

Fertilization in the Ewe following Multiple Ovulation and Uterine Insemination

A. O. Trounson and N. W. Moore

Department of Animal Husbandry, University of Sydney Farms,
Camden, N.S.W. 2570.

Abstract

Two sites of uterine insemination were compared with natural service for the recovery and fertilization of sheep ova following multiple ovulation induced with an equine anterior pituitary extract (HAP) given after a period of progesterone treatment. Freshly ejaculated semen was placed directly into either the lumen of the body of the uterus or extremities of the uterine horns 24–30 h after HAP treatment.

There was little difference between the two sites of uterine insemination in recovery and fertilization rates of ova. Recovery rates were slightly lower (6–9%) following uterine insemination, but fertilization rates were considerably higher than in naturally mated ewes (96·5 v. 59·2%). Overall, uterine insemination resulted in the recovery of 20% more fertilized ova than after natural service.

Progesterone–HAP treatment gave a marked degree of control over the time of ovulation and it is suggested that uterine inseminations 24 h after the conclusion of treatment be used when large numbers of ova of known age are required at predetermined times.

Introduction

In the ewe failure of fertilization to both natural service and artificial insemination frequently occurs following multiple ovulation, particularly when relatively large doses of gonadotrophin are used to induce multiple ovulation. The reasons for fertilization failure are obscure and in a series of studies in which large numbers of fertilized ova were required at predetermined times, several methods of insemination were used in an attempt to obtain high rates of fertilization of multiple-ovulated ova.

Materials and Methods

A total of 201 mature Merino ewes were used in the studies and all ewes were treated with progesterone and an equine anterior pituitary extract (HAP) to induce multiple ovulation at predetermined times. Progesterone was administered daily (12 mg/day) by intramuscular injection for 12–30 days and HAP at total doses of 48–68 mg was given as three equal subcutaneous injections on consecutive days commencing on the day before the final injection of progesterone. HAP was prepared as described by Moore and Shelton (1964), and with the treatment regime used in the studies the majority of ewes could be expected in oestrus on the day after the final HAP injection (Moore 1970).

Eighty-five ewes were destined for natural mating and after treatment they were run with harnessed entire rams. When first detected in oestrus and at 12 h after first detection each ewe was hand-mated to at least two entire rams that were known to produce semen of high fertilizing capacity. The remaining 116 ewes were surgically inseminated. They were run with harnessed entire or vasectomized rams and regardless of the time of oestrus all ewes were inseminated 24–30 h after the final HAP injection. Inseminations were carried out under local anaesthesia (Xylocaine). The uterus was exposed by midventral laparotomy and 0·02 ml of freshly ejaculated semen was

placed into the lumen of the body of the uterus or extremities of each uterine horn by means of finely drawn sterile glass pipettes.

At 2, 3-4 or 6 days after surgical insemination or natural mating laparotomies were carried out under general anaesthesia (Nembutal). The numbers of recently formed corpora lutea were recorded and ova were recovered by flushing the Fallopian tubes and uterine horns with one of several culture media. Following recovery the ova were examined as fresh specimens and apparent normal cleavage was used as the criterion of fertilization.

Results

Of the 85 ewes destined for natural service 14 were not observed in oestrus after treatment and they have been excluded from the data shown in Table 1. A further 17 ewes of those surgically inseminated did not exhibit oestrus, but data for these ewes are included in Table 1. All of the 170 ewes that were in oestrus following treatment were first served within 2 days of the final HAP injection.

Table 1. Effect of method of insemination on the recovery and fertilization of ova following multiple ovulation

Method and site of insemination	No. of ewes	Time of ovum recovery ^A	Mean no. of ovulations	Percentage of ova:		No. of ewes with fertilization:		
				Recovered	Fertilized	Full	Partial	None
Natural mating	23	2	11.4	70.0	49.5	12	2	9
	21	3 and 4	9.6	79.6	53.9	6	9	6
	27	6	9.8	69.7	73.4	19	4	4
Total	71		10.1	72.4	59.2	37	15	19
Surgical: body of uterus	23	2	9.7	74.9	96.4	19	4	0
	27	3 and 4	8.5	53.7	95.9	25	2	0
	7	6	9.1	57.8	100.0	7	0	0
Total	57		9.1	63.4	96.6	51	6	0
Surgical: uterine horns	23	2	8.3	73.2	96.4	20	1	2
	8	3 and 4	12.6	73.3	90.5	4	4	0
	28	6	9.1	59.1	99.3	27	1	0
Total	59		9.2	66.6	96.4	51	6	2
Grand total	187		9.5	68.0	80.5	139	27	21

Table of χ^2 values

Source of deviation	Degrees of freedom	χ^2 value:	
		Recovery	Fertilization
Methods of insemination	2	12.64**	260.65***
Times of recovery	2	11.96**	12.85**
Interaction	4	32.28***	28.39***
Total	8	56.88	301.89

** $P < 0.01$. *** $P < 0.001$.

^A Days after mating or uterine insemination.

