J. Reprod. Fert. (1974) **36,** 203–205

DEVELOPMENT OF THE PENETRATION ACTIVITY OF MOUSE EPIDIDYMAL SPERMATOZOA *IN VIVO* AND *IN VITRO*

A. PAVLOK

Czechoslovak Academy of Sciences, Institute of Physiology and Genetics of Farm Animals, Liběchov, Czechoslovakia

(*Received* 13*th July* 1973)

The development of the fertilizing capacity of mammalian epididymal spermatozoa has been studied mainly in rabbits (see Nishikawa & Waide, 1952; Bedford, 1966; Fulka & Koefoed-Johnsen, 1966; Orgebin-Crist, 1967) and occasionally in rats (Blandau & Rumery, 1964) and hamsters (Horan & Bedford, 1972). Although the results of these studies were not absolutely identical, even in the same species, it is generally agreed that the most proximal site from which fertile spermatozoa can be isolated is the corpus epididymidis and, in exceptional cases, the distal parts of the caput (Blandau & Rumery, 1964; Orgebin-Crist, 1967; Paufler & Foote, 1968).

This paper describes the results of a study of the penetration activity of mouse epididymal spermatozoa. As distinct from preceding studies, in which the fertilizing capacity of spermatozoa was tested only *in vivo*, we simultaneously attempted its evaluation by means of two *in-vitro* fertilization methods. Activation of the proteolytic enzymes in the apical part of the spermatozoa for penetration of the zona pellucida is regarded as the most important factor in the fertilization of intact ovulated oocytes, while oocytes deprived of the zona pellucida can be used to test the ability of spermatozoa to penetrate the vitelline membrane.

Experiments were carried out with adult female and male F_9 hybrids, aged 8 to 18 weeks, from inbred C57B110ScSn/Ph females and A/Ph males. Individual segments of the epididymis (Plate 1) were successively removed, starting with the caput, and taking care to avoid contamination by spermatozoa from the more distal segments. On removal, the segments were immediately placed in 50 μl culture medium in a watch-glass, under a layer of liquid paraffin (Pavlok & McLaren, 1972). The spermatozoa were released into the medium by gentle pressure on the tissue with dissecting needles. The sperm concentration in the stock suspension ranged from 20 to 50×10^6 spermatozoa/ml.

Superovulation was induced with 8 to 10 i.u. PMSG (Antex-Leo, Copenhagen) administered in two doses separated by a 12-hr interval. An injection of 25 i.u. HCG (Praedyn-Spofa) was given 45 to 50 hr after the first dose. Females to be fertilized *in vivo* were anaesthetized with ether 2 to 3 hr after presumed superovulation, laparotomy was performed and inseminations were carried out by introducing 10 to 15 μl sperm suspension into both uterine horns. The

females were killed 6 to 6½ hr after insemination and the ova were isolated, mounted and fixed. Fertilization was effected *in vitro* by the method employed by Pavlok & McLaren (1972), using bovine serum albumin Cohen fraction V (Koch-Light), in a concentration of 0·4 to 0·6 mg/ml, instead of freeze-dried serum albumin. Incubation was carried out at 37·5° C in a 95% air+5% CO_2 atmosphere and was terminated after 5 hr (zona-free ova) or 6 hr (intact ova). The methods used for fixation, staining and evaluation have already been described (Pavlok & McLaren, 1972).

Table 1 shows the results of the fertilization of mouse ova by spermatozoa from various segments of the epididymis, using three different methods. In general, irrespective of the fertilization method, this study confirmed the findings in other mammalian species, that the fertility of epididymal spermatozoa diminishes progressively from the distal to the proximal segments of the epididymis.

