FERTILIZING LIFE OF MOUSE SPERMATOZOA IN THE FEMALE TRACT

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(Received 16th January 1968)

Summary. The duration of fertilizing capacity of mouse spermatozoa in the female tract was investigated by insemination of mice following the injection of PMSG and HCG or *post partum*. It was found that the fertilizing life of mouse spermatozoa in the female tract is about 12 hr rather than 6 hr as previously reported.

The duration of fertilizing capacity of mouse spermatozoa in the female reproductive tract was reported to be 6 hr by Merton (1939), and this figure has been quoted by many authors (Parkes, 1960; Bishop, 1961; Hartman, 1962; Chang, 1965). Since Merton's estimate for the fertilizing life of mouse spermatozoa was much shorter than the times determined for other rodents [14 hr in the rat (Soderwall & Blandau, 1941), 22 hr in the guinea-pig (Yochem, 1929; Soderwall & Young, 1940)] and non-rodent species [30 hr in the rabbit (Hammond & Asdell 1926), 126 hr in the ferret (Chang, 1965), 144 hr in the horse (Day, 1942; Burkhardt, 1949)] and because of the lack of detailed data in Merton's report (1939), the following experiments were performed to reassess the duration of the fertilizing life of mouse spermatozoa in the female tract.

Initially, an attempt was made to determine the fertilizing life of mouse spermatozoa in the female tract of mice during the oestrous cycle. Mice determined by their vaginal smear to be in pro-oestrus were inseminated at various times during the evening of expected oestrus. Some of them did not ovulate and the eggs recovered from others appeared to have been shed over a wide range of time as judged by the appearance of the eggs and the state of the cumulus clot. Thus we resorted to using injected or post-partum mice for this study.

In the first set of experiments, ninety-eight mature virgin Swiss-Webster albino females (23 to 30 g) were divided into seven groups according to the time of insemination following an ovulating injection of human chorionic gonadotrophin (HCG, A.P.L.). Each group received 3 i.u. of pregnant mare serum (PMS, Equinex) in 0.06 ml saline solution and the same dose of HCG 48 hr later. Since ovulation takes place about 10 to 12 hr after injection of HCG in this strain of mice under the same treatment (Marston & Chang, 1964), the time of expected ovulation was assumed to be 12 hr after injection of HCG.

Spermatozoa from the vas deferens and cauda epididymidis of one or two males or from the uterus of a recently mated female were suspended in 0.5 to 1.0 ml of TC 199 (Difco) at room temperature, and used to inseminate five to six females, employing a technique similar to that of Dziuk & Runner (1960). Only those suspensions with adequate sperm numbers and a high degree of motility were used for insemination. Groups of females were inseminated 12, 9, 8, 6, 3 or 0 hr after injection of HCG or at 2 hr before injection of HCG, corresponding to 0, 3, 4, 6, 9, 12 or 14 hr, respectively, before the expected time of ovulation.

A second set of experiments consisted of fifteen females obtained during gestation. They were periodically inspected until delivery and divided into three groups. One group was inseminated at 9 to 10 hr, another at 14 hr and a third group at 22 hr *post partum*. Since ovulation occurs at 19 to 22 hr *post partum* in the mouse (Merton, 1939), 22 hr was taken as the expected time of ovulation. Thus the times of insemination were 12 to 13, 8, or 0 hr before the expected time of ovulation.

TABLE 1
FERTILIZING LIFE OF MOUSE SPERMATOZOA IN THE FEMALE TRACT

Time of insemination*	No. of females used		Total eggs examined	Fertilized eggs	Percentages of fertilized eggs in all females
	Total	With fertile eggs			
Hours after HCG injection					
-2	12	2	87	5	6
0	12	3	110	19	17
0†	10	5	165	24	15
+3	12	7	109	28	26
+3†	10	6	171	29	17
+6†	10	10	150	108	72
+8	10	10	133	84	63
+9†	10	9	146	127	87
+12	12	12	130	110	85
Hours <i>post partum</i>					
9 to 10	4	1	41	2	5
14	5	4	61	32	52
22	6	6	75	63	84

* Ovulation usually occurs about 12 hr after injection of HCG or 22 hr after parturition.

† Inseminated with uterine spermatozoa.

Eggs were recovered about 10 hr after the expected time of ovulation by flushing the oviducts with saline solution (0.9% NaCl) by means of a syringe with a 28 gauge needle. The recovered eggs were mounted, fixed, stained according to Marston & Chang (1964) and examined under a phase-contrast microscope. They were classified as fertilized only if the fertilizing spermatozoon or male pronucleus was found in the vitellus. Grossly degenerate and fragmented eggs were not included in the data.

From the results presented in Table 1 it can be seen that the highest percentages of eggs fertilized in all the females were 84 to 87% when insemination

was within 3 hr of the expected time of ovulation. The percentages decreased sharply (17 to 26%) in mice inseminated about 9 hr before the expected time of ovulation, but a few eggs (5 to 17%) were fertilized after insemination at 12 to 14 hr before the expected time of ovulation.

Since relatively high proportions of mice inseminated about 8 hr before the expected time of ovulation in both cases had fertilized eggs at autopsy (i.e. 59% and 80%, respectively), and since fertilized eggs were obtained from mice inseminated as early as 12 to 14 hr before the expected time of ovulation, it is evident that the fertilizing life of spermatozoa in the female tract of the mouse is much longer than 6 hr as reported by Merton (1939). As mice have been observed not to ovulate earlier than 10 hr after injection of HCG (Edwards & Gates, 1959), the present study can be inferred to show that the fertilizing life of mouse spermatozoa in the female reproductive tract can last for 12 hr although the proportion of spermatozoa which can maintain their fertilizing capacity apparently decreased after 4 hr in the female tract.

Ovulation in the mouse occurs 1 to 4 hr after the onset of oestrus (Snell, Hummel & Abelman, 1944) and the highest fertilization rate occurs with insemination at the time of ovulation as shown in the present study. Since there is no significant difference in the fertilization rate between sperm recovered from the epididymis and the uterus (Krzanowska, 1966; and present study), it appears that either capacitation of mouse spermatozoa in the female tract is not required or requires only 1 hr as suggested by Braden & Austin (1954).

This study was supported by a grant from the Ford Foundation and a grant (GM-14370) from the United States Public Health Service.

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