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## Effect of diluent type and storage time on the pregnancy rate of ewes inseminated laparoscopically with chilled ram semen

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### ABSTRACT

Five trials involving 879 ewes were conducted in November, December 1993, February, March and April 1994. Ewes were synchronised with CIDR™ type G devices for 14 days and in the November and December trials ewes received 400 iu PMSG (Pregnenol) at time of device removal. Semen from Dorset x Romney rams was diluted to a concentration of  $200 \times 10^6$  sperm/ml in either a milk diluent or with a synthetic ram semen diluent RSD-1 supplemented with carboxymethyl cellulose (CMC) to prevent sedimentation. The diluted semen was cooled to 15°C and packaged in 0.25 ml straws. Semen was used either on day of collection (D 0 = 6-18 hr after collection) or the following day (D 1 = 30-42 hr). Ewes were inseminated intrauterine using a laparoscopic technique on a time basis 53-56 hr after CIDR removal. Pregnancy was determined by ultrasonic scanning 45-50 days later.

There was no significant difference between diluents (milk = 57.3% and RSD-1 + CMC = 57.8%). There was a significant effect of length of storage at 15°C (D 0 = 62.2% vs D 1 = 52.3%;  $P < 0.01$ ) and a significant effect of season (between trials;  $P < 0.001$ ) with an increase from November (39.5%) up to March (72.3%). There was also a seasonal difference in the proportion of ewes showing oestrus prior to insemination (November = 32.3% to April 80.8%;  $P < 0.001$ ) and oestrous status at time of insemination had a significant effect on pregnancy rate (66.6% oestrus vs 41.0% not oestrus;  $P < 0.001$ ). There was no significant difference between operators.

These results indicate acceptable levels of fertility can be obtained with the intrauterine insemination of chilled stored semen up to 42 hr after collection. They also highlight the important effects that season and ewe synchrony can have in insemination programmes.

**Keywords:** Semen diluents; laparoscopic AI; chilled semen; storage time; oestrous status; pregnancy rate.

### INTRODUCTION

A new synthetic ram semen diluent (RSD-1) has been developed to extend the life of ram semen stored at 15°C (Upreti *et al.*, 1991, 1995). However this increased storage life has not been reflected in improved conception rates after insemination (Smith *et al.*, 1993). Sedimentation of semen during chilled storage has been shown to effect fertility (Rodriguez-Gil and Rigour, 1995). Modification of RSD-1 by the addition of carboxymethyl cellulose (CMC) was performed to prevent sedimentation and the effects on fertility following insemination was monitored.

### MATERIALS AND METHODS

Five trials involving 879 ewes were conducted in November, December 1993, February, March and April 1994. Ewes were synchronised with EASIBREED CIDR™ type G (InterAg, Hamilton, NZ) devices for 14 days and in the November and December trials ewes received 400 iu PMSG (Pregnenol; Heriot Developments Pty Ltd. Australia) at time of device removal. Ewes were joined with harnessed vasectomised rams (5%) at time of device removal.

Semen from Dorset x Romney rams was diluted to a concentration of  $200 \times 10^6$  sperm/ml, in either a milk diluent or with a synthetic diluent (RSD-1) supplemented with CMC (2.0% w/v) cooled to 15°C, packaged in 0.25 ml straws and stored at 15°C until use. Semen from up to 10 rams was pooled prior to dilution in four of the trials, while in the February trial semen from individual rams was used. Semen was used

either on day of collection (D 0 = 6-18 hr after collection) or the following day (D 1 = 30-42 hr). Ewes were inseminated intra-uterine using a laparoscopic technique on a time basis 53-56 hr after CIDR removal. Each ewe received approximately 0.1 ml of semen in each uterine horn. Inseminations were performed by up to four operators per trial with one operator involved in all trials. Oestrous status at the time of insemination was determined by the presence or absence of tugging marks. Pregnancy was determined by ultrasonic scanning 45-50 days later.

### Statistical Analysis.

Chi squared analysis of the proportion of ewes treated that were detected in oestrus and the proportion of ewes inseminated that were detected as pregnant were performed.

### RESULTS AND DISCUSSION

There was a significant ( $P < 0.001$ ) difference between trials in pregnancy rate with an increase from November (39.5%) up to March (77.7%). This was related to the seasonal difference seen in the proportion of ewes showing oestrus prior to insemination which increased from November (35.5%) to April (80.8% Table 1).

