J. Reprod. Fert. (1971) **26,** 1–13

EFFECTS OF SITE OF INSEMINATION, SPERM MOTILITY AND GENITAL TRACT CONTRACTIONS ON TRANSPORT OF SPERMATOZOA IN THE EWE

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(*Received* 13*th March* 1970, *revised* 15*th December* 1970)

Summary. Three experiments were conducted to examine the effects of site of insemination, sperm motility and contractions of the genital tract on the transport of spermatozoa in the ewe.

In the first experiment, the proportion of ewes from which spermatozoa were recovered from the Fallopian tubes was reduced, both in ewes receiving oxytocin (1·0 or 10·0 i.u., intramuscularly) and in those in which only shallow insemination could be achieved.

The second experiment examined the effects of sperm motility (live versus dead spermatozoa), inhibition of genital tract contractions (halothane anaesthesia: −, +) and stimulation of genital tract contractions (oxytocin injections: −, +). Sperm motility was found to be the most important factor affecting transport through the cervix. Oxytocin had little effect, but following insemination with immotile spermatozoa, inhibition of genital tract contractions reduced the number of spermatozoa recovered from both the cranial cervix and Fallopian tubes.

The effects of site of insemination (external versus internal cervical os) and sperm motility (live versus dead spermatozoa) were examined in the third experiment. Few spermatozoa were found between the mucosal folds of the cervix when immotile spermatozoa were used. Large numbers of spermatozoa were recovered from both the cervix and Fallopian tubes after insemination at the level of the internal cervical os, particularly following the use of motile spermatozoa.

The results demonstrate the importance of sperm motility, particularly in relation to the establishment of the cervical population of spermatozoa.

INTRODUCTION

The physiological mechanisms responsible for the transport of spermatozoa through the ovine cervix are poorly understood. Starke (1949) and Mattner & Braden (1963) found that spermatozoa can reach the Fallopian tubes within

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6 to 8 min of coitus, and such evidence supports the view that contractions of the genital tract account for the passage of spermatozoa through the lower segments. In contrast, the studies of Quinlan, Maré & Roux (1932), Green & Winters (1935), Lopyrin & Loginova (1939) and Dauzier & Wintenberger (1952) indicate that several hours elapse before spermatozoa are found in the Fallopian tubes. Their data suggest that the passage of spermatozoa through the cervix requires approximately 30 min and is effected by the inherent motility of the male gamete.

Because of this conflict, the experiments described herein were designed to examine the relative importance of several factors thought to influence the transport of spermatozoa through the ovine genital tract with particular reference to the cervix.

MATERIALS AND METHODS

Sheep and management

Mature Merino ewes were randomly allocated to treatments on a within-draft basis and inseminated 2 to 16 hr after the onset of oestrus.

Semen

Ejaculates of good initial motility were collected from two mature Merino rams by artificial vagina, pooled and used for insemination without dilution. When required (Exps 2 and 3), spermatozoa were killed (absence of motility, positive nigrosin–eosin staining reaction) by plunging the semen into liquid nitrogen, thawing, and re-freezing.

Technique of insemination

Normal inseminations (external cervical os) were performed with the aid of a duck-billed speculum and headlight. Mucus present in the vagina was removed before insemination. In Exp. 1, the plastic inseminating pipette was inserted as far as possible into the cervix of each ewe and the depth of cervical penetration recorded. In Exps 2 and 3, the pipette was inserted to a uniform depth of approximately 0·5 to 1·0 cm.

A sterile glass pipette with a fine glazed tip was used for inseminations at the level of the internal cervical os (Exp. 3). Following mid-ventral laparotomy under local anaesthesia, the uterus was penetrated just caudal to the internal bifurcation, and the semen deposited near the utero-cervical junction.

In Exps 1 and 2, a dose of 0·1 ml of semen containing 300 to 400×10^6 spermatozoa was used. The inseminate volume was varied in Exp. 3 to allow injection of a constant number of spermatozoa (800×10^6).

