IJAE
Vol. 121, n. 1 (Supplement): 169, 2016
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

# The amniotic membrane from the human placenta contains different subregions with different morpho-functional features

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Human Amniotic Membrane (AM) has been attributed anti-inflammatory, anti-angiogenic, anti-fibrotic and anti-microbial effects (1-2). A number of clinical studies have used AM for applications in regenerative medicine, but it has yet to be elucidated whether the different areas of the AM have the same plasticity and differentiation potential. Thus, the aim of our study was to map the AM from normal human placenta. AMs were obtained from 24 healthy women undergoing caesarean section. Four areas were considered with respect to the umbilical cord: the first area, closer to the umbilical cord, was named the central area; the second, in the middle, was the intermediate area; the third was the peripheral area (all the three areas were part of the chorion frondosum) and the fourth, the reflected area, corresponded to the chorion laeve. Our results demonstrate the presence of a multi-layered epithelium in different areas of AM, except for the intermediate area, with a number of budding or detaching cells as well as of apoptotic cells, especially in the central area. By means of immune-histochemistry and quantitative analysis with MetaMorph analysis software, we evaluated the in situ expression of different proteins observing that the peripheral area has the highest OCT-4, c-KIT and SOX-2 expression, well known indicators of pluripotency, and the highest levels of CREB and p-CREB, well known for its key role in proliferation, differentiation and apoptosis, whereas mainly the central area expresses high levels of $\alpha$-fetoprotein, suggesting that it could be more prone to hepatic differentiation. Interestingly, amniotic epithelial cells (AECs) from the peripheral area display the highest quantity of granules in the cytoplasm, as shown in electron microscopy preparations. In addition, in vitro cultured AECs derived from the reflected area demonstrate the highest clonogenic capacity, whereas those from the intermediate area show the highest osteogenic potential, when induced to osteogenic differentiation. Our study is the first to propose the morpho-functional mapping of the AM as a useful tool to identify areas with different stemness properties and plasticity. This could increase the efficiency of human AM application within a therapeutic context.

This work was supported by funds granted from MIUR to Prof. Roberta Di Pietro and Lucia Centurione and by Fondazione Poliambulanza-Istituto Ospedaliero, Brescia, Italy to Prof. Ornella Parolini.

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Keywords

Human term placenta; amniotic membrane; amniotic epithelial cells; placenta stem cells.