Overall, surgical insemination depressed recovery rates by some 6-9%, but its effect was modified by the time elapsing between insemination and recovery of ova. In naturally mated ewes the time elapsing between mating and recovery had little

effect upon recovery rates whereas following surgical insemination recovery rates decreased as the time elapsing between insemination and recovery was increased.

A much higher proportion of ova recovered after surgical insemination were fertilized than those recovered after natural mating, but there was no effect of site of surgical insemination on fertilization rates. Overall, delaying the time of recovery increased the proportion of recovered ova that were fertilized in the naturally mated group. Within naturally mated ewes fertilization rates rose by some 24% from day 2 to day 6 after mating ($P < 0.05$). In 19 of the 71 naturally mated ewes that were served none of the recovered ova were fertilized and in a further 15 ewes one or more ova were unfertilized, whereas in the 116 surgically inseminated animals complete and partial failure of fertilization was observed in only 2 and 12 ewes respectively (34 of 71 v. 14 of 116; $P < 0.001$).

As evidenced by stage of development of ova at the time of recovery, the method of insemination appeared to have no marked effect upon the time at which fertilization occurred. Irrespective of method of insemination the vast majority of ova recovered on day 2 were of two cells, on day 3 and 4 of 8–16 cells and on day 6 they were morulae of some 20–30 cells.

In the surgically inseminated ewes failure to exhibit oestrus did not appear to be associated with any gross changes in the time of ovulation or of fertilization. The stage of development of ova recovered from the 17 ewes that did not exhibit oestrus was similar to that in ewes that did exhibit oestrus.

Discussion

The decrease in the proportion of ova recovered following surgical insemination, particularly when three or more days elapsed between insemination and recovery, provides further evidence to support the conclusions of Killeen and Moore (1971) that handling of the reproductive tract of the ewe at about the time of ovulation results in rapid transport of ova through the tract.

A delay in the time of recovery was associated with an increase in fertilization rates in the naturally mated group, but it is difficult to visualize a direct effect on fertilization of time of recovery. A more likely explanation lies in the preferential loss or disintegration of unfertilized ova. However, it has been shown that unfertilized ova can be recovered in a relatively intact state as late as 15 days after oestrus (Bindon 1969), but major differences exist between that study and the present one. The ewes used by Bindon had not received any hormone treatment prior to oestrus and all had been mated to vasectomized rams. Further, far too few animals were involved to accurately assess the proportion of ova recovered at various times after oestrus.

The very high fertilization rates achieved by uterine insemination clearly indicated that the vast majority of multiple-ovulated ova were fertilizable. Fertilization failure following multiple ovulation and natural mating would appear to be primarily due to faulty transport of spermatozoa to the site of fertilization and perhaps more specifically to failure of spermatozoa to penetrate the cervix in sufficient numbers to result in high rates of fertilization.

The somewhat lower recovery rates observed in surgically inseminated ewes were more than compensated for by the very high fertilization rates. Of 1061 ova shed by surgically inseminated ewes, 666 were recovered and fertilized (62.8%),

whereas in naturally mated ewes 308 of 718 (42·9%) ova shed were recovered and fertilized. This difference would have been even more marked had the 14 ewes that were destined for natural mating, but failed to exhibit oestrus, been considered. Within the surgically inseminated ewes failure to exhibit oestrus did not affect ovulation, recovery rates or fertilization rates. Clearly, there are distinct advantages to be gained from surgical insemination, but the advantages can only be fully realized following treatment which provides a marked degree of control over the time of ovulation and allows inseminations to be carried out at predetermined times without reference to the detection of oestrus. Within this laboratory progesterone and HAP treatment, followed by surgical insemination into the extremities of the uterine horns 24 h after the final injection of HAP, has been adopted as a routine procedure when fertilized sheep ova of known age are required at predetermined times.

Acknowledgments

Grateful acknowledgment is made to Ms Anne Pugh, Ms Sally Hockings, Mr Ken Old and Mr John Ellsmore for technical assistance. The study was supported by a grant from the Australian Research Grants Committee.

References

- Bindon, B. M. (1969). Fate of the unfertilized sheep ovum. *J. Reprod. Fertil.* **20**, 183–4.
- Killeen, I. D., and Moore, N. W. (1971). The morphological appearance and development of sheep ova fertilized by surgical insemination. *J. Reprod. Fertil.* **24**, 63–70.
- Moore, N. W. (1970). Fertilization in ewes treated with progesterone and equine anterior pituitary extract. *J. Endocrinol.* **46**, 121–2.
- Moore, N. W., and Shelton, J. N. (1964). Response of the ewe to a horse anterior pituitary extract. *J. Reprod. Fertil.* **7**, 79–87.