Table 1. Fertility of epididymal spermatozoa under different fertilization conditions

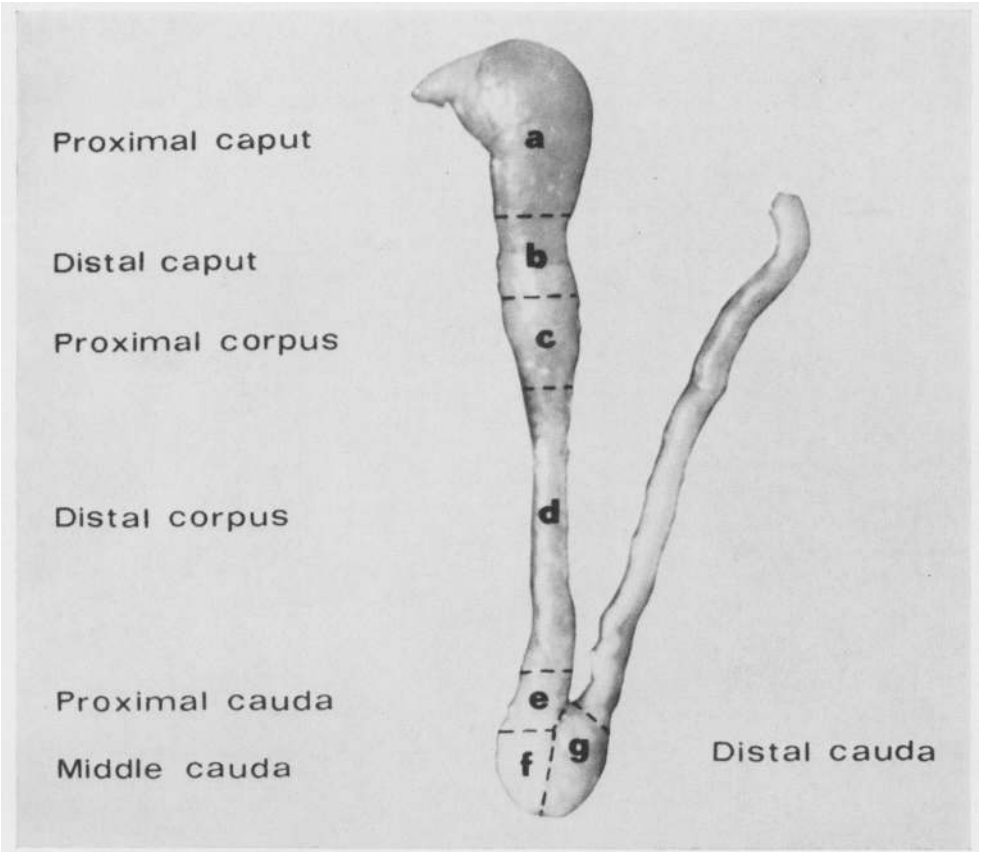
Segment	*Fertilized*			*Single differences* (t *test*)		
	In vivo (*A*)	In vitro (*intact ova*) (*B*)	In vitro (*zona-free ova*) (*C*)	*A*×*B*	*A*×*C*	*B*×*C*
b	0 (132)	0 (152)	18·2 (126)	0	++	++
c	24·4 (160)	2·6 (194)	50·0 (134)	++	+	+++
d	34·2 (111)	2·9 (172)	70·1 (127)	++	++	+++
e	51·8 (112)	22·7 (189)	64·7 (133)	++	0	+++
f	64·5 (154)	59·4 (175)	77·9 (127)	0	0	0
g	62·2 (148)	67·0 (188)	66·7 (117)	0	0	0

The values show the percentage of eggs fertilized, followed in parentheses by the number of eggs examined.
0, Non-significant; +, $P<0·05$; ++, $P<0·025$; +++, $P<0·001$.

Comparison of fertility with reference to the fertilization method showed no significant differences with spermatozoa from the middle and distal parts of the cauda epididymidis ('f', 'g'). Fertilization by spermatozoa from the more proximal segments was significantly less successful, particularly in the case of intact ova fertilized *in vitro*. The lower percentage of ova fertilized *in vitro* compared with those fertilized *in vivo* may be due to the inability to provide spermatozoa *in vitro* with the same conditions for ensuring their capacitation and survival as those which exist *in vivo*.

