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THE FERTILIZING LIFE OF SPERMATOZOA IN THE RABBIT OVIDUCT

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Summary
An experiment was designed to estimate the fertilizing life of spermatozoa in the rabbit oviduct. Mature, estrous rabbit does were artificially inseminated with 100 x 10^6 spermatozoa. Six hours after insemination, one uterine horn was ligated just below the uterotubal junction, and the contralateral oviduct was ligated just above the uterotubal junction. Human chorionic gonadotropin (HCG) was administered so as to induct ovulation at approximately 10, 13, 16, or 22 hr. after insemination. At 22 hr. after the estimated time of ovulation, the rabbits were slaughtered. The spermatozoa and ova were recovered by flushing the oviduct and uterotubal junction segments of the female tract. The ova were examined microscopically and the incidence of fertilization, the number of blastomeres, and the occurrence of abnormal ova were noted. A proportion of the ova was fixed and stained with lacmoid, and the spermatozoa in and on the ova were counted.

As the interval between insemination and ovulation increased from 10 hr. to 16 hr., there was a significant decline in the mean number of spermatozoa attached to the ova. This decline coincided with the sharp decrease in fertilization observed during the same period. The decline in the number of spermatozoa attached to the ova may reflect both the depletion of spermatozoa in the oviducts with time and a general deterioration of the ability of aged spermatozoa to contact and penetrate ova.

It is concluded that the fertilizing life of spermatozoa in the rabbit oviduct is approximately 13 to 16 hours. As such, the fertilizing life of spermatozoa in the oviduct may be shorter than in other regions of the rabbit female tract.

Introduction
The functional significance of spermatozoa lies in their ability to fertilize ova. Upon completion of spermatogenesis, spermatozoa are immature, immotile, and incapable of fertilization. After being passively transported into the epididymis, the spermatozoa undergo a process of gradual maturation during which they increase their potential for motility and fertilization (Bedford, 1966; Orgebin-Crist, 1967). In the cauda epididymidis of the rabbit, spermatozoa may retain their potential for fertilization up to 49 days (Tesh and Glover, 1969).

After being deposited in the female tract, spermatozoa acquire the ability to fertilize ova by residing in the reproductive tract for a given period of time (capacitation). Survival of spermatozoa in the rabbit female tract is quite limited. Spermatozoa retain their fertilizing capacity only up to about 32 hr. in the female tract (Hammond and Asdell, 1926; Tesh, 1969). Motility is maintained slightly longer than the capacity for fertilization and has been observed up to 50 hr. in the rabbit uterus (Noyes and Thibault, 1962).

Few attempts have been made to study
spermatozoan survival in different regions of the female reproductive tracts of rabbits. Since the oviduct is the site of fertilization, estimation of the fertilizing life of spermatozoa within this structure is of special interest. The following experiment was undertaken to determine the fertilizing life of spermatozoa in the rabbit oviduct. An additional facet of this study was concerned with the role of the uterotubal junction in spermatozoan survival. An attempt was made to determine whether conditions for spermatozoan survival were more favorable in the uterotubal junction than in the oviduct.

Materials and Methods

Collection and evaluation of Semen. Semen was collected twice weekly with an artificial vagina from six New Zealand White bucks of proven fertility. To minimize spermatozoan aging in the male tracts, the semiweekly collections were initiated 1 month prior to the first insemination. Aliquots were drawn from each semen specimen for multiple hemacytometer estimates of sperm concentration. The percent and quality of sperm motility were assessed. Only specimens exhibiting good forward motion by at least 75% of the spermatozoa were used for insemination. Immediately after this examination and within 0.5 hr. of collection, generally two, or sometimes three specimens were pooled and the semen was used for insemination.

