FERTILIZATION *IN VITRO* OF MOUSE AND HAMSTER EGGS AFTER THE REMOVAL OF FOLLICULAR CELLS

H. MIYAMOTO AND M. C. CHANG

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

(Received 25th January 1972, accepted 17th March 1972)

Summary. The removal of follicular cells decreased the proportion of mouse eggs penetrated by epididymal spermatozoa, but did not affect the proportion of hamster eggs penetrated, whether the spermatozoa were taken from the epididymis or from the uterus.

In the rabbit, the cumulus oophorus is said to be dispersed by hyaluronidase released from the sperm acrosome (Austin, 1961) and corona radiata cells are removed by a tubal factor (Swyer, 1947). It has been postulated that the presence of cumulus oophorus may decrease the incidence of polyspermy and increase the chance of fertilization, and that the corona radiata cells may play a rôle in the orientation of spermatozoa approaching the eggs (Austin, 1961).

After the dissolution of the cumulus oophorus by treatment with hyaluronidase and removal of the corona radiata by manual shaking, the denuded rabbit eggs can be fertilized *in vitro* (Bedford & Chang, 1962). Recently, it was reported that removal of the cumulus oophorus and the corona radiata from rabbit eggs does not lower the proportion of eggs fertilized *in vitro* (Chang, Hanada & Hunt, 1971; Fraser, Dandekar & Vaidya, 1971). By contrast, Cross & Brinster (1970) reported that the removal of the follicular cells from the mouse eggs reduced the fertilization rate inseminated with spermatozoa recovered from the uterus. Iwamatsu & Chang (1970), however, reported that the percentage of eggs penetrated by spermatozoa preincubated with bovine follicular fluid was lower in mouse eggs with follicular cells than in those without follicular cells.

This experiment was designed to determine if the follicular cells surrounding the eggs have a particular rôle to play in the fertilization *in vitro* of mouse and hamster eggs.

Mature female CD-1 mice were subjected to the procedure of superovulation and their eggs were recovered according to the procedures reported elsewhere (Edwards & Gates, 1959; Miyamoto & Chang, 1972). One group of eggs in cumulus clot was placed in a watch glass containing 0.5 ml of 0.003% hyaluronidase in a medium containing bovine serum albumin, sodium pyruvate (Toyoda, Yokoyama & Hosi, 1971; Miyamoto & Chang, 1972) plus 21.58 mM-sodium lactate; the other group was placed in another watch glass containing no hyaluronidase. After the dissolution of the cumulus oophorus and the corona radiata,

the hyaluronidase-treated eggs were washed five times while the eggs in cumulus clot were washed twice in order to remove oviducal fluid. A sperm suspension was prepared by mincing cauda epididymidis of a mature male CD-1 mouse in a watch-glass containing 1 ml medium. About 0.1 ml of sperm suspension was added to 0.1 ml medium containing eggs under paraffin oil in a watch glass. After the further addition of about 0.2 to 0.3 ml medium, the suspension was thoroughly mixed, and incubated at 37° C in an atmosphere of 5% CO₂ in air for 5 to 7 hr.

Mature female golden hamsters were also subjected to a procedure of super-ovulation (Yanagimachi & Chang, 1964) and were killed 15 to 17 hr after the injection of HCG for the recovery of eggs. The medium used for hamster eggs consisted of an equal volume of Tyrode's solution and heated hamster blood serum (at 56° C for 50 to 60 min). The hamster eggs were similarly treated with hyaluronidase and washed as in the case of the mouse eggs. Hamster sperm suspension was prepared by placing spermatozoa obtained either from the cauda epididymidis or from the uterus of a hamster mated 3 to 4 hr previously in 1.5 ml Tyrode's solution. The procedure for *in-vitro* fertilization was the same as for mouse eggs. After incubation for 5 to 7 hr, the eggs were taken out, mounted on a slide and examined. They were then fixed with neutral formalin and stained for the assessment of fertilization (Miyamoto & Chang, 1972).

From the results presented in Table 1, it can be seen that, after the removal of cumulus oophorus and the corona radiata cells, the denuded mouse eggs can be penetrated *in vitro* by epididymal spermatozoa and the hamster eggs can also be penetrated *in vitro* either by epididymal spermatozoa or by uterine spermatozoa. However, the proportion of penetrated eggs (89 versus 68%) and that of eggs undergoing fertilization (85 versus 63%) were significantly higher ($P < 0.01$) in the mouse eggs with follicular cells than in the denuded mouse eggs. This confirms the results reported by Cross & Brinster (1970) who found a relatively higher proportion of fertilized eggs amongst those with follicular cells than amongst the denuded eggs (90 versus 46%). Whether the relatively low proportion of penetrated eggs amongst the denuded mouse eggs is due to the lack of energy sources supplied by the follicular cells (Biggers, Whittingham & Donahue, 1967; Donahue & Stern, 1968), to lack of progesterone produced by follicular cells for the induction of the acrosome reaction (Edwards, Steptoe & Purdy, 1970) or to the rapid loss of fertilizability in the mouse eggs after the removal of follicular cells as in the rabbit eggs (Chang & Bedford, 1962), is difficult to say at present. Since a relatively high proportion of mouse eggs with follicular cells was fertilized in chemically defined media (Cross & Brinster, 1970; and present study) while a low proportion of mouse eggs with follicular cells was fertilized by preincubated spermatozoa in the presence of bovine follicular fluid (Iwamatsu & Chang, 1970), it may be assumed that the capacitation of spermatozoa and the fertilization of mouse eggs *in vitro* are more dependent on the medium used than on the presence or absence of follicular cells. There was no significant difference in the proportions of hamster eggs fertilized, whether they were denuded or not (87 to 88% versus 84 to 91%) and whether epididymal or uterine spermatozoa were used (87 to 91% versus 84 to 88%). This confirms the statement by Yanagimachi (1969) that cumulus oophorus

