A COMPARISON OF THE TRANSPORT OF SPERMATOZOA IN SPAYED AND ENTIRE EWES

A. J. ALLISON*

*Department of Animal Husbandry, The University of Sydney,
Sydney, N.S.W. 2006, Australia*

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Summary. The pattern of transport of spermatozoa in spayed ewes treated with 10 mg progesterone for 12 days followed by 24 µg ODB was compared with that in entire animals at two times of recovery following artificial insemination or natural mating. Spayed ewes had fewer spermatozoa in all sections of the tract than did entire ewes for all treatment combinations. The numbers recovered from the cervix and vagina were higher at 2 than at 24 hr after insemination, while the converse was true for the Fallopian tubes. More spermatozoa were recovered from the Fallopian tubes of naturally mated than of artificially inseminated ewes but the difference was not evident in other regions of the tract. Histological examination of the uterine endometrium indicated an early stage of atrophy in the spayed ewes. Thus, hormonal regimens which will induce behavioural oestrus of 'normal' dimensions in spayed ewes will not maintain the functional integrity of the uterus and the transport of spermatozoa within the tract is grossly impaired.

INTRODUCTION

Oestrogen is necessary for the establishment and maintenance of a population of spermatozoa within the cervix of the ewe (Allison & Robinson, 1972) and a balance of ovarian steroids appears also to be involved. The numbers of spermatozoa recovered from the cervix, uterus and Fallopian tubes of progesterone-oestrogen-treated spayed ewes were many fewer than those reported in entire ewes. In the only other published report on the transport of spermatozoa in spayed ewes (Mattner & Braden, 1963), the numbers recovered compared favourably with those obtained from entire ewes under similar circumstances (Mattner, 1963). However, spermatozoa were recovered 3½ to 4 hr after natural or forced mating whereas the data reported by Allison & Robinson (1972) concerned recovery 24 hr after artificial insemination. In this study, therefore, the transport of spermatozoa in spayed and entire ewes was compared following natural mating or artificial insemination.

* Present address: Invermay Agricultural Research Centre, Private Bag, Mosgiel, New Zealand.

MATERIALS AND METHODS

Experimental design

Twenty medium wool Merino ewes were ovariectomized 7 weeks before the start of the experiment. Twenty similar entire ewes were used as controls. One spayed ewe died during the course of the experiment.

The ewes were randomized into a factorial experimental design along the following lines:

Type of ewe	Spayed versus entire
Time of recovery (hours)	2 versus 24
Type of mating	Artificial insemination versus natural mating
Factorial: $2 \times 2 \times 2$; $n=5$; $N=40$.	

Hormone administration

Spayed ewes were subjected to two 'artificial' oestrous cycles (Allison & Robinson, 1972) and the experimental oestrus was induced by similar treatment, namely 10 mg progesterone/day for 12 days, followed 48 hr later by a single injection of 25 μ g oestradiol benzoate (ODB).

For experimental convenience, the cycles of the entire ewes were synchronized with 12 daily injections of 10 mg progesterone followed by 500 i.u. PMSG given at the time of the last injection. The ewes were used at the next cycle following this treatment.

Insemination and mating

Oestrus was detected by harnessed vasectomized rams. Semen for artificial insemination was collected by artificial vagina and ejaculates were pooled following a visual assessment of sperm density and motility, using only semen of high quality. Insemination was carried out with 0.15 ml undiluted semen estimated to contain not less than 300×10^6 spermatozoa. Natural mating was by hand service to each of two rams within 5 min. Entire ewes were inseminated or mated on detection of oestrus and spayed ewes 24 hr after the injection of oestrogen. They were run in a small pen until just before slaughter.

Slaughter of ewes and recovery of spermatozoa

Ewes were killed at the laboratory by intracardiac injection of 3 ml 60% sodium pentobarbitone solution. The reproductive tract was immediately removed and flushed. In general, procedures of flushing, storage and counting were as described by Allison & Robinson (1972) except that the cervixes were dissected away from the rest of the tract, immediately frozen in liquid nitrogen vapour at -193°C and subsequently stored at -20°C . Immediately before counting, each cervix was divided into caudal, mid and cranial sections. Each section was dissected into six to eight longitudinal strips which were placed into a vial containing 10 ml saline. The strips were left to thaw for approximately 30 min and then vigorously shaken before withdrawal of an aliquot for counting. Methods of counting were as described previously except that, for ease of counting, uterine washings were concentrated by centrifugation at 3500 rev/min for 10 min.

