

TRANSPORT OF SPERMATOZOA AND APPARENT FERTILIZATION RATE IN YOUNG AND MATURE MERINO EWES

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Summary

Transport of spermatozoa and apparent fertilization rate were compared, under natural and hand-mating conditions, in 1½ years-old nulliparous (young) and 63 years-old parous (mature) Merino ewes.

Significantly more ova recovered from young ewes did not have sperm attached to the zona pellucida. Ewes fell into two distinct populations, those having < 208 spermatozoa and those having > 800 spermatozoa in the two oviducts combined. In a significantly higher proportion of young ewes < 208 sperm were recovered from the oviducts. There were no significant differences between young and mature ewes in the numbers of sperm in the cervix and uterus, but in young ewes with < 208 in the oviducts there were less sperm in the cervix and uterus than in young ewes with > 208 in the oviducts.

Mating system did not have a significant effect on any of the parameters.

It was concluded that fertilization failure, due to inefficiency of sperm transport into and through the cervix is a possible cause of the higher incidence of failure to lamb in young ewes.

I. INTRODUCTION

There are a number of reports, reviewed by Mullaney and Brown (1970), indicating a greater incidence of failure to lamb after joining in young ewes than in older ewes. Failure to lamb may be due to many causes, of which one is fertilization failure resulting from deficient transport of spermatozoa. We have examined the hypothesis that transport of spermatozoa is less efficient in young than older Merino ewes. Lang and Oh (1968) have presented evidence to support this hypothesis in Romney ewes.

Ewes were either run with entire rams continuously until slaughter (natural-mating), or allowed only one service (hand-mating). It was assumed that naturally-mated ewes would be served more than once, and since cervical populations of spermatozoa are related to the number of services (Mattner and Braden 1967), it was expected that numbers of cervical spermatozoa would be greater in naturally-mated than in hand-mated ewes. Hand-mating also permitted accurate timing of the interval between service and slaughter.

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II. MATERIALS AND METHODS

(a) *Animals and management*

From the beginning of February 1968, 30 nulliparous Merino, ewes aged about 1½ yr (young ewes) and 30 parous Merino ewes aged about 63 yr (mature ewes) were grazed together at the University of N.S.W. Field Station, Wellington, in central western N.S.W. In mid-March 1968, the ewes were weighed after a 16 h fast from food and water and then were assigned, within age groups, to either a naturally-mated or hand-mated group in a random stratified allocation on the basis of live weight. Naturally-mated groups were placed in two adjoining 0.4 ha paddocks, while hand-mated groups were placed together in a third paddock. The feed available in all paddocks was predominantly irrigated "Sudax".

Four fertile Merino rams were used during the experiment, one being run with each of the naturally-mated groups, while the other two were used for the hand-mated groups. The rams were interchanged regularly so that each ram spent an equal time with each group. In an attempt to equilibrate spermatozoa numbers per ejaculate (Lightfoot 1968), the rams had spent the four days immediately preceding the start of the study with the flock ewes which were being joined at that time. Two vasectomised rams were run with the hand-mated groups. All rams were fitted with 'Sire-Sine' harnesses and crayons.

All ewes were yarded twice each day at approximately 0630 and 1730 hours. Ewes in the naturally-mated groups which had been marked by the rams were noted. In the hand-mated groups, ewes that were marked by the vasectomised rams were drafted off and allowed one service by a ram. The procedure described by Lightfoot, Croker and Neil (1967) was followed.

(b) *Slaughter and recovery of reproductive tracts*

Ewes were slaughtered by bleeding at approximately 0900 hours each day. Naturally-mated ewes first noted to have been marked by a ram at 0630 hours or 1730 hours were killed approximately 26.5 h or 39.5 h later respectively. Hand-mated ewes were killed approximately 24.5 or 38.5 h post-coitus, depending on whether they were detected in oestrus at 0630 or 1730 hours respectively.

Before removal of the reproductive tract, the uterotubal and uterocervical junctions, as well as the anterior vagina, were ligated with forceps. The ovaries were examined and the number of corpora lutea or large follicles was recorded.

The techniques of Mattner and Braden (1963) were used to determine the numbers of spermatozoa in the cervix, uterus and in each oviduct. Recovered ova were examined for spermatozoa adhering to the zona pellucida.

(c) *Statistical analyses*

Age and mating effects were examined for significance by the chi-square test, and by analysis of variance after angular transformation of percentages. Estimated numbers of spermatozoa were transformed by logarithms (using $\log(n + 1)$ to allow for zero values). The variances of the transformed data were tested for homogeneity by analysis of variance (Rao 1952).

III. RESULTS

(a) *General*

The mean liveweights of the young and mature ewes of 34.2 ± 0.7 and 41.5 ± 1.1 kg respectively, were significantly different ($P < 0.001$, t-test).

During the experimental period of 19 d, 18 mature ewes (9 naturally-mated, 9 hand-mated) and 26 young ewes (12 naturally-mated, 14 hand-mated) mated and were slaughtered.

(b) *Examination of ovaries and ova*

All mature ewes and 24 young ewes had ovulated. One large Graafian follicle was found in the ovaries of each of the two young ewes which had not ovulated. The apparent ovulation rates in young and mature ewes were 1.04 and 1.78 respectively, the proportion of mature ewes with double ovulations being greater than that of young ewes ($X_1^2 = 21.7$, $P < 0.0005$).