TABLE 1
FERTILIZATION OF MOUSE AND HAMSTER EGGS *in vitro* AFTER REMOVAL OF FOLLICULAR CELLS

Species	Spermatozoa	Eggs with (+) or without (-) follicular cells	No. of experiments	Total no. of eggs examined	Total no. of penetrated eggs	% eggs penetrated (range)	Total no. of eggs undergoing fertilization	% eggs undergoing fertilization (range)
Mouse	Epididymal	+	7	289	257	89 (70 to 98)	246	85 (60 to 97)
		-	8	296	201	68 (48 to 84)	186	63 (40 to 79)
Hamster	Epididymal	+	8	208	189	91 (77 to 100)	183	88 (65 to 100)
		-	9	214	186	87 (68 to 97)	181	85 (59 to 96)
	Uterine	+	5	140	117	84 (59 to 97)	110	79 (59 to 97)
		-	5	151	133	88 (71 to 100)	124	82 (62 to 100)

and corona radiata cells have no power to induce the capacitation of hamster epididymal spermatozoa. Since the absence of follicular cells does not hinder the penetration of spermatozoa *in vitro* in the rabbit (Chang *et al.*, 1971; Fraser *et al.*, 1971) and in the hamster (the present study), while the presence of follicular cells appears to facilitate the penetration of mouse eggs (Cross & Brinster, 1970; present study), the possibility of a species difference concerning the rôle of follicular cells for fertilization cannot be excluded.

This work was supported by grants from the U.S. Public Health Service (GM 14370) and the Ford Foundation.

REFERENCES

- AUSTIN, C. R. (1961) *The mammalian egg*. Blackwell Scientific Publications, Oxford.
- BEDFORD, J. M. & CHANG, M. C. (1962) Fertilization of rabbit ova *in vitro*. *Nature, Lond.* **193**, 898.
- BIGGERS, J. D., WHITTINGHAM, D. G. & DONAHUE, R. P. (1967) The pattern of energy metabolism in the mouse oocyte and zygote. *Proc. natn. Acad. Sci. U.S.A.* **58**, 560.
- CHANG, M. C. & BEDFORD, J. M. (1962) Fertilizability of rabbit ova after removal of the corona radiata. *Fert. Steril.* **13**, 421.
- CHANG, M. C., HANADA, A. & HUNT, D. M. (1971) Fertilization of denuded rabbit eggs *in vitro* by sperm recovered from the uterus or vagina. *Nature, Lond.* **232**, 343.
- CROSS, P. C. & BRINSTER, R. L. (1970) *In vitro* development of mouse oocytes. *Biol. Reprod.* **3**, 298.
- DONAHUE, R. P. & STERN, S. (1968) Follicular cell support of oocyte maturation: production of pyruvate *in vitro*. *J. Reprod. Fert.* **17**, 395.
- EDWARDS, R. G. & GATES, A. H. (1959) Timing of the stages of maturation division, ovulation, fertilization and the first cleavage of adult mice treated with gonadotrophin. *J. Endocr.* **18**, 292.
- EDWARDS, S., STEPTOE, P. & PURDY, J. (1970) Fertilization and cleavage *in vitro* of preovulator human oocytes. *Nature, Lond.* **227**, 1307.
- FRASER, L. R., DANDEKAR, P. V. & VAIDYA, R. A. (1971) *In vitro* fertilization of tubal rabbit ova partially or totally denuded of follicular cells. *Biol. Reprod.* **4**, 229.
- IWAMATSU, T. & CHANG, M. C. (1970) Further investigation of capacitation of sperm and fertilization of mouse eggs *in vitro*. *J. exp. Zool.* **175**, 271.
- MIYAMOTO, H. & CHANG, M. C. (1972) The importance of serum albumin and metabolic intermediates for capacitation of spermatozoa and fertilization of mouse eggs *in vitro*. *J. Reprod. Fert.* (In press).
- SWYER, G. (1947) A tubal factor concerned in the denudation of rabbit ova. *Nature, Lond.* **159**, 873.
- TOYODA, Y., YOKOYAMA, M. & HOSI, T. (1971) Studies on the fertilization of mouse eggs *in vitro*. I. *In vitro* fertilization of eggs by fresh epididymal sperm. *Jap. J. Anim. Reprod.* **16**, 147.
- YANAGIMACHI, R. (1969) *In vitro* capacitation of hamster spermatozoa by follicular fluid. *J. Reprod. Fert.* **18**, 275.
- YANAGIMACHI, R. & CHANG, M. C. (1964) *In vitro* fertilization of golden hamster ova. *J. exp. Zool.* **156**, 361.