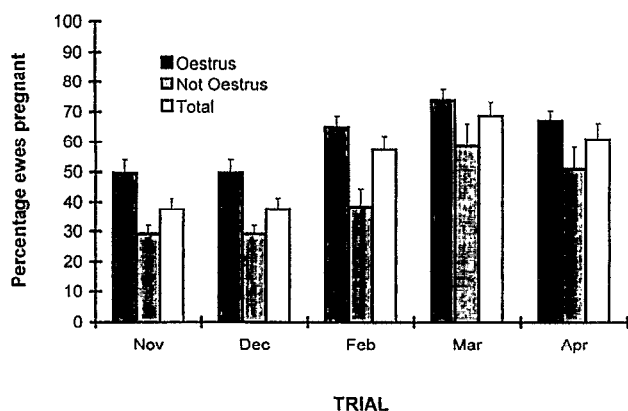
This change can be explained by the seasonal differences in time to onset of oestrus following synchronisation treatment and the variation between years previously reported (Smith *et al.*, 1991).

Oestrous status at the time of insemination had a significant effect on pregnancy rate in each of the five trials although the magnitude of that effect diminished between the non-breeding (Nov. 32%,  $P < 0.001$ ) and breeding season (April 17%,  $P < 0.05$ ) (Table 1 and Figure 1).

**TABLE 1.** Effect of season on the proportion of ewes detected in oestrus at the time of insemination and on the pregnancy rate.

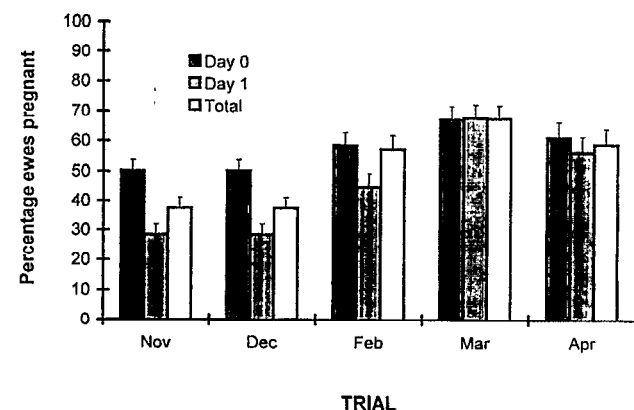
Trial	Nov	Dec	Feb	March	April	Total
No. Ewes AI	129	127	249	130	244	879
No. Ewes oestrus at AI (%)	41 (32.3)	53 (41.7)	177 (71.1)	101 (77.7)	197 (80.8)	569 (64.7)
Percent ewes pregnant						
Oestrus	61.0	50.9	68.9	70.3	68.0	66.6
Not oestrus	29.6	36.5	37.5	79.3	51.1	41.0
Total	39.5	41.7	59.8	72.3	64.8	57.6

**FIGURE 1:** Effect of oestrous status at time of insemination on the pregnancy rate of ewes inseminated with chilled semen in each trial. Values are means  $\pm$  se predicted from the appropriate regression model.



There were significant effects of storage time in the November, December and February trials but not in the March and April trials with semen inseminated on D 0 having a higher pregnancy rate than semen inseminated on D 1 (Figure 2). This effect was related to the oestrous status of the ewe with little difference between days for oestrous ewes and for D 0 to be better than D 1 in the non-oestrous ewes. This

**FIGURE 2:** Effect of time of semen storage at 15°C on the pregnancy rate of ewes inseminated in each trial. Values are means  $\pm$  se predicted from the appropriate regression model.



**TABLE 2.** Interactions between semen and oestrous status of ewes at time of insemination for each trial. Values presented are percentage of ewes pregnant and number of ewes inseminated ( ).

Time of Storage	Day 0			Day 1		
	Oestrous	Not	Total	Oestrous	Not	Total
Trial (sig) <sup>1</sup>						
November (*)	64.3 (28)	24.3 (37)	41.5 (65)	53.9 (13)	33.3 (51)	37.5 (64)
December (*)	70.4 (27)	47.5 (40)	56.7 (67)	30.8 (26)	23.5 (34)	26.7 (60)
February (NS)	75.2 (109)	41.0 (39)	66.2 (148)	58.8 (68)	33.3 (33)	50.5 (101)
March (NS)	64.0 (50)	85.7 (14)	68.7 (64)	76.5 (51)	73.3 (15)	75.8 (66)
April (NS)	70.7 (99)	56.5 (23)	68.0 (122)	65.3 (98)	45.8 (24)	61.5 (122)
Overall (NS)	70.6 (313)	45.1 (153)	62.2 (466)	61.7 (256)	36.9 (157)	52.3 (413)

<sup>1</sup>(Sig) indicates the level of significance of the interaction between storage time and oestrous status.

interaction was significant ( $P < 0.05$ ) in the November and December trials (Table 2).

