P-205: Non-invasive metabolomics analysis of spent culture media predicts embryo viability

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1. **Abstract title:**

Non-invasive metabolomics analysis of spent culture media predicts embryo viability.

2. **Study question:**

Are there metabolites in the spent media that can act as biomarkers to predict embryo implantation?

3. **Summary answer:**

A set of culture-media specific biomarkers were identified that highly predicted implantation potential in several fertility centers.

4. **What is known already:**

A critical step in an IVF cycle is the selection of the best embryo to be transferred, since treatment success strongly depends on this choice. The standard of care tool for embryo selection has been morphokinetics criteria, which has low predictability to ascertain implantation potential. During the last 20 years, the alternative has been PGT (Preimplantation Genetic Testing), an invasive method that requires embryo biopsy. For this reason, novel non-invasive embryo screening methods are required for the optimization of embryo selection such as AI-morphokinetics, metabolomics and non-invasive PGT.

5. **Study design, size, duration:**

This retrospective study was done on 197 samples collected in three different clinics, using two different culture media (Sage n=129, and Vitrolife n=68), over a period of two years. Eighty-two of the spent media come from embryos that implanted (P) and 115 from non-implanted embryos (NP). Embryos were obtained and cultured using routine IVF practices. 20 - 40 µL of spent media were collected after incubation between day 3 and 5.

6. **Participants/materials, setting, methods:**

Patients undergoing infertility treatment in 3 clinics were included in this study. Spent media were frozen at -20ºC after collection. Metabolites were extracted from the spent media samples (20 µL) using a ultrafiltering approach to remove molecules >3KDa and then run in a UPLC-Fusion

Orbitrap MS/MS system which determined the abundance of metabolites in each sample. Different statistical techniques were applied to reduce the huge number of metabolites found to those most informative ones.

7. **Main results and the role of chance:**

More than 5,550 metabolites were identified and measured.

A first analysis was performed with 129 samples of Sage media (30 from pregnant (P) and 99 from non-pregnant cycles (NP)). A Sage-specific MPI (Metabolite Pregnancy Index) was built with the most informative metabolites to determine pregnancy potential. The non-supervised analysis (not telling the software which sample is pregnant and which is not) identified 100% for P and 63% of for NP. Using supervised analysis, 100% of P samples were identified and 81% of NP samples.

A second analysis was performed with 74 samples of Vitrolife media coming from two different clinics. With the data from the first clinic samples (n=37: 20 P and 17 NP), a Vitrolife-specific MPI (Metabolite Pregnancy Index) was built with the most informative metabolites to determine pregnancy potential. For the first clinic, the non-supervised analysis showed an ability to predict embryo viability of 100% for P and 88% for NP samples. A blind analysis with a third batch of Vitrolife samples from a second center (n=31: 19P+12NP) showed 78% of P samples were identified and 61% of NP samples.

8. **Limitations, reasons for caution:**

The study was retrospective, but for one of the centers it was blinded and still yielded high implantation predictability. Nevertheless, a prospective clinical trial is underway. A small limitation of the test is that each culture media has a different subset of informative biomarkers. Other media are being validated.

9. **Wider implications of the findings:**

This metabolomics tests is non-invasive, inexpensive, does not required any change in embryology protocol, and can be combined with NI-PGT and or morphokinetics, having the potential in itself or in combination with other methods to significantly improve embryo selection without causing embryo damage.

10. **Study funding/competing interest(s):**

Overture Life.

11. **Trial registration number:** Non applicable.