Recovery and counting of spermatozoa

The techniques used for recovery and enumeration of spermatozoa from the Fallopian tubes following laparotomy (Exp. 1) and from the Fallopian tubes, uterus and cervix following slaughter by intracardiac injection (Exp. 2), were as described by Lightfoot & Salamon (1970). However, in Exp. 3, after slaughter, the Fallopian tubes and uterus were treated as above, and the cervix was flushed

twice with 10 ml of saline from the uterine end. It was then divided into caudal, mid and cranial segments of equal length and the segments were placed in Bouin's fixative. From each segment, transverse sections (6 μm) were cut, mounted, stained (haematoxylin–eosin) and examined microscopically (×400) for spermatozoa. When large numbers of spermatozoa were present, counting became difficult and so a scoring system (Table 8) was adopted.

Statistical analyses

Data were examined by the appropriate analysis of variance, after transformation according to the function $\log_{10}$ (number of spermatozoa +2). Differences between cervical segments (i.e. caudal, mid and cranial) were examined by the procedure for split-plot experiments (Cochran & Cox, 1957).

EXPERIMENTAL DESIGN AND RESULTS

Experiment 1: *The effects of oxytocin and depth of insemination on the number of spermatozoa in the Fallopian tubes* 2 *hr after insemination*

Three groups each consisting of twenty ewes received either 0, 1·0 or 10·0 i.u. of oxytocin (Syntocinon, intramuscularly) at the time of insemination. Spermatozoa were recovered by flushing the Fallopian tubes *in vivo* 2 hr after insemination.

TABLE 1

EXP. 1. THE EFFECTS OF OXYTOCIN AND DEPTH OF INSEMINATION ON THE NUMBER OF SPERMATOZOA RECOVERED FROM THE FALLOPIAN TUBES 2 HR AFTER INSEMINATION

Treatment	*No. of ewes examined*	*No. of ewes yielding sperm.*	*Mean no. of sperm.*
Dose of oxytocin (i.u.)			
0	20	7	1948
1	20	5	71
10	20	2	2
Depth of insemination			
Shallow: < 1 cm	32	6	34
Medium: 1 to 3 cm	21	4	35
Deep: > 3 cm	7	4	5520
Overall	60	14	674

Only fourteen (23%) of the sixty ewes examined yielded spermatozoa (Table 1). Of the ewes receiving either 0, 1·0 or 10·0 i.u. oxytocin, spermatozoa were recovered from 35, 25 and 10% respectively (0 versus 1·0 and 10·0 i.u., $\chi_1^2 = 2{\cdot}28$, $0{\cdot}1 < P < 0{\cdot}2$). The administration of oxytocin also depressed the mean numbers of spermatozoa recovered.

Both the proportion of ewes yielding spermatozoa (57% versus 19%; $\chi_1^2 = 3{\cdot}17$, $0{\cdot}05 < P < 0{\cdot}1$) and the mean number of spermatozoa recovered

Table 2

EXP. 2A. THE EFFECTS OF SPERM MOTILITY, HALOTHANE ANAESTHESIA AND OXYTOCIN ON THE NUMBERS OF SPERMATOZOA RECOVERED FROM THE CERVIX, UTERUS AND FALLOPIAN TUBES OF EWES SLAUGHTERED 30 MIN AFTER INSEMINATION

Treatment no.	*Treatment*			*No. of ewes*	*Cervix*			*Uterus*	*Fallopian tubes*
	Sperm motility	*Anaesthesia*	*Oxytocin*		*Caudal*	*Mid*	*Cranial*	*Mean no. of sperm.*	*Mean no. of sperm.*
					10^{-3} × *Mean no. of sperm.*				
1	+	−	−	8	4678 *6·368* (8)	171 *4·243* (8)	165 *2·977* (6)	1604 (2)	17 (2)
2	+	−	+	8	6904 *6·736* (8)	484 *4·950* (8)	10 *3·344* (7)	18 (1)	15 (2)
3	+	+	−	8	7823 *6·538* (8)	569 *3·929* (7)	11 *3·193* (7)	256 (2)	9 (1)
4	+	+	+	8	6417 *6·544* (8)	801 *5·192* (8)	6 *3·111* (7)	19 (2)	39 (2)
5	−	−	−	8	1598 *4·772* (8)	9 *2·903* (6)	10 *2·082* (4)	331 (2)	52 (2)
6	−	−	+	8	151 *4·640* (8)	2 *1·783* (4)	23 *2·137* (4)	0 (0)	62 (2)
7	−	+	−	8	935 *5·068* (8)	2 *1·462* (3)	1 *0·717* (1)	18 (2)	0 (0)
8	−	+	+	8	527 *5·076* (8)	1 *1·474* (3)	1 *1·459* (3)	27 (2)	0 (0)

Logarithmic means in italics. The number of ewes yielding spermatozoa are shown in parentheses.

