P-144: A metabolomics approach to identify aneuploid embryos.

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

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1. **Abstract title:**

A metabolomics approach to identify aneuploid embryos.

2. **Study question:**

Can euploid embryos be identified in a non-invasive manner by measuring the concentration of specific metabolomic biomarkers in spent culture media?

3. **Summary answer:**

Specific metabolites in embryo spent culture media are correlated with euploidy.

4. **What is known already:**

Implantation potential and euploidy decrease with advancing maternal age. Preimplantation Genetic Testing (PGT) has been used to avoid the transfer of aneuploid embryos, which will not result in a successful pregnancy. However, embryo biopsy is invasive and operator-dependent resulting in wide variability in pregnancy rates depending on the center. Although DNA can be detected in spent media, correlation with trophectoderm biopsy vary widely between studies. Recently, metabolomics studies on spent culture media have shown great potential to ascertain implantation potential. In this study we aim to determine if euploidy can also be ascertained by non-invasive metabolomics analysis of spent media.

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

This study includes spent media samples collected before trophectoderm biopsy from embryos that were later analyzed by PGT using NGS. Among them, 60 out of 116 were classified by PGT as aneuploid, 42 as euploid and 14 as mosaic. Spent media samples were blindly analyzed to find metabolites differentially abundant in the different embryo groups, leading to the definition of a method that allows to select euploid embryos for transfer without damaging viable embryos,

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

Patients undergoing PGT were included in this study. Spent media samples were collected just before biopsy, ultrafiltered to remove molecules >3KDa and run through a UPLC- Fusion Orbitrap MS/MS system at 500,000 FWHM mass resolution. Different machine learning techniques were

applied to reduce the huge number of metabolites to a limited number of informative biomarkers for euploidy.

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

The analysis of mass spectrometry (MS) led to the identification of a sub-set of biomarkers whose concentration differed between aneuploid, euploid and mosaic embryos. The concentration of this subset of biomarkers was increased in aneuploid embryos and reduced in euploid and mosaic embryos. Fifty-four out of the 60 aneuploid embryos (90%) showed a reduced concentration metabolites profile specific of aneuploidy and different from euploidy.

The challenge of this study relies on the wide range of types of aneuploidy found in blastocysts, from monosomies to trisomies for each possible chromosome, alone or in combination of abnormalities. Each aneuploidy may produce different metabolic alterations, affecting very diverse and multiple biochemical pathways in different ways. Given the diversity of the aneuploid group, the sub-set of identified biomarkers are probably definitory of a viable metabolic activity and can be hardly found by chance.

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

The study was retrospective but a prospective clinical trial is underway. In addition, all the samples were collected in the same clinic using the same culture media. The test needs to be validated in multiple clinics and culture media.

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

90% aneuploidy ascertainment without biopsy is as high as the highest reported NI-PGT results. Further advantages of metabolomics is that is cheaper than NI-PGT by NGS and does not require change in embryology protocols. Combined with markers for embryo viability other than euploidy might yield further improvements on embryo selection.

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

Intramural.

11. **Trial registration number:** not applicable.