Preparation and Insemination of Does. Twenty-two adult, female, estrous rabbits of the New Zealand White strain weighing 3.3 to 4.8 kg were used in this study. The appearance of the vaginal orifice was used as the index of estrus. Those rabbits exhibiting a swollen, deep red to purple, relaxed vaginal orifice were considered to be in full estrus. To preclude the possibility of induction of ovulation by mechanical stimulation of the anterior vagina during artificial insemination (cf. Bishop, 1933; Sawyer, 1949), the does were anesthetized with sodium pentobarbital (Diabutal, Diamond Laboratories) prior to insemination. To induce the desired level of anesthesia, the sodium pentobarbital was slowly injected into the marginal ear vein at a dosage of approximately 25 mg per kilogram body weight. Following induction of anesthesia, 100 x 10⁶ spermatozoa in 0.2 to 0.6 ml semen were deposited in the anterior vagina near the cervix.

To induce ovulation, the does were injected intravenously with 50 IU of HCG (A. P. L., Ayerst Laboratories). Five does received HCG at the time of insemination and served as controls. In the three remaining groups, 6 does received HCG at 3 hr., six does received HCG at 6 hr., and five does received HCG at 12 hr. after insemination. Assuming ovulation occurs 10 hr. after administration of HCG (Harper, 1961), this experimental design allowed testing of the fertility of spermatozoa that had resided in the female tract for periods of 10 to 22 hr. before ovulation.

Ligation and Transection of Reproductive Tracts. To prevent continual transport of spermatozoa into the oviducts from lower regions of the reproductive tract, each tract was ligated and transected near the uterotubal junctions at 6 hr. after insemination. After anesthetizing the rabbit with sodium pentobarbital, a 6 cm abdominal incision was made midventrally. Through this incision, one uterine horn was ligated with silk suture (3-0) approximately 1.5 cm below the uterotubal junction. Just above the ligature, the horn was transected by cautery (figure 1). Similarly, the centralateral oviduct was doubly ligated approximately 1.0 cm and 1.5 cm above the uterotubal junction. The oviduct was transected between the two ligatures (figure 1), and the abdomen was subsequently closed. Throughout the surgical procedure, special care was taken to avoid undue injury to the blood supply of the uterus and oviducts.

Recovery and Examination of Ova and Spermatozoa. At 32 hr. after HCG injection (i.e., 22 hr. after the expected time of ovulation), the animals were slaughtered with an overdose of sodium pentobarbital. The reproductive tracts were excised, dissected from surrounding fat and tissues, and cleaned of

Figure 1. Ligation site at laparotomy and transection sites at autopsy.
The tracts were then transected approximately 1 cm below and 1 cm above the uterotubal junctions, and each oviduct was divided into two segments of equal length (figure 1). The sections of the tracts were then flushed from each end with 1.0 ml of Tyrode's solution.

The flushings were subsequently examined for ova. Using cleavage and extrusion of the second polar body as criteria for fertilization, the eggs were examined using both conventional and phase contrast microscopy. The incidence of fertilization, the number of blastomeres, and any abnormalities associated with the ova were noted. A proportion of the ova were fixed in acetic-alcohol (1:3 vol/vol), stained with 1% lacmoid in 45% acetic acid, and examined at 160 and 400 magnifications (Chang, 1952). The spermatozoa in the mucin layer, zona pellucida, and perivitelline space were counted.

Following these examinations, 1.0 ml of each flushing was placed in a siliconized tube and centrifuged for 10 min. at 1,500 g. Immediately after centrifugation, 0.8 ml of the supernatant were drawn off and the spermatozoa in the remaining 0.2 ml solution were counted using a Fuchs Rosenthal Ultraplane hemacytometer. From these counts the number of spermatozoa in each section of the tracts were estimated.

Statistical analyses were made using the fourfold contingency tests of Mainland and Murray (1952) or Student's t-test.

Results

Effect of Ligation and time of Ovulation on Fertilization Rate. Thirty-two hours after injection of HCG, 196 ovulation points were observed on the ovaries of the 22 does used in this study (table 1). The number of ovulation points in both ovaries of each doe ranged from four to 13 with a mean of 8.9 ± 2.8. Defining ova recovery rate as 100 \[\frac{\text{number of ova recovered}}{\text{number of ovulation points}}\], the 163 ova recovered from the 22 does represented a recovery rate of 83%.

