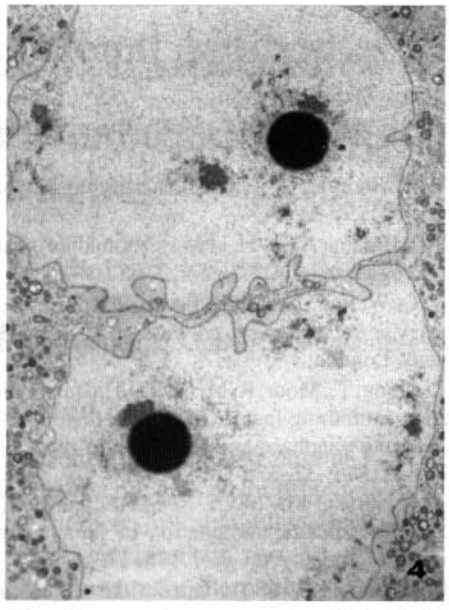

Fig. 4. Giant immature oocyte produced by fusion of two normal oocytes. Germinal vesicles are closely apposed, nucleoli are clearly visible. EM, magnification 5000x.

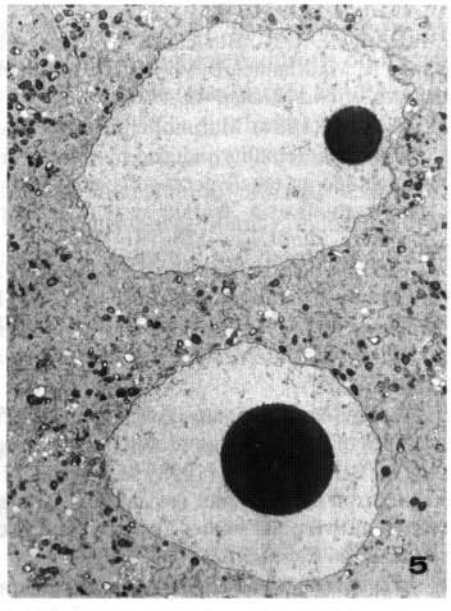

Fig. 5. When the above oocytes are cultured *in vitro* and then parthenogenetically activated, two polar bodies are extruded and in the cytoplasm two pronuclei are visible. EM, magnification 5000x.

medium containing colchicine and fixed for cytogenetic analysis. Although the quality of the preparation obtained was rather poor, the four cells had reached metaphase and contained apparently 60 chromosomes. The occurrence of three pronuclei and two second polar bodies in giant oocytes is, to our knowledge, unique.

In order to analyze the phenomenon of giant embryos in more detail, giant mouse oocytes were produced by polyethylene-glycol-induced fusion of two immature oocytes, which were thereafter cultured *in vitro* (Table 1).

Table 1. Production of giant mouse oocytes by fusion and their culture in vitro

Total no. of oocytes	Stage of maturation after culture (%) (12 h)		
	Without PB	With one PB	With two PBs
116	44 (38)	36 (31)	36 (31)

PB: first polar body

In total we have produced 116 giant cells containing two germinal vesicles (Fig. 4). When cultured *in vitro*, 44 (44/116, 38%) cells did not extrude the first polar body and they almost exclusively contained a single metaphase plate (39/44). The oocytes of the second category extruded two first polar bodies and they also contained two metaphase II plates (36/116, 31%), whilst in the last group only one polar body was evident and these cells contained only a single metaphase II plate (36/116, 31%). When parthenogenetically activated with ethanol, the oocytes of the first group were largely fragmented. On the other hand, 70% (14/20) of oocytes in the second group extruded two second polar bodies and contained two pronuclei (Fig. 5). The remaining oocytes were typically fragmented. The oocytes of the last group extruded a single polar body and contained one pronucleus (9/14, 64%). Five oocytes were fragmented. The oocytes examined under the electron microscope (EM), i.e. those just after the induction of fusion or at 6 h after activation, were morphologicaly normal.

Discussion

In our laboratory, we have occasionally found large embryos, but we did not study their origin and developmental potential consistently. For example, we reported the incidence of one giant two-cell embryo in a mouse female treated with a low dose of mitomycin C at the preovulatory stage of oogenesis (Jacquet and Pire, 1984). Development of this giant embryo was quite normal in culture and it reached the blastocyst stage at the same time as other embryos. This giant blastocyst was transferred for a few hours into medium containing colchicine, and prepared according to the technique of Tarkowski (1966). Cytogenetic analysis confirmed its triploid nature. Since that time, we have also found a few giant two-cell embryos in the mouse not treated with chemicals.

