

P-170: Non-invasive detection of metabolically impaired euploid blastocysts with low implantation potential.

Authors: Cabello-Pinedo S, Abdulla H, Seth-Smith M, Escriba M, Crespo J, Horcajadas JA, Munné S.

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1. Abstract title:

Non-invasive detection of metabolically impaired euploid blastocysts with low implantation potential.

2. Study question:

As not all euploid embryos implant, is it possible to identify non-implanting euploid blastocysts from their spent media metabolomics profile?

3. Summary answer:

Analysis of the spent media metabolomics profile could predict about 30% implantation failure of euploid embryos, coinciding with the upper limit of PGT-A success.

4. What is known already:

Embryo implantation potential and euploidy rates decrease with advancing maternal age. Preimplantation Genetic Testing (PGT) is been used in routine clinical practice to avoid the transfer of aneuploid embryos with minimal chance of resulting in a viable pregnancy. However, even the best PGT-A published results do not exceed 70% ongoing pregnancy rates. Little is understood of the causes of failure of euploid embryos to implant, and methods to discriminate between viable euploid embryos and non-viable euploid embryos that will not result in a successful pregnancy have not been developed yet.

5. Study design, size, duration:

This study includes spent media samples collected before trophectoderm biopsy from embryos that were later analyzed by PGT and transferred. To develop the algorithm, this study used a dataset that included 37 samples collected using Vitrolife media that were classified as pregnancy (P) or non-pregnancy (NP) according to embryo implantation outcome after transfer. The algorithm was then tested on spent media of 42 euploid blastocysts.

6. Participants/materials, setting, methods:

For the training subset (n=37), patients undergoing infertility treatments were included. For the validation stage (n=42), patients whose embryos were analyzed through PGT were recruited. In both cases, spent media samples (20-40 μ l) were collected after incubation between days 3 and 5 and just before biopsy. Before the analysis, samples were diluted with distilled water and ultrafiltered to remove molecules >3KDa and run through a UPLC- Fusion Orbitrap MS/MS system to determine metabolite concentration.

7. Main results and the role of chance:

Samples from the training subset were processed, detecting more than 5,550 metabolites in spent media samples. Several statistical techniques were applied to reduce this large number of metabolites to the most informative ones for pregnancy outcome. This led to the definition of a Vitrolife specific MPI (Metabolite Pregnancy Index), which has proved to be highly predictive of both P and NP embryos. This technique was tested in a completely different data subset showing an ability to identify 78% of P samples and 61% of NP samples.

For the validation part of the study, metabolic profile of culture media coming from euploid embryos (n=42) was studied in order to assess whether the implantation failure of some euploid embryos may be due to metabolic impairment. A 30.95% of euploid embryos in the validation subset showed a metabolomic profile predicting poor implantation potential despite being euploid.

Although further research is necessary, these results open the possibility of applying metabolomics to differentiate, within euploid embryos, those that are viable from the ones that will not result in a successful pregnancy.

8. Limitations, reasons for caution:

The study was retrospective but a prospective clinical trial is underway. In addition, all the samples were collected using the same culture media and the metabolomic algorithm is media specific. The study needs to be validated in multiple culture media and test its outcome comparing it with more clinical data.

9. Wider implications of the findings:

This study represents an initial step in the development of non-invasive methods based on metabolomics approach, that allow identification of viable embryos, not only based on ploidy status. The optimization of embryo selection techniques, without altering embryology protocols, may result in an improvement in pregnancy outcomes in women undergoing ART.

10. Study funding/competing interest(s):

Intramural.

11. Trial registration number: not applicable.