SPERMATOZOA IN THE GENITAL TRACT OF THE EWE

III. THE ROLE OF SPERMATOZOAN MOTILITY AND OF UTERINE CONTRACTIONS IN TRANSPORT OF SPERMATOZOA

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[*Manuscript received June* 27, 1963]

Summary

Laparotomy was performed on 28 oestrous ewes and either live or dead spermatozoa were deposited in the fallopian tubes or in one uterine horn.

Dead spermatozoa appeared to be transported from the cranial extremity of one uterine horn to the body of the uterus and into the contralateral uterine horn as readily as were live spermatozoa but did not pass from the uterus to the fallopian tubes as freely as did the latter. Live spermatozoa passed from the uterus into the cervical canal more freely than did dead spermatozoa but only a small number, relative to the number present in the uterus, was recovered from the caudal portion of the cervix.

After spermatozoa were deposited in both fallopian tubes, small numbers were recovered from the uteri when live (but not dead) spermatozoa were introduced.

The importance of the cervix and the uterotubal junction in limiting the number of spermatozoa that pass in either direction is discussed in relation to the continuity of transport from the cervix to the fallopian tubes in the vaginally inseminated ewe. Observations on the survival of spermatozoa in the uterus and fallopian tubes are also presented.

I. INTRODUCTION

Previous studies (Mattner 1963) indicated that slow progression of spermatozoa from the cervix to the fallopian tube of the ewe may occur for 24 hr or more after coitus, irrespective of the occurrence or failure of initial rapid spermatozoan transport. Slow, continuous transport of spermatozoa to the tubes is likely to be of considerable importance in maintaining a population of viable spermatozoa within the fallopian tubes of the inseminated ewe over a prolonged period, for the life of the spermatozoa in the tube is evidently short (Quinlan, Maré, and Roux 1933; Edgar and Asdell 1960).

Rapid transport of spermatozoa appears to be a result of strong uterine contractions such as are induced by mating (Reynolds 1930; Millar 1952; Van Demark and Hays 1952) or by mechanical stimulation of the cervix or the external genitalia (Beshlebnov 1938; Krehbiel and Carstens 1939). During this phase of transport, spermatozoan motility probably contributes little toward the passage of spermatozoa through the genital tract of the ewe since inert particles were found in the fallopian tubes of some ewes within 15 min after suspensions of such particles were deposited in the anterior vagina (Mattner and Braden 1963). However, the response of the uterus to the stimulation of mating or manipulation of the cervix is only transient and uterine activity returns to the prestimulation level within a few minutes (Van Demark

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and Hays 1952). It is likely, therefore, that spermatozoan motility may then be of greater importance in the passage of spermatozoa through the genital tract. Thus Dauzier (1955) found no evidence to indicate that dead spermatozoa reached the fallopian tubes after they were deposited in the base of the ovine uterus during laparotomy.

In the anaesthetized ewe, rhythmic uterine contractions are inhibited for only 10–15 min after minor surgery on the uterine wall (Polovceva 1940). Therefore, in the present study, semen was injected directly into the uterus or the fallopian tubes to enable observations to be made on the distribution of live and of dead spermatozoa within the genital tract that had not been stimulated by mating or by the manipulations involved in vaginal insemination. This procedure also enabled observations to be made on the survival of spermatozoa in the uterus and fallopian tubes.

II. Materials and Methods

Twenty eight Merino ewes, 5–8 years old, were used. Laparotomy was performed during oestrus with the ewes under light anaesthesia (5% "Pentothal", intravenous). In each animal, induction of anaesthesia, laparotomy, insemination, and closure of the abdominal wall occupied less than 8 min and all animals were able to stand unaided 20 min after induction of anaesthesia. No disinfectants were used but normal hygeine was observed throughout all surgical procedures. A microsyringe was used for the injection of semen. The tip of a blunt-ended hypodermic needle was inserted through the abdominal ostium 3 cm into the ampulla for tubal insemination, or through the uterine wall into the lumen of one uterine horn, 0·5–1 cm caudal to the uterotubal junction, for uterine insemination. Semen was collected from rams by electroejaculation immediately before use. Ejaculates were used only if preliminary examination (Mattner and Voglmayr 1962) indicated that more than 90% of the spermatozoa were progressing actively. As soon as possible after insemination, a portion of the remaining semen was diluted 1 : 1000 with warm buffer solution (15 g $Na_2HPO_4.12H_2O$: 3 g KH_2PO_4 : 1000 ml distilled water) and the numbers of motile and of morphologically abnormal spermatozoa were determined using phase-contrast microscopy ($\times$192–480). Slides warmed to 36–37°C on a thermostatically controlled stage were used for the motility determinations.