The frequency of giant eggs has not been recorded in our laboratory, but it is generally very low. According to Austin and Braden (1954), it would be about 0.1% in mice and rats. Austin and Braden (1954) also reported the presence of two female pronuclei in one rat giant zygote, and Austin and Bishop (1957) described also one rat giant zygote showing two second metaphase spindles. It seems that two types of giant ova, mononuclear and binuclear, exist. In a detailed study performed on Chinese hamster oocytes and zygotes, Funaki and Mikamo (1980) found that all giant one-cell

zygotes had only a single female pronucleus. In rabbits and Chinese hamsters, the incidence is apparently higher, i.e. about 0.5% (Austin and Braden, 1954; Funaki and Mikamo, 1980). Furthermore, in the Chinese hamster, all digynic triploid zygotes that were analyzed had developed from giant oocytes, showing that in this species the giant oocytes seem to contribute considerably to spontaneous triploidy (Funaki and Mikamo, 1980). As suggested by these authors, the nuclear-cytoplasmic ratio of giant triploid eggs may be more favorable, at least for preimplantation development, than that of any other type of triploidy. Giant diploid oocytes could be an important source of human digynic triploids, in spite of the prevailing view that suppression of the first polar body is the predominant cause. As we mentioned above, the incidence of giant oocytes is very low. The induced cell-to-cell fusion may clarify the problem of giant oocytes (Gulyas, et al., 1984; Nogues et al., 1995). However, in this case two immature oocytes must be fused and thereafter cultured *in vitro*. The fusion of two metaphase II oocytes will exclude the possibility of the chromosome movement and their dislocation during maturation. We believe that our observations clearly show that the use of abnormally sized oocytes, for example in human IVF and related technologies, is extremely risky and in most cases may result in abnormal chromosomal configurations of embryos produced. Moreover, the transparency of human eggs is much lower comparing to the mouse oocytes and eggs, and thus to achieve perfect evaluation would be even more complicated. It has been reported recently that binuclear oocytes can be also found in humans. When cultured *in vitro*, they mature up to the second metaphase with two polar bodies and two haploid sets of chromosomes (Rosenbusch and Schneider, 1998). Thus, we believe that the use of oocytes that are evidently larger comparing to normal eggs is rather risky. This does, however, not exclude those cases when normally sized oocytes contain two germinal vesicles or some abnormalities related to the suppression of the first or second polar body expulsion.

References

Austin, C. R. (1961) *The Mammalian Egg*. Blackwell Scientific Publications, Oxford.

Austin, C. R., Amoroso, E. C. (1959) The mammalian egg. *Endeavour* **18**, 130-143.

Austin, C. R., Bishop, M. W. H. (1957) Fertilization in mammals. *Biol. Rev.* **32**, 296-349.

Austin, C. R., Braden, A. W. H. (1954) Anomalies in rat, mouse and rabbit eggs. *Aust. J. Biol. Sci.* **7**, 537-542.

Austin, C. R., Walton, A. (1960) Fertilization. In: *Marshall's Physiology of Reproduction*, ed. Parkes, A. S., pp. 310-416, Longmans, London.

Fulka, Jr., J., Jung, T., Moor, R. M. (1992) The fall of biological maturation promoting factor (MPF) and histone H1 kinase activity during anaphase and telophase in mouse oocytes. *Mol. Reprod. Dev.* **32**, 378-382.

Fulka, Jr., J., Horska, M., Moor, R. M., Fulka, J., Kanka, J. (1996) Oocyte-specific modulation of female pronuclear development in mice. *Dev. Biol.* **178**, 1-12.

Funaki, K., Mikamo, K. (1980) Giant diploid oocytes as a cause of digynic triploidy in mammals. *Cytogenet. Cell Genet.* **28**, 158-168.

Gulyas, B. J., Wood, M., Whittingham, D. G. (1984) Fusion of oocytes and development of oocyte fusion products in the mouse. *Dev. Biol.* **101**, 246-250.

Jacobs, P. A., Angell, R. R., Buchanan, I. M., Hassold, T. J., Matsuyama, A. M., Manuel, B. (1978) The origin of human triploids. *Ann. Human Genet.* **42**, 49-57.

Jacquet, P., Pire, P. (1984) Morphological and cytogenetic studies of dominant lethality induced by mitomycin C and cyclophosphamide in female germ cells: the use of Robertsonian translocations as a "marker system" to identify the zygote pronuclei. *Mutat. Res.* **128**, 181-194.

Jonasson, J., Therkelsen, A. J., Lauritsen, J. G., Lindsten, J. (1972) Origin of triploidy in human abortuses. *Hereditas* **71**, 168-171.

Kajii, T., Niikawa, N. (1977) Origin of triploidy and tetraploidy in man: 11 cases with chromosome markers. *Cytogenet. Cell Genet.* **18**, 109-125.

Nogues, C., Ponsa, M., Egozcue, J., Vidal, F. (1995) Cytogenetic studies of oocyte fusion products. *Zygote* **3**, 27-29.

Rosenbusch, B., Schneider, M. (1998) Maturation of a binuclear oocyte from the germinal vesicle stage to metaphase II: formation of two polar bodies and two haploid chromosome sets. *Human Reprod.* **13**, 1653-1655.

Tarkowski, A. K. (1966) An air-drying method for chromosome preparations from mouse eggs. *Cytogenetics* **5**, 394-400.