TABLE 1
ESTIMATED NUMBERS OF SPERMATOOA RECOVERED FROM VARIOUS DIVISIONS OF THE REPRODUCTIVE TRACT OF SPAYED AND ENTIRE EWES AT DIFFERENT TIMES AFTER MATING AND ARTIFICIAL INSEMINATION

Main effects	Division of the reproductive tract					
	Vagina		Cervix		Uterus	
	Arithmetic mean ($\times 10^3$)	$\log_{10} (x+2)$	Arithmetic mean ($\times 10^3$)	$\log_{10} (x+2)$	Arithmetic mean ($\times 10^3$)	$\log_{10} (x+2)$
Type of ewe (n = 19 to 20)						
Spayed	35094	5.652	7522	6.045	7	2.461
Entire	88530	6.554	27516	7.112	84	3.932
<i>P</i>		<0.01		<0.001		<0.05
Time of recovery (n = 19 to 20)						
2 hr	123275	7.285	30771	7.247	23	3.168
24 hr	349	4.920	4267	5.910	68	3.405
<i>P</i>		<0.001		<0.001		N.S.
Type of mating (N = 19 to 20)						
Artificial insemination	47773	5.783	18584	6.346	55	2.826
Natural mating	75851	6.422	16454	6.811	36	3.747
<i>P</i>		N.S.		<0.05		N.S.
Position of cervix (n = 39)						
Caudal			10464	6.170		
Mid			5751	5.674		
Cranial			1408	5.285		
<i>P</i>				<0.01		

N.S., not significant.

Analysis of the data for cervical counts was by a split plot technique (Cochran & Cox, 1957).

Uterine histology

After flushing, a sample of the uterus was taken from one uterine horn midway between the external bifurcation and the uterotubal junction and placed in 12.5% formalin. Subsequently, each sample was dehydrated, embedded in paraffin wax and cross-sections were cut at 6 μm . Duplicate cross-sections were stained with haematoxylin and eosin or periodic acid-Schiff's reagent (PAS), and examined under a light microscope. The data recorded were: (i) height of luminal epithelial cells (intercotyledonary area); (ii) height of glandular epithelial cells (mid-gland region); (iii) width of myometrium; (iv) width of longitudinal muscle; and (v) degree of coiling of glands.

All measurements (in μm) were made at four separate locations using an eyepiece micrometer. The number of gland coils was estimated by counting the number in contact with an arbitrary line extending perpendicularly from the myometrium to the luminal epithelium.

Histological data were subjected to standard analyses of variance.

RESULTS

Ewes in oestrus

Fifteen of nineteen spayed ewes and all of the entire animals were in oestrus at the time of insemination.

Numbers of spermatozoa recovered

Table 1 presents the main effects and Table 2 the significant interactions.

Spayed ewes had significantly fewer spermatozoa than entire ewes in all divisions of the tract ($P < 0.05$ to $P < 0.001$). Significantly fewer spermatozoa were in the vagina and cervix at 24 hr than at 2 hr ($P < 0.001$), while significantly more were in the Fallopian tubes. Natural mating was associated with larger mean numbers of spermatozoa recovered from the cervix than were recovered following artificial insemination ($P < 0.05$). This was due primarily to the higher numbers present 24 hr after insemination (type of mating $\times$ time of recovery interaction; $P < 0.01$; Table 2A).

Numbers of spermatozoa recovered from the Fallopian tubes were also increased following normal mating ($P < 0.001$) although mean numbers recovered from the uterus were apparently unaffected.

Overall, there was a gradient of mean numbers of spermatozoa in the cervix, with most in the caudal and least in the cranial sections ($P < 0.01$). However, numbers recovered from the cranial cervix were similar at both times of recovery, whereas populations of spermatozoa within the mid and caudal sections dropped sharply between 2 and 24 hr ($P < 0.001$; Tables 2B and 3). The distribution of spermatozoa within the cervix also differed greatly between spayed and entire ewes. In the former, a much lower proportion of the population recovered 2 hr after insemination was in the cranial and mid thirds of

the cervix and very few spermatozoa were maintained in any section 24 hr after insemination ($P < 0.01$; Tables 2C and 3).

