Short Communication

Preimplantation Development of Giant Triploid Zygotes in the Mouse

(oocytes / fusion / triploid zygotes)

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Abstract. Giant oocytes or two-cell embryos have been reported in various mammalian species. They may arise during multiplication of oogonia, after fusion of two oogonia or, more probably, when nuclear division is not accompanied by cytoplasmic division. The ultimate fate of these giant embryos is not well known. In our laboratory, giant two-cell mouse embryos have been occasionally observed. Recently, we observed two giant one-cell zygotes in the same species. Both showed two female pronuclei and one male pronucleus, as well as two second polar bodies localized at opposite poles of the embryo. These two giant zygotes showed normal viability and developmental capacity. Their triploid nature was confirmed by cytogenetic analysis. In order to study this interesting phenomenon in more detail, we produced giant oocytes containing two germinal vesicles by cell fusion and cultured them *in vitro*. About one third of them extruded two first polar bodies; in the second group only one polar body was observed, whilst the last group was without polar bodies. When parthenogenetically activated, the consistent answer analogical to that observed in "in vivo" oocytes was only observed when oocytes with two polar bodies were activated. The implication for IVF technologies is discussed.

Triploid embryos can originate in different ways. Thus, diandric embryos arise from the fertilization of an oocyte by two spermatozoa, while digynic embryos may have various origins: (a) fertilization of an egg derived from a binuclear (germinal vesicle, GV) oocyte; (b) suppression of the formation of either the first or the second polar body; (c) fertilization of giant oocytes containing one large metaphase II (two joined metaphases II) or two individual metaphase II chromosome sets.

The suppression of polar body extrusion was proposed as the main causal mechanism of digynic triploidy (Jonasson et al., 1972; Kajii and Nikawa, 1977; Jacobs et al., 1978), while the giant oocytes have been generally ignored.

Giant oocytes were described in rabbit, rat, mouse (Austin and Braden, 1954; Austin and Walton, 1960), cotton-rat (Austin and Amoroso, 1959) and Chinese hamster (Funaki and Mikamo, 1980). They may arise during multiplication of oogonia when nuclear division is not accompanied by cytoplasmic division, or from fusion of two oogonia; the former possibility being more probable (Austin, 1961). Giant two-cell embryos have also been reported in mammals and, according to Austin (1961), their ultimate fate is unknown. However, the discovery of two four-cell Chinese hamster embryos as well as of one rat morula and one rat blastocyst has been reported more recently (Funaki and Mikamo, 1980), showing that, at least in these species, giant eggs are able to sustain normal preimplantation development.

Material and Methods

Giant embryos were obtained from Balb/c female mice which were induced to superovulate by i.p. injection of 5 I.U. pregnant mare serum gonadotropin (PMSG) followed after 48 h by 5 I.U. human chorium gonadotropin (hCG) (both hormones were obtained from Intervet, Folligon, International B. V., Boxmeer, the Netherlands). Females were caged with males immediately after hCG injection and checked on the following morning for the presence of vaginal plugs. Positive females were killed 20 h post hCG (one-cell embryos) or 42 h post hCG (two-cell embryos). One-cell-staged embryos were released from the ampulla into Yamada's culture medium containing 1 mg/ml hyaluronidase, whilst two-cell-staged embryos were collected by flushing the oviducts with hyaluronidase-free medium. After rinsing, embryos were cultured to the expected interval of the first or third cleavage (37°C, 5% CO_2 in air) before being

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Abbreviations: dbcAMP – dibutyryl cyclic AMP, hCG – human chorionic gonadotropin, IVF – *in vitro* fertilization, PMSG – pregnant mare serum gonadotropin.

transferred into medium containing 100 ng/ml colchicine, and the metaphase spreads were prepared according to the technique of Tarkowski (1966).

Oocyte-oocyte fusion

This procedure was described in detail by Fulka, Jr., et al. (1992). Briefly, the oocytes were isolated from large antral follicles of PMSG-treated females and manipulated in M2 medium containing bovine serum albumin (BSA) (4 mg/ml) and dibutyryl cyclic AMP (dbcAMP) (150 μg/ml). Cumulus cells were removed by extensive pipetting. Zona pellucida was dissolved by pronase treatment (0.5% in PBS). Pairs of oocytes were agglutinated in phytohemagglutinin solution (200 μg/ml) and thereafter incubated in polyethylene-glycol solution for 45 s. After an extensive washing, the oocytes were cultured in M199 containing BSA (4 mg/ml), gentamicin (25 μg/ml) and Na-pyruvate (0.2 mM). The oocytes were evaluated after 12 h in culture (orcein staining, phase contrast) or activated by two electric pulses (1 kV, 100 μs) before further culture for another 6 h. To exclude the possibility of the eventual negative effect of different treatments, freshly fused eggs and some activated giant cells were fixed in 2.5% glutaraldehyde and 0.6% paraformaldehyde in 0.06 cacodylate buffer, and examined under the JEOL JEM CXII 100 electron microscope (EM) (Fulka, Jr., et al., 1996). All chemicals were purchased from Sigma (Sigma-Aldrich, Prague, Czech Republic).

