Fertilization of rat oocytes cultured *in vitro* from various stages of maturation

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In a previous study, it was shown that nuclear maturation from the germinal vesicle stage to division of the first polar body in rat oocytes could be completed in a defined medium, but that the proportion of oocytes penetrated and cleaved was low (Niwa & Chang, 1975), perhaps because of some fault in the maturation process. The present study was to determine the number of oocytes penetrated and cleaved *in vitro* when they were cultured at various stages during maturation *in vivo*.

White rats of the Wistar strain were used. Immature females (21–25 days old) were induced to superovulate by a s.c. injection of 10 i.u. PMSG (Serotropin: Teikoku-Zoki Co.) 48 hr before an i.p. injection of 10 i.u. HCG (Puberogen: Sankyo Co.). Oocytes were recovered at various times after the HCG injection according to the procedures described by Niwa & Chang (1975). Nuclear maturation of rat oocytes *in vitro* and *in vivo* is completed by 13 hr after injection of HCG (Niwa & Chang, 1975). Oocytes recovered at and 2, 4, 6, 8 or 10 hr after the HCG injection were therefore cultured for 13, 11, 9, 7, 5 and 3 hr, respectively, in a CO₂ incubator (5% CO₂ in air at 37°C) before exposure to preincubated spermatozoa. Two or three females were used in each group and 30–40 oocytes from each female were cultured in 0.4 ml medium covered with paraffin oil (Nakarai Chemical Co.) in a tissue-culture dish (35 × 11 mm: Toyoshima Seisakusho). Spermatozoa were obtained from the cauda epididymidis of adult rats (200–300 days old) and preincubated at a concentration of 0.3–0.6 × 10⁶ spermatozoa/ml for 5–5½ hr in a CO₂ incubator. The composition and the method of preparation of the medium for sperm suspension, culture of the oocytes and insemination were as described by Toyoda & Chang (1974).

After incubation for 12, 24 or 35½–36 hr, the oocytes were mounted *in toto*, fixed, stained with 0.25% lacmoid in 45% acetic acid (Chang, 1952) and then examined for evidence of fertilization. The oocytes containing an enlarged sperm head(s) or a male pronucleus(ei), or which had cleaved with a fertilizing sperm tail(s) in the vitellus, were considered to be undergoing fertilization.

Table 1. Fertilization *in vitro* of rat oocytes matured in culture from various stages of maturation *in vivo*

Time of collection of oocytes (hr after HCG)	Period of culture (hr)	Time of examination (hr after insemination)	No. of oocytes examined	No. of oocytes undergoing fertilization				
				Total	With abnormality*	With male and female pronuclei	Meta- to telophase of 1st cleavage	2-Celled
0	13	12	74	72	28	44	0	0
0	13	24	77	51	23	28	0	0
0	13	35½–36	80	60	38	3	7	12
2	11	35½–36	90	72	46	5	8	13
4	9	35½–36	96	78	48	10	3	17
6	7	35½–36	101	90	50	20	3	17
8	5	35½–36	110	100	31	19	3	47
10	3	35½–36	60	58	4	6	0	48
14	0	35½–36	71	71	0	2	1	68

* Oocytes with enlarged or absent sperm head, only one pronucleus, fragmented or dispersed nuclei.

From the results presented in Table 1, it can be seen that the proportion of oocytes undergoing fertilization was very high (66–100%) in those recovered at any stages of maturation and cultured

in vitro. Although 66–97% of the oocytes recovered after culture for 13 hr were undergoing fertilization 12–36 hr after insemination, the number undergoing normal fertilization was reduced (37%) with increasing duration of incubation.

Of the oocytes recovered 2–10 hr after HCG injection, 80–97% were undergoing fertilization, which was, however, abnormal in most oocytes (56–64%) recovered 2–6 hr after HCG injection. Only 18–22% of oocytes matured *in vitro* from 2–6 hr after injection of HCG cleaved, but 47–83% of those matured *in vitro* from 8–10 hr after HCG injection did so. All the oocytes fully matured *in vivo* and ovulated were undergoing normal fertilization and 96% were at the two-cell stage.

In the previous study, only 18–22% of oocytes from CD strain rats were penetrated and undergoing fertilization after maturation *in vitro* (Niwa & Chang, 1975). The improved fertilization rate for oocytes of rats in the present study is unlikely to be due to the strain difference and is most probably related to technical improvement. However, the improved rate of cleavage for oocytes recovered 8–10 hr after HCG injection is related to the stage of maturation reached *in vivo*, since first anaphase is reached by 8 hr after injection of HCG (Niwa & Chang, 1975). It therefore appears that the final stage of fertilization was inhibited if the oocytes were cultured in a defined medium from the stages before anaphase I, although sperm penetration of oocytes matured *in vitro* from any stages of maturation was possible.

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