THE PASSAGE OF SPERMATOOZOA THROUGH THE CERVIX OF OVARIECTOMIZED EWES TREATED WITH PROGESTERONE AND OESTROGEN

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Summary. Thirty spayed ewes were used in a 2×3 split-plot factorial experiment ($n = 5$) in which oestrus was induced with 30 or 90 μg oestradiol benzoate (OB) following a period of progesterone priming. They were inseminated 36 hr after oestrogen and the numbers of spermatozoa in the caudal, mid- and cranial regions of the cervix (sub-plots) were estimated 1, 12 and 24 hr later. At each interval of time and for each region of the cervix, fewer spermatozoa were recovered from the ewes treated with 30 μg OB than from those treated with 90 μg ($P < 0.05$).

INTRODUCTION

Allison & Robinson (1972) demonstrated the importance of the balance of ovarian steroids in the initiation and maintenance of a cervical population of spermatozoa following artificial insemination of ovariectomized ewes in which oestrus was induced by oestrogen after priming with progesterone. They suggested that an imbalance of progesterone and oestrogen could affect the transport of spermatozoa by altering either the quantitative and qualitative characteristics of the cervical secretions or the motility of the tract.

This paper reports an extension of that work.

MATERIALS AND METHODS

Experimental design

A split-plot factorial design was used, with the following form.

Main plots: dose of OB for induction of oestrus—30 or 90 μg ; time of recovery of spermatozoa—1, 12 or 24 hr after insemination; factorial 2×3 ; $n = 5$; $N = 30$.

Sub-plots: region of the cervix from which spermatozoa were recovered—caudal, mid- or cranial; $30 \times 3 = 90$ observations.

Several months after ovariectomy, thirty mature medium-wool Merino ewes

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were injected intramuscularly daily for 12 days, each with 10 mg progesterone in oil (Robinson, 1959), commencing 16 days before the date for insemination. A single injection of either 30 μg or 90 μg oestradiol benzoate (OB) was given 60 hr after the final injection of progesterone. This was not necessarily the optimum dose for preparation of the tract for sperm transport (Allison & Robinson, 1972), but it was selected because of the well established time/dose relationships between times of injection of such a daily dose of progesterone followed by oestrogen and the onset and termination of oestrus. The 90- μg dose of OB is adequate to induce oestrus of about 48 hr duration commencing about 18 hr after injection, while the 30- μg dose may be expected to induce oestrus of about 28 hr duration commencing about 22 hr after injection (Scaramuzzi, Lindsay & Shelton, 1971). Ewes were inseminated 36 hr after the OB injection without reference to teaser rams, i.e. towards the middle of the induced oestrous period.

Two ewes were lost from the experiment by mischance. No attempt was made to replace them because of the necessity to conduct all operations at the pre-determined time.

Artificial insemination

Pooled semen, collected by artificial vagina from three rams and of high quality as assessed visually, was used. For practical purposes, a uniform dose of 0.1 ml containing 450 to 500 $\times 10^6$ spermatozoa was introduced under the papilla at the cervical os. Care was taken to avoid differences in depth of insemination. Stress was avoided as far as possible and after insemination the ewes were kept in a small pen before slaughter.

Recovery of spermatozoa

Ewes were killed by an intracardiac injection of sodium pentobarbitone solution (Lethabarb: V.S. Supplies Ltd, Camperdown, New South Wales). The reproductive tract was immediately removed and ligated at the utero-cervical junction. The cervix was dissected away from the rest of the tract, using thoroughly cleaned instruments for each animal, and was stored in a plastic bag in a deep-freeze at -15°C . Subsequently, each frozen cervix was divided into three equal portions—caudal, mid- and cranial. Recovery and estimation of numbers of spermatozoa in each portion utilized the techniques described by Lightfoot & Salamon (1970) and Allison & Robinson (1970). This involved the cutting of the portions of the cervix into longitudinal strips and shaking vigorously with 10 ml saline to dislodge the spermatozoa before counting. This technique is more efficient than flushing. Lightfoot, Croker & Neil (1967) made counts of spermatozoa after successive flushings of the cervix and in the first flushing recovered only 59% of the total number of spermatozoa recovered. By contrast, R. J. Lightfoot (unpublished data), on checking the shaking technique using successive washings, recovered in the first washing more than 90% of the total number of spermatozoa recoverable. A. J. Allison (unpublished results) also checked the technique and in all of ten cervixes found the washing from a second shaking to contain less than 2% of the numbers of spermatozoa present in the first.

The percentage of inseminated spermatozoa recoverable by this technique from the cervix shortly after insemination is much higher than that recoverable by flushing (Table 1).

Analysis of the data

A split-plot analysis of variance was applied, after adding 2 to each count to avoid zero values, using a $\log_{10}$ transformation. Missing values were inserted, with consequent adjustment of degrees of freedom. Correlation coefficients were calculated for numbers of spermatozoa in successive regions of the tract at each interval of time. The three regions of the cervix were regarded as a linear series for the purpose of analysis.

