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Sperm Transport in the Reproductive Tract of the Female Rabbit: I. The Rapid Transit Phase of Transport[1,2]

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ABSTRACT

Rabbit spermatozoa were recovered from the upper ampulla and the fimbrial and ovarian surfaces of all females examined at 1 min post coitum (p.c.). As many as 1,000 spermatozoa could be recovered, on occasion, from the upper ampulla, fimbria and ovary of individual animals within the first 15 min p.c. After artificial intravaginal insemination, the incidence of rapid transport and distribution of sperm in the female tract were the same as in mated females. The rapid transit phase of sperm transport is an asymmetric phenomenon, occurring predominantly in the left side of the female tract of both mated and artificially inseminated does. Most spermatozoa were not motile after rapid transport and in more than 90% of these, the membranes overlying the acrosome were disrupted. After rapid transport, spermatozoa were located almost exlcusively in the uppermost regions of the oviduct. Most of the spermatozoa were cleared to the peritoneal cavity between 15 min and 4 h p.c. and none appeared to reenter the lower levels of the female tract. The sustained migration of motile spermatozoa from the uterus into the oviduct was first detected at 90 min p.c., when small populations of motile sperm were recovered from the lower isthmus of all animals examined. These observations show clearly that rapid passive transfer of rabbit spermatozoa to the upper oviduct and peritoneal cavity is a regular sequel to mating. Most sperm in this vanguard are dead and are cleared to the peritoneal cavity before ovulation and thus can have no direct role in fertilization.

INTRODUCTION

Sperm transport in female mammals following mating has been frequently described as occurring in sequential phases (e.g., Hafex, 1975). During an initial phase of rapid transport, the female tract transfers spermatozoa from the site of insemination to the oviducts at much faster rates than can be attained by the propulsive activity of the sperm flagellum. Such rapid transport appears to be a ubiquitous phenomenon among mammals known to differ markedly in other aspects of their reproductive biology. In species where considerable quantities of semen pass into the uterus, e.g., the rat, mouse, guinea pig, hamster, dog, pig and horse, spermatozoa are present in the oviducts within minutes of ejaculation (Blandau, 1969; Bedford, 1972). The same is true for mammals whose cerivx effectively restricts the passage of seminal plasma, since spermatozoa have been recovered from the upper oviduct within 5–6 min of mating in sheep (Starke, 1949; Mattner and Braden, 1963), 2–3 min in cattle (Van Demark and Moeller, 1951) and 5 min after vaginal insemination of humans (Settlage et al., 1973, 1975).

Rapid sperm transport has not been demonstrated heretofore in the rabbit, even though this species has been used frequently for such studies after natural mating. Braden's (1953) observations of a 3 h delay before sperm enter the rabbit oviduct are at present the standard citation, although Chang (1952) and El-Banna and Hafez (1970) reported sperm recovery from the oviduct within 1 h after mating and Morton and Glover (1974) recovered spermatozoa from the oviduct in 2 of 4 does 30 min post coitum (p.c.). Spermatozoa have been

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recovered from rabbit oviducts 15 min after artificial intravaginal insemination (Krehbiel et al., 1972), but this early recovery has been attributed to stimuli associated with handling of the animals (Lodge et al., 1972; Mandle, 1972). The concept of rapid sperm transport in the rabbit has to be reconciled with the demonstrated infertility of does whose oviducts are ligated above the uterotubal junction before 3 h after mating (Adams, 1956; Greenwald, 1956). This finding is one basis for Thibault's doubt that there is a rapid sperm transport in rabbits, cattle or sheep (Thibault, 1972).

Procedures developed recently for recovery of spermatozoa from the oviduct (Overstreet and Cooper, 1975) enable us to demonstrate, consistently, sperm transport to the upper oviduct and peritoneal cavity of the female rabbit within 1 min of coitus. Our observation that the vast majority of rabbit spermatozoa do not survive rapid transport resolves the apparent conflict between our findings and those of previous ligation studies in the rabbit. It also raises a fundamental question as to the functional significance of rapid sperm transport for fertility in rabbits.

MATERIALS AND METHODS

Animals and Insemination

Each of 20 New Zealand White does was mated once to 1 of 3 stud bucks of proven fertility. The reproductive tracts of 5 does were examined for spermatozoa at 1 min, 15 min, 90 min and 4 h p.c. When does were examined for spermatozoa within 1 min p.c., the effects of handling and administration of anesthetic were obviated by previous attachment of an i.v. catheter (via a marginal ear vein) for infusion of 0.9% NaCl (Normal Saline, Abbott). This did not affect mating behavior (Fig. 1). At the moment of ejaculation, a stop watch was started and the buck was removed from the presence of the doe. A lethal dose of sodium pentobarbital (Nembutal, Abbott) was injected via the catheter at 20–30 seconds p.c. The unconscious doe was taken to an adjacent 37°C controlled environment room where her abdomen was opened and both oviducts were clamped simultaneously with hemostats. The total elapsed time from ejaculation to application of the first hemostat was never longer than 1 min, 15 seconds. Does sacrificed 15 min, 90 min and 4 h after mating were placed in a restraining box and taken to the 37°C room where all subsequent procedures were carried out.

