IN VITRO DEVELOPMENT OF GROWING OOCYTES FROM FETAL AND EARLY POSTNATAL MOUSE OVARIES

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Shortly after birth in the mouse ovary a group of oocytes initiate and progress synchronously through growth and increase their diameter from about 12 to 80 µm during the first 3 weeks. It is thought that these oocyte acquire the meiotic competence to resume meiosis at 14-16 days according to an autonomous program (Canipari et al., 1984) and external signals provided by somatic cells (Chesnel et al., 1994). Previous studies have shown that growing mouse oocytes can be obtained from long term **in vitro** culture of fetal ovary fragments (for references, see De Felici and Dolci, 1987). The objective of the present study was to verify whether such culture system could be employed with early post natal ovaries and would allow the development of follicles and the acquisition of meiotic competence by oocytes.

MATERIALS AND METHODS

Ovaries obtained from 13.5-17.5 days post coitum (dpc) mouse CD-1 embryos or 1-10 days post partum (dpp) mice, were torn into small fragments (0,5-1 mm) and placed into culture at 37°C in 5% CO_2 in air. The culture medium was modified E-MEM supplemented with 5 % horse serum and 2.5 % fetal calf serum (De Felici and Dolci, 1991). Histological analysis of ovaries and ovary fragments was performed in semi-thin (1 µm) sections of Epon embedded samples prepared following standard methods. Oocytes were obtained from ovaries by puncturing with 25-gauge needles. At the end of the culture period, oocytes were isolated from the fragments by pipetting through a pulled glass pipette and allowed to mature, as revealed by germinal vesicle break down (GVBD), for 15-18 hr in 100 µl drops of M16 medium under paraffin oil. In some experiments the meiotic status of GV oocytes was evaluated after Hoechst 33258 staining according to the degree of heterochromatin association with the nucleolus using the classification of Mattson and Albertini (1990).

RESULTS

Follicles of approximately 30 and 100 µm diameter (type 3b, type 4 and type 5a, Pedersen and Peters, 1968), were present in the ovaries of 6 and 8-10 day-old mice, respectively. The largest uncultured oocytes isolated from ovaries of these ages have a diameter <50 µm and remained arrested in germinal vesicle (GV) stage in culture, indicating that they were meiotically incompetent. The oocytes from 6, 8 and 10 dpp fragments underwent extensive growth *in vitro* and reached approximately the same maximum diameter (60-70 µm) in 16, 14 and 10 days of culture, respectively. Some of the ooctes were enclosed in preantral large follicles (diameter about 150 µm, type 5b, Fig. 1A). There was no significant difference in the percentage of oocytes with diameter ≥65 µm to undergo GVBD; 50-60 % were GVDB-competent in all groups. Oocytes ≤60 µm remained arrested at the GV stage.

Fragments of ovaries from mice younger than 6 days of age or from embryos were grown *in vitro* to the same total chronological age (22 days) as those described above. However, although large number of healthy growing oocytes were obtained only a few of them were able to reach a diameter ≥60 µm (Fig. 2). These oocytes were unable to undergo GVBD and were often enclosed by 1-2 layers of follicle cell-like somatic cells (Fig. 1B). Most of them were found to contain stage III and IV GV.

CONCLUSIONS

The results demonstrate that the *in vitro* culture system of ovary fragments allows oocytes from 6-day-old mice onward to become GVBD-competent. Follicle development up to large preantral stage is also possible. The reason for the failure of oocytes from younger mice and fetal ovaries to gain meiotic competence remains to be elucidated. The possibility that it may depend by lack of external signals which should be provided by the surrounding somatic cells is currently under study.