(5520 versus 34) were higher for the ewes in which semen could be deposited further than 3 cm into the cervix.

Experiment 2A: The effects of sperm motility and contractions of the genital tract on the distribution of spermatozoa throughout the genital tract 30 min after insemination

A 2 × 2 × 2 factorial design (n = 8, N = 64) was used to examine the following factors:

(1) Sperm motility — Live versus dead spermatozoa
(2) Inhibition of genital tract contractions — Nil versus halothane anaesthesia
(3) Stimulation of genital tract contractions — Nil versus oxytocin

TABLE 3

EXP. 2A. SUMMARY OF ANALYSIS OF VARIANCE FOR NUMBERS OF SPERMATOZOA RECOVERED FROM THE CERVIX

Source of variation		*d.f.*	*Variance ratio*
Sperm motility	(A)	1	69·20***
Halothane anaesthesia	(B)	1	1·25
Oxytocin	(C)	1	0·60
A × B		1	1·09
A × C		1	1·17
B × C		1	0·36
A × B × C		1	0·61
Error 1 (Ewes within A × B × C)		56	2·67†
Cervical segment	(D)		
Linear		1	252·87***
Quadratic		1	19·61***
A × D			
A × linear		1	0·06
A × quadratic		1	8·58**
Pooled (non-significant) first, second and third order interactions		12	1·11
Error 2		112	1·41†

** $P < 0·01$; *** $P < 0·001$.
† Error mean square.

The effects of halothane anaesthesia and oxytocin on spontaneous contractions of the oestrous ovine genital tract *in vivo* have been evaluated by Lightfoot (1970a, b). Halothane anaesthesia resulted in complete inhibition of cervical and uterine contractions in 79% and 43%, respectively, of the ewes examined, but slight residual activity was evident in the remaining cases. The inhibition was not overcome by vaginal stimulation, but oxytocin (800 mU, intravenously) produced a contractile response of lesser intensity and duration than that recorded in conscious ewes.

In the present experiment, the relevant ewes were inseminated approximately 5 min after establishing anaesthesia (surgical level) and killed while still

TABLE 4

EXP. 2A. EFFECTS OF SPERM MOTILITY, HALOTHANE ANAESTHESIA AND OXYTOCIN ON THE NUMBERS OF SPERMATOZOA RECOVERED FROM THE CERVIX, UTERUS AND FALLOPIAN TUBES OF EWES SLAUGHTERED 30 MIN AFTER INSEMINATION

Treatment		*No. of ewes*	*Cervix*			*Uterus*	*Fallopian tubes*
			Caudal	*Mid*	*Cranial*	*Mean no. of sperm.*	*Mean no. of sperm.*
			10^{-3} × *mean no. of sperm.*				
Sperm motility	+	32	6·455 *6·547* (32)	507 *4·579* (31)	48 *3·156* (27)	474 (7)	20 (7)
	−	32	803 *4·889* (32)	3 *1·905* (16)	9 *1·599* (12)	94 (6)	28 (4)
Halothane anaesthesia	−	32	3333 *5·629* (32)	166 *3·470* (26)	52 *2·635* (21)	488 (5)	36 (8)
	+	32	3926 *5·807* (32)	343 *3·014* (21)	5 *2·120* (18)	80 (8)	12 (3)
Oxytocin	−	32	3759 *5·687* (32)	188 *3·134* (24)	46 *2·242* (18)	552 (8)	19 (5)
	+	32	3500 *5·749* (32)	322 *3·350* (23)	10 *2·513* (21)	16 (5)	29 (6)
Overall		64	3629 *5·718* (64)	255 *3·242* (47)	28 *2·377* (39)	284 (13)	24 (11)

Logarithmic mean in italics. The number of ewes yielding spermatozoa are shown in parentheses.