As the interval between insemination and ovulation increased from 10 hr. to 16 hr., there was a sharp decline in the fertilization rates in both oviducts (table 1). In the group ovulating 13 hr. after insemination, the fertilization rates decreased significantly from those of the corresponding oviducts in the 10-hr. group. In the groups ovulating 13 hr. and 16 hr. after insemination, the fertilization rates in the sides ligated below the uterotubal junction differed significantly between the two groups. These observations suggest that the fertilizing capacity of spermatozoa is impaired when spermatozoa have resided in the oviducts for 13 hours. Moreover, it appears that spermatozoa lose their fertilizing capacity after residing in the oviduct for 13 to 16 hours.

Within the group ovulating 10 hr. after insemination (control group), the fertilization rates in the two oviducts were not significantly different. However, within the group ovulating

<table>
<thead>
<tr>
<th>Time of ovulation after insemination (hr)</th>
<th>No. of rabbits</th>
<th>Site of ligationa</th>
<th>Total no. of ovulation points</th>
<th>No. of eggs recovered</th>
<th>No. of eggs fertilized</th>
<th>Fertilization rate (%)</th>
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<tbody>
<tr>
<td>10</td>
<td>5</td>
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aAbove UTJ refers to ligation of the oviduct approximately 1.0 cm above the uterotubal junction; below UTJ refers to ligation of the uterine horn approximately 1.5 cm below the uterotubal junction.

bFigures with different superscripts differ significantly at P < 0.01 (b is different from c and d, but not from bc, etc.).
13 hr. after insemination, an enhanced fertilization rate was observed on the side in which the ligature had been placed below the uterotubal junction.

**Cleavage Rate and Egg Transport.** All ova were recovered 32 hr. after HCG injection or approximately 22 hr. after ovulation. In the groups in which ovulation followed insemination by 10 hr. and 13 hr., the mean number of blastomeres per fertilized ovum was 4.3 and 4.3, respectively. In the group in which ovulation followed insemination by 16 hr., only one four-cell egg was recovered. All fertilized ova appeared normal, having two polar bodies and equally cleaved blastomeres.

On the side in which the ligature has been placed on the uterine horn just below the uterotubal junction, 18% of the ova were recovered from the ampullary segment, 77% from the isthmic segment, and 5% of the ova were recovered from the uterotubal junction segment. In the other side (ligation above the uterotubal junction), 21% of the ova were recovered from the ampulla and 79% were recovered from the isthmus.

**Effect of Ligation and Time of Ovulation on the Number of Spermatozoa Attached to the Ova.** A total of 97 ova, recovered from 12 does, were fixed in acetic-alcohol, stained with lacmoid, and examined for spermatozoa (table 2). The percentage of eggs with spermatozoa in the mucin layer, in the zona pellucida, or in the perivitelline space was significantly reduced in the group ovulating 16 hr. after insemination as compared with the 13 hr. groups. On the side in which the ligature has been placed above the uterotubal junction, significantly fewer spermatozoa were on or in ova in the 16-hr. group than in the 13 hr. group, and in the 13-hr. group than in the 10-hr. group (table 2). Within the group ovulating 13 hr. after insemination, more spermatozoa were attached to the ova on the side of the tract ligated below the uterotubal junction than on the side ligated above the uterotubal junction (table 2). The large decline in the number of spermatozoa attached to the ova as the interval between insemination and ovulation increased from 13 hr. to 16 hr. coincided with the sharp decline in the fertilization rate during this same period (tables 1, 2).

**Number of Spermatozoa Recovered from the Female Tracts.** At 32 hr. after injection of HCG (i.e., approximately 22 hr. after ovulation), the does were slaughtered, the reproductive tracts were flushed, and the flushings were examined for spermatozoa. Since the spermatozoa were recovered 22 hr. after ovulation, little can be concluded concerning the numbers of spermatozoa present in the oviducts at the time of ovulation. It seems safe to infer, however, that the recovery of spermatozoa from some of the oviducts 22 hr. after ovulation indicates that many more spermatozoa were present in the oviducts at the time of ovulation. The numbers of spermatozoa recovered from the tracts were highly variable from one tract to another.