Rapid sperm transport following artificial insemination was investigated in 3 does selected for estrous receptivity in the presence of a buck which was allowed to mount but not to mate. Each doe was inseminated *per vaginum* with a single ejaculate by the method of Adams (1962) and was examined 15 min later in the manner previously described.

Division of the Reproductive Tract: The Sequence of Clamping

Preliminary experiments showed that sperm redistribution artifacts occur when the oviduct is divided with hemostats. Such artifacts are due to oviductal contractions, induced by clamping, which redistribute the tubal contents. The sequence of clamping and the site at which the first hemostat is applied are therefore of critical importance for determining serum distributions in the oviduct. Since our initial observations indicated that rabbit spermatozoa were recovered almost exclusively from the upper oviduct after rapid transport, this compartment was the first to be isolated at 1 and 15 min postinsemination. Therefore, in each of these animals, the ampullar isthmic junction (AIJ) was identified first and a hemostat was clamped across the ampulla, midway between the AIJ and the infundibulum to isolate the upper ampulla, fimbria and ovary from the remainder of the tract (Fig. 2). The second hemostat was clamped across the isthmus approximately 2 cm above the uterotubal junction (UTJ) and the lower segment of the isthmus was isolated from the uterus by clamping the third hemostat on the intramural region of the isthmus at the UTJ. The fourth hemostat was applied across the isthmus just below the AIJ to divide the remaining oviduct into upper isthmic and lower ampullar compartments (Fig. 2). Each uterine horn was then clamped below the UTJ and above the internal os of the cervix and a single hemostat was clamped across the vagina, 3–5 cm below the exocervix. When does were sacrificed after the phase of rapid transport at 90 min and 4 h p.c., the first hemostat clamped on the oviduct was placed across the isthmus 2 cm above the UTJ and the second across the UTJ; the third divided the ampulla into halves and the fourth was clamped just below the AIJ. The lower levels of the tract were clamped as previously described. These operations were carried out simultaneously on both sides of the tract and required about 20–50 seconds to complete.

Sperm Recovery at 37°C

Since many of the observations in this study were made of living spermatozoa, we considered it essential to carry out all manipulations and observations at 37°C. The necessity for careful environmental control has been illustrated in our studies of rabbit spermatozoa recovered from the oviduct. When the lower isthmus is flushed at room temperature rather than at 37°C, the proportion of motile spermatozoa is lower and the incidence of damage to the sperm head membranes is increased (Overstreet and Cooper, 1975).

Different sets of instruments were used for sperm recovery from the oviducts and ovaries (by JWO) and uteri, cervices and vaginae (by GWC). The lower tract was excised as a unit by cutting below the vaginal and above the cranial uterine hemostats. Each ovary and oviduct was removed together and wrapped in saline-moistened paper towels until processed. The ovary and fimbria were each isolated and placed in separate Petri