TABLE 5

EXP. 2A. EFFECT OF HALOTHANE ANAESTHESIA ON THE NUMBERS OF SPERMATOZOA RECOVERED FROM THE CERVIX, UTERUS AND FALLOPIAN TUBES OF EWES SLAUGHTERED 30 MIN AFTER INSEMINATION WITH IMMOTILE SPERMATOZOA (TREATMENTS 5 TO 8 IN TABLE 2)

Treatment	*No. of ewes**	*Cervix*			*Uterus*	*Fallopian tubes*
		Caudal	*Mid*	*Cranial*	*Mean no. of sperm.*	*Mean no. of sperm.*
		10^{-3} × *Mean no. of sperm.*				
Conscious ewes	16	875 *4·706* (16)	5 *2·343* (10)	16 *2·110* (8)	166 (2)	57 (4)
Anaesthetized ewes	16	731 *5·072* (16)	1 *1·468* (6)	1 *1·088* (4)	23 (4)	0 (0)

Logarithmic means in italics. The number of ewes yielding spermatozoa are shown in parentheses.

* Oxytocin treatments pooled.

under anaesthesia. Oxytocin (Syntocinon, 1·0 i.u. in 1 ml) was injected intravenously 1 min before, and again 15 min after insemination. All ewes were slaughtered 30 min after insemination and the various parts of the genital tract were ligated within 3 min of slaughter.

Marked differences in the position and activity of the genital tracts were observed at slaughter. Halothane anaesthesia, without oxytocin, resulted in complete relaxation of the tract and the absence of spontaneous contractions. In contrast, uteri in conscious ewes that received oxytocin lay in the high-retracted position and exhibited pronounced contractile activity.

The effects of all treatments on the numbers of spermatozoa recovered from the genital tract are shown in Table 2, the relevant analysis of variance in Table 3 and treatment main effects in Table 4.

Cervix. Of the three factors examined, only sperm motility had a significant effect ($P < 0{\cdot}001$) on the mean number of spermatozoa recovered from the cervix. Few spermatozoa were found after insemination with immotile, compared with motile, cells (Table 4).

Split-plot analysis (Table 3) revealed a significant interaction ($P < 0{\cdot}01$) due to an effect of sperm motility on the relative numbers of spermatozoa recovered from adjacent cervical segments. Insemination with motile cells led to an almost linear reduction in the number recovered from successively ascending segments, whereas the use of immotile cells resulted in a steep fall in sperm numbers between the caudal and mid regions, with little further reduction in the cranial segment.

Following insemination with immotile spermatozoa, but not with motile cells, halothane anaesthesia inhibited the progression of spermatozoa through the cervix (Table 5).

Analysis of the data for inseminations with immotile spermatozoa only, showed that the interaction was significant (halothane anaesthesia × cervical segment, linear; $P < 0{\cdot}05$).

Of the thirty-two ewes inseminated with immotile cells, all animals, both conscious and anaesthetized, yielded spermatozoa from the caudal segment of the cervix. In the mid-cervical segment, however, anaesthesia reduced the proportion of ewes yielding spermatozoa from 63% to 38%, and in the cranial segment, from 50% to 25%.

Uterus and Fallopian tubes. Spermatozoa were recovered from the uterine flushings in only thirteen (20%) of the sixty-four ewes examined. The results (Tables 2, 4 and 5) suggest that among ewes in which transport occurred, the quantitative passage of spermatozoa to the uterus was depressed by halothane anaesthesia, by injections of oxytocin, and by insemination with immotile spermatozoa.

Spermatozoa were found in tubal flushings from only eleven (17%) of the sixty-four ewes and there were no obvious differences between any of the treatment main effects shown in Table 4. Examination of the data for inseminations with immotile cells only, however, revealed that no spermatozoa were recovered from the Fallopian tubes of the sixteen anaesthetized ewes, whereas a mean of 226 tubal spermatozoa were recovered from four of the conscious animals (Table 5).