Histology

A summary of the histological observations is presented in Table 4. The height of the luminal epithelium of spayed ewes was less than that in the entire

TABLE 2
ESTIMATED NUMBERS OF SPERMATOZOA RECOVERED FROM THE
CERVIX OF SPAYED AND ENTIRE EWES AT DIFFERENT TIMES
AFTER MATING AND ARTIFICIAL INSEMINATION

<i>Significant interactions</i>			
A. <i>Type of mating</i> $\times$ <i>time of recovery</i> F=7.57; d.f.=1; $P < 0.01$			
<i>Time of recovery</i>	<i>Type of mating</i>		
	<i>Artificial insemination</i>	<i>Natural mating</i>	
2 hr	34019 (7.242)	27524 (7.252)	
24 hr	3150 (5.450)	5383 (6.371)	
B. <i>Position of cervix</i> $\times$ <i>time of recovery</i> F=19.59; d.f.=2; $P < 0.001$			
<i>Time of recovery</i>	<i>Cervical region</i>		
	<i>Caudal</i>	<i>Mid</i>	<i>Cranial</i>
2 hr	20211 (7.114)	9594 (6.320)	1176 (5.369)
24 hr	717 (5.251)	1908 (5.042)	1641 (5.216)
C. <i>Type of ewe</i> $\times$ <i>position of cervix</i> F=6.23; d.f.=2; $P < 0.01$			
<i>Type of ewe</i>	<i>Cervical region</i>		
	<i>Caudal</i>	<i>Mid</i>	<i>Cranial</i>
Spayed	6080 (5.719)	1896 (4.756)	150 (4.462)
Entire	15148 (6.613)	9698 (6.590)	2670 (6.084)

Values expressed as mean numbers of spermatozoa $\times 10^3$
($\log_{10} (x+2)$ in parentheses)

animals but differences were more evident in the subepithelial stroma (Pl. 1, Figs 1 to 4). Within the stroma, oedema was markedly reduced and the nuclei appeared more compact. This also was the case in the stroma of the glandular region in the spayed animals.

Glandular proliferation was not so far advanced in spayed as in entire ewes, shown by the lower epithelial cell heights and a smaller number of gland coils ($P < 0.01$ to $P < 0.001$). There was also a marked diminution in cytoplasm, apparent crowding together of nuclei, and an almost complete absence of

TABLE 3
ESTIMATED NUMBERS OF SPERMATOOA RECOVERED FROM THE CAUDAL, MID AND CRANIAL PORTIONS OF THE CERVIX
OF SPAYED AND ENTIRE EWES AT DIFFERENT TIMES AFTER MATING AND ARTIFICIAL INSEMINATION

Time of recovery	Type of mating	Cervical region of spayed ewe				Cervical region of entire ewe			
		Caudal	Mid	Cranial	Total	Caudal	Mid	Cranial	Total
2 hr	A.I. N.M.	9072 13361	5056 1480	252 106	14380 14947	32994 25415	19613 12225	1883 2461	54490 40100
Mean (n' = 10)		11217	3268	179	14664	29205	15919	2172	47295
24 hr	A.I. N.M.	47 635	61 619	89 143	197 1397	677 1507	2895 4057	2530 3803	6102 9367
Mean (n' = 9 to 10)		341	340	116	797	1092	3476	3167	7735

Values expressed as mean numbers of spermatozoa $\times 10^3$ (n = 5).
A.I., artificial insemination; N.M., natural mating.

mitotic figures (Pl. 1, Figs. 5 to 8) which were frequent in samples from entire ewes (Pl. 1, Fig. 9). There were no apparent differences between spayed and entire ewes in the amount of PAS-positive material.

The widths of the myometrium and of the longitudinal muscles were reduced in spayed ewes but no other histological differences were apparent within this region.

TABLE 4

HISTOLOGICAL MEASUREMENTS ON THE UTERI OF SPAYED AND ENTIRE EWES AT DIFFERENT TIMES AFTER SAMPLING

Main effects	Histological parameter measured				
	Height of luminal epithelial cells (μm)	Height of glandular epithelial cells (μm)	Width of myometrium (μm)	Width of longitudinal muscle (μm)	No. of gland coils*
Type of ewe (N=19 to 20)					
Spayed	28.8†	20.0	2071	419	8.7
Entire	24.4	13.9	1627	307	6.8
P	N.S.	<0.001	<0.001	<0.05	<0.01
Time of sampling (N=19 to 20)					
2 hr	23.6	13.6	1585	296	7.0
24 hr	25.2	14.2	1667	318	6.6
P	N.S.	<0.05	<0.001	N.S.	N.S.

N.S., not significant.

* Number of glands in contact with an arbitrary line from the myometrium to the luminal epithelium.

† Values expressed as mean measurement at four locations within each sample.