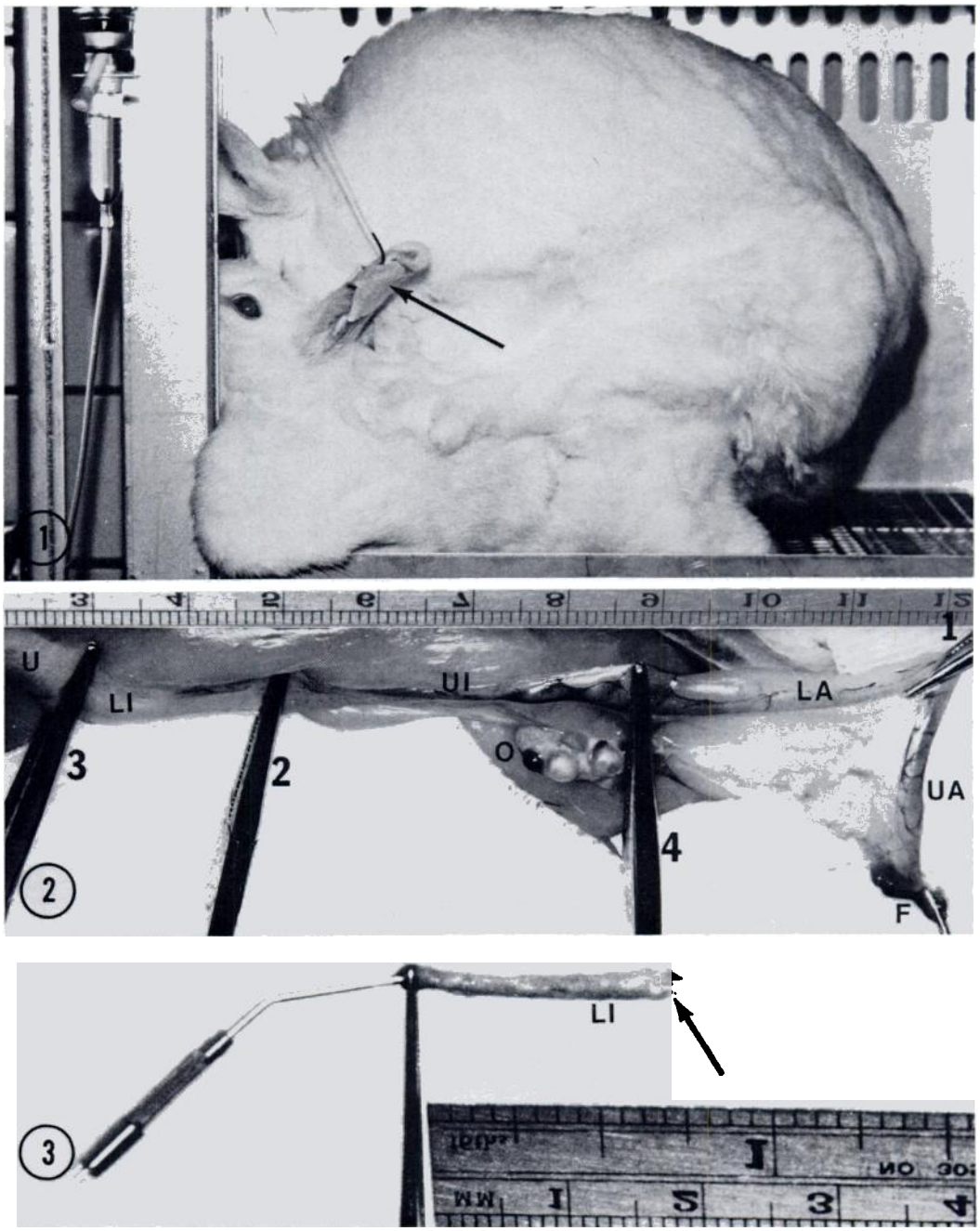

FIG. 1. Estrous doe; moment of buck's ejaculation thrust. Does assayed for sperm transport 1 min p.c. were fitted with a Butterfly-23 Infusion Set (Abbott) for infusion of normal saline and Nebutal administration 30 seconds p.c. The Butterfuly cannula was held in a marginal ear vein with surgical adhesive tape (arrow). × 0.25.

FIG. 2. Exposed oviduct: The sequence of hemostat clamping was #1, 2, 3, 4, for does assayed 1 and 15 min p.c. and #2, 3, 1, 4 for does assayed 90 min and 4 h p.c. Hemostat #1 isolated the upper ampullar (UA), fimbrial (F) and ovarian (O) compartments from the lower ampulla (LA). Hemostats 2 and 3 isolated a 2 cm segment of the lower isthmus (LI) from the uterus (U), at the level of the uterotubal junction (hemostat 3). Hemostat 4 was placed just below the ampullar isthmic junction to isolate the upper isthmus (UI) from the lower ampulla. × 1.36.

FIG. 3. Rabbit spermatozoa were isolated from oviductal compartments by flushing 5% serum Tyrode's and 0.5% SDS through blunted infusion needles (left) into 10 μl micropipettes (right). Arrow indicates the uterine end of a 2 cm lower isthmic segment (LI); the infusion needle is held in place with forceps. × 1.95.

dishes (35 X 10 mm, Falcon Plastics) and spermatozoa were recovered from their surfaces by slowly washing each with 0.5 ml of 0.5% sodium dodecyl sulfate (SDS) [Lauryl Sulfate, Sigma] in distilled water. These washings were transferred to 0.5 ml polyethylene centrifuge tubes. SDS is a negatively charged detergent which, in the concentration used, dissolves the ovarian and mucosal surfaces, leaving intact the disulfide-bonded rabbit sperm heads (Calvin and Bedford, 1971).