When dead spermatozoa were required for insemination, semen was frozen and thawed rapidly. The absence of live spermatozoa was determined by direct microscopy and by nigrosin–eosin staining (Hancock 1952).

Ewes were killed and spermatozoa were recovered from the genital tracts, and the numbers estimated, by the methods described by Mattner and Braden (1963) except that warm buffer solution was used, in place of saline, to flush the genital tracts.

III. Observations

(*a*) *Tubal Insemination*

At laparotomy approximately 0·01 ml semen containing 10–40 million spermatozoa was injected into each fallopian tube in 16 oestrous ewes.

Experiment 1

Freshly collected semen, in which 90–95% of the spermatozoa were motile and less than 1% were tailless, was injected in five ewes, and semen in which all spermatozoa were dead and less than 5% were tailless was injected in five other ewes. Each ewe was killed 30 min after the insemination and immediately after the abdominal wall was opened, the genital tract was ligated at the cranial end of the fallopian tube and at the cranial end of each uterine horn, approximately 0·5 cm caudal to the uterotubal junction. The genital tract was then removed and the fallopian tubes and uterus divided and flushed with warm buffer solution.

In the five ewes that were inseminated with semen containing live spermatozoa, 42% (S.E. ± 7) of the introduced spermatozoa were recovered from the fallopian tubes and neither the morphology nor the motility of the recovered spermatozoa differed materially from that exhibited originally by spermatozoa in the semen used for the insemination. In each instance, the increase in non-motile and in tailless forms was less than 5 and 1%, respectively. Spermatozoa were also recovered from the uterus in each of the above ewes, the mean number being 3500 (S.E. ± 1500) and the percentages of motile and tailless spermatozoa did not differ from those recorded for spermatozoa recovered from the fallopian tubes. In the five ewes that were inseminated with semen containing dead spermatozoa, 46% (S.E. ± 6) of the introduced spermatozoa were recovered from the fallopian tubes. Comparing recovered spermatozoa with those in the insemination sample, there was less than 2% increase in the proportion of tailless spermatozoa in each instance. Spermatozoa were not found in the uterus in any of these five ewes.

Experiment 2

Freshly collected semen was introduced into both fallopian tubes in six ewes. In each ewe, one fallopian tube was removed surgically 3 hr after insemination. Two hr later (5 hr after insemination) the animal was killed and ligatures were placed at each end of the remaining fallopian tube, and at the cranial end of the cervix before the genital tract was removed, divided, and flushed.

For an *in vitro* control, semen was incubated in excised fallopian tubes. Approximately 0·01 ml of freshly collected semen (from four different rams), in which approximately 90–95% of the spermatozoa were motile and less than 1% were tailless, was injected into the ampulla in four pairs of excised fallopian tubes that had previously been flushed with 20 ml of warm buffer solution. These control tubes were ligated at both ends and incubated at 37°C in buffer solution. Spermatozoa were flushed from one of each pair of tubes after 3 hr and from the remaining tubes after 5 hr incubation.

The mean of the number of spermatozoa recovered from the fallopian tubes of the six ewes expressed as a percentage of the number introduced, was 5·7% (S.E. ± 1·4) at 3 hr and 3·7% (S.E. ± 1·3) at 5 hr. There was a statistically significant increase in the proportion of non-motile and of tailless spermatozoa in the fallopian tubes with time (see Table 1). In every instance, the proportion of non-motile and of tailless spermatozoa was greater in spermatozoa recovered from the fallopian tube 3 hr after insemination than in the original semen sample but was less than in

spermatozoa recovered from the remaining fallopian tube 5 hr after insemination (the probability that this would occur in 6 out of 6 ewes by chance is $(\frac{1}{2})^6$, i.e. $P = 0{\cdot}0156$). Few abnormalities, other than tailless forms, were observed in spermatozoa recovered at 3 hr, but at 5 hr up to 10% of the spermatozoa appeared to be disintegrating: in these, both the head and tail were no longer highly refractile to light and in many the tail appeared to be shortened and its outline was indistinct.

Spermatozoa were recovered from the uterus in 5 of the 6 ewes, the mean number for all six ewes being 3600 (S.E. $\pm$ 1400). However, in contrast to spermatozoa recovered from the fallopian tubes at either 3 or 5 hr after insemination, approximately 95% of the spermatozoa recovered from the uterus were motile and less than 5% were tailless.

TABLE 1

SURVIVAL OF SPERMATOZOA IN THE FALLOPIAN TUBES OF OESTROUS EWES

Time in Tube (hr)	No. of Observations	Percentage of Spermatozoa Motile		Percentage of Spermatozoa Tailless	
		Mean	S.E.	Mean	S.E.
0*	6	92	1	2	–
3	6	20	4	21	5
5	6	7	3	39	6

* Observation from samples as inseminated.

