DEVELOPMENT OF MOUSE EGGS FERTILIZED *IN VITRO* BY EPIDIDYMAL SPERMATOZOA

H. MIYAMOTO AND M. C. CHANG

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

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Summary. About 64% of 854 superovulated mouse eggs were fertilized *in vitro* by epididymal spermatozoa and were capable of cleaving in chemically defined media. Approximately 10% of such eggs fertilized *in vitro* or *in vivo* could develop to the blastocyst stage in culture. When two-cell eggs fertilized *in vitro* and *in vivo* were transferred to the oviducts of pseudopregnant mice, only 13% and 16%, respectively, developed into normal fetuses.

The possibility of development of mouse eggs fertilized *in vitro* by spermatozoa recovered from the uterus has been reported by Whittingham (1968), Mukherjee & Cohen (1970) and Cross & Brinster (1970). It has also been reported that mouse eggs can be fertilized *in vitro* by epididymal spermatozoa in the presence of bovine follicular fluid (Iwamatsu & Chang, 1969, 1970, 1971) or in its absence (Toyoda, Yokoyama & Hosi, 1971), but whether such eggs can develop in culture and even mature into fetuses following transfer to recipients has not been investigated. This paper reports the results of experiments designed to determine the proportion of mouse eggs fertilized *in vitro* by epididymal spermatozoa in the absence of bovine follicular fluid, and the proportion of fertilized eggs which developed into blastocysts following further culture. The possibility of normal development following the transfer of such eggs to the oviducts of recipients was also investigated.

Superovulation in mature female Swiss albino mice, weighing 21 to 25 g, was induced by the intraperitoneal injection of 5 i.u. PMSG followed by 5 i.u. HCG 44 to 48 hr later. The mice were killed 14 to 16 hr after the injection of HCG, their oviducts were removed and placed under light paraffin oil (viscosity, 125/135) in a watch-glass kept at 37° C, and eggs in the cumulus clot were released into the oil. The paraffin oil was previously equilibrated with 5% CO₂ in air in the presence of a small volume of saline.

A sperm suspension was prepared by mincing cauda epididymidis of a mature Swiss albino mouse in a watch-glass containing 1 ml of a medium which was slightly modified, by the addition of 21.58 mM-sodium lactate, to that used by Toyoda *et al.* (1971). About 0.2 ml sperm suspension was added to the egg clot under the oil. After adding another 0.2 to 0.3 ml medium, the eggs and spermatozoa were thoroughly mixed and then incubated at 37° C in an atmos-

phere of 5% CO₂ and air. The final concentration of spermatozoa in each watch-glass was 1 to $5 \times 10^3/\text{mm}^3$.

After incubation for 6 to 7 hr, the eggs were taken out and washed in 2 ml of a culture medium and then cultured under oil in a small droplet of culture medium of the same composition as that used by Whitten & Biggers (1968), except that the concentration of sodium pyruvate was 0.55 mM instead of 0.33 mM. After culture for 24 hr, all the eggs were washed twice in the medium, the cleaved eggs were counted and only two-cell eggs were cultured for another 72 hr in fresh medium. The number of eggs which had developed into morulae or blastocysts were recorded. In some experiments, one-cell eggs fertilized *in vivo* from mated animals were similarly cultured as controls.

TABLE 1
DEVELOPMENT OF MOUSE EGGS FERTILIZED *in vitro* WITH EPIDIDYMAL SPERMATOA AND THEN FURTHER CULTURED FOR 24 TO 96 HR

Eggs	Exp.	No. of eggs cultured	No. of two-cell eggs obtained 24 hr later (%)	No. of morulae obtained 96 hr later (%)	No. of blastocysts obtained 96 hr later (%)
Fertilized <i>in vivo</i>	1	62	49 (79)	8 (16)	4 (8)
	2	122	83 (68)	6 (7)	13 (16)
	3	80	56 (70)	4 (7)	3 (5)
	4	98	68 (69)	10 (15)	2 (3)
Total		362	256 (71)	28 (11)	22 (9)
Fertilized <i>in vitro</i>	1	131	78 (60)	6 (7)	10 (13)
	2	144	72 (50)	8 (11)	6 (8)
	3	89	56 (63)	3 (5)	7 (13)
	4	140	91 (65)	10 (11)	6 (6)
	5	106	66 (62)	4 (6)	4 (6)
	6	149	108 (72)	19 (18)	18 (17)
	7	95	77 (81)	8 (10)	5 (6)
Total		854	548 (64)	58 (11)	56 (10)

Female Swiss albino mice made pseudopregnant by mating with vasectomized males were used as recipients. When vaginal plugs were found in the morning, transfer operations were performed in the afternoon. The eggs that had cleaved into two cells in culture after fertilization *in vitro* or *in vivo* were transferred into the ampullar portion of the left oviduct according to the method described by Tarkowski (1959). Each recipient received ten eggs and was killed on the 17th day after transfer in order to examine the uterine contents.

It can be seen from Table 1 that 71% of the eggs fertilized *in vivo* cleaved after culture for 24 hr and 64% of eggs cleaved following insemination with epididymal spermatozoa and further culturing for 24 hr. Following the transfer of 180 eggs fertilized *in vivo* into the oviducts of eighteen recipient mice and eighty eggs fertilized *in vitro* into the oviducts of eight recipient mice, twenty-nine normal fetuses (16%, from six pregnant mice) and ten (13%, from two pregnant mice), respectively, were obtained. Thus, there was very little difference in the proportion of development in culture (Table 1) or following

transfer between the eggs fertilized *in vivo* and those fertilized by epididymal spermatozoa *in vitro* under our experimental conditions.

Mukherjee & Cohen (1970) reported that 37% of mouse eggs fertilized *in vitro* by uterine spermatozoa could develop into blastocysts in culture. We found that only 10% of the eggs fertilized *in vitro* by epididymal spermatozoa could develop into blastocysts in culture even though we used the same medium. In the transfer of eggs fertilized *in vitro*, Whittingham (1968) obtained a 20% development, Cross & Brinster (1970) observed a 3.2% development, and we obtained a 13 to 16% development when two-cell eggs were transferred into the oviduct. Mukherjee & Cohen (1970), however, obtained a 48% development when eggs which had been fertilized *in vitro* and cultured to the blastocyst stage were transferred. Thus, it appears that the great variation obtained by different investigators is due to the diversity of procedures and techniques. There is no doubt, however, that the normal development of mouse eggs fertilized *in vitro* by epididymal spermatozoa is possible, although the potential for development still awaits further improvement.

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