THE MATURATION *IN VITRO* OF MOUSE OOCYTES COLLECTED AT VARIOUS TIMES AFTER AN INJECTION OF PMSG

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An injection of 5 i.u. PMSG to 21-day-old mice greatly increases the number of large follicles in the ovary but does not cause any morphological change in the oocyte (Merchant & Chang, 1971; Cross, 1973). If an injection of HCG is given 48 hr later, the oocytes pass rapidly through the remaining stages of meiosis and reach the second metaphase in 12 to 14 hr (Edwards & Gates, 1959). Oocytes which are mechanically released from the follicles and cultured in an appropriate medium without any hormonal stimulation reach second metaphase in 14 to 17 hr (Donahue, 1968; Cross & Brinster, 1970). The present report deals with the fate of oocytes left in the follicles and their developmental potential at various times after an injection of PMSG.

Mouse oocytes have been matured *in vitro* by many workers (Edwards, 1965; Biggers, Whittingham & Donahue, 1967; Donahue, 1968; Cross & Brinster, 1970; Cross, 1973) and the method followed here is similar to that of Donahue (1968). Oocytes were obtained at various times (Table 1) from Swiss mice that had been injected intraperitoneally with 5 i.u. PMSG (NIAMD-PMSG-1) at 21 days of age. Each ovary was placed in a watch-glass containing medium (Krebs–Ringer salt solution containing 0.25 mM-pyruvate, 1 mg/ml glucose and 3 mg/ml bovine serum albumin; Donahue, 1968) under oil and the follicles were punctured to release the oocytes. Before culture, a few oocytes from each animal were examined under a phase-contrast microscope for signs of nuclear maturation or other gross changes in the cytoplasm. The remaining oocytes were pooled for each time period and were washed once and cultured in drops of medium under oil at 37°C in 5% CO₂ in air. After 18 hr culture, the oocytes were transferred to glass slides, compressed gently under a coverslip and examined under a phase-contrast microscope. The slides were then fixed in 10% neutral formalin overnight and stained with aceto-carmin. The stages of maturation, as shown in Table 1, were determined in accordance with the photographs of Donahue (1968).

Preliminary observations showed that no spontaneous nuclear maturation occurred up to 120 hr; all the oocytes observed had intact germinal vesicles. Spontaneous ovulation, as judged by the number of corpora lutea, was less than 1%; mature females normally ovulate within 24 hr (Edwards & Fowler, 1960) or even within 14 hr (Kaufman & Whittingham, 1972) of an injection of PMSG.

Oocytes collected between 72 and 120 hr showed an increasing tendency to

become detached from the surrounding cumulus cells and most of such oocytes which had lost their cumulus cells had large granules in the cytoplasm, indicating that degeneration had started. Most or all of the oocytes collected 120 hr after PMSG treatment had such granules in the cytoplasm.

Table 1. Maturation *in vitro* of mouse oocytes collected at various times after an injection of PMSG

Time after PMSG injection (hr)	No. of animals	No. of oocytes	Condition of oocytes				
			Grossly degenerated*	Germinal vesicle†	Metaphase I	Anaphase and telophase	Metaphase II
48	4	196	3.1% (6)	5.1% (10)	7.1% (14)	2.0% (4)	82.7% (162)
60	4	219	5.0% (11)	4.1% (9)	9.6% (21)	1.4% (3)	79.8% (175)
72	4	184	15.8% (29)	7.6% (14)	29.3% (54)	1.6% (3)	45.7% (84)
84	4	243	13.2% (32)	13.2% (32)	25.1% (61)	—	48.5% (118)
96	4	181	18.8% (34)	40.9% (74)	17.1% (31)	—	23.2% (42)
108	4	208	19.7% (41)	56.7% (118)	9.6% (20)	1.0% (2)	13.0% (27)
120	4	232	34.5% (80)	65.5% (152)	—	—	—

* The stage of nuclear maturation could not be determined.

† A few oocytes at the condensed chromatin stage were also included.

The results of the experiments are shown in Table 1. The highest percentage (82.7%) of oocyte nuclear maturation was obtained when oocytes were collected 48 hr after the injection of PMSG. This percentage is similar to that of 88% obtained by Donahue (1968) and of 90.7% by Cross & Brinster (1970). There was a substantial reduction in the number of oocytes collected after 72 hr which completed nuclear maturation in culture. The number of degenerated oocytes increased only marginally but those arrested at metaphase I increased considerably. There was no increase in the number of oocytes which remained at the germinal vesicle stage. Thus, the reduction in the percentage of oocytes which completed maturation was not due to failure to enter the division cycle but mainly because of the arrest at metaphase I. According to Donahue (1968), there are two stages at which significant numbers of oocytes are arrested other than that which occurs naturally at metaphase II; they are the germinal vesicle (5 to 10%) and metaphase I (4%) stages.

The oocytes cultured 96 hr after the PMSG injection showed a different trend. The reduction in the percentage which completed maturation was mainly because of the failure of a substantial number of oocytes to enter the division cycle rather than to arrest at metaphase I. The percentage of grossly degenerated oocytes had also increased considerably. After 108 hr, only 13% completed the development while those retaining intact germinal vesicles increased from 40.9% to 56.7%. Even those oocytes which completed nuclear

maturation did not look normal since all had large granules in the cytoplasm. None of the oocytes collected 120 hr after PMSG treatment proceeded beyond the germinal vesicle stage.

The loss of the developmental potential of the oocytes left in the follicles after PMSG injection is a gradual process which occurs between 72 and 96 hr later. Although no quantitative estimation has been made, it is obvious that cumulus cells must play a very important rôle in this process. Removal of cumulus cells before culture has been shown to reduce the number of oocytes maturing *in vitro* (Kennedy & Donahue, 1969; Cross & Brinster, 1970) although Cross (1973) could not find any effect.

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