Comparison of cervical, transcervical and laparoscopic insemination of ewes with chilled stored and frozen ram semen

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ABSTRACT

A technique for transcervical artificial insemination (TAI) of the ewe has been reported as an alternative to laparoscopic intrauterine insemination (LAI). This study evaluated the techniques under New Zealand conditions.

Romney ewes (365) were treated in February for 14 days with CIDR® devices and inseminated (50-56 h) after device removal. Semen was diluted in a milk based diluent to concentrations of 800 x 10^6/ml for cervical insemination (CAI) (0.2 ml) and 200 x 10^6/ml for TAI and LAI (0.25 ml straws) and cooled to 15°C until insemination either 12 - 18 hr (D 0) or 36-42 hr (D 1) after collection. Frozen semen (100 x 10^6 sperm; >50% motile, in 0.25 ml straws) from the same rams was used via the TAI and LAI methods. All inseminations were on a time basis about 52 h after CIDR removal. Oestrous status of ewes at AI and the depth of TAI were recorded. Pregnancy was determined by ultrasonic scanning 50 days post insemination. Two additional trials were conducted in Nov-Dec (214 ewes) and March (115 ewes) in which TAI and LAI were compared using D 0 semen.

In the main trial LAI (63%) was superior to TAI (40%) ($P<0.05$) while CAI (50%) was intermediate.

Within the TAI method full penetration of the cervix (achieved in 51% of the ewes) had higher conception results than partial penetration (52% vs 30%, $P<0.05$). The full penetration rates with chilled semen were no better than CAI while partial penetration was lower ($P<0.05$). Oestrous status at time of AI influenced the results with conception rates of oestrous ewes being higher (60.7% v 39.7%; $P<0.001$). TAI penetration rate was not influenced by oestrous status. Results from the two additional trials support those from the main trial in relation to chilled semen. With frozen semen there were no significant differences between TAI or LAI (64.1 vs 66.7) nor between levels of penetration with TAI (full 68%, partial 58%).

Under the conditions of these experiments TAI did not prove to be a suitable alternative for LAI. The poor results of partial TAI compared to CAI with chilled semen probably reflects the difference in sperm numbers per inseminate used with the two methods and suggests that success of the TAI technique may be concentration dependant. This is supported by the data with the frozen semen. However this data (frozen semen) is at variance with previous published information on the TAI technique.

Key words: Ram semen; insemination techniques; trans-cervical (Guelph); pregnancy rate.

INTRODUCTION

The use of AI as a technique to increase the rate of genetic improvement and the dispersal of improved genotypes has had limited application within the New Zealand sheep flock. Reasons advanced for this have been the high cost of insemination relative to the value of the animal produced using laparoscopic insemination and the variable results and constraints on semen availability when using fresh semen via the cervical method. A transcervical technique has been recently developed and proposed as a viable alternative insemination method (Halbert *et al.*, 1990a, b; Buckrell *et al.*, 1992). This technique was compared to the existing techniques of laparoscopic intrauterine insemination (Killeen and Caffery, 1982) and cervical insemination with both fresh (chilled) and frozen ram semen under New Zealand conditions.

MATERIALS AND METHODS

Animals. A series of three trials were conducted November-December 1993 (214 Coopworth ewes), February 1994 (365 Romney ewes) and March 1994 (115 Dorset x Romney ewes).

All ewes were synchronised with EASI-BREED™ CIDR® type G devices (InterAg, Hamilton) inserted for 14 days. Those in the November-December trial received 400 iu PMSG (Pregnecol - Heriot Development Pty Ltd, Australia) at the time of device removal. Ewes were inseminated between 50 and 56 hr after CIDR removal and the semen from 10 Dorset x Romney rams was used in all trials. In the first and third trial pooled semen was used while that from individual rams was used in the February trial. Semen was diluted in a milk diluent cooled to 15C and held at that temperature until it was used either 5-8 hr (Day 0) or 29-32 hr (Day 1) after collection. In trial 2 (February) frozen semen from some of the same rams was also used (80 ewes).

Ewes were allocated to treatment groups on the basis of age and liveweight at the time of CIDR insertion.

Insemination techniques. Three insemination techniques were compared in the February trial, while only two systems were tested in the other trials. The techniques evaluated were:

(a) Cervical (CAI) in which 0.2 ml of chilled semen at a concentration of 800 x 10^6 sperm/ml was placed in the external os of the cervix with the ewe restrained in a standing position.

(b) Laparoscopic (LAI) - where 0.25 ml of semen in straws at concentrations of 200 x 10^6/ml (chilled) and 400 x 10^6/ml (frozen) is injected into the lumen of the uterine horn visualised via a laparoscope (Killeen and Caffery 1982).