The upper ampulla, blotted free of blood, was cannulated at its ovarian end with a 10 μl micropipette (Yankee Micropet, Clay Adams) into which 5–10 μl of tubal fluid could usually be collected by capillary action. If less than 10 μl of free fluid was obtained, a 5% mixture (v/v) of rabbit serum (previously stored at −20°C in Tyrode's solution pH 7.4) was introduced into the uterine end of the segment. The serum Tyrode's mixture was delivered via the blunted needle of a Butterfly-23 Infusion Set (Abbott) held in place with forceps (Fig. 3). The tubing of this set was mounted in a micropipette controller (Clinac Micropipetter, Lapine) to regulate fluid delivery. After the 10 μl sample was collected, the needle of a second infusion set was inserted into the uterine end of the upper ampulla and 0.5 ml of 0.5% SDS was flushed through the segment into a 0.5 ml polyethylene centrifuge tube. All oviductal segments were flushed over glass slides and any fluid leaked was added to the final SDS flush. Lower ampullar segments were sampled in the same manner. Upper and lower isthmic segments were cannulated at their uterine ends with 10 μl micropipettes inserted 1–2 mm into the tubal lumen. About 10 μl of serum Tyrode's mixture had to be introduced into each isthmic segment in order to collect 10 μl samples of their contents, after which 0.5 ml of 0.5% SDS was flushed and collected, as previously described.

The lower levels of the female tract were sampled as follows: the uterine horns were draped across the barrel of a 5 ml syringe in order to hold the cervices above the vaginae. The body of the vagina was opened and its fluid contents were aspirated. The cervices were cut apart, separated from the vaginae at the fornices and blotted free of blood; care was taken to avoid touching the external folds of the exocervices. Each cervix was held with forceps above a collecting tube (75 X 12 mm, Kimble), a Pasteur pipette was inserted past the external os and 1 ml of serum Tyrode's mixture was flushed through the endocervix. The cervix was then divided at the level of the fornix into exocervix and endocervix and each was placed in a tube containing 1% SDS. Uterine horns, blotted free of blood, were cut from the caudal uterine hemostats, the cut ends were held above collecting tubes and 1 ml of serum Tyrode's mixture was injected with a tuberculin syringe into each uterus, just below the cranial hemostat. This flush often did not pass freely from the horn, in which case it was expressed into the collecting tube with forceps. Finally, 1 ml of 0.5 % SDS was injected into each horn and collected. Left and right uterine horns and corresponding segments of the oviducts were stored separately in normal saline after flushing until their dimensions could be measured with calipers.

Phase Contrast Microscopy at 37° C and Estimation of Total Sperm Numbers

Spermatozoa were observed in the 37° C controlled environment room with dry phase contrast optics at 100–400X magnifications. They were scored as motile if any sign of flagellar movement was noted and each was classified according to the state of the membranes overlying the acrosomal region of the head (Overstreet and Cooper, 1975).

Ten μl samples of the flushes from each level of the tract were spread between a glass slide and coverslip (#1, 18 X 18 mm) and scanned until 100–200 consecutive spermatozoa were scored for motility and morphology. Oviductal samples often had fewer than 100 spermatozoa, as did uterine samples recovered 1 and 15 min p.c. Up to 50 μl of the 1 ml serum Tyrode's samples of the latter were scanned before adding the remainder to the SDS flush of the uterus. All spermatozoa in the 10 μl flushes of each segment of the oviduct were counted. The 0.5 ml SDS flushes of each oviducal segment and washings of the ovary and fimbria, collected in polyethylene centrifuge tubes, were centrifuged for 1 min in a Beckman 152 Microfuge and after resuspension in 10 μl of 0.5% SDS, a direct microscopic count was made of sperm heads.

The number of spermatozoa in cervical and uterine samples was estimated by multiple hemocytometer counts of sperm heads, after suitable dilutions with 1% SDS. Endocervical and uterine sperm populations recovered in serum Tyrode's were counted separately from those recovered in SDS, except for those uterine samples noted above, which had few spermatozoa. Each exocervix and endocervix was repeatedly flushed with 1% SDS, before being discarded. Spermatozoa recovered by SDS treatment were dissociated into heads and tails by repeated aspiration through a Pasteur pipette prior to counting. The efficiency of these procedures for recovery of sperm from the oviducts and lower tract was assessed in preliminary experiments which included an additional flush with 0.5% SDS. More than 90% of the total sperm recovered from each region were consistently found in the serum Tyrode's and first SDS flushes.

Statistical Analysis of the Data

Owing to the small sample sizes and great variability in sperm numbers recovered among does, statistical analysis of the data was carried out using the nonparametric Randomization Test (Siegal, 1956). This test is 100% efficient in comparison with the Student t Test. The Randomization Test for matched pairs was used to study the asymmetry of rapid sperm transport within animals. All other unpaired statistical comparisons were based upon the Randomization Test for unmatched pairs, unless otherwise indicated. A 5% significance level was applied to all comparisons. Because of the small sample sizes, the tables contain the median and the range, which are more useful measures of central tendency and dispersion than the mean and standard error of the mean. Occasionally, spermatozoa were recovered from a single animal in a group or sperm numbers in a series were based on 2 values only. In these cases, the tables display the mode. In the tables, the symbol ≈ denotes a statis-

TABLE 1. Number of spermatozoa recovered from 5 adjacent regions of the female rabbit reproductive tract during the first 4 h post coitum.

