Keywords: Array-CGH, Embryo biopsy, Preimplantation, Mosaicism, NGS, blastocyst, cleavage stage embryos.

TITLE (25):

Concordance rates of day-3 (D3) and trophectoderm biopsies (D5) using Next Generation Sequencing (NGS)

List the authors:

STUDY QUESTION (25):

Is the chromosomal status of embryos on cleavage stage and blastocyst stage equal?

What are the concordance rates per embryo and per chromosome using NGS?

SUMMARY ANSWER (25):

After comparison of NGS results on D3 and D5 embryo biopsies, the confirmation rate for cleavage stage embryo biopsies on D5 trophectoderm biopsies was XXX

WHAT IS KNOWN ALREADY (100):

The comparison of the confirmation rates of D3 embryo biopsy and trophectoderm biopsy, showed similar high concordance rates with whole blastocyst results for both biopsy strategies following array-CGH protocol on previous publications (Mir ,2016)

This blinded study was conducted to re-analyse 109 embryos previously diagnosed as aneuploid by array-CGH. PGS was performed using array-CGH on D3 (n=50) or D5 (n=59). Reanalysis of whole blastocysts was carried out with same array-CGH protocol .

The PGS result was confirmed in the whole blastocyst in 49/50 (98 %) abnormal embryos after D3 biopsy and 57/59 (96.6 %) abnormal embryos after trophectoderm biopsy.

STUDY DESIGN, SIZE AND DURATION (75):

Retrospective blinded observational study including 35 patients (118 embryos) undergoing PGS in IVI Abu Dhabi from August 2016 to January 2017 has been performed to validate the chromosomal status of D5 embryos previously diagnosed on D3 with NGS technology. Double biopsy (D3 and D5) was performed to all embryos that reached blastocyst stage on D5 and were not selected for transfer (including surplus euploid embryos that could not be vitrified according to the UAE law).

PARTICIPANTS, SETTING, METHODS (75):

Infertile patients with normal karyotype undergoing PGS with fresh oocytes, ≥5 MII or more than 4 embryos to biopsy on D3, age between 18 and 45 years old, with a body mass index between 19 and 30. For the cases of D3 embryo biopsy, only embryos with five or more nucleated blastomeres and less than 25 % fragmentation degree were biopsied and for blastocyst biopsy only hatching blastocyst were biopsied.

MAIN RESULTS AND THE ROLE OF CHANCE (200)

Concordant rates for segmental and whole – chromosome aneuploidies determined between D3 (blastomere) and D5 (trophectoderm) biopsy with NGS technology have been blinded evaluated. The concordance rate between D3 and D5 for the same embryo was 96 (81,4%) of 118 for the detection of segmental aneuploidies and 94 (79,7%)of 118 for whole-chromosome aneuploidies.

The concordance rates between blastomere and trophectoderm biopsies were calculated independently of the type of the aneuploidy detected.

We calculated two type of concordance rates: the concordance rate per analyzed chromosome, where we considered the total number of chromosomes independently if they were called as euploid or aneuploidy (24 chromosomes per embryo); and the concordance rate per aneuploid chromosome, where we considered only the detected aneuploidies.

The overall concordance rate per analyzed chromosome (24 chromosomes x 118 embryos) was 99.3% (2813/2832 chromosomes). For whole chromosomes aneuploidy, the concordance rate was 99.5% (2819/2832) and for segmental aneuploidies was 99.8% (2826/2832).

False positives per embryo diagnosis were 14 out of 118 (11,9%). From which 6/118(5.1%) were segmental and 8/118 (6.8%) were per whole chromosome.

False positives per chromosome were 13/2832 (0,5%) for whole chromosome and 6/2832 (0,2%) for segmental. Total false positive per chromosome was 0,7%

LIMITATIONS , REASONS FOR CAUTION (50):

The limitations of the study are related to the difficulty to discriminate the origin of the discrepancies, either attributed to the presence of mosaicism or to technical artefacts mostly incorporated during whole genome amplification.

Single cell genetic technologies do not provide 100% accuracy and are still biased by amplification artefacts

WIDER IMPLICATIONS OF THE FINDINGS (50)

Is important to know the limitation of D3 and D5 biopsies from biological and technical point of view to consider the proper stage of embryo biopsy. The reported mosaicism incidence in preimplantation embryos is between 4 and 90%. Further studies are needed to elucidate the real rate of mosaicism.

STUDY FUNDING/COMPLETING INTERESTS:

This study received no funding and there are no conflicts of interests to be declared.