P-582 High level of concordance between invasive and non-invasive preimplantation genetic testing for aneuploidies (niPGT-A) at day5 and day6-7

A. Biricik[1], V. Bianchi[2], F. Lecciso[1], M. Surdo[1], M. Manno[2], V. Saino[1], E. Cotroneo[1], F. Fiorentino[1], F. Spinella[1]

[1]"Eurofins Genoma", Preimplantation Genetic Diagnosis, Roma, Italy ; [2]Future for Family, Policlinico Città di Udine, Udine, Italy

Study question: To explore ploidy concordance between invasive and non-invasive PGTA (niPGT-A) at different embryo culture time.

Summary answer: High level (>84%) of concordance rate for ploidy and sex, sensitivity (>88%), and specificity (76%) were obtained for both day6/7 samples and day5 samples.

What is known already: The analysis of embryo cell free DNA (cfDNA) that are released into culture media during in vitro embryo development has the potential to evaluate embryo ploidy status. However, obtaining sufficient quality and quantity of cfDNA is essential to achieve interpretable results for niPGT-A. More culture time is expected to be directly proportional to the release of more cfDNA. But embryo culture time is limited due to in-vitro embryo survival potential. Therefore, it is important to estimate the duration of the culture that will provide the maximum cfDNA that can be obtained without adversely affecting the development of the embryo.

Study design, size, duration: A total of 105 spent culture media (SCM) from day5-day7 blastocyst stage embryos have been included in this cohort study. The cfDNA of SCM samples were amplified and analyzed for niPGT-A by NGS analysis. The SCM samples were divided into 2 subgroups according the embryo culture hours (Day5 and Day6/7 group). The DNA concentration, informativity and euploidy results have then been compared with their corresponding embryos after trophectoderm biopsy (TE) and PGT-A analysis by NGS

Participants/materials, setting, methods: Embryos cultured until Day3 washed and cultured again in 20µl fresh culture media until embryo biopsy on Day5, 6, or 7. After biopsy SCM samples were immediately collected in PCR tubes and conserved at -20°C until whole genome amplification by MALBAC® (Yicon Genomics). The TE and SCM samples were analyzed by next-generation sequencing (NGS) using Illumina MiSeq® System. NGS data analysis has been done by Bluefuse Multi Software 4.5 (Illumina) for SCM and TE samples

Main results and the role of chance: Only the SCM samples which have an embryo with a conclusive result were included in this cohort (n=105). Overall 97.1% (102/105) of SCM samples gave a successful DNA amplification with a concentration ranging 32.4-128.5ng/µl. Non-informative (NI) results including a chaotic profile (>5 chromosome aneuploidies) were observed in 17 samples, so 83.3%(85/102) of SCM samples were informative for NGS data analysis. Ploidy concordance rate with the corresponding TE biopsies (euploid vs euploid, aneuploid vs aneuploid) was 84.7% (72/85). Sensitivity and specificity were 92,8% and 76,7%, respectively with no significant difference for all parameters for day 6/7 samples compared with day 5 samples. The false-negative rate was 3.5% (3/85), and false-positive rate was 11.7% (10/85).

Limitations, reasons for caution: The sample size is relatively small. Larger prospective studies are needed. As this is a single-center study, the impact of the variations in embryo culture conditions can be underestimated. Maternal DNA contamination risk cannot be revealed in SCM, therefore the use of molecular markers would increase the reliability.

Wider implications of the findings: Non-invasive analysis of embryo cfDNA analyzed in spent culture media demonstrates high concordance with TE biopsy results in both early and late culture time. A non-invasive approach for aneuploidy screening offers important advantages such as avoiding invasive embryo biopsy and decreased cost, potentially increasing accessibility for a wider patient population.

Trial registration number: Not applicable

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