(c) Transcervical (Guelph TAI) - where 0.25 ml of semen in straws at same concentrations as for the LAI was attempted to be deposited in the uterine lumen. The ewe was restrained in a crate in dorsal recumbency, the cervix located and a thin metal insemination probe manipulated through the cervical folds (Buckrell *et al.*, 1994). A maximum time limit of 5 minutes was set to achieve full penetration of the cervix. If full penetration had not been achieved in this time semen was deposited in the cervix (partial penetration).

Operators. Two inseminators with considerable experience in both cervical and laparoscopic methods and who had practised with the transcervical system for a period of 9 months to ensure high rate of penetration and correct placement of the insemination probe performed all the inseminations.

Oestrous status. Vasectomised rams (5%) were joined with the ewes at the time of CIDR removal and the presence or absence of tupping marks recorded at the time of insemination. Those with tupping marks were categorised as being "oestrus" and those without as "not oestrus".

Pregnancy. All ewes were real time ultrasound scanned at about 50 days post-insemination to determine pregnancy status.

Analysis of data. The proportion of the ewes inseminated that were classified as pregnant (to that insemination) for the various treatments were subjected to Chi Square analysis. Each trial was analysed separately as well as an overall analysis being performed.

RESULTS

The pregnancy data for the ewes inseminated with chilled semen in the three trials is summarised in Table 1.

Overall there was a significant ($P<0.001$) effect of insemination technique with the LAI (57.2%) being superior to the TAI (34.0%). In the February trial the CAI system produced intermediate results (50.4%) and was similar to that achieved with full penetration in the TAI method (52.0%). There was a significant ($P<0.01$) effect of penetration success in the TAI method with full penetration (46.2%) better than partial penetration (21.0%).

Oestrous status of the ewe at the time of insemination had a significant ($P<0.001$) effect on pregnancy rate overall (oestrus 56.5% vs not oestrus 38.1%, Table 2). This was less pronounced in the TAI ewes. The proportion of ewes in oestrus increased from the first to the third trial. Storage time

TABLE 1: Effect of system of insemination and the degree of transcervical penetration on pregnancy rate of ewes inseminated with chilled semen expressed as percentage of the number of ewes inseminated (x).

Trial	Semen Storage	Insemination Technique							
		Cervical	Guelph Transcervical					Laparoscopic	
			Full		Part		Total		
Nov-Dec	Day 0		38.9	(59)	10.0	(40)	27.3	(99)	54.5 (55)
	Day 1								36.7 (60)
	Subtotal								50.8 (115)
Feb	Day 0	53.7 (67)	60.0	(10)	31.6	(19)	41.4	(29)	67.3 (55)
	Day 1	46.9 (66)	46.7	(15)	28.6	(14)	37.9	(29)	55.9 (41)
	Subtotal	50.4 (133)	52.0	(25)	30.2	(33)	39.7	(58)	62.9 (96)
March	Day 0		59.1	(22)	25.9	(27)	40.8	(49)	67.4 (31)
	Day 1								73.5 (34)
	Subtotal								70.7 (65)
Total		50.4 (133)	46.2	(106)	21.0	(100)	34.0	(206)	57.2 (269)

TABLE 2: Effect of oestrous status of ewes at insemination with chilled semen on pregnancy rates with different insemination techniques and trials. Values are % ewes pregnant (number ewes inseminated)

Trial	Insemination Technique								Ewes in Oestrus %
	Cervical		TAI (Guelph)		Laparoscopic		Total		
	Oestrus	Not	Oestrus	Not	Oestrus	Not	Oestrus	Not	
Nov-Dec	–	–	29.4 (51)	24.0 (50)	68.0 (25)	39.8 (88)	42.1 (76)	34.0 (138)	35.5
Feb	62.9 (62)	38.9 (72)	32.0 (25)	45.5 (33)	69.8 (63)	35.5 (31)	60.7 (150)	39.7 (136)	52.4
March	–	–	52.5 (40)	11.1 (9)	69.4 (49)	75.0 (16)	61.8 (89)	52.0 (25)	78.1
Total	62.9 (62)	38.9 (72)	37.9 (116)	30.4 (92)	69.3 (137)	42.9 (135)	56.5 (315)	38.1 (299)	51.3

TABLE 3: Effect of insemination technique and oestrous status of ewes at time of insemination on pregnancy rate after insemination with frozen semen in the February trial

Insemination Technique	Oestrus status					
	Oestrus		Not		Total	
Transcervical (Guelph)	70.8	(24)	53.3	(15)	64.1	(39)
Lapaoscopic	81.0	(21)	50.0	(18)	66.7	(39)
Total	75.5	(45)	57.5	(33)	65.4	(78)

Values are percentage of ewes pregnant (number of ewes inseminated)

of semen did not have a significant overall effect but pregnancy rates tended to be lower with Day 1 semen than with Day 0 semen. Results with the frozen semen (Table 3) indicated no effect of insemination technique but there was an effect ($P<0.05$) of oestrous status at AI.

