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Embryoid body in vitro formation from human amniotic fluid stem cells (AFSCs): an ultrastructural study

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Amniotic fluid stem cells (AFSCs) harbour the potential to differentiate into cells of any of the three germ layers and to form embryoid bodies (EBs) without inducing teratoma formation (De Coppi et al., 2007; Antonucci et al., 2012). However, no previous findings have been reported regarding embryoid body in vitro development and ultrastructural organization. Thus, this was the aim of our study. Amniotic fluid samples were obtained from women undergoing amniocentesis for prenatal diagnosis at 16-19 weeks of pregnancy after written informed consent and the local ethical committee approval. Human AFSCs were cultured up to the 8th passage and analysed with RT-PCR for the expression of pluripotency markers. Some cellular pools were cultured in suspension in uncoated Petri dishes (hanging drop method) to obtain EB formation. After 5 days of culture, the appearance of EBs of different size was observed with phase contrast microscopy and monitored up to 10-15 days of culture. In parallel, EB standard embedding in paraffin for light microscopy or in epoxy resin for transmission electron microscopy was performed. RT-PCR analysis revealed the presence of classical markers of pluripotency (OCT4, NANOG, SOX2) in AFSCs at the 2th-8th passage, whereas specific markers of the three embryonic germ layers were detected in EB specimens. Morphological assays of three-dimensional aggregates demonstrated the presence of solid structures only at the beginning of the culture whereas signs of apoptotic cell death accompanied by the secretion of an amorphous substance were soon detected. These features were preliminary to the development, at later culture time intervals, of an inner hollow cavity surrounded by a crown of flat cells displaying a number of electron dense granules and highly resembling trophoblastic cells.

References

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Key words
Embryoid body, amniotic fluid stem cells, ultrastructural characterization.