drafting may have contributed to the reduced level of synchronisation in this trial.

For the purposes of analysing effects of time from CIDR withdrawal to oestrus on conception rates, ewes were grouped (across ages, treatments and days) according to whether they came into oestrus 12-30, 36-48, or 54-72 hours post-CIDR withdrawal, or were not observed in oestrus. Table 2 shows that the time from CIDR withdrawal to oestrus significantly ($P<0.01$) influenced conception rates, with the highest conception rates being in ewes which exhibited oestrus 12-30 hours after the withdrawal of CIDRs.

The effects of the interval from oestrus to AI on conception rates are also shown in Table 2. Conception rates were

FIGURE 1: Number of Ewes Cycling: Post CIDR Removal.

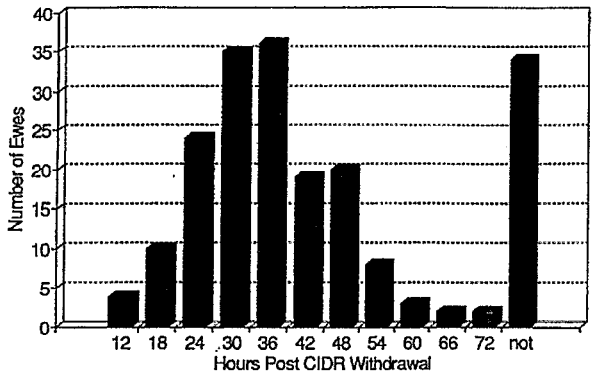

TABLE 2: Effect of time from CIDR withdrawal to oestrus and from oestrus to AI on conception rates.

Time (h)	No. of ewes	Conception rate	Significance (P)
CIDR withdrawal to oestrus			
12 to 30	73	0.47 ± 0.24[a] (62%)[b]	
36 to 48	75	0.24 ± 0.23 (56%)	
54 to 72	15	-0.69 ± 0.54 (33%)	
Not observed	34	-1.02 ± 0.39 (26%)	0.004
Oestrus to AI			
-12 to -1	48	-0.25 ± 0.29 (43%)	
0 to 6	49	0.12 ± 0.29 (53%)	
12 to 18	52	0.99 ± 0.31 (73%)	
24 to 30	14	-2.32 ± 0.53 (50%)	
Not observed	34	-1.02 ± 0.39 (26%)	0.001

[a] Logit-transformed mean±s.e.
[b] Retransformed mean (% conceiving).

highest in ewes inseminated 12 to 18 hours after oestrus and increases or decreases in this time interval reduced conception rates. This trial was undertaken with Romney ewes and the results may differ for fine wool ewes or other sheep breeds.

In summary, the results of this study suggest that conception rates to cervical AI with fresh semen are maximised when the interval from CIDR withdrawal to AI is 48 to 54 hours. This is a slightly higher interval then the current industry recommendations of 45-48 h (Harvey, 1989; Shackell, 1991), but the difference in conception rates is likely to be small. However, reducing the interval to 42 hours produces an appreciable decline in conception rates.

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