Region	Median number of sperm/animal at each time (range)*							
	1 min		15 min		90 min		4 h	
Ovary[a]	101 (1–563)	≈	298 (2–2,000)	>	0 (0–1,046)	≈	0 (0–1)	
Fimbria[a]	5 (3–478)	<	124 (5–4,000)	>	39 (0–601)	≈	0 (0–109)	
Oviduct[b]	5 (0–194)	≈	55 (0–2,000)	≈	18 (2–822)	<	1,331 (37–5,235)	
Uterus	0.37×10^4 (0.002 – 5.86)	≈	0.06×10^4 (0.002 – 7.49)	<	1.53×10^5 (0.42 – 2.90)	<	1.45×10^6 (0.02 – 2.24)	
Cervix[c]	0.56×10^7 (0.06 – 1.78)	≈	1.70×10^7 (0.04 – 3.60)	≈	1.54×10^7 (0.02 – 2.32)	≈	2.10×10^7 (0.03 – 3.22)	

*Data are expressed as the median (range) number of sperm recovered in the serum Tyrode's and SDS flushes of each region. Five females were sampled/time of interest. Significant or insignificant differences in sperm numbers within regions at adjacent recovery times are denoted by the symbols >, < and ≈, as defined in the Materials and Methods. Cervical sperm numbers at 4 h p.c. were significantly greater than those at 1 min p.c., but not those at 15 min p.c.

[a]Spermatozoa recovered in 0.5% SDS alone.

[b]Based on the sum of sperm recovered from the lower and upper segments of the isthmus and ampulla of each oviduct.

[c]Spermatozoa recovered from exocervix and endocervix.

tically nonsignificant difference. Significant differences and their direction are denoted by the greater than or less than symbols, > and <, respectively.

RESULTS

One min after ejaculation, spermatozoa were present in one or both oviducts of all does examined (Tables 1 and 2). In 3/10 animals, more than 1,000 spermatozoa were recovered by 15 min p.c. from the ampulla, fimbria and ovary, with spermatozoa from the latter representing an unknown proportion of those transported to the peritoneal cavity. The numbers of spermatozoa recovered from the oviduct at 1 min p.c. were not significantly different from that recovered at 15 min p.c. (Table 1), but the variation in sperm recovery between does was so great that it is not clear whether rapid transport had ended by 1 min p.c. The time required for sperm passage from the uterus to the upper ampulla appears to be of short duration, as relatively few spermatozoa were recovered from the isthmus and lower ampulla within 15 min of insemination (Tables 2, 5). The sequence of hemostat clamping employed at 1 and 15 min p.c. was designed specifically to pick up spermatozoa in transit through the lower compartments of the oviduct (see Materials and Methods). We emphasize that throughout the 4 h interval p.c., the upper isthmus and lower ampulla remained essentially free of spermatozoa (Table 2) and that spermatozoa were not redistributed to the lower levels of the oviduct after having been propelled directly to the upper ampulla.

When sperm transport 15 min after natural mating and artificial insemination was compared, sperm numbers were not significantly different in the uterus or in the oviduct. However, a greater number of spermatozoa was found in the cervix of does receiving artificial insemination. Rapid transport to the upper tract after natural mating (1 min, 15 min) and artificial insemination (15 min) was asymmetric, greater sperm numbers being recovered from the oviduct, fimbria and ovary on the left side (8/9 compartments: Sign Test, P = 0.039; see also Tables 4, 5). Sperm numbers in the cervix and uterus were not different on the two sides.

In 1/7 animals examined at 1–15 min p.c., motile spermatozoa were recovered from the ampulla and of these, only 8% were motile. A median of 98% of sperm recovered from the ampulla at 1–15 min p.c. had visible disrup-

TABLE 2. Regional distribution of spermatozoa in the rabbit oviduct during the first 4 h p.c.

Region	1 min		15 min		90 min		4 h	
	No. of animals with sperm	Total no. of sperm recovered	No. of animals with sperm	Total no. of sperm recovered	No. of animals with sperm	Total no. of sperm recovered	No. of animals with sperm	Total no. of sperm recovered
Ovary	5	838	5	3,745	1	1,046	2	2
Fimbria	5	522	5	7,516	3	680	2	112
Upper ampulla	3	218	4	2,227	1	801	3	3
Lower ampulla	1	3	1	1	0	0	0	0
Upper isthmus	0	0	0	0	0	0	3	27
Lower isthmus (2 cm)	1	2	0	0	5	68	5	11,819

5 animals were examined at each time of interest. Spermatozoa from fimbrial and ovarian surfaces were recovered in SDS; isthmic and ampullar spermatozoa represent the total number recovered in serum Tyrode's and SDS.

tions of the membranes over the acrosomal region of the head and a median of 15% had dissociated into heads and tails (Table 3). The motility and integrity of spermatozoa recovered from the cervix and uterus at 1 and 15 min p.c. were also poorer than that of spermatozoa recovered from the vagina at these times. By 90 min p.c., sperm viability in the uterus had significantly improved and was similar to that of the vaginal population (Table 3). In contrast, the viability of spermatozoa in the cervical lumen remained at the initial lower level throughout the remaining phases of transport (Overstreet et al., 1978).