In the mature ewes, 25 of a possible 32 ova (78 per cent) were recovered whereas in young ewes, 23 of a possible 25 (92 per cent) were recovered, there being no significant difference between the age groups ($X_1^2 = 1.12$, $0.25 < P < 0.50$).

For naturally and hand-mated mature ewes, the proportions of recovered ova with spermatozoa attached to the zona pellucida were 1/12 (92 per cent) and 13/13 (100 per cent) respectively; corresponding proportions for young ewes were 6/10 (60 per cent) and 8/13 (62 per cent). Analysis of variance of the percentages indicated a highly significant ($P < 0.001$) age effect, but no mating effect, due to fewer of the ova from young ewes having sperm attached to the zona pellucida.

TABLE 1

Effect of age of ewe and mating system on the number of spermatozoa recovered from the cervix, uterus and each oviduct

Division of genital tract	Mean ($\pm$ standard error) after logarithmic transformation (with antilog)			
	Young ewes		Mature ewes	
	Naturally-mated (12 ewes)	Hand-mated (14 ewes)	Naturally-mated (9 ewes)	Hand-mated (9 ewes)
Cervix	5.49 ± 0.37 (308,000)	5.78 ± 0.22 (607,000)	4.99 ± 0.36 (97,600)	5.96 ± 0.23 (905,000)
Uterus	3.27 ± 0.61 (1,840)	3.10 ± 0.47 (1,270)	3.65 ± 0.47 (4,410)	4.50 ± 0.18 (31,900)
Oviduct	2.53 ± 0.34 (335)	2.39 ± 0.31 (245)	3.31 ± 0.29 (2,060)	3.37 ± 0.10 (2,340)

(c) Recovery of spermatozoa

Mean counts of spermatozoa in regions of the genital tract are presented in Table 1. Analysis of variance of transformed counts in both the cervix and uterus showed that variances were homogeneous, and that neither age nor system of mating had significant effects on cervical or uterine counts of spermatozoa. However, analysis of variance of counts in the oviducts revealed a significant ($P < 0.05$) age effect on heterogeneity of variance, which remained even after removal from the analysis of ewes with no spermatozoa in the oviducts.

Inspection of the spermatozoa counts in the two oviducts combined revealed that ewes fell into two distinct populations, those having < 208 spermatozoa and those having > 800 spermatozoa. There were ten young ewes and only one mature ewe in the < 208 spermatozoa class. None of the ova recovered from oviducts containing < 208 spermatozoa had spermatozoa in the zona, whilst all ova from both young and mature ewes in the > 800 spermatozoa class showed spermatozoa in their zonae. Mean numbers of spermatozoa in the uterus and cervix of young ewes in these classes are shown in Table 2. In analyses of variance, using the method for heterogeneous variances (Snedecor 1956), oviducal spermatozoa class was a significant effect on both uterine and cervical spermatozoa numbers. Mean cervical and uterine counts of spermatozoa were significantly larger in the > 208 class than in the < 208 class (t test for heterogeneous variances and unequal sub class numbers, Snedecor 1956).

TABLE 2

Mean transformed counts Of spermatozoa, $\pm$ standard error, in the cervix and uterus of young ewes in which either < 208 or > 208 spermatozoa were recovered from the oviducts

Spermatozoa in the oviducts	Cervix	Uterus
< 208	4.79 ± 0.26	1.05 ± 0.28
> 208	6.10 ± 0.08	4.30 ± 0.03

IV. DISCUSSION

The results of this study suggest that fertilization failure may contribute to the higher incidence of failure to lamb in young Merino ewes. Some support for this conclusion is provided by Salamon and Robinson (1962) who found, on the basis of ewes in oestrus and artificially inseminated, a 15-20 per cent increase in lambing performance of six-tooth and full-mouth ewes compared with maiden ewes.

Both fertilization failure and embryonic mortality could have contributed to the difference observed by Salamon and Robinson (1962), but Edey (1969) concluded that there is no clearcut age effect on basal embryonic loss in ewes.

The high proportion of young ewes in which ova showed no spermatozoa in the zona pellucida is probably a consequence of the high proportion of these ewes in which numbers of spermatozoa in the oviduct were zero or < 208 . Quinlivan

and Robinson (1967) concluded that chances of fertilisation were improbable when numbers of spermatozoa in the oviducts were < 200 . Our analyses indicate that low oviducal spermatozoa numbers are related to low uterine and cervical spermatozoa numbers (Table 2). Hence we conclude that transport of spermatozoa into and through the cervix is inefficient in some ewes. Lang and Oh (1968) also concluded that transport of spermatozoa was more variable in young than in mature Romney ewes.

Mattner (1966) and Mattner and Braden (1969a, b) consider that the physical and rheological properties of cervical mucus and the motility of spermatozoa are mainly responsible for the movement of spermatozoa into and through the cervix. It seems possible that the production and/or properties of mucus are different in young and mature ewes. Since secretion and viscosity of mucus appear to be controlled by oestrogens (Lindsay and Francis 1968), inefficient transport of spermatozoa may be due to lower oestrogen production in some young ewes as postulated by Edgar (1962).

Contrary to expectation, larger numbers of spermatozoa were not recovered from the cervixes of naturally-mated than from hand-mated ewes. It is possible that the slightly longer interval between mating and slaughter in the naturally-mated ewes was the reason, since Lang and Oh (1968) reported a sudden decrease in sperm numbers in the reproductive tract between 36 and 48 h post-coitus.

V. ACKNOWLEDGMENT

T. G. Kennedy was in receipt of an Australian Wool Board Post-Graduate Research Scholarship.

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