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Entosis Occurs in Human Embryo Implantation.

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Abstract:

Introduction: The incapability of the embryo to clear the luminal epithelial cells to reach the stroma could lead to implantation failures. Although apoptosis is the proposed mechanism for the removal of endometrial epithelial cells (EEC), recent research points out that a non-apoptotic cell-in-cell invasion mechanism named entosis would lead to the clearance of the EEC lining. Emerging evidences in murine models strongly suggest that entosis could drive the first stages of human implantation. Thus, it is proposed that trophoblast cells of human blastocysts could internalize EEC, throughout the activation of Rho-ROCK pathway in EEC. The main objective of this research was to confirm the occurrence of entosis in human embryo implantation and the implication of Rho-ROCK pathway in this mechanism.

Methods: Primary human EEC obtained from endometrial biopsies of egg donors attending our clinic were cocultured in vitro with human trophoblastic spheroids (JAR) and human aneuploid blastocysts (in suspension and adherent conditions) to asses internalization of EEC inside trophoblastic cells. EEC and trophoblast cells were differentially stained using Cell-Tracker fluorescent probes. Internalization phenomena was evaluated by confocal microscopy analysis and 3D reconstruction models. To study the role of Rho-ROCK pathway on internalization, EEC were treated with 10uM of ROCK inhibitor (Y-27632). Inhibited and control stained EEC were cocultured with JAR spheroids. After 24 hours, JAR spheroids were isolated. Effect of ROCK inhibition was assessed by counting fluorescent signal of EEC and checking gene expression of ROCK1, Vimentin and hCG by qPCR on isolated JAR spheroids.

Results: Confocal microscopy analysis and 3D reconstruction models confirmed entosis of EEC by trophoblast cells, meaning that EEC were internalized by JAR spheroids and trophoblast cells of human blastocysts. After the analysis of fluorescent signals associated to EEC in isolated JAR spheroids from cocultures in suspension, we found that inhibition of ROCK in EEC lead to a decrease in EEC internalization. Expression of vimentin in isolated JAR spheroids was also decreased, indicating lower number of EEC internalized.

Conclusion: Our data support that entosis occurs in human embryo implantation and Rho-ROCK pathway may be implicated in this process. Up to our knowledge, our study is the first to confirm the occurrence of entosis in human embryo implantation. Support: PFIS (PI/00009), APOTIP/2018/010, Miguel Servet Contract (CPII18/00002) and ISCIII FIS project (PI17/00931).

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Category (Complete): 11.2 - Basic Reproductive Biology: Implantation, Endometrium
Presentation Preference (Complete): Either Oral or Poster
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