There were no significant effects of diluent type in any trial. However there were significant interactions between diluent type and oestrous status in three of the trials (Table 3). Overall the tendency was for the RSD-1 + CMC to have higher pregnancy rates than milk in the oestrous ewes although the magnitude of the effect varied between trials.

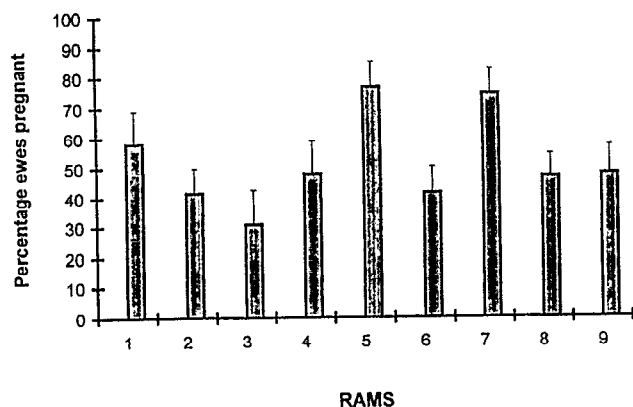
**TABLE 3:** Interactions between diluent type and oestrous status of ewes at time of insemination for each trial. Values presented as percentage of ewes pregnant and number of ewes inseminated ( ).

Diluent	Milk			RSD-1 + CMC		
	Oestrous	Not	Total	Oestrous	Not	Total
Trial (sig) <sup>1</sup>						
November (*)	55.6 (18)	30.4 (46)	37.5 (64)	65.2 (23)	28.6 (42)	41.5 (65)
December (*)	57.1 (21)	46.2 (39)	50.0 (60)	46.9 (32)	25.7 (35)	35.8 (67)
February (NS)	71.4 (63)	42.3 (26)	62.9 (89)	67.5 (114)	34.8 (46)	58.1 (160)
March (NS)	69.4 (49)	75.0 (16)	70.8 (65)	71.2 (52)	84.6 (13)	73.9 (65)
April (**)	59.8 (97)	60.9 (23)	60.0 (120)	76.0 (100)	41.7 (24)	69.4 (124)
Overall (*)	64.1 (248)	46.0 (150)	57.3 (398)	68.5 (321)	36.3 (160)	57.8 (481)

<sup>1</sup>(Sig) indicates the level of significance of the interaction between diluent and oestrous status.

In the February trial there was a significant ( $P < 0.01$ ) effect of ram with individual pregnancy rates ranging from 30% to 77% (Figure 3) although all semen met a criteria of greater than 88% progressively motile sperm at the completion of processing. These results are similar to those recorded for cervical insemination of chilled semen from different rams (Smith et al., 1995).

**FIGURE 3:** Effect of individual rams on the pregnancy rate of ewes in the February trial. Values are means  $\pm$  sem predicted from the appropriate regression model.



There were no significant differences between inseminators in any of the trials although some inexplicable higher order interactions involving inseminators were significant.

These results indicate that acceptable levels of fertility can be obtained with the intra-uterine insemination of chilled stored semen held at 15°C for up to 42 h after collection.

However the lack of effect of diluent type on fertility despite large differences in sperm survival (motility) upon incubation at 38°C in favour of the RSD-1 + CMC diluent (data not reported) supports our previous findings (Smith *et al.*, 1993). These data and the fact that no correlations were obtained between fertility and maintenance of motility upon incubation for individual rams (February trial; incubation data not reported) highlights the urgent need for improved *in vitro* assessment techniques of semen quality.

The results also highlight the major effect that season can exert on the pregnancy rates to AI. While a considerable amount of the seasonal effect can be attributed to that associ-

ated with the oestrous status of the ewe at insemination, the effect of season on ram semen "quality" cannot be ignored.

## ACKNOWLEDGMENTS

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