Table 1. Recovery of spermatozoa from the cervix of ewes by flushing or shaking techniques at various times after insemination

Reference	Technique	Interval from insemination (hr)	% Spermatozoa recovered *
Quinlivan & Robinson (1969)	Flushing	1	0.5
Mattner, Entwistle & Martin (1969)	Flushing	4	2.3
Lightfoot & Restall (1971)	Flushing	0.5	1.2
Allison (1972)	Shaking	2	5.5

* Of numbers inseminated.

Table 2. Estimated corrected mean numbers of spermatozoa recovered from three regions of the cervix in ewes

Time after insemination (hr)	Dose of OB (μ g)	Estimated mean no. of spermatozoa recovered from regions of cervix			
		Caudal ($\times 10^3$)	Mid- ($\times 10^3$)	Cranial ($\times 10^3$)	Total* ($\times 10^3$)
1	30	2680	51	3	73
	90	4724	552	6	252
12	30	135	71	17	54
	90	374	1087	180	418
24	30	85	135	31	71
	90	177	174	63	125

Means derived from reversion of $\log_{10}(x+2)$ values. Each value is the mean of five observations.

* These values are not the Σ Caudal, Mid-, and Cranial values presented but the reconverted values of the Σ log means used in the analysis.

RESULTS

Table 2 presents the estimated (reconverted) mean numbers of spermatozoa recovered from each of the three regions of the tract for each treatment combination. Table 3 is an analysis of variance.

There was a highly significant overall decline in the mean numbers of spermatozoa recovered from the caudal to cranial regions of the cervix ($P < 0.001$). This

relationship altered with the passage of time, the numbers becoming more evenly distributed (time $\times$ region interaction; $P < 0.001$). At 12 and 24 hr, the mid-cervical section contained relatively more spermatozoa than did either of the other regions ($P < 0.05$). At each interval of time and for each region of the tract, fewer spermatozoa were recovered from the ewes treated with 30 μ g OB than those treated with 90 μ g ($P < 0.05$).

Table 3. Split-plot analysis of variance of data for numbers of spermatozoa recovered from three regions of the cervix in ewes after $\log_{10}(x+2)$ transformation

Source of variation	Degrees of freedom	Mean square
Main plots		
Dose of OB (D)	1	6.99*
Time after insemination (T)	2	0.35
Interaction (D $\times$ T)	2	0.77
Error	22	1.40
Total	27	
Sub-plots		
Region of cervix (R)	(2)	
linear	1	26.45***
quadratic	1	1.62*
Interactions		
R $\times$ D	2	0.37
R $\times$ T	4	4.90***
R $\times$ D $\times$ T	4	0.26
Error	44	0.28
Total	56	
Grand total	83	

* $P < 0.05$; *** $P < 0.001$.

Table 4. Table of correlation coefficients for numbers of spermatozoa in three regions of the cervix at three intervals from insemination

Region of cervix	Interval from insemination					
	1 hr		12 hr		24 hr	
	Caudal	Mid	Caudal	Mid	Caudal	Mid
Mid	0.57†		0.87***		0.91***	
Cranial	0.02	0.24	0.64*	0.83**	0.73**	0.83***

All values are positive.

† $P < 0.10$; * $P < 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

The nature of dispersal of spermatozoa through the cervix is shown in Table 4. At 1 hr after insemination, the numbers present in the cranial third were not correlated with those in the caudal or mid-thirds. By 12 hr, the numbers in successive regions were highly correlated.

DISCUSSION

Mattner & Braden (1969) have shown that only some 50% of live spermatozoa are recovered by flushing techniques; the rest remain in the crypts. The vigorous shaking used in our technique strips off much of the cervical epithelium with the associated spermatozoa and results in recovery of the majority of the spermatozoa, judging from the relatively high recoveries shown in Table 1 and the low numbers of spermatozoa recoverable in subsequent shakings. Certainly, the proportion recovered is sufficient for use in comparative studies such as that described in this paper.

This study confirms the finding of Allison & Robinson (1972) that the total number of spermatozoa recovered from the cervix of spayed ewes is affected by the dose of oestrogen involved in the induction of oestrus. It extends it in that it shows the changing pattern of distribution of spermatozoa in the caudal, mid- and cranial regions of the cervix with the passage of time and the potential effect on this of factors such as change of endocrine status.

The mean numbers of spermatozoa 1 hr after insemination were relatively high in the caudal region of the cervix, of the order of 3 to 5×10^6 , and declined markedly towards the cranial third in which the mean was only 3 to 6×10^3 . At 12 hr, the pattern was significantly altered, with relatively larger numbers in the mid- and cranial regions, particularly in the ewes treated with the higher dose of OB, in which a difference of approximately tenfold between the two treatments attained significance ($P = 0.05$). It could be argued that the higher dose of oestrogen facilitated passage of spermatozoa through the cervix. By 24 hr, the distribution of spermatozoa between the three regions of the cervix was more even, with no significant differences between treatments.

The data provide strong evidence against rapid transport through the cervix in that there was no correlation between the numbers of spermatozoa in the caudal and cranial regions 1 hr after insemination. By 12 hr, there was a positive correlation and at 24 hr, the correlations between the numbers present in the three regions were even higher.

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