Experiment 2B: The effects of natural service versus artificial insemination on the distribution of spermatozoa throughout the genital tract 30 min after semen deposition

In order to compare sperm transport following natural service with that resulting from artificial insemination in the control treatment (live spermatozoa, no anaesthesia, no oxytocin) of the main experiment (2A), an additional eight ewes from the same flock were allowed one natural service with a ram and slaughtered 30 min later. The ewes were allocated to the natural service treatment during the conduct of the main experiment and successive ewes were served by either one of the two rams on an alternate basis.

TABLE 6

EXP. 2B. EFFECT OF ARTIFICIAL INSEMINATION VERSUS NATURAL SERVICE ON THE NUMBERS OF SPERMATOZOA RECOVERED FROM THE CERVIX, UTERUS AND FALLOPIAN TUBES 30 MIN AFTER SEMEN DEPOSITION

Method of insemination	*No. of ewes*	*Cervix*			*Uterus*	*Fallopian tubes*
		Caudal	*Mid*	*Cranial*	*Mean no. of sperm.*	*Mean no. of sperm.*
		$10^{-3}\times$ *Mean no. of sperm.*				
Artificial insemination	8*	4678	171	165	1604	17
		6·368	*4·243*	*2·977*		
		(8)	(8)	(6)	(2)	(2)
Natural service	8	22714	1120	281	406	45
		7·001	*5·471*	*4·485*		
		(8)	(8)	(8)	(2)	(5)

Logarithmic means in italics. The number of ewes yielding spermatozoa are shown in parentheses.

* Ewes from Treatment 1, Exp. 2A (Table 2).

Significantly more spermatozoa ($P < 0{\cdot}05$) were recovered from the cervix of ewes following natural service than following artificial insemination (Table 6); Similar numbers of spermatozoa were found in the uterus for both treatments; however, more ewes yielded tubal spermatozoa following natural service.

Experiment 3: The effects of site of insemination and sperm motility on the distribution of spermatozoa throughout the genital tract 2 hr after insemination

The experiment was of factorial design (2×2, $n = 11$, $N = 44$) and examined the effects of site of insemination (external cervical os versus internal cervical os) and sperm motility (live versus dead spermatozoa) on the distribution of spermatozoa throughout the genital tract 2 hr after insemination.

Spermatozoa in flushings from the tract. The mean number of spermatozoa recovered per ewe was approximately 119×10^6 or 15% of the 800×10^6 inseminated. Most of this difference may be attributed to expulsion and/or drainage of mucus and spermatozoa from the vagina between insemination and slaughter as reported by Conley & Hawk (1969). Phagocytosis of spermatozoa, and incomplete recovery during flushing, would also have contributed to the loss.

The results (Table 7) show that the numbers of spermatozoa recovered from the vagina 2 hr after insemination were independent of both sperm motility and site of insemination, but the numbers of spermatozoa recovered from the cervix were strongly influenced by these factors. Semen deposition at the uterine end of the cervix resulted in higher numbers than normal insemination ($P < 0{\cdot}001$) and motile spermatozoa were more effective than immotile cells ($P < 0{\cdot}01$). The data suggest that following insemination at the level of the external cervical os, motile spermatozoa were more effective in entering the cervix, whereas after insemination at the level of the internal cervical os, large numbers of both motile and immotile spermatozoa entered and traversed the cervix in the caudal direction. However, the motile cells were more effectively retained within the lumen of the cervix.

TABLE 7

EXP. 3. THE EFFECTS OF SITE OF INSEMINATION AND SPERM MOTILITY ON THE DISTRIBUTION OF SPERMATOZOA IN THE GENITAL TRACT 2 HR AFTER INSEMINATION

Site of insemination	*Sperm motility*	*No. of ewes*	*Vagina*	*Cervix*	*Uterus*	*Fallopian tubes*
			$10^{-3} \times$ *Mean no. of sperm.*			
External cervical os	+	11	105206	2919	6	0·604
			7·909	*6·064*	*2·581*	*1·920*
	−	11	135318	846	2	0·499
			8·027	*5·358*	*1·606*	*1·177*
Internal cervical os	+	11	114460	22746	7373	71·367
			7·156	*7·138*	*6·281*	*4·516*
	−	11	74642	3094	5226	5·875
			7·298	*6·196*	*6·003*	*2·860*

Logarithmic means in italics.

