FERTILIZING LIFE OF GOLDEN HAMSTER SPERMATOZOA IN THE FEMALE TRACT

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Summary. Hamster spermatozoa recovered from the epididymis or the uterus were deposited into the uterine horns at various times before ovulation. It was found that the fertilizing life of hamster spermatozoa in the female tract is about 13 hr.

The fertilizing life of the spermatozoa of the commoner laboratory animals in the female tract varies according to the species involved. In farm animals, the fertilizing life of spermatozoa in the female tract of the pig (du Mesnil du Buisson & Dauzier, 1955; Pitkjanen, 1960), sheep (Green, 1947; Dauzier & Wintenberger, 1952) and cattle (Laing, 1945; Vandeplassche & Paredis, 1948; Tarosz, 1961) is about 24 to 48 hr. The following experiments were performed to ascertain the fertilizing life of golden hamster spermatozoa in the female tract.

Mature golden hamsters, *Mesocricetus auratus*, were kept in an animal room under conditions of constant temperature (22° C) and artificial lighting (19.00 to 07.00 hours darkness). Some females were kept under reversed light and dark conditions (03.00 to 15.00 hours darkness) for the recovery of uterine spermatozoa. All females were examined daily for the presence of a post-oestrous discharge and then caged according to the day of their oestrous cycle. Only animals exhibiting a regular, 4-day oestrous cycle were used in this experiment.

A suspension of epididymal spermatozoa was prepared by mincing one cauda epididymidis with scissors in 4 ml Hanks' solution. For the preparation of suspensions of ejaculated spermatozoa, females kept under normal or reversed light conditions were killed 10 to 15 min after mating with two males, and the mass of spermatozoa expressed from the uterus were placed in 2 ml Hanks' solution.

The females to be inseminated were anaesthetized with ether at appropriate times and the uteri were exposed through a mid-ventral incision. About 0.2 ml of the sperm suspension was injected into the lumen of each uterine horn by means of a 23-gauge needle attached to a tuberculin syringe. Groups of females were inseminated at appropriate times before or after the onset of oestrus, which was ascertained by their lordosis in the presence of active males. The time of ovulation was assumed to be about 8 hr after the onset of lordosis (Harvey, Yanagimachi & Chang, 1961).

Eggs were recovered about 10 hr after the expected time of ovulation by flushing the oviducts with saline using a 30-gauge needle, and were mounted, fixed, stained and examined for the assessment of fertilization. The eggs that had either a swollen sperm head or male pronucleus in the vitellus with the tail of a fertilizing spermatozoon were recorded as eggs undergoing fertilization.

From Table 1 it can be seen that the highest fertilization rates (92 to 100%) occurred when insemination took place within $8\frac{1}{2}$ hr of the expected time of ovulation. These rates decreased from 51% to 66% if spermatozoa were in the female tract for 10 to 12 hr. The rate of fertilization decreased sharply (20% to 24%) when spermatozoa were present for about 13 hr, but a few eggs (2% to 3%) were still fertilized when insemination took place 13 to $15\frac{1}{2}$ hr before the expected time of ovulation. None of seventy-seven eggs was fertilized when spermatozoa were deposited in the uterus 16 to $16\frac{1}{2}$ hr before the expected time of ovulation.

TABLE 1
FERTILIZING LIFE OF HAMSTER SPERMATOZOA IN THE FEMALE TRACT

Time of insemination		No. of females used		Total no. of eggs examined	No. of eggs undergoing fertilization (%)
Hours before (+) or after (−) the onset of oestrus	Hours before expected time of ovulation†	Total	With fertile eggs		
−5 to −5.5	2.5 to 3	4	4	57	57 (100)
−5 to −5.5*	2.5 to 3	6	6	96	96 (100)
−2 to −2.5*	5.5 to 6	6	6	93	91 (98)
−0.5 to −1*	7 to 7.5	6	6	100	100 (100)
0 to +0.5	8 to 8.5	5	5	77	71 (92)
+2 to +2.5*	10 to 10.5	6	6	92	53 (58)
+3.5 to +4	11.5 to 12	6	6	92	61 (66)
+3.5 to +4*	11.5 to 12	5	5	80	41 (51)
+4.5 to +5	12.5 to 13	8	6	115	27 (24)
+4.5 to +5*	12.5 to 13	6	5	97	19 (20)
+6 to +6.5	14 to 14.5	6	2	104	2 (2)
+5 to +6*	13 to 14	7	3	119	4 (3)
+7 to +7.5*	15 to 15.5	6	2	95	2 (2)
+8 to +8.5*	16 to 16.5	5	0	77	0 (0)

* Inseminated with uterine spermatozoa.

† Ovulation usually occurs about 8 hr after the onset of oestrus.

There was no striking difference between spermatozoa recovered from the epididymis and from the uterus 15 min after mating, although a greater proportion of eggs was fertilized by epididymal spermatozoa than by uterine spermatozoa (61% versus 43%) in animals inseminated 8 to 13 hr before ovulation. This indicates that the secretions from the male accessory glands do not play an important rôle in maintaining the fertilizing life of spermatozoa. Since the fertilizable life of hamster eggs after ovulation is about 12 hr (Yanagimachi & Chang, 1961), the sharp drop in the fertilization rate following insemination 13 hr before ovulation is not due to the loss of fertilizability of eggs. It may indicate a uniform deterioration of spermatozoa in the female tract. The high fertilization rate obtained by insemination $2\frac{1}{2}$ to 3 hr before ovulation confirms the reports that hamster spermatozoa reach the site of fertilization within 1 hr of copulation (Yanagimachi & Chang, 1963) and that

the capacitation of hamster spermatozoa can be achieved within 2 to 4 hr in the female tract (Strauss, 1956; Chang & Sheaffer, 1957; Yanagimachi, 1966).

In view of the fact that the fertilizing life of spermatozoa in the female tract is 12 hr in the mouse (McGaughey, Marston & Chang, 1968), 13 hr in the hamster (present study), 14 hr in the rat (Soderwall & Blandau, 1941), and 22 hr in the guinea-pig (Soderwall & Young, 1940), while the fertilizable life of eggs after ovulation is 15 hr in the mouse (Marston & Chang, 1964), 12 hr in the hamster (Yanagimachi & Chang, 1961), 12 hr in the rat (Blandau & Jordan, 1941) and 20 hr in the guinea-pig (Blandau & Young, 1939), it appears that the fertile lives of rodent spermatozoa and eggs in the female tract are similar in a given species. In other species, the fertilizing life of spermatozoa in the female tract is 30 hr in the rabbit (Hammond & Asdell, 1926), 126 hr in the ferret (Chang, 1965) and 144 hr in the horse (Day, 1942; Burkhardt, 1949), while the fertilizable life of eggs after ovulation is 6 to 8 hr in the rabbit (Hammond, 1934; Chang, 1952), 30 to 36 hr in the ferret (Hammond & Walton, 1934; Chang & Yanagimachi, 1963) and an estimated time of 2 to 20 hr in the mare (Berliner, 1959), i.e. in these species, the fertilizing life of spermatozoa is much longer than the fertilizable life of eggs. Such differences may be evolved according to their different patterns of reproduction.

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