THE FERTILIZING LIFE OF SPERMATOZOA IN THE CAUDA EPIDIDYIMIDIS OF MICE AND HAMSTERS

C. M. LUBICZ-NAWROCKI, NANCY I. F. LAU AND M. C. CHANG

*Worcester Foundation for Experimental Biology,
Shrewsbury, Massachusetts 01545, U.S.A.*

(Received 1st May 1973)

Summary. Mouse and hamster spermatozoa from the cauda epididymidis were deposited into the female tract at various periods following ligation of the corpus epididymidis. It was found that the number of spermatozoa in the cauda epididymidis decreased sooner in the hamster than in the mouse but the initial decrease in fertilizing ability occurred much earlier in the mouse than in the hamster. The fertilizing life of spermatozoa in the cauda epididymidis is approximately 25 days in both species.

Spermatozoa develop their fertilizing ability during passage through the epididymis (Young, 1931; Bedford, 1966; Orgebin-Crist, 1967; Horan & Bedford, 1972) but gradually lose their ability to fertilize or to produce viable embryos after prolonged storage in the cauda epididymidis (Hammond & Asdell, 1926; Tesh & Glover, 1969; Lubicz-Nawrocki & Glover, 1973). Hammond & Asdell (1926) found that following bilateral ligation of the corpus epididymidis, most of the male rabbits became infertile after 38 days when the number of pregnancies and the size of litters were taken as criteria of fertility. Recently, Tesh & Glover (1969) reported that complete loss of fertilizing ability of rabbit spermatozoa occurs between 42 and 49 days after ligation of the corpus epididymis. The following experiments were designed to determine the fertilizing life of mouse and hamster spermatozoa and to ascertain the number of spermatozoa in the cauda epididymidis after its isolation from proximal regions by means of ligation.

In order to age spermatozoa in the male tract, bilateral ligation of the corpus epididymidis was performed in mature CD-1 mice (23 to 26 g) and golden hamsters (125 to 145 g). In male mice, the corpus epididymidis was exposed through a central abdominal incision. Two ligatures were placed between the caput and cauda epididymis and the intervening segment was removed. The testis and epididymis were manipulated back into the scrotum and testes were palpated at various times until the end of treatment to check that they remained in the scrotum. In hamsters, an incision was made in the scrotum and a single ligature was placed around the distal portion of the corpus epididymidis.

In order to ascertain the number of spermatozoa in the cauda epididymidis after its isolation, the same number of male animals as used in the fertility tests (Table 1) were killed at the same post-operative intervals.

Table 1. Effect of ligation of the corpus epididymidis on the number of spermatozoa in the cauda epididymidis and their fertilizing ability in the mouse and hamster

	Days of ligation	No. of males	No. of sperm. $\times 10^6$ / cauda epididymidis (Mean $\pm$ S.E.)	% of motile sperm.	No. of sperm. inseminated (Mean $\pm$ S.E.)	Females with fertilized eggs / females inseminated	No. of eggs recovered†	No. of eggs fertilized	% of eggs fertilized
Mouse	0	5	15.8 $\pm$ 0.8	70 to 90	10.8 $\pm$ 0.5	5/5	154	145	94.1
	10	5	13.9 $\pm$ 0.7	50 to 80	9.2 $\pm$ 0.5	3/5	113	28	24.8
	17	5	15.0 $\pm$ 1.2	10 to 70	8.9 $\pm$ 1.3	2/5	90	9	10.0
	25	6	13.5 $\pm$ 1.0	≤ 10	8.9 $\pm$ 0.7	1/6	131	8	6.1
	32	5	8.9 $\pm$ 1.1*	~ 1	6.0 $\pm$ 0.1	0/5	89	0	0.0
Hamster	0	3	103.5 $\pm$ 6.4	60 to 80	9.6†	5/5	60	60	100.0
	10	3	79.4 $\pm$ 6.2*	50 to 70	8.7	5/5	59	59	100.0
	14	3	41.2 $\pm$ 6.8	30 to 40	8.8	5/5	59	43	74.5
	21	5	13.3 $\pm$ 1.2	20 to 30	9.2	5/5	60	12	20.0
	25	5	8.5 $\pm$ 0.6	10 to 20	9.4	1/5	64	2	3.1

* Significantly different from the controls ($P < 0.05$); Student's t test.

† Female mice were induced to superovulate.

‡ Female hamsters in each group were inseminated with aliquots of one sperm suspension.

To test the fertilizing ability of aged mouse spermatozoa after various periods, animals were killed and from each a sperm suspension was prepared by macerating the cauda epididymidis from both sides in a watch-glass containing 0.15 ml Medium TC 199 (Difco Laboratories). The percentage of motile spermatozoa was estimated under a phase contrast microscope and the concentration of spermatozoa was determined using a haemocytometer. Superovulation was induced in mice by the intraperitoneal injection of 5 i.u. PMSG during metoestrus followed by 5 i.u. HCG 46 hr later. Females were anaesthetized with sodium pentobarbitone (Diabital; Diamond) and intrauterine insemination was performed by injecting 0.05 ml sperm suspension through the cervix of one female (Dziuk & Runner, 1960) using a 23-gauge needle attached to a tuberculin syringe. For the hamster, three or five male animals were used in each experiment to ensure that adequate numbers of spermatozoa were available for insemination. In each group, one sperm suspension was prepared by macerating the caudae in 3.5 ml sterilized Hanks' solution. After a rapid estimation of sperm numbers, the suspension was diluted with Hanks' solution, where necessary, to a final concentration of approximately 40×10^6 spermatozoa/ml. Since the hamster has a bipartite uterus where the cervix is divided by a septum (Hunter, 1969), sperm samples were injected directly into the uterine horns. Five females in oestrus were anaesthetized with sodium pentobarbitone and the uterine horns were exposed through a mid-ventral incision. Aliquots (0.2 ml) of sperm suspension were injected into each uterine horn by means of a 23-gauge needle attached to a tuberculin syringe and the sperm motility was ascertained.