Few spermatozoa were recovered from the upper oviduct by 4 h p.c. (Table 2). The number of sperm recovered from the fimbriae declined significantly between 90 min and 4 h p.c., apparently due to their clearance to the peritoneal cavity. A small number of motile spermatozoa was recovered from the lower isthmus of all animals examined 90 min p.c. (Tables 2–4). This first appearance of motile spermatozoa in the lower isthmus is the result of a second phase of sperm entry into the oviduct following mating. The details of this succeeding phase of sperm migration in the female tract are the subject of a separate report (Overstreet et al., 1978).

DISCUSSION

Location and Viability of Spermatozoa After Rapid Transport

This study demonstrates unequivocally that rapid sperm transport occurs after mating in the rabbit, as in all other mammalian species studied adequately (Blandau, 1969; Bedford, 1972). One reason this was not detected previously is that no attempt was made to recover sperm from the fimbriae and ovarian surfaces (Braden, 1953; Krehbiel et al., 1972; Morton and Glover, 1974). Spermatozoa transported during the rapid transit phase are located almost exclusively in the most cranial regions of the oviduct (Tables 1, 2). This immediate sequestration of spermatozoa in the upper oviduct after rapid transport clearly differs from the subsequent gradual migration of motile sperm into the lower isthmus (Overstreet et al., 1978). We emphasize that spermatozoa are not found at the site of fertilization (lower ampulla) immediately after mating and that those sperm in the upper ampulla are cleared to the peritoneal cavity after rapid transport. The few spermatozoa remaining in the ampulla during the first 4 h p.c. are for the most part nonmotile and have disrupted head membranes.

Most of the rabbit spermatozoa involved in rapid transport do not survive it. This mortality seems to be the net result of an initial reduction in viability at the cervix followed by a further decline during passage through the lower oviduct (Table 3). The death of these spermatozoa did not result from the recovery procedures, since a high percentage of viable spermatozoa were recovered simultaneously from other regions of the tract (Table 3) and from the ampulla itself at later intervals (Overstreet et al., 1978). Reduced sperm motility in the oviduct has been observed in sheep (Quinlan et al., 1932) and mice (Bryan, 1974) as well as in guinea pigs, rats, golden hamsters, Chinese hamsters and Libyan jirds (Austin and Bishop, 1958). Ahlgren's (1969) recovery of only nonmotile spermatozoa from the oviducts of 2 women 1 h after coitus is also consistent with the present results.

The fact that rapid transport is lethal to the majority of spermatozoa may explain the low fertility after postcoital ligation of the lower oviduct in rabbits (Adams, 1956; Greenwald, 1956), rats (Leonard, 1950. Sharma et al., 1969) and hamsters (Yanagimachi and Chang, 1963). We know that most spermatozoa entering the rabbit oviduct after rapid transit are confined for hours in the isthmus within 2 cm of the uterotubal junction (Overstreet et al., 1978). A close reading of Adams' report (1956) indicates that he probably excluded this population from the oviduct in his study. These facts clearly illustrate why ligation studies are not a critical means of determining the sequence of events during sperm transport.

Our results seem relevant to the continuing debate on the initial events of sperm transport in sheep and cattle. Thibault and colleagues (Thibault, 1972; Thibault et al., 1975) have stated that rapid sperm transport does not occur in cattle and express doubt as to its existence in sheep (Thibault and Winterberger-Torres, 1967). Their conclusions are based primarily on the absence of spermatozoa in histological sections of the isthmus and lower ampulla sampled 2 or more hours after mating. A similar conclusion could have been drawn in the present study if we had examined only the isthmus and lower ampulla immediately p.c. (Table 2) or if the entire tract had been ex-

TABLE 3. Motility and viability of spermatozoa recovered from the reproductive tract of the female rabbit between 1 min and 90 min after a single mating.