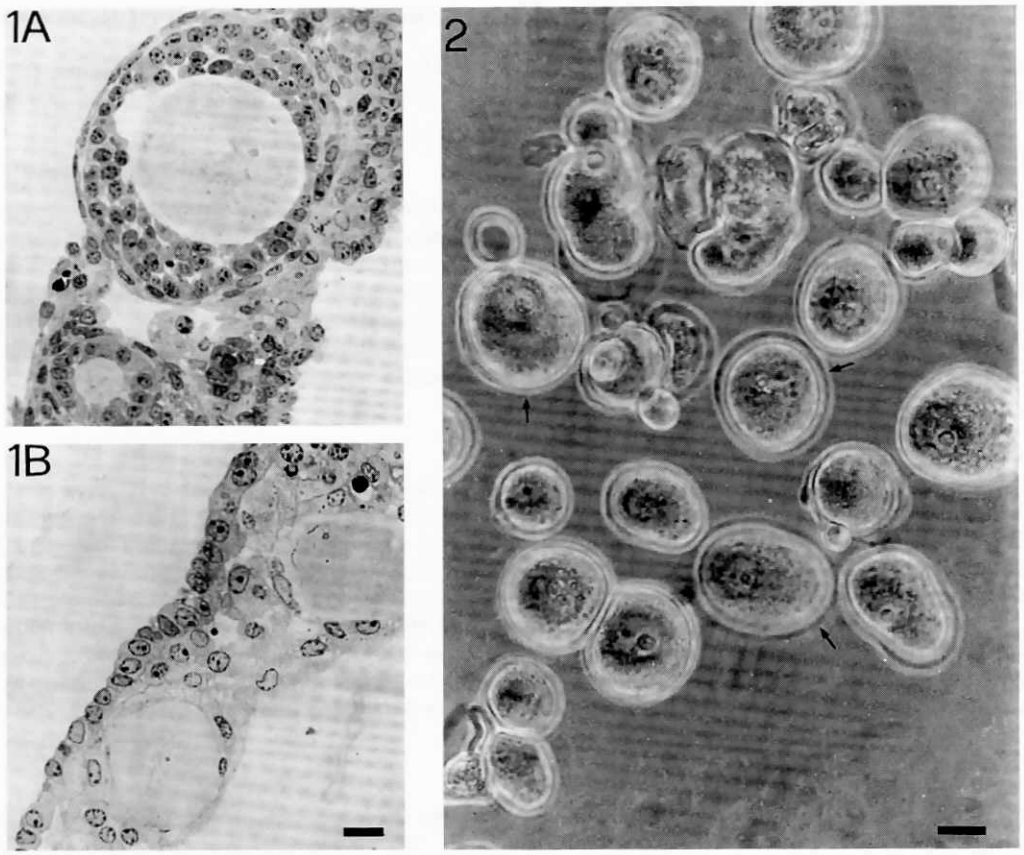

Fig. 1. A) Type 5b follicle grown in culture of ovary fragments of 7-day-old mice; oocytes enclosed by 1 layer of follicle cell-like somatic cells obtained from culture of fragments of fetal ovary. Bar approximately 20 μm.

Fig. 2. Oocytes isolated from fragments of 16.5 dpc fetal ovaries after 3 weeks of culture; Note the presence of zona pellucida (arrows) and diffrent oocyte sizes. Bar approximately 20 μ.

References

Canipari, R., Palombi, F., Riminicci, M., and Mangia, F. (1984). early programming of maturation comptence in mouse oogenesis. Dev. Biol. 102: 519-524.
Chesnel, F., Wigglesworth, K., and Eppig, J. (1994). Acquisition of meiotic competence by denuded mouse oocytes: partecipation of somatic cell product (s) and cAMP. Dev. Biol. 161: 285-295.
De Felici, M., and Dolci, S. (1991). Leukemia inhibitory factor sustains the survival of mouse primordial germ cells cultured on TM4 feeder layers. Dev. Biol. 147: 281-284.
De Felici, M., and Dolci, S. (1987). Cellular interactions of mouse fetal germ cells in in vitro systems. In: Current Topics in Developmental Biology, (McLaren, A., Siracusa, G., eds), Vol. 23, pp. 147-162, Academic Press.
Mattson B. A., Albertini D. F. (1990). Oogenesis: chromatin and microtubule dynamics during meiotic prophase. Mol. Repr. Dev. 25: 374-383.
Pedersen, T., and Peters, H. (1968). Proposal for a classification of oocytes and follicles in the mouse ovary. J. Repro. Fert. 17: 555-557.