Eggs were flushed from the uterine tubes approximately 40 hr after insemination and mounted *in toto* (Chang, 1952); the eggs were examined under a phase contrast microscope and the fertilizing ability of mouse and hamster spermatozoa was estimated in terms of the percentage of eggs that had cleaved and contained a spermatozoon.

Table 1 shows that the mean number of spermatozoa in the hamster cauda epididymidis decreased significantly ($P < 0.05$) within 10 days after ligation of the corpus epididymidis whereas, in mice, a significant change in sperm numbers occurred only after 25 to 32 days had elapsed ($P < 0.05$; Table 1). Following ligation of the corpus epididymidis in mice and hamsters, the decrease in the numbers of motile spermatozoa was similar after comparable post-operative periods.

Fertility tests showed that fertilizing ability was unaffected in hamsters until 14-day-old spermatozoa were used, whilst in mice it decreased drastically within 10 days. Thereafter, the decrease continued at a slower rate than in hamsters but fertilizing ability in mouse and hamster spermatozoa was very low after 25 days.

Our observations in mice and hamsters are consistent with the findings in rabbits that fertility declines after prolonged storage of spermatozoa in the cauda epididymidis (Hammond & Asdell, 1926; Tesh & Glover, 1969). It seems that the initial loss of fertilizing ability occurs sooner in the mouse than in the hamster, although complete loss of fertilizing ability occurs after approximately 25 days in both species. In the rabbit, the fertilizing ability of the

spermatozoa was unaffected until 28 days (Tesh & Glover, 1969). As with the results in rabbits (Hammond & Asdell, 1926; Tesh & Glover, 1969), impaired fertilizing ability in mouse and hamster spermatozoa was not related to the decreased numbers of motile spermatozoa.

It is of interest that the total number of spermatozoa in the mouse cauda epididymidis is much lower than in the hamster (16×10^6 versus 104×10^6 ; Table 1) and that sperm numbers decrease slowly in the mouse compared with the hamster after ligation of the duct. There is some indication that after ligation and transection of the corpus epididymidis in rabbits, significant depletion of spermatozoa from the cauda epididymidis does not occur for several weeks (Tesh & Glover, 1969). It seems unlikely that transection of the duct in addition to ligation might retard loss of spermatozoa from the cauda epididymidis since the same procedure in hamsters results in a significant decrease in sperm numbers after 10 days which is similar to that found after ligation alone (C. M. Lubicz-Nawrocki, unpublished observations).

It appears that loss of fertilizing ability by members of a sperm population is not an 'all or none' phenomenon and the fertilizing life of spermatozoa in the cauda epididymidis varies in different species. Whether ageing of mouse and hamster spermatozoa also increases embryonic mortality, as has been reported to occur in rabbits (Tesh & Glover, 1969), must await further study.

This work was financed by a grant (HD 03472) from the U.S. Public Health Service and a grant from the Ford Foundation. Thanks are due to Mrs Virginia Kelleher for typing the manuscript.

REFERENCES

- BEDFORD, J. M. (1966) Development of fertilizing ability of spermatozoa in the epididymis of the rabbit. *J. exp. Zool.* **163**, 319.
- CHANG, M. C. (1952) Fertilizability of rabbit ova and the effects of temperature *in vitro* on their subsequent fertilization and activation *in vivo*. *J. exp. Zool.* **121**, 351.
- DZIUK, P. J. & RUNNER, M. N. (1960) Recovery of blastocysts and induction of implantation following insemination of immature mice. *J. Reprod. Fert.* **1**, 321.
- HAMMOND, J. & ASDELL, S. A. (1926) The viability of the spermatozoa in the male and female reproductive tracts. *Br. J. exp. Biol.* **4**, 155.
- HORAN, A. H. & BEDFORD, J. M. (1972) Development of the fertilizing ability of spermatozoa in the epididymis of the Syrian hamster. *J. Reprod. Fert.* **30**, 417.
- HUNTER, R. H. F. (1969) Capacitation in the golden hamster with special reference to the influence of the uterine environment. *J. Reprod. Fert.* **20**, 223.
- LUBICZ-NAWROCKI, C. M. & GLOVER, T. D. (1973) The influence of the testis on the survival of spermatozoa in the epididymis of the golden hamster, *Mesocricetus auratus*. *J. Reprod. Fert.* **34**, 317.
- ORGEBIN-CRIST, M.-C. (1967) Maturation of spermatozoa in the rabbit epididymis: fertilizing ability and embryonic mortality in does inseminated with epididymal spermatozoa. *Annls Biol. anim. Biochim. Biophys.* **7**, 373.
- TESH, J. M. & GLOVER, T. D. (1969) Ageing of rabbit spermatozoa in the male tract and its effect on fertility. *J. Reprod. Fert.* **20**, 287.
- YOUNG, W. C. (1931) A study of the function of the epididymis. III. Functional changes undergone by spermatozoa during their passage through the epididymis and vas deferens in the guinea-pig. *J. exp. Biol.* **8**, 151.