Region	No. of animals with sperm		Median percentage of sperm/animal (range)*									
			Motile sperm[a]			Nonviable sperm[b]			Disrupted sperm[c]			
	1–15 min	90 min	1–15 min		90 min	1–15 min		90 min	1–15 min		90 min
Ampulla	7	1	0[d] (0–8)		1	98 (86–100)		96	15 (0–50)		27
Isthmus	0	5	...		94 (71–100)	...		0[d] (0–8)	...		0
Uterus	4**	5	25 (0–61)	<	90 (39–94)	57 (29–100)	>	12 (4–41)	7 (2–100)	>	0[d] (0–1)
Endocervix	10	5	37 (24–53)	≈	51 (27–56)	43 (32–63)	≈	36 (28–48)	2 (1–4)	≈	3 (1–4)
Vagina	10	5	72 (62–91)	≈	86 (23–86)	23 (9–27)	≈	10 (9–26)	2 (0–5)	≈	0 (0–2)

*Ten animals were sampled at 1–15 min and 5 at 90 min. Insufficient data were available for statistical comparisons in the isthmus and ampulla.

**One animal contained a single, nonmotile, disrupted, dissociated sperm.

[a]Spermatozoa showing any sign of flagellar activity.

[b]Immotile spermatozoa with visible disruption of the head membranes, plus those which had dissociated into heads and tails.

[c]Sperm which had dissociated into heads and tails; based on counts of tails only.

[d]Mode.

TABLE 4. Asymmetry of rapid transport through the female rabbit reproductive tract following natural mating.

Region	1 min			15 min			90 min		
	Left side		Right side	Left side		Right side	Left side		Right side
Ovary	101 (1–563)	>	2 (0–5)	298 (1–2,000)	>	1 (0–42)	0[b] (0–498)*	≈	0[b] (0–548)
Fimbria	15 (3–478)	>	0[b] (0–3)	124 (2–4,000)	≈	38 (0–547)	40 (0–527)	>	1 (0–74)
Ampulla	21 (0–194)	>	1 (0–2)	53 (0–2,000)	>	3 (0–46)	0[b] (0–800)	≈	0[b] (0–1)
Isthmus	0	≈	0[b] (0–2)	0	≈	0	12 (0–21)	≈	12 (0–18)
Uterus	0.253×10^3 (0.015 – 57.00)	≈	1.554×10^3 (0.004 – 4.55)	2.74×10^2 (0.01 – 671)	≈	2.76×10^2 (0.01 – 78)	9.20×10^4 (2.20 – 11.10)	≈	6.30×10^4 (2.00 – 23.50)
Cervix[a]	2.64×10^6 (0.27 – 10.32)	≈	3.00×10^6 (0.11 – 7.50)	9.48×10^6 (0.19 – 11.90)	≈	6.56×10^6 (0.23 – 24.09)	7.76×10^6 (0.17 – 14.00)	≈	7.66×10^6 (0.05 – 9.69)

Data are expressed as median number (range) of spermatozoa/region. Five animals were sampled/time of interest.

[a]Exocervix and endocervix.

[b]Mode.

*Spermatozoa were recovered in a single animal and in regions on both sides.

TABLE 5. Regional distribution, asymmetry of transport, motility and viability of spermatozoa recovered from the reproductive tract of the female rabbit 15 min after intravaginal artificial insemination.

Region	No. of animals with sperm	Sperm recovery[a]			Sperm motility and viability[b]	
		Left side		Right side	Motile sperm	Nonviable sperm
Ovary	3	105 (47–177)	>	1 (0–2)	...	...
Fimbria	3	88 (42–323)	>	0[c] (0–1)	...	...
Ampulla	3	45 (25–214)	>	0[c] (0–4)	8 (0–18)	87 (73–100)
Isthmus	1	1[c] (0–1)		0[c] (0–1)	0	100
Uterus	3	15 (0–58)	<	37 (10–73)	* (15–67)	* (33–46)
Cervix	3	1.66×10^7 (1.36 – 1.74)	≈	1.74×10^7 (1.15 – 1.98)	26 (19–41)	60 (41 – 83)
Vagina	3	...		...	76 (71–77)	20 (14–23)

Data are expressed as median (range) sperm number or percentage motile or nonviable. Motile sperm were those showing any sign of flagellar activity. Nonviable sperm were those which were immotile with visibly disrupted head membranes.

[a]Sperm recovered in serum Tyrode's and SDS flushes.

[b]Sperm recovered in serum Tyrode's alone.

[c]Mode.

*Motility was assessed in only 2 animals.

amined after 4 h p.c., i.e., when sufficient time had elapsed for sperm clearance from the upper oviduct.

Histological studies are further confounded by the phenomenon of asymmetry of transport in the left versus right oviduct. Such asymmetry is apparent in our data on rapid transport (Tables 4, 5) as well as in our study of sperm migration to the oviduct at the time of fertilization (Overstreet et al., 1978). A similar asymmetry in the rate of ovum transport in the rabbit (Longley et al., 1968; Polidoro et al., 1973) is thought to depend on the shorter length of the left oviduct (Polidoro et al., 1973), a fact we have also recorded (for 82 does examined, mean length ± SEM [range] in cm of right oviduct = 12.5 ± 0.2 [9.3 − 17.1], left oviduct = 11.4 ± 0.2 [8.9 − 16.0]). The possible relationship between this anatomical asymmetry and sperm transport remains to be explored.

