The effect of cold shock on mouse oocyte maturation *in vitro*

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Summary. Maintenance of mouse ovaries in ice-cold medium for 1, 2 or 4 h before removing the oocytes did not alter the incidence of spontaneous maturation or increase the number of oocytes degenerating, fragmenting or forming 2 equal blastomeres in culture.

Introduction

In a study on rabbit oocyte maturation *in vitro* (Smith, Tyler & Erickson, 1978) one excised ovary was kept in ice-cold culture medium while follicles from the other were individually dissected and measured before culture of the oocytes. Although this is a standard method for maintaining ovaries for biochemical determinations and follicle cell culture (for example, see Erickson, Challis & Ryan, 1974; Ledwitz-Rigby *et al.*, 1977; Salomon, Yanovsky, Mintz, Amir & Lindner, 1977), it is possible that such treatment could damage the oocytes generally and/or cause fragmentation or parthenogenetic cleavage. Cold shock is known to cause parthenogenetic development of ovulated oocytes (see Graham, 1974, for a review). We have therefore examined the effect of cold shock on excised mouse ovaries which were cooled for various periods before removal of the oocytes for culture.

Materials and Methods

Random-bred Swiss (CD-1) mice, 6–10 weeks of age, were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Massachusetts, U.S.A.). The mice were killed by cervical dislocation and the ovaries were excised immediately. The ovaries, 4 from 4 different mice for each treatment, were placed in an embryological watchglass containing 3 ml culture medium at 37°C under paraffin oil or into a test tube (Falcon Plastics) containing cold culture medium (3 ml) at 4°C and maintained on ice for 1, 2 or 4 h. Chilled ovaries were then placed in watchglasses containing medium at 37°C for dissection.

Oocytes were released into the watchglass by puncturing antral follicles and those with the normal appearance of the germinal vesicle stage were washed once and cultured as described by Brinster (1963). Oocytes were cultured at 37°C for 16–20 h in an atmosphere of 5% CO₂ in air and 100% humidity. The medium used throughout was a standard mouse ovum culture medium (Biggers, Whitten & Whittingham, 1971) modified slightly to contain bovine serum albumin (Sigma Chemical Company) (4 mg/ml) and sodium pyruvate (0.055 mg/ml).

After culture, oocytes were classified with the aid of a dissecting microscope (×100) as mature (polar body extruded), meiotically inactive (germinal vesicle retained), or as having resumed but not completed the first meiotic division (no nucleus or polar body present). Oocytes showing vacuolization, cytolysis, necrosis, fragmentation, 2 blastomeres equal in size, or loss of spherical shape were scored as degenerate. To visualize chromosomal configurations, cultured oocytes were placed on microscope slides, fixed in acetic acid: absolute ethanol (3:1 v/v) and stained with Giemsa (Harleco) at a 1:50 dilution with distilled water (for details see Rafferty, 1970). For each of the various treatments 20–30 oocytes from 2 of the total number of experiments were fixed, stained, examined and photographed with a Zeiss Photomicroscope II. Since inspection showed the results of each treatment

to be identical in all replicates, the data were pooled. The calculations described by Maxwell (1961) for 2×4 contingency tables were used to obtain overall χ^2 values for the different categories of oocytes presented in Table 1.

Table 1. The effect of cold shock on mouse oocyte maturation *in vitro*

Treatment	No. of exps.	No. of ovaries	Total no. cultured	Oocytes			
				No. not degenerating after 16–20 h (%)*	No. with PB (%)†	No. with GVB (%)†	No. with GV (%)†
Control	4	16	205	163 (80)	146 (90)	16 (9)	1 (1)
Cold for 1 h	4	16	185	155 (84)	144 (93)	10 (6)	1 (1)
Cold for 2 h	3	12	163	131 (80)	124 (95)	7 (5)	—
Cold for 4 h	3	12	154	126 (82)	113 (90)	12 (9)	1 (1)
				$\chi^2_3 = 1.303\dagger$	$\chi^2_3 = 3.425\dagger$	$\chi^2_3 = 2.923\dagger$	

PB, polar body; GV, germinal vesicle; GVB, germinal vesicle breakdown.

* Of oocytes cultured.

† Of oocytes which did not degenerate.

‡ $P > 0.3$.

Results and Discussion

Table 1 indicates that the incubation of mouse ovaries in cold medium for as long as 4 h had no effect on the spontaneous maturation of oocytes in culture and that increasing the duration of cold treatment did not increase the proportion of oocytes degenerating in culture. Furthermore, cold shock did not induce fragmentation or abnormal cleavage (scored as degenerate) and the incidence of these forms among the degenerating oocytes was very low. The cytological studies showed that all oocytes examined at the polar body stage contained eccentric metaphase chromosome configurations and chromosomes dispersed within the polar bodies (Pl. 1, Fig. 1). Some metaphase II oocytes showed dividing polar bodies containing a spindle and telophase chromosomes (Pl. 1, Fig. 3). The oocytes scored as having polar bodies had therefore truly undergone the first maturation division rather than extruding a cytoplasmic fragment. The few oocytes examined which had undergone germinal vesicle breakdown but not extruded a polar body were at the metaphase I stage (Pl. 1, Fig. 2). We conclude that oocytes from chilled ovaries are not affected in terms of their ability to undergo spontaneous maturation *in vitro*, and are not induced to undergo fragmentation or parthenogenetic cleavage. Future studies correlating oocyte maturation *in vitro* with various aspects of follicular biochemistry can therefore be expected to provide valid results when ovarian tissue is maintained on ice before processing.

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PLATE I

Fig. 1. Control mouse oocyte cultured for 16–20 h, fixed and stained with Giemsa. The oocyte has reached metaphase of the 2nd meiotic division. Note the polar body containing chromosomes. $\times 660$.

Fig. 2. Oocyte from mouse ovary maintained for 2 h on ice. The oocyte has resumed but not completed the 1st meiotic division in culture. Note the prominent metaphase I chromosomes. $\times 540$.

Fig. 3. Oocyte from a mouse ovary chilled for 4 h. The oocyte has reached metaphase II in culture. $\times 610$.

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