

Non-invasive test for embryo competent selection by quantification of cell-free nucleic acids in embryo culture micro drop

Abstract

Objective

In order to increase the success rates of IVF cycles, improved methods for embryo selection to produce a baby are required. We determined the cell-free nucleic acids (cfNA) levels in human embryo spent culture media and we evaluated their possible use as biomarkers for embryo competent selection.

Design

Human fertilized oocytes were individually cultured from zygote to blastocyst stage. A total of 60 spent culture media were collected on day 3 (6-8 cells) and day 5/6 blastocyst stage.

Materials and Methods

MicroRNAs (miRNAs) such as MiR-21 and Let-7b were extracted from drops with the QIAamp kit and quantified by RT-qPCR using TaqMan technology. Cell-free DNA (cfDNA) was quantified using Bio-Rad Supermix SYBR Green. Statistical analyses defined relationship between nucleic acid content and embryo outcome.

Results

We demonstrate that the embryo culture medium samples, during in vitro early embryo development, contained embryonic cfDNAs and miRNAs. The concentration values of cfDNA are lower in the culture medium in which emerge top quality embryo compared to no top ($p < 0.05$). In the embryos that reached good blastocyst quality and leading to pregnancy, the variation in the

cfDNA concentration between day 3 and day 5/6 is significantly decreased significantly and drastically of 88% (22.16 ng/ml and 2.75 ng/ml at day 3 and day 5/6 respectively). This variation is very low between day 3 and day 5/6 in the no good blastocyst quality (6.46 ng/ml and 3.78 ng/ml at day 3 and day 5/6 respectively, 41% decrease). Relate to the expression of the miRNAs which identified in spent media and was correlated with embryo outcome, MiR-21 and Let-7b were more highly concentrated in both day-3 and day-5 media samples when compared with day-0 samples (cycle threshold=33 and 34 versus 39.5, respectively).

Conclusion

Under in vitro IVF/ICSI conditions, changes in the nucleic acids levels in the embryo culture medium on day 3 and day 5/6 predict the embryo quality and may be used as a new potential biomarker for selecting top quality embryos. Our data strongly open the possibility to develop a new quick and low-cost test for the selection of the embryos viable with the highest implantation potential.