Many more spermatozoa were found in uterine flushings following semen deposition at the internal cervical os than after normal insemination ($P < 0{\cdot}001$). Only 5 to 7×10^6 of the 800×10^6 spermatozoa that were deposited at the utero-cervical junction remained in the uterus and of the total spermatozoa recovered from the tract, approximately 80% had passed caudally to the vagina.

Results for the Fallopian tubes were similar to those for the cervix in that both insemination at the internal cervical os ($P < 0{\cdot}001$) and the use of motile cells ($P < 0{\cdot}01$) resulted in significantly more spermatozoa being recovered. Spermatozoa were found in the Fallopian tubes of seven and four of the eleven ewes in each treatment following normal insemination with motile and immotile spermatozoa respectively. Only one ewe (inseminated with immotile cells) failed to yield spermatozoa in the tubal flushings following semen deposition at the internal cervical os. There were no significant interactions.

Spermatozoa in the cervical sections. Results for the numbers of spermatozoa observed in transverse sections from the caudal, mid and cranial regions of the cervix (after flushing) are presented in Table 8. When immotile cells were used, no spermatozoa were found between the mucosal folds following insemination

at the external os, and only very low numbers after insemination at the utero-cervical junction. Consequently, an analysis of variance was performed on the data pertaining to inseminations with motile cells only. In this case, large numbers of spermatozoa were observed in the cervical sections, particularly after semen deposition at the utero-cervical junction ($P < 0{\cdot}01$). With both methods of insemination, the numbers of spermatozoa fell from the caudal to the cranial end of the cervix but the rate of decline was steeper following semen deposition at the external cervical os (site of insemination × position in cervix, linear; $P < 0{\cdot}01$).

TABLE 8

EXP. 3. THE EFFECTS OF SITE OF INSEMINATION AND SPERM MOTILITY ON THE NUMBERS OF SPERMATOZOA COUNTED IN TRANSVERSE SECTIONS CUT FROM THE CAUDAL, MID AND CRANIAL SEGMENTS OF THE CERVIX AFTER FLUSHING

Site of insemination	*Sperm motility*	*Cervix*			*Total*
		Caudal	*Mid*	*Cranial*	
External cervical os	+	3·18	2·27	0·73	6·18
	−	0·00	0·00	0·00	0·00
Internal cervical os	+	3·55	2·91	2·82	9·28
	−	0·36	0·00	0·27	0·63

The data are mean classification scores (see below) from eleven ewes/treatment.

No. of spermatozoa counted in sections	*Classification score*
0	0
1 to 9	1
10 to 99	2
100 to 999	3
⩾ 1000	4

DISCUSSION

In general, the results of the experiments reported here support the findings of Phillips & Andrews (1937), Schott & Phillips (1941), Starke (1949), Mattner & Braden (1963) and Mattner (1963a) that spermatozoa can reach the Fallopian tubes of the ewe within 30 min of their deposition in, or near, the external cervical os. The results do not agree with reports by Quinlan *et al.* (1932), Green & Winters (1935), Kelley (1937), Lopyrin & Loginova (1939), Dauzier & Wintenberger (1952), Dauzier (1958) and Edgar & Asdell (1960) which suggest that several hours usually elapse before spermatozoa can be found in the Fallopian tubes. Apart from obvious differences in techniques used for detecting spermatozoa, the conflicting results could be due to the fact that rapid transport occurs only in a proportion of ewes and the proportion may be lower following artificial insemination than natural service. Rapid transport was evident in only 25% of the (control treatment) ewes in Exp. 2A, and in no more than 13% of the ewes studied by Lightfoot & Salamon (1970). Even after 2 hr,

spermatozoa were found in the tubes of only 35% and 64% of the control ewes examined in Exps 1 and 3 respectively and the numbers of cells recovered were highly variable and often quite low.

