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Ultrastructural analysis of mouse blastocysts cultured in vitro under different oxygen concentrations

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During the last years, embryo development in vitro was studied in different culture conditions and oxygen (O2) concentrations. Higher developmental blastocyst (Bl) rates were obtained with embryos cultured under a physiological O2 tension (5%), respect to those cultured under atmospheric O2 conditions (20%) [1], but the mechanisms responsible for this, during the preimplantation embryogenesis remain unclear. This study aimed to evaluate the effect of physiologic or atmospheric O2 tension on the ultrastructure of mouse Bl. In vivo, Bl were flushed out of the uterus after natural fertilization (controls). In vitro fertilization (IVF) was performed using KSOM medium and Bl were then cultured under an O2 tension of 5% and 20% for 5 days [2]. After collection, Bl were washed in PBS, fixed in 2.5% glutaraldehyde/PBS and subjected to standard preparative for transmission electron microscopy (TEM) [3]. Morphometric analysis was done on ultrathin sections. The cells of the trophoblast (TE) formed a single, continuous layer of flattened cuboidal cells. In all the group, both inner cell mass (ICM) and TE showed the presence of extensive regions of less dense, granular cytoplasm. Microvilli were distributed on the apical surface, projecting toward the zona pellucida. Nuclei were delimited by integral nuclear membranes and contained dispersed euchromatin with patches of heterochromatin. Cells in mitotic division, with well-defined chromosomes, were occasionally identified. Isolated mitochondria and vacuoles were numerous. Mitochondria, in both ICM and TE, had an elongated and tubular shape, delimited by a double electron-dense membrane. The numerical density of mitochondria was lower in vitro than in vivo, especially under 20% O2. Interestingly, this alteration in density in vitro was associated to an increased vacuolization, both at 5% and 20% O2. These results indicated that alterations in the Bl ultrastructure, especially at 20% O2, can be connected to the O2 concentration and can motivate the higher developmental rates obtained at lower O2 concentration.

References

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Key words

Blastocyst, IVF, oxygen concentration, TEM, ultrastructure.

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