The Mechanism of Rapid Sperm Transport

The arguments for and against transport of rabbit spermatozoa by contractions of the female reproductive tract have been discussed by Bedford (1971) and Dandekar et al. (1972). Notwithstanding the conflicting evidence from previous studies, our observations clearly demonstrate passive transfer of spermatozoa to the ampulla, infundibulum and ovary immediately following insemination. The increased contractile activity of the uterus (Fuchs, 1972) and oviducts (Westman, 1926), which is associated with mating, seems certain to play the major role in the rapid transport process. Uterine contractions during coitus in the rabbit have been shown to be mediated by adrenergic sympathetic nerves (Fuchs, 1972), probably as a spinal reflex (Cross, 1958). Mechanical distention of the vagina produces a similar pattern of uterine contraction (Ferguson, 1941; Cross, 1958; Fuchs et al., 1965) and artificial insemination of estrous does also is followed by rapid sperm transport indistinguishable from that following mating (Tables 4, 5).

There is no evidence for significant release of oxytocin after mating in the rabbit (Sharma and Chaudhury, 1970) and this has been ruled out as a cause of postcoital uterine contraction in this species (Fuchs, 1972). Oxytocin release is associated with mating in cattle (Hays and Van Demark, 1953a), sheep (Roberts and Share, 1968), goats (McNeilly and Ducker, 1972) and humans (Fox and Knags, 1969). However, careful examination of the postcoital pattern of uterine contraction in cattle (Van Demark and Hays, 1952) reveals a strong resemblance to that produced by epinephrine injection (Hays and Van Demark, 1953b) and experiments with goats (McNeilly and Ducker, 1972) and sheep (Lightfoot, 1970) cast doubt on the role of oxytocin in rapid sperm transport in these species.

Studies of the electrical activity of the tract of conscious rabbits suggest that simultaneous contractions of the vagina, cervix, uterus and oviduct follow sympathetic stimulation (Ruckebusch, 1975). Although local contractions of the oviduct are propagated primarily toward the uterus after mating (Westman, 1926), ascending contractions from the uterus result in activation of the lower isthmus (Ruckebusch, 1975), with propagation of contractile waves into the oviduct (DeMattos and Coutinho, 1971). These coordinated contractions may propel uterine spermatozoa to the peritoneal cavity.

On the basis of the present studies, we cannot conclude with certainty whether rapid sperm transport in the rabbit is a single event or a series of events, but it seems very likely that this phase of transport has ended by 2–5 min p.c. when uterine contractions cease (Fuchs, 1972). A subsequent refractory state of the rabbit uterus coincides with the rapidly rising levels of ovarian steroids secreted in response to mating (Hilliard et al., 1964, 1974; Waterston and Mills, 1976). During this period of uterine relaxation, motile spermatozoa migrate from the endocervix into the uterus and lower isthmus of the oviduct (Overstreet et al., 1978) and continue to do so after uterine contractions resume 1–3 h p.c. (Fuchs, 1972, 1974).

The Significance of Rapid Sperm Transport for Fertility

The vast majority of rabbit spermatozoa do not survive rapid transport through the female reproductive tract and certainly play no direct role in fertilization. Indeed, those arriving in the upper ampulla immediately after mating are cleared from the oviduct within a few hours and motile spermatozoa only reach the site of fertilization (the lower ampulla) in significant numbers after ovulation (Overstreet et al., 1978). The uterotubal junction and isthmus of

the rabbit oviduct do not impede the rapid transit of spermatozoa after mating. However, for at least 10 h subsequently, sperm migration through the isthmus is restricted until ovulation commences (Overstreet et al., 1978). This blockage to sperm ascent may be important in the formation of a sperm reservoir (Harper, 1973a, b) and/or in reducing polyspermic fertilization (Braden and Austin, 1954). The oviduct of the estrous rabbit may possess the inherent capability to restrict the upward migration of motile spermatozoa, with rapid transport being a superimposed event which is unimportant for fertility. Alternatively, a change in patency of the lower oviduct may follow rapid sperm transport. Since restriction of sperm ascent is similarly efficient in non-ovulatory does for at least 12 h after artificial intravaginal insemination (Overstreet and Cooper, 1978), postcoital changes in ovarian steroid secretion seem unlikely to mediate a change in tubal patency. It seems possible, however, that a functional barrier could arise in the lower oviduct as a consequence of contact with spermatozoa, seminal plasma constituents (Freund, 1973) or substances released as the sperm cells are disrupted during rapid transport.

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