FERTILIZATION OF CHINESE HAMSTER EGGS IN VITRO

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Fertilization of eggs *in vitro* has been described in three mammalian species—the rabbit (Dauzier, Thibault & Wintenberger, 1954; Chang, 1959; Suzuki & Mastroianni, 1965; Brackett & Williams, 1965), Syrian hamster (Yanagimachi & Chang, 1964; Barros & Austin, 1967) and laboratory mouse (Whittingham, 1968). Penetration by spermatozoa and pronucleus formation have also been observed in rat eggs, following dissolution of the zona pellucida by chymotrypsin (Toyoda & Chang, 1968).

In preliminary attempts to fertilize eggs of the Chinese hamster, *Cricetulus griseus*, *in vitro*, no success was achieved using fresh spermatozoa, but 10% of eggs (8 of 80) were penetrated by spermatozoa which had previously been incubated with Syrian hamster cumulus clot. This treatment was used in a larger study in August 1968, as described here.

Immature and maturing Chinese hamsters, 5 to 8 weeks old, were injected intraperitoneally with 5 i.u. PMSG (Equinex, Ayerst) followed 48 to 52 hr later by 10 i.u. HCG (A.P.L., Ayerst). Tubal eggs were recovered from females killed 15½ to 16½ hr after HCG and, from earlier studies, these were considered to be not more than 2 hr post-ovulation. Follicular eggs were obtained from females where ovulation had not begun or only a few follicles ruptured at this time, and from one female killed 14½ hr after HCG. Syrian hamster cumulus clot was used for all pre-treatments of spermatozoa. It was obtained from superovulated mature females (Greenwald, 1962) killed on Day 4 of the cycle, 14½ to 15½ hr after injection of 10 i.u. HCG; about half the cumulus from one oviduct was used for each preparation.

Chinese hamster spermatozoa were stripped from the vas deferens into mineral oil and added to 1 ml of either Tyrode's solution or TC medium Ham F10 containing 1% bovine serum albumin. A drop of the suspension, 1 to 2 times the volume of the cumulus and containing approximately 4 to 6 million spermatozoa/ml, was then added to the Chinese hamster eggs or to Syrian hamster material for pre-incubation. Eggs for culture with the pre-treated spermatozoa were recovered from Chinese hamster females 2 to 3 hr later and added to the pre-incubation mixture. They were easily distinguished from the Syrian hamster eggs by their smaller size, as well as a different appearance of the

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cytoplasm, polar bodies and chromatin. All incubations were carried out at 37 to 38° C under mineral oil equilibrated with 5% CO₂ in air. Chinese hamster eggs were examined after 5½ to 8 hr in contact with spermatozoa.

The highest number of penetrated eggs was observed after incubation of tubal eggs with pre-treated spermatozoa in F10+albumin (Table 1). Fertilizing spermatozoa were present on 45% of eggs in the ten successful cultures. Eggs undergoing normal fertilization had a second polar body, enlarged perivitelline space and two pronuclei. None of the eggs showed a sperm flagellum in the cytoplasm. Usually the anterior end of the sperm midpiece was attached to the vitelline membrane while the sperm tail and part of the midpiece lay outside the zona pellucida (Pl. 1, Fig. 1). Seven activated eggs, all from culture in Tyrode's solution, showed no trace of a male pronucleus or sperm structure. Fifteen other eggs, with no flagellum attached to them, contained what appeared to be both male and female pronuclei (Pl. 1, Fig. 2) and had probably been penetrated by spermatozoa. Incomplete entry of the fertilizing spermatozoon and absence of the sperm tail from cleaving eggs had been observed after fertilization *in vivo*, although in a smaller proportion of eggs (Pickworth, Yerganian & Chang, 1968).

Two or more 'subnuclei' replaced the normal female pronucleus in some of the activated eggs (Pl. 1, Fig. 3), suggesting that scattering of chromosomes had occurred. Fertilizing spermatozoa were present on sixteen of the twenty eggs showing this abnormality, and male pronuclear appearance was normal. Pronucleus formation was well advanced in most fertilized eggs. Enlarging sperm heads were found in six monospermic eggs, three of which contained large female pronuclei and therefore showed an abnormal sequence of development. One polyspermic egg was found after culture in Tyrode's solution, and thirteen (23% of activated eggs) in F10+albumin. All but one polyspermic egg came from cultures with pre-incubated spermatozoa.

No Syrian hamster eggs were penetrated by Chinese hamster spermatozoa, nor were spermatozoa adhering to the zona pellucida after incubation.

Syrian hamster cumulus was used for pre-treatment of spermatozoa in this study to conserve the more limited supply of Chinese hamsters for the main incubations. Excellent motility was observed with the Syrian hamster material. Incubation with tubal or follicular contents from other species has been shown to induce capacitation of Syrian hamster spermatozoa *in vitro*, resulting in more rapid penetration of eggs added to the culture after this period (Barros,

EXPLANATION OF PLATE 1

Phase contrast. Figs. 1 and 2: fresh specimens, compressed; ×268. Figs. 3 and 4: fixed, stained with lacmoid; ×201.

FIG. 1. Egg of normal penetrated appearance showing second polar body, male (left) and female (right) pronuclei; anterior end of sperm flagellum attached to the vitelline membrane.

FIG. 2. Egg showing second polar body and pronuclei comparable with Fig. 1; no sperm flagellum attached at the time of examination.

FIG. 3. Penetrated egg showing sperm midpiece and male pronucleus (a) but containing two small female pronuclei adjacent to the second polar body (b).

FIG. 4. Egg showing a partially enlarged sperm head (a), second polar body (a, upper right) and large female pronucleus (b).

PLATE 1

(Facing p. 372)

TABLE 1
FERTILIZATION OF CHINESE HAMSTER EGGS IN VITRO; EFFECT OF PRE-INCUBATION OF SPERMATOOZA

Medium	Source of eggs	Sperm. pre-inc. (hr)	No. of cultures		No. of eggs				
			Total	With penetrated eggs	Total	Activated*	Fertilizing sperm. present	Fertilized? no sperm. flagellum	No male component
F10 with 1% b.s.a.	Oviduct	—	9	1	97	6	6	—	—
	Oviduct	2 to 3	21	10	192	51	42 (22%)	9	—
	Follicles	2 to 3	3	—	25	—	—	—	—
Tyrode's solution	Oviduct	2 to 3	17	11	168	29	17 (10%)	6	6
	Follicles	2 to 3	10	—	71	1	—	—	1

* By spermatozoa or other stimulus.

1968; Yanagimachi, 1969). In the Chinese hamster there is probably an interval of 2 hr or more between ovulation and sperm penetration *in vivo* (Pickworth *et al.*, 1968). No timing of sperm entry was attempted in the present series *in vitro*. However, it seems likely that important changes in the spermatozoa were initiated during pre-incubation, and the difference between results from fresh and pre-incubated spermatozoa may have arisen from a short fertilizable life of the eggs in culture.

Both fresh and pre-incubated spermatozoa showed good motility in culture with Chinese hamster tubal eggs. By contrast, the activity of spermatozoa with Chinese hamster follicular contents was severely reduced, and restricted to a narrow border zone around the preparation. The effect of follicular material from untreated mature females was not investigated.

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