Motility was observed in 24% (S.E. $\pm$ 5) of the spermatozoa recovered from the four fallopian tubes that were incubated *in vitro* for 3 hr. No motility was observed in spermatozoa recovered from three of four fallopian tubes that were incubated for 5 hr but in the remaining tube approximately 0·2% of the spermatozoa were motile. However, the morphology of the spermatozoa was not materially affected by either the incubation or by the flushing procedure. In each instance (at both 3 hr and 5 hr recoveries) the increase in the percentage of tailless forms was less than 2%.

(*b*) *Uterine Insemination*

In 12 oestrous ewes, 0·02 ml semen containing 20–40 million spermatozoa was introduced into the cranial end of the left uterine horn. Six ewes (group A) were inseminated with freshly collected semen from 1 of 6 ejaculates. A further ewe was inseminated from each ejaculate after the spermatozoa had been killed by rapid freezing of the semen. Thus there were six ewes (group B) that were inseminated with semen containing dead spermatozoa only. Each ewe was killed 4 hr after insemination and ligatures were placed at either end of each fallopian tube and at the cranial end of

the cervix. The genital tract was then removed and divided into the four sections that were flushed with buffer solution in the following order: right fallopian tube, right uterine horn, body of the uterus together with the left uterine horn, and the cervix. The cranial 1 cm of the cervix was excised and discarded before the caudal portion was flushed. The percentages of motile and tailless forms in the recovered spermatozoa were estimated to the nearest 5%.

The mean of the number of spermatozoa recovered from the entire uterus expressed as a percentage of those introduced was 70% (S.E. $\pm$ 7) following insemination of freshly collected semen (group A) and 78%(S.E. $\pm$ 8) following insemination with semen containing dead spermatozoa (group B). Spermatozoa were recovered from the right uterine horn in all ewes from both group A and group B. The mean of the number of spermatozoa in the right uterine horn expressed as a percentage of the total number recovered from the uterus was 24% (S.E. $\pm$ 6) in ewes from group A and 21% (S.E. $\pm$ 5) in ewes from group B and the difference between these means was not significantly different by *t*-test.

Spermatozoa were present in the caudal portion of the cervix and in the right fallopian tube in each ewe from group A, the mean numbers being 779,000 (S.E. $\pm$ 137,000) and 1840 (S.E. $\pm$ 470), respectively. For the six animals, the ratio of the number of spermatozoa in the uterus to the number in caudal portion of the cervix was 70 : 1 (range 40 : 1 to 140 : 1) and the ratio of the number of spermatozoa in the right uterine horn to that in the right fallopian tube was 3800 : 1 (range 1800 : 1 to 6000 : 1). Spermatozoa were not recovered from either the caudal portion of the cervix or the right fallopian tube in four of the six animals in group B. In the two remaining ewes, the number of spermatozoa recovered from these sites were 10,000 and 200, and 60,000 and 250, respectively. For these ewes, the ratio of the number of spermatozoa in the uterus to the number in the caudal portion of the cervix was 3600 : 1 and 900 : 1, respectively, and the ratio of the number of spermatozoa in the right uterine horn to that in the right fallopian tube was 35,000 : 1 and 86,000 : 1, respectively.

The percentages of motile and tailless forms in spermatozoa recovered from the caudal portion of the cervix, from the uterus, and from the right fallopian tube in animals from group A are shown in Table 2. In each of the six animals there was no material difference in the percentage of either motile or tailless forms between spermatozoa recovered from the uterus and those from the caudal portion of the cervix, the percentages being similar to those originally recorded for spermatozoa in the ejaculate from which the insemination sample was drawn. In 5 of 6 ewes, spermatozoa recovered from the right fallopian tube exhibited a greater proportion of non-motile forms than did those recovered from the uterus and the difference between the respective mean percentages obtained in the six animals (14%, see Table 2) was significant by the *t*-test ($0{\cdot}02 < P < 0{\cdot}05$). There was also a greater incidence of tailless forms in spermatozoa recovered from the right fallopian tube than in those recovered from the uterus in 4 of 6 animals. However, the mean difference between the two percentages obtained in the six animals (4%, see Table 2) was not significant by the *t*-test ($0{\cdot}05 < P < 0{\cdot}1$).

IV. Discussion

In the present experiments, anaesthesia persisted for only a few minutes in each ewe. It is unlikely to have influenced the results, for deeper and more prolonged anaesthesia seemed to have little or no effect on the tonus of the uterine musculature (Polovceva 1940; Dauzier 1955) or on the progression of spermatozoa in the uterus and fallopian tubes (Dauzier 1958).