The finding that spermatozoa from segments 'b', 'c' and 'd' were capable of penetrating zona-free ova *in vitro* at a higher rate than intact ova *in vitro* or *in vivo* could be due to several causes. During sperm maturation, the mechanism determining their ability to penetrate the vitelline membrane presumably develops somewhat ahead of that which enables them to penetrate the zona pellucida. The problem may, however, be far more complex and the extent of the experiment is too small to allow of a definitive conclusion. Blandau & Rumery (1964) and Orgebin-Crist (1967) assumed that penetration of the egg

PLATE 1



The mouse epididymis, approx. × 5, showing the individual segments ('a' to 'g') from which spermatozoa were obtained.

(*Facing p.* 204)

membranes by spermatozoa depended on their form of motion, which is quite different in the proximal and distal segments of the epididymis. Developmental changes in the character of their motility were studied in detail and confirmed by Fray, Hoffer & Fawcett (1972). In our experiments, a steady decrease in the number of spermatozoa with progressive motility in the more proximal segments was already observed from segment 'e'. The proportion of spermatozoa with progressive motility in segment 'a' was very small (less than 10%) and they stopped moving only a few minutes after their isolation from the epididymis. The fertility of the spermatozoa in this segment was, therefore, not tested.

Fulka & Koefoed-Johnsen (1966) and Orgebin-Crist (1967) found that, after intrauterine insemination in rabbits, the number of spermatozoa from the distal segments of the epididymis which penetrated to the oviducts and to the surface and perivitelline space of the ova was far greater than the number from the proximal segments. Good motility is evidently more important for sperm penetration of the uterotubal junction and the membranes of intact ova than for penetration of the vitelline membrane, without the zona pellucida.

Fulka & Koefoed-Johnsen (1966), Bedford (1967) and Orgebin-Crist (1969) achieved a partial increase in the fertilizing capacity of spermatozoa from the proximal segment of the corpus or from the caput epididymidis after ligation of the epididymal duct. On repeating these experiments in hamsters, Horan & Bedford (1972) found no positive effect on fertility, although they observed partial improvement of motility. Sperm maturation in the epididymis thus seems to be connected partly with the morphologically and functionally different segments of the epididymal duct and partly with the time the spermatozoa remain in the epididymis, irrespective of the segment. The available evidence indicates that the relevant properties of spermatozoa determining their full fertilizing capacity develop largely autonomously.

REFERENCES

Bedford, J. M. (1966) Development of fertilizing ability of spermatozoa in the epididymis of the rabbit. *J. exp. Zool.* **163,** 319.

Bedford, J. M. (1967) Effects of duct ligation on the fertilizing ability of spermatozoa from different regions of the rabbit epididymis. *J. exp. Zool.* **166,** 271.

Blandau, R. J. & Rumery, R. E. (1964) The relationship of swimming movements of epididymal spermatozoa to their fertilizing capacity. *Fert. Steril.* **15,** 571.

Fray, C. S., Hoffer, A. P. & Fawcett, D. W. (1972) A re-examination of motility patterns of rat epididymal spermatozoa. *Anat. Rec.* **173,** 301.

Fulka, J. & Koefoed-Johnsen, H. H. (1966) The influence of epididymal passage in rabbits on different spermatozoan characteristics including fertilizing capacity. *A. Rep. R. Vet. Agric. Coll. Steril. Inst.* p. 213.

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.

Nishikawa, Y. & Waide, Y. (1952) Studies on the maturation of spermatozoa. I. Mechanism and speed of transition of spermatozoa in the epididymis and the functional changes. *Bull. natn. Inst. agric. Sci., Tokyo,* Series G, No. 3, 68.

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.

Orgebin-Crist, M. C. (1969) Studies on the function of the epididymis. *Biol. Reprod.* Suppl. **1,** 155.

Paufler, S. K. & Foote, R. H. (1968) Morphology, motility and fertility of spermatozoa recovered from different areas of ligated epididymides. *J. Reprod. Fert.* **17,** 125.

Pavlok, A. & McLaren, A. (1972) The rôle of cumulus cells and the zona pellucida in fertilization of mouse eggs *in vitro*. *J. Reprod. Fert.* **29,** 91.