Regardless of the occurrence or failure of rapid transport, there can be little doubt from Exps 2 and 3 that sperm motility is an essential prerequisite for the quantitative distribution of spermatozoa throughout the genital tract. Active sperm-tail flagellation was important for the initial entry of large numbers of spermatozoa into the cervix and their progression in reasonable numbers to the mid and cranial segments. In addition, the results of Exp. 3 have shown that motility is required for spermatozoa to penetrate between the deeply divided mucosal folds of the cervix. Immotile spermatozoa did not penetrate as they were easily removed by flushing, confirming the recent observation of Mattner & Braden (1969). Thus, the formation and retention of the sperm reservoir in the ovine cervix is primarily dependent on the motility of the spermatozoon—a view in accord with the conclusions of Mattner (1966).

The evidence suggesting participation of vaginal, cervical and/or uterine contractions in the transport of spermatozoa through the ovine cervix is well documented by Mattner & Braden (1963) and Mattner (1963b) but their observations on the transport of immotile particles are in conflict with those of Dauzier (1953). The present results (Exps 2 and 3) show quite clearly that, at least in a proportion of ewes, low numbers of immotile spermatozoa can ascend through the cervix. Further, as immotile spermatozoa do not penetrate between the mucosal folds, it can be assumed that they pass through the cervix mainly by way of the central lumen of the canal where presumably a more fluid environment exists due to the caudal drainage of uterine secretions. Uterine and/or cervical contractions appear to be responsible for this phase of sperm transport as inhibition of the contractile activity of the genital tract by halothane anaesthesia significantly reduced the number of spermatozoa recovered from the mid and cranial regions of the cervix.

Participation of uterine contractions in the movement of spermatozoa from the uterus to the Fallopian tubes, even though sperm motility appeared to be quantitatively more important, was supported by the results of Exp. 3. Considerable numbers of immotile spermatozoa were recovered from the Fallopian tubes 2 hr after their deposition at the utero-cervical junction. This observation confirms findings of a similar nature reported previously by Dauzier (1958) and Mattner (1963b). Some caution is necessary in the interpretation of experiments involving uterine insemination, however, as the technique may stimulate an abnormal pattern of uterine contractions possibly due either to physical manipulation of the uterus or to the action of pharmacologically active substances that occur in ram semen, such as prostaglandins. Nevertheless, additional evidence that myometrial activity supplements sperm motility in this phase of transport is seen in the results of Exp. 2A. Following cervical insemination with immotile cells, no spermatozoa were found in tubal flushings from anaesthetized ewes, whereas several conscious ewes yielded tubal spermatozoa (Table 5).

The effects of administering oxytocin on the movement of spermatozoa or inert material through the female reproductive tract have been studied in several species and the subject has been discussed in reviews by Cross (1959)

and Fitzpatrick (1966). Evidence in the sheep, however, is limited, but reports by Thibault & Wintenberger-Torres (1967) and Lang & Oh (1968) do not support the concept that oxytocin hastens the ascent of spermatozoa through the ewe's reproductive tract. The results of Exps 1 and 2A reported here, further indicate that, although low doses appear to have little effect, high doses of oxytocin in the oestrous ewe are detrimental to the transport of spermatozoa through the genital tract. Similarly, low doses of oxytocin administered at the time of insemination appear to have little effect on fertility in the ewe (Jones, 1968; Jones, Martin & Lapwood, 1969; Salamon & Lightfoot, 1970) but high doses (5 to 20 i.u.) reduce both fertilization (Lightfoot & Salamon, 1970) and lambing rates (Salamon & Lightfoot, 1970).

In conclusion, the present study indicates that, although contractions of the ovine genital tract supplement sperm motility in effecting transport of spermatozoa through the uterus and, to a lesser extent, the cervix, active flagellation by the sperm-tail appears essential for the establishment of the cervical population of spermatozoa and their subsequent distribution throughout the uterus and Fallopian tubes in physiological numbers.

ACKNOWLEDGMENTS

We are indebted to Professor T. J. Robinson and Dr S. Salamon, Department of Animal Husbandry, University of Sydney, and to Professor C. W. Emmens, Department of Veterinary Physiology, University of Sydney, for their interest and criticisms. Syntocinon was generously donated by Sandoz, Australia Pty Ltd. This work was supported in part by grants from the Australian Research Grants Committee and the Australian Wool Board.

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