Spermatozoa were recovered from the right uterine horn of each ewe 4 hr after either live spermatozoa or dead spermatozoa were deposited in the uterus near the left uterotubal junction. The number of spermatozoa at this site, relative to the total number in the uterus, was also similar with either live or dead spermatozoa. Nevertheless, spermatozoa were recovered from the right fallopian tube in only 2 of the 6

Table 2

Percentages of motile and tailless spermatozoa in the caudal portion of the cervix, the uterus, and the right fallopian tube 4 hr after deposition of semen in the left uterine horn of oestrous ewes

Insemination samples contained 90–95% motile and <5% tailless spermatozoa

Division of Genital Tract	Motile Spermatozoa Recovered (%)		Tailless Spermatozoa Recovered (%)	
	Mean	S.E.	Mean	S.E.
Cervix	93	1	5	1
Uterus	92*	1	5	1
Right fallopian tube	78*	4	9	3

* Differences significant by *t*-test, see text.

ewes that were inseminated with dead spermatozoa and, in each instance, the ratio of the number at this site to the number in the right uterine horn was smaller than that in each of the six ewes that were inseminated with live spermatozoa. It is evident, therefore, that spontaneous contractions of the unstimulated uterus are able to transport spermatozoa in both cranial and caudal directions within the uterus independently of spermatozoan motility, but are of less importance than spermatozoan motility in the passage of spermatozoa from the uterus to the fallopian tubes.

It has been postulated that, in the vaginally inseminated animal, the cervix and the uterotubal junction function as barriers against the passage of large numbers of spermatozoa to the fallopian tubes (Austin and Braden 1952; Braden 1953). The results obtained in the present studies indicate that motile spermatozoa are able to traverse the cervix and the uterotubal junction in either the caudal or the cranial direction. Spermatozoa were not recovered from the uterus in any of the five ewes in

which dead spermatozoa were deposited in the fallopian tubes. On the other hand, when live spermatozoa were deposited in the fallopian tubes, spermatozoa were found in the uterus in 10 of the 11 sheep, though the number was small (approximately 0·04% at ½ hr and 0·3% at 5 hr) compared with the number present in the fallopian tubes. Similarly, when deposited in the uterus, live spermatozoa passed more freely from the uterus to the caudal portion of the cervix than did dead spermatozoa but the number of spermatozoa recovered from the caudal portion of the cervix was small compared to the number in the uterus (approximately 1·4% at 4 hr). Thus it appears that the cervix and the uterotubal junction may be considered as simple mechanical barriers that are able to maintain a gradient in spermatozoan concentration, directed either cranially or caudally, between the cervical canal and the body of the uterus on the one hand and between the uterine horn and the fallopian tube on the other. Since motile spermatozoa can evidently progress in either cranial or caudal directions through these partial barriers, the effective direction of spermatozoan progression through the uterotubal junction is probably determined by the relative concentration of motile spermatozoa prevailing at either end of the structure. Similarly, the respective concentrations of motile spermatozoa currently present in the cervical canal and in the body of the uterus probably determine the outcome of spermatozoan progression between these two regions of the genital tract at any given time.

In the vaginally inseminated ewe, the restrictive action of the cervix and of the uterotubal junction seems to be associated with the maintenance of a gradient that would favour continual progression of spermatozoa toward the fallopian tubes. The number of spermatozoa in the cervix of the mated ewe seems to be maximal at about 15 min after coitus and although it soon begins to decline, it always remains greater than the number in the uterus (Mattner 1963). The restriction on spermatozoan progression from the cervical canal into the vagina or the uterus probably prevents too rapid dispersal of the established spermatozoan population. The present results also support those of Dauzier (1955) in indicating that the uterotubal junction of the ewe prevents rapid passage of spermatozoa from the uterus into the fallopian tube. It appears likely, therefore, that the cervix and the uterotubal junctions and the motility of the spermatozoon itself, are together responsible for the maintenance of a continuous progression of spermatozoa from the cervix to the site of fertilization (the fallopian tubes).

The concurrent observations on the survival of spermatozoa within the genital tract of the ewe are in agreement with the findings of Quinlan, Maré, and Roux (1933) and Edgar and Asdell (1960). They indicate that continual progression of spermatozoa to the fallopian tubes from the caudal divisions of the genital tract is necessary if a population of viable spermatozoa is to be maintained in the fallopian tubes over a reasonably long period after coitus. A rapid increase in the proportion of non-motile spermatozoa in the fallopian tubes occurred after tubal insemination and was accompanied by a slower, but nevertheless substantial, increase in the proportion of morphologically abnormal forms. Destruction of spermatozoa in the uterus and the cervix was evidently much slower.

V. References

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