DISCUSSION

Spayed ewes had fewer spermatozoa than entire ewes in all sections of the tract for all treatment combinations. Numbers of spermatozoa in the cervix in spayed ewes were similar to those reported by Mattner (1963) and Mattner & Braden (1963, 1967) for spayed and entire ewes, respectively. These numbers, however, represented only some 30% and 10% of the numbers recovered from entire ewes 2 and 24 hr, respectively, after insemination. Flushing techniques used by Mattner & Braden (1963) for the recovery of cervical spermatozoa result in approximately 50% of the total numbers being recovered (Mattner, 1968). A much higher rate of recovery than 50% is obtained as a result of the method of recovery employed in this experiment, which partially accounts for the high numbers of cervical spermatozoa recovered in entire ewes.

Although spermatozoa entered the caudal cervix of the spayed ewes in large numbers, relatively few were found in the mid and cranial thirds and subsequently in the uterus and Fallopian tubes. Reduced numbers in the uterus and Fallopian tubes are almost certainly a reflection of low numbers in the cervix and, in particular, in the cranial third.

Evidence of an increase in numbers of spermatozoa in the uterus and Fallopian tubes from 2 to 24 hr after insemination is in agreement with that in

earlier reports (Mattner, 1963; Quinlivan & Robinson, 1967, 1969). The observation that natural mating had no appreciable effect on vaginal numbers of spermatozoa even 2 hr after insemination was unexpected. Ewes artificially inseminated received about 300×10^6 spermatozoa, while animals naturally mated twice would be expected to have had an initial vaginal pool far in excess of this number. Obviously, a high proportion of the inseminate was lost within 2 hr of mating.

Despite the lack of a demonstrable difference at 2 hr in numbers of spermatozoa in the vagina and cervix of naturally mated as compared with artificially inseminated ewes, natural mating resulted in higher cervical populations at 24 hr in both spayed and entire ewes. There is no obvious explanation for this finding.

Comparative failure of sperm transport in spayed ewes is probably due to a combination of factors—cervical secretions, motility of the reproductive tract and phagocytosis of spermatozoa within the tract. The quantitative production of cervical mucus during oestrus is comparable for entire ewes and spayed ewes treated with 25 µg ODB (Allison, 1972). Consequently, it is not easy to implicate differences in production of cervical mucus in the relatively inefficient sperm transport in spayed ewes. Subtle differences in qualitative characteristics of the mucus, however, may be involved.

The rôle of uterine motility in the transport of spermatozoa in the ewe is uncertain (Lightfoot & Restall, 1971). The pattern of contractility is modified at oestrus (Lightfoot, 1969) and can be altered by treatment with oestrogen (Brinsfield, 1968). There is a distinct possibility that the uterine motility of the progesterone–oestrogen-treated spayed ewe differed from that of the normal oestrus ewe, so affecting the pattern of sperm transport.

The phagocytic response to spermatozoa within the uterus of spayed ewes had been studied in entire animals by Brinsfield, Hawk & Righter (1964) and by Mattner (1969). It is possible that an inadequate endocrine regimen in the spayed ewes may have contributed to the inefficient maintenance of the cervical population, particularly in the cranial and mid sections, due to leucocytic invasion from the uterus (Mattner, 1968).

The nature of the structural involution of the reproductive tract in the absence of the ovaries has been reviewed by Parkes & Deanesly (1966) who state that, in general, administration of oestrogens to the spayed animal will produce most, if not all, of the extra-ovarian histological and physiological changes

EXPLANATION OF PLATE 1

Histology of the uterine endometrium from entire and spayed ewes. $\times 280$.

Figs 1 and 2. Luminal epithelium and subepithelial stroma from entire ewes at oestrus.

Figs 3 and 4. Luminal epithelium and subepithelial stroma from spayed ewes in which oestrus was induced. Compared with entire animals, stromal oedema is markedly reduced in spayed ewes and there is a marked compaction of nuclei.

Figs 5 and 6. Mid-gland region. Samples from entire ewes at oestrus.

Figs 7 and 8. Mid-gland region from spayed ewes in which oestrus was induced. In these samples, there is a reduction in glandular epithelial cell height, crowding together and compaction of nuclei, and an apparent loss of cytoplasm.

Fig. 9. Mitotic figures (arrowed) in the upper gland region in a sample from an entire ewe at oestrus.

PLATE 1

(Facing p. 422)

associated with the normal oestrous cycle. Examination of Table 4 and Plate 1 indicates that the uterus of the spayed ewe treated with 10 mg progesterone/day followed by 25 μ g ODB is not maintained in a normal physiological state when compared with that of the entire animal. The reduction in all histological parameters measured may have been due to inadequate oestrogen. It appears that the uteri of the spayed ewes were undergoing an early stage of atrophy, although all had been injected with ovarian steroids from the day of ovariectomy. Thus, hormonal regimens which will result in behavioural oestrus of normal duration (Fletcher, 1968) will not maintain the functional integrity of the uterus.

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