**Discussion**

Because the oviduct is the site of fertiliza-
tion, measurement of the fertilizing life of spermatozoa within this structure is of special interest. Previous attempts have been made to assess the fertilizing life of spermatozoa in the rabbit oviduct. Chang and Pincus (1964) found that when 20 to 46 million spermatozoa were deposited into the oviducts of rabbits 28 to 30 hr. before ovulation, the proportion of eggs fertilized was 7.3%. Employing similar techniques, Adams (1968) found that the deposition of 4 to 10 million spermatozoa into each oviduct approximately 13, 20, 25, or 30 hr. before ovulation resulted in fertilization rates of 73%, 74%, 47% and 30%, respectively.

It is difficult to evaluate the significance of these studies. It may be argued that the conditions of these experiments do not reflect the physiological state generally found in the oviducts after coitus. The number of spermatozoa deposited in the oviducts in these studies was 1,600 or more times the maximum mean number of spermatozoa normally found in the oviducts after coitus (cf. Braden, 1953). Moreover, freshly ejaculated spermatozoa that have been deposited in the oviduct may be functionally different from spermatozoa that have been transported to the oviduct from the vagina after coitus. Spermatozoa reaching the oviducts after vaginal insemination have been subjected to various barriers and stresses during transit through the vagina, cervix and uterus. Transport and capacitation of spermatozoa in lower regions of the reproductive tract may alter the ability of spermatozoa to survive in the oviduct (cf. Dukelow and Williams, 1967; Soupart, 1967). On these bases one might expect that vaginally inseminated spermatozoa would retain their fertilizing capacity a shorter time in the oviduct than freshly ejaculated spermatozoa that have been placed in the oviducts.

In another study, Dukelow and Williams (1967) reported that capacitated spermatozoa survive only a short time in the rabbit oviduct. When 12-hr.-old uterine spermatozoa (2.5 X 10^4) were deposited in the oviduct 6 hr. and 10 hr. after HCG injection, 4.5% and 81% of the eggs were fertilized. When 8 hr. uterine spermatozoa were deposited in the oviduct 6 hr. and 10 hr. after HCG injection, 41% and 92% of the eggs were fertilized. From these observations, Dukelow and Williams concluded that the oviductal milieu is detrimental to capacitated spermatozoa.

In the present study, a new approach was used to assess the functional life of spermatozoa in the rabbit oviduct. By ligation of the tract near the uterotubal junctions at 6 hr. after insemination, further transport of spermatozoa into the oviducts was prevented. The induction of ovulation at known intervals after insemination allowed estimation of the fertilizing life of spermatozoa in the oviducts.

Braden (1953) found that the mean number of spermatozoa in the oviducts rose to approximately 5,000 at 6 hr. after mating. After this the mean number remained relatively constant. In the present experiment, ligation near the uterotubal junction was delayed until 6 hr. after insemination to allow the number of spermatozoa in the oviducts to reach near-maximum levels. When ovulation occurred 10 hr. after insemination, adequate numbers of fertile spermatozoa were present in the oviducts to effect maximal fertilization (table 1). As the interval between insemination and ovulation increased, the fertilization rate declined sharply. This decline in fertilization rate was most likely due to the progressive loss of the fertilizing ability of spermatozoa, rather than to the progressive depletion of oviductal spermatozoa.

