

THE TRANSPORT OF SPERMATOZOA IN THE RABBIT DOE BEFORE AND AFTER OVULATION

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Summary

The number of spermatozoa in the fallopian tubes and in the uterus was recorded at 2, 3, and 4 hr *post coitum* (*p.c.*) in two groups of rabbits mated about 10 hr before (group A) or about 3 hr after ovulation (group B). Mean numbers of spermatozoa recovered from the fallopian tubes 2, 3, or 4 hr after mating tended to be greater in group B than in group A ($P < 0.4$, $P < 0.1$, and $P < 0.4$, respectively). However, the ratio of the number of spermatozoa in the fallopian tubes to the number in the uterus was significantly higher in group B than in group A animals ($P < 0.001$). In rabbits killed 2 hr *p.c.*, four of the six in group B but none of the six in group A had spermatozoa in the ampullae of the fallopian tubes. The results indicate that the rate of spermatozoan transport in the mated female is higher after than before ovulation.

Penetration of eggs by spermatozoa was not observed in any of those recovered from the group B does killed 2 and 3 hr *p.c.* and in only one of the 48 eggs from does killed at 4 hr *p.c.*

I. INTRODUCTION

In both the rat (Braden and Austin 1954) and hamster (Yanagimachi and Chang 1963) transport of spermatozoa within the female tract is more rapid after ovulation than before ovulation, whereas in the rabbit Yanagimachi and Chang (1963) found no difference in the rate of transport before and after ovulation. However, the results of Braden (1953) and Braden and Austin (1954) suggested a difference in transport of spermatozoa in the rabbit before and after ovulation. However, the results were obtained at different times and could not strictly be compared.

In the present study, the effect of time of mating in relation to ovulation was therefore re-examined, and animals in which ovulation was induced by copulation with a vasectomized male were used, assuming that ovulation occurred 10 hr after mating (Hammond 1925; Harper 1963).

II. MATERIALS AND METHODS

A total of 36 mature does was randomized into two groups of 18 animals. Four males of proven fertility and two vasectomized males were used for mating. Does from one group (group A) were mated with an entire male and killed at 2, 3, and 4 hr *post coitum* (*p.c.*). Does from group B were mated with an entire male 13 hr

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after copulation with a vasectomized male and killed at 2, 3, and 4 hr *p.c.*, i.e. about 5, 6, and 7 hr, respectively, after ovulation.

Each doe was removed to the cage of the male for service, but care was taken to subject the animal to as little stress as possible and to return the doe to her cage immediately after mating. Generally, does received two services when mated with either vasectomized or entire males, but 6 of the 18 does mated during the post-ovulatory period would accept only one service by the entire male.

TABLE I

DISTRIBUTION OF SPERMATOZOA IN THE GENITAL TRACTS OF RABBIT DOES MATED BEFORE
(GROUP A) OR AFTER (GROUP B) OVULATION

The mean number of spermatozoa in the fallopian tubes, expressed as a percentage of the number in the uterus, is given in parenthesis

Time after Mating (hr)	Division of Genital Tract	No. of Does per Group	Mean No. of Spermatozoa \pm S.E.	
			Group A	Group B
2	Fallopian tubes	6	531 \pm 211	1,350 \pm 447
	Uterus		929,000 \pm 194,000 (0.07)	692,000 \pm 136,000 (0.21)
3	Fallopian tubes	6	2,330 \pm 1,080	8,670 \pm 1,530
	Uterus		1,263,000 \pm 402,000 (0.24)	1,254,000 \pm 267,000 (0.87)
4	Fallopian tubes	6	6,100 \pm 2,220	14,140 \pm 4,740
	Uterus		2,750,000 \pm 480,000 (0.22)	1,613,000 \pm 336,000 (0.85)

The animals were killed by cervical dislocation. The reproductive tract was immediately exposed and the fallopian tubes were divided into ampulla and isthmus in all animals killed 2 and 3 hr *p.c.* The fallopian tubes were not divided in animals killed 4 hr *p.c.* Spermatozoa were recovered and counted by the method described by Mattner and Braden (1963).

Eggs from rabbits mated about the time of ovulation were examined by phase-contrast microscopy.

III. RESULTS

Mean number of spermatozoa in the fallopian tubes tended to be greater and the number in the uteri lower in group B than in group A does [$P < 0.4$, $P < 0.1$, $P < 0.4$, respectively, for 2, 3, and 4 hr *p.c.* (Table 1)]. When the number of spermatozoa in the fallopian tubes of each was expressed as a percentage of those in the uterus, the ratios were consistently higher in group B than in group A (Table 1). An analysis of variance was made of the logarithms of these ratios (Table 2). There was a significant difference between the animals mated before

and those mated after ovulation. There was a significant reduction of the ratio with time ($P < 0.01$).