The successful full penetration rate for the TAI ewes varied from trial to trial (November-December 59.6%; February 43.1% and March 44.9%). The success of penetration was not influenced by the oestrous status of the ewe.

Two operators performed the insemination procedure and there was no operator difference in any of the insemination techniques. Records of the time and labour involved in the procedures indicated that throughput per inseminator was half (15-18 per hr) for the TAI method compared to that for LAI (30 ewes per hour) although the TAI method required only half the labour input.

DISCUSSION

The data obtained in this trial indicates that the TAI is currently not a suitable substitute for laparoscopic insemination with chilled ram semen in that it gave lower pregnancy rates and the effort (labour, time and operator skill and training) required was at least equal to that required for the laparoscopic technique.

The lower pregnancy rates with the TAI system were not entirely due to failure to achieve full penetration and thus intrauterine deposition of the semen. The pregnancy rates for full TAI were consistently lower than that for the LAI. This is similar to findings reported for comparison of the systems using frozen semen (Windsor *et al.*, 1994).

The rate of successful penetration achieved in this study is considerably lower than that reported by Buckrell *et al.* (1994) and that achieved during the training procedures (70-85%) but is comparable with that of Windsor *et al.* (1994). All of these reports have penetration rates considerably higher than that reported for other methods (Fuki and Roberts 1987; Eppleston, 1992).

The poorer pregnancy rates following partial penetration of the cervix with TAI in comparison to that obtained with deposition of semen at the cervical os with CAI is undoubtedly due to differences in the semen concentrations used for the two techniques and is in agreement with the findings of Windsor *et al.* with frozen semen.

The relatively high pregnancy rates obtained with frozen semen in comparison to that with chilled semen by both TAI and LAI may also be related to the dose of semen used and could explain the smaller differences between full (68%) and partial (60%) penetration seen with the frozen semen. The frozen semen used in this trial had a very high (67%) post-thaw motility and thus these ewes would be receiving 1.8 x more motile sperm than the ewes inseminated with chilled semen.

The variation between trials in rate of penetration could indicate either seasonal or time since lambing effects. However, the level of full penetration achieved appears to be the major limitation to the adoption of the technique as in our trials there were no benefits of TAI relative to LAI in terms of time, labour or operator skills (level of training required).

Further research into means of increasing the rate and ease of cervical penetration would appear to be necessary for this technique to achieve general acceptance.

ACKNOWLEDGEMENTS

Mr C. Harris for care and treatment of animals in trials 1 and 3; Ms A. Death and staff at Flock House for assistance with trial 2; Miss J.K. Smith for assistance with animal treatment and semen handling in all trials.

The work was funded by the New Zealand Meat Research and Development Council (Project 91 MT 66/3.2).

REFERENCES

Buckrell, B.C.; Bushbeck, C.; Gartley, C.J.; Kroetsch, T.; McCutcheon, W.; Martin, J.; Penner, W.K. and Walton, J.S. 1992. A breeding trial using transcervical technique for artificial insemination in sheep. *Proceedings of the 12th International Congress Animal Reproduction (The Hague) Vol.* **3:** 1531-1536.

Buckrell, B.C.; Bushbeck, C.; Gartley, C.J.; Kroetsch, T.; McCutcheon, W.; Martin, J.; Penner, W.K. and Walton, J.S. 1994. Further development of a transcervical technique for artificial insemination in sheep using previously frozen semen. *Theriogenology* **42:** *601-611.*

Eppleston, J. 1992. Studies on the fertility of frozen-thawed ram semen. *Ph.D. Thesis, the University of New South Wales,* Australia.

Fuki, Y. and Roberts, E.M. 1978. Further studies on non-surgical intrauterine technique for artificial insemination in the ewe. *Theriogenology* **10:** 381-393.

Halbert, G.W.; Dobson, H.; Walton, J.S. and Buckrell, B.C. 1990(a). A technique for transcervical intrauterine insemination of ewes. *Theriogenology* **33:** 993-1010.

Halbert G.W.; Dobson, H.; Walton, J.S.; Sharpe, P. and Buckrell, B.C. 1990(b). Field evaluation of a technique for transcervical intrauterine insemination of ewes. *Theriogenology* **33:** 1231-1243.

Killeen, I.D. and Caffery, G.J. 1982. Uterine insemination of ewes with the aid of a laparoscope. *Australian Veterinary Journal* **59:** 95.

Windsor, D.P.; Szell, A.Z.; Bushbeck, C.; Edward, A.Y.; Milton, J.T.B. and Buckrell, B.C. 1994. Transcervical artificial insemination of Australian Merino ewes with frozen-thawed semen. *Theriogenology* **42:** 147-158.