It is true that the population of spermatozoa in the oviduct diminishes with time. Spermatozoa are lost from the oviduct via phagocytosis by polymorphonuclear leukocytes. Moreover, there appears to be a continual loss of spermatozoa from the oviduct into the peritoneal cavity (Ahlgren, 1969). In the present study, however, evidence suggests that adequate numbers of spermatozoa probably remained in the oviducts a sufficient time to effect maximal fertilization. When spermatozoa were recovered 22 hr. after ovulation (32 to 44 hr. after insemination), up to several hundred spermatozoa were found in some of the oviducts in all groups. It is highly probable that much larger numbers of spermatozoa were present in the oviducts at the time of ovulation. Several spermatozoa were observed on unfertilized ova even when ovulation occurred 16 hr. and 22 hr. after insemination. It appears from this observation that although spermatozoa were present in the vicinity of the ova, they were incapable of fertilization. A great reduction in the number of spermatozoa attached to the ova was found as the interval between insemination and ovulation increased from 13 hr. to 16 hours. This reduction probably reflects to some extent the depletion of spermatozoa in the oviduct. Moreover, this reduction may reflect a general deterioration both of the ability of the aged spermatozoa to contact the surfaces of the ova and of the
capacity of spermatozoa to penetrate ova.

Other studies suggest that spermatozoa may be retained in the rabbit oviduct for a considerable time around the time of ovulation. Braden (1953) found a negligible number of spermatozoa in the oviducts up to 3 hr. after mating. Yet when Adams (1956) and Greenwald (1956) ligated oviducts near the uterotubal junction at 2 hr. after insemination, 16% and 19% of the ova were fertilized. These studies suggest that although small numbers of spermatozoa were initially present in the oviducts, some viable spermatozoa were still present in the oviducts at the time of ovulation. 8 hr. after ligation. Harper (1973) reported that when ligation at the uterotubal junction was performed approximately 10 hr after insemination, 790 to 10,465 spermatozoa were obtained from the oviducts 5 to 10 hr. after ligation.

In the present study, when fertilization occurred, it appeared to occur normally. Although fertilization of ova by aged spermatozoa has resulted in slower cleavage rates in vitro (Maurer et al., 1969), this effect was not apparent in the present study. All ova were recovered approximately 22 hr. after ovulation.

Most of the fertilized ova had four blastomeres, but a few ova had eight blastomeres. Since the most advanced rabbit eggs normally undergo the second cleavage division at about 16 hr. after ovulation and the third division at 22 hr. (Lewis and Gregory, 1929), cleavage rates in this experiment appeared normal. The morphological appearance of the fertilized ova also seemed normal. Ligation did not appear to adversely affect egg transport in the oviducts.

In the group ovulating 13 hr. after insemination, an enhanced fertilization rate was observed on the side in which the ligature had been placed below the uterotubal junction. Coincident with this enhanced fertilization was a significantly larger number of spermatozoa attached to the ova. It has been suggested that the rabbit uterotubal junction functions as a reservoir for spermatozoa (EI-Banna and Hafez 1970). If this is so, this enhanced fertilization capacity may be due to the continual migration of spermatozoa from the uterotubal junction into the oviduct after ligation. Ligation of the other oviduct above the uterotubal junction prevented further migration of spermatozoa into the oviduct from the uterotubal junction. The possibility also exists, however, that spermatozoa retain their fertilizing ability longer in the uterotubal junction than in the oviduct. If such is the case, most oviductal spermatozoa in the group ovulating 13 hr. after insemination may have lost their capacity for fertilization by the time ovulation occurred, while many spermatozoa migrating into the oviduct from the uterotubal junction may yet have retained their fertilizing ability.

It appears from these observations that spermatozoa retain their fertilizing capacity only approximately 13 to 16 hr. in the rabbit oviduct. That rabbit spermatozoa retain their fertilizing ability up to 30 to 36 hr. in the entire female tract (Hammond and Asdell, 1926; Tesh and Glover, 1966; Seitz et al. 1970) suggests that the fertilizing life of spermatozoa is more limited in the oviduct than in other portions of the female tract. Future research is required to assess the survival of spermatozoa in other regions of the female reproductive tract and under different hormonal condition. More complete information on the composition of the luminal fluids should lead to a clearer understanding of the factors affecting spermatozoan survival in the female tract.

**Literature Cited**