Spermatozoa were not found in the ampullae of the fallopian tubes in any of the group A does killed 2 hr *p.c.*, though they were present in each isthmus. Each isthmus from the six group B does killed 2 hr *p.c.* likewise contained spermatozoa,

TABLE 2
ANALYSIS OF VARIANCE OF THE LOGARITHMIC RATIOS OF THE TUBAL SPERMATOZOA TO
THE UTERINE SPERMATOZOA

Source of Variation	Degrees of Freedom	Mean Square	F
Matings pre- v. post-ovulatory	1	4.1739	17.17***
Time of recovery of spermatozoa (<i>p.c.</i>)	2		
2 v. 3 v. 4 hr			
Linear	1	2.4779	10.18**
Quadratic	1	0.7791	3.21
Interaction	2	0.0479	0.20
Error	30	7.2926	—

** $P < 0.01$. *** $P < 0.001$.

but in addition, 5 of the 12 ampullae also had spermatozoa (the mean number recovered was 50). At 3 hr *p.c.* this difference between groups A and B had largely disappeared: small numbers of spermatozoa (15–200) were present in five ampullae from group A and seven from group B.

TABLE 3
EXAMINATION OF EGGS FROM RABBITS MATED ABOUT 3 HR AFTER OVULATION

Interval from Ovulation to Slaughter (hr)	No. of Eggs Recovered	No. of Eggs in Cumulus	No. with Corona	No. Denuded	No. with Spermatozoa on Zona
5	49	49	0	0	0
6	45	22	34	11	3
7	48	0	18	30	12

Eggs were recovered from each of the group B does. In every instance, the eggs recovered from the does killed 2 hr *p.c.* had intact cumuli oophori, and the eggs were found in clusters (Table 3). One cluster of four eggs had several spermatozoa embedded in the peripheral matrix of the cumulus but no spermatozoa were seen between the cumulus cells. Of the 49 eggs recovered, 44 were located in the ampulla. In does killed 3 hr *p.c.* the corona radiata was still present in 76% of the eggs recovered. Spermatozoa were observed in either the cumulus oophorus or corona radiata in only

two of the 45 recovered eggs; in addition, three denuded eggs had spermatozoa attached to the zona pellucida. Only 13 of the 45 eggs recovered were located in the ampulla. At 4 hr *p.c.* the percentage of denuded eggs had increased to 63% and there were no eggs with intact cumuli oophori. In each doe some of the eggs had spermatozoa attached to the zona pellucida and three eggs had spermatozoa in the corona. Only one of the eggs was seen to be penetrated by spermatozoa; it possessed two polar bodies and two small pronuclei.

IV. DISCUSSION

Spermatozoa were present in the isthmus of the fallopian tubes in all of 12 rabbits killed 2 hr *p.c.* This finding is in contrast with the observations of Heape (1905), Parker (1931), Florey and Walton (1932), and Braden (1953), who all reported that between 3 and 4 hr were required for spermatozoa to reach the lower half of the fallopian tubes in the rabbit. However, Chang (1952) claimed that in a few instances he recovered spermatozoa from the upper half of the fallopian tube at 1 hr *p.c.* Adams (1956) and Greenwald (1956) ligated the fallopian tubes of rabbits $1\frac{1}{4}$ and 2 hr, respectively, after normal mating and found that a small proportion of the eggs were subsequently fertilized. The failure of the earlier workers to find spermatozoa in the fallopian tubes before 3–4 hr was probably due, in part, to the method of recovery or detection used. Natural mating rather than artificial insemination was used because of the effect of physiological stress in the initial period after mating on the rate of transport of spermatozoa (Mattner 1963). Unfortunately this also meant that no control could be exercised over the number of spermatozoa that were inseminated. The lower numbers of spermatozoa found in the uterus of the group B does were no doubt a reflection of the lower numbers deposited in the vagina, for one-third of the does accepted only one service.

Although spermatozoa were not present in the ampullae of any of the does killed 2 hr after normal mating, between 15 and 150 spermatozoa were found at this site in 4 of the 6 animals killed 2 hr after a post-ovulatory mating. In the rat, Braden and Austin (1954) found the number of spermatozoa present in the fallopian tubes increased with the time interval between ovulation and mating. They suggested that an increase in sperm transport occurred as oestrus progressed. A similar finding was reported by Yanagimachi and Chang (1963) in the hamster. In rabbits Yanagimachi and Chang assessed spermatozoa transport by the number of eggs fertilized when the fallopian tubes had been ligated 2 hr *p.c.* A much lower percentage of eggs was fertilized when ligation was performed after ovulation but this could equally have been due to an effect on the survival or the capacitation of spermatozoa in the fallopian tubes.

Austin and Braden (1954) using rabbits mated about the time of ovulation found that, although none of the 19 eggs examined at 4 hr *p.c.* were penetrated by spermatozoa, 4 of 26 eggs examined 5 hr after mating had been penetrated. The present observations are in agreement with their conclusion: only 1 of the 48 eggs examined 4 hr after mating was penetrated. The delay between arrival of spermatozoa around the eggs and sperm penetration is attributed to the need for the process of "capacitation" (Austin 1952). When six rabbits were killed 17 hr after natural

mating 40 eggs were recovered and all were penetrated by spermatozoa (Turnbull, unpublished results).

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