Results

Mouse giant oocytes, zygotes and cleaving embryos can be occasionally isolated either from ovaries or from oviducts. Recently, we have found two giant one-cell embryos, and they showed a very particular feature: they both contained two female pronuclei and one male pronucleus, as well as two second polar bodies located at opposite poles of the embryo (Fig. 1). One of these two zygotes was immediately transferred into medium supplemented with colchicine, and the examination of chromosomes of the first mitotic metaphase revealed the presence of three separated haploid chromosome sets. The second zygote was maintained in normal culture medium and its development occurred in the same way as in normal embryos, up to the four-cell stage, where anomalies in the size of blastomeres as well as in their contact between each other were detected (Figs. 2 and 3). The embryo was transferred for a few hours into fresh

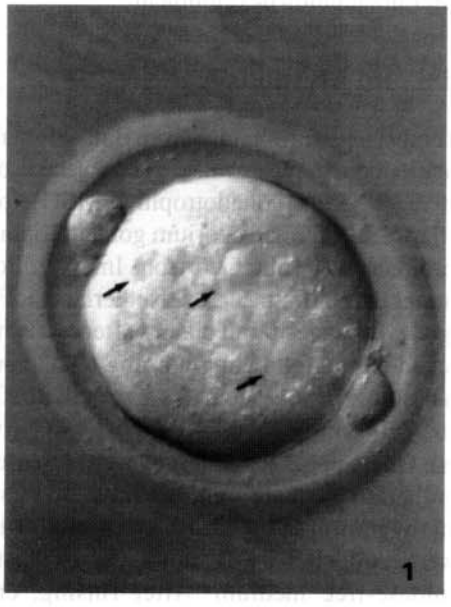

Fig. 1. Giant one-cell zygote showing two second polar bodies and three pronuclei (arrows). Magnification 600x.

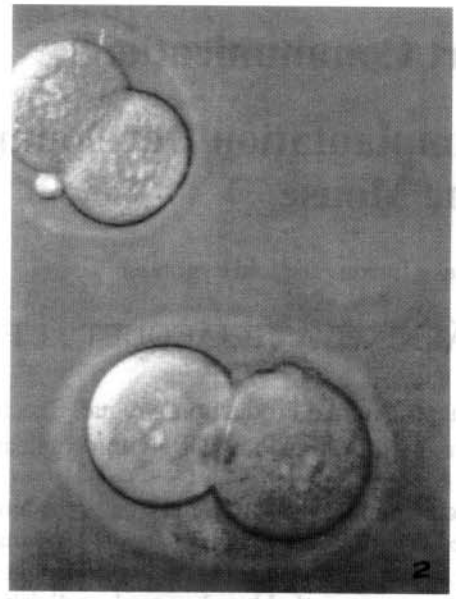

Fig. 2. Giant and normal embryos at the two-cell stage. Magnification 600x.

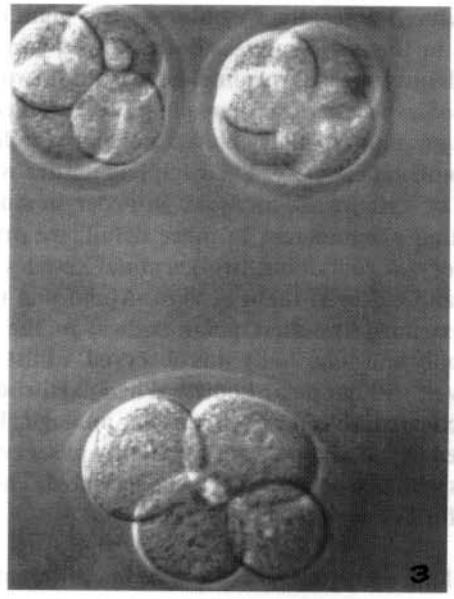

Fig. 3. Giant and normal embryos developed to the four-cell stage in culture. Magnification 600x.