ABSTRACTS

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Management of poor prognosis patients undergoing IVF: Should we offer PGS in difficult cases?

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Introduction: A number of complex entities, including advanced maternal age (AMA), recurrent miscarriages (RM) and recurrent IVF failures (RIF), may increase the risk of numerical chromosomal abnormalities of the embryos. There is limited data regarding to implantation and pregnancy rates for these poor prognosis patients undergoing comprehensive chromosomal screening (CCS) by array comparative genomic hybridisation (aCGH). The aim of the present study was to analyse if the use of aCGH for PGS improves the implantation and pregnancy rates with reduced spontaneous abortions in poor prognosis patients with complex fertility backgrounds and to establish a generalised management scheme in these complex cases. We further aimed to analyse if there are differences in the aneupl oidy rates between embryos cultured in all-in-one time-lapse incubation (TLI) systems and standard incubators (SI) in PGS cycles.

Materials and Methods: PGS was performed for the couples with at least one of the following factors; maternal age of 36 years or more, at least three previous miscarriages, at least three previous IVF failures and normal karyotype. A control group of poor prognosis patients wit h AMA, RM and/or RIF and who did not choose to undergo PGS was included in the analysis to compare the clinical results with the PGS group. Sixty six patients were included in the PGS group and informed consent was obtained from all the couples prior to ea ch PGS cycle. A total of 205 embryos were biopsied on day 5/6 of development. Whole genome amplification (SurePlex, Invitrogen) and aCGH was performed (24Sure v3 array kit, Invitrogen). The implantation and pregnancy rates for each group were analysed.

Results: PGS results showed that 78% of the embryos obtained from poor prognosis patients were aneuploid. Twenty three patients with transferable embryos had one or two with the mean of 1.3 embryo transfers. The number of embryos transferred was slightly hig her in the control group (1.6). The implantation rate for the PGS group was 11% higher compared to the control group (p=0.1). The pregnancy loss rates for both PGS (28%) and control groups (23%) were similar.
The aneuploidy rate was further investigated in these embryos cultured in a TLI system (69) and in a conventional incubator (157). The rate of aneploidy was exactly the same for the embryos cultured in a TLI system (72%) and in a conventional incubator (72%).

Conclusions: In this study, we have shown that poor prognosis patients develop a higher number of chromosomally abnormal embryos. This incidence of high abnormal embryos may explain the low pregnancy rates due to IVF failure and miscarriages. This study showed that offering PGS to poor prognosis patients may lower the psychological distress and reduce the financial difficulties that the patients may undergo. We have shown that applying PGS to these patients to select a euploid blastocyst eliminates the unnecessary embryo transfer that may not resu lt in pregnancy or lead to a miscarriage as well as improving the IVF outcome.

First NGS Based Comprehensive Chromosome Screening Data From Turkey

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NGS has been widely used in all genetic fields and recently it has become an encouring tool for reproductive genetics. As a center with high expertise in FISH analysis we have been obs erving a switch to NGS based PGS applications recently which has been more dramatic compare to array based technologies we have been performing before. The purpose of this report is to document Mikrogen Genetic Laboratory's experience on NGS based compreh ensive chromosome screening and to present the data applied in our center between July 2015 - March 2016.

Material&Methods: All single cells were collected in 2µl PBS solution. Whole genome amplification procedure was performed with SurePlex DNA Amplification Kit (Illumina, Inc). Amplified samples for

NGS were processed with VeriSeq PGS kit (Illumina). Whole procedure was performed according to VeriSeq PGS workflow (Illumina, Inc.). The following bioinformatics analysis was accomplished with a pre-release version of BlueFuse Multi for NGS (Illumina, Inc.) (http://www.illumina.com/products/veriseq-pgs.html).

Results: In total of 110 patients 336 embryos were screened using NGS (Next-Generation Sequencing). 10 of these patients were diagnosed wi th chromosomal translocations. 108 (%32) of these 336 embryos were normal. 65 (%19) aneuploidies, 125(%37) complex aneuploidies,13 (%4) mosaicism and 9(%2) partial deletion /duplications were observed. 16(%5) embryos were not determined due to ampli fication failure. Rather than 13,16,17,18,21,22,X,Y chromosomes detected by FISH, NGS identified aneuploidies in other chromomes as well. If aneuploidies were detected by FISH methodology 55% of the embryos would be normal. However, NGS showed that 34% of the embryos were normal. NGS was able to detect chromosomal aneuploidies which can not be detected by FISH in 21% of the embryos. We also observed that NGS is a robust technology for unbalanced translocation detection (Figure 1). NGS technology is valuabl e for translocation screening as the erros of other chromosomes can be determined at the same time.

Conclusion : Chromosomal copy number assessment based on NGS is very recent technology and offers encouraging advances towards improved PGS. Especially increased dynamic range enabling enhanced detection of mosaicism in multicellular samples (Fiorentino et. al., 2014). Cinical usage of NGS technolgy in IVF field becomes widespread due to robust methodology. Unbalanced translocation screening with NGS technology will be very important milestone in PGD applications.

References :

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First experience with the 24Sure+ in PGD of balanced translocations and other selected structural aberrations

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Introduction: About 9 % of couples worldwide have experience with infertility. Structural chromosome abnormalities occur with frequency of 0, 19 % in the human population. Reciprocal translocations are the most common structural abnormalities in human and they occur with frequency 0, 6 % in newborns. In our study we use aCGH as a screening method in PGD of reciprocal translocation and chromosome rearrangements.

Material and methods: 12 couples with chromosomal rearrangements from 4 IVF centres in Czech Republic were included into study. 63 trophectoderm and 9 blastomere samples (total 72) were analysed by aCGH (24Sure+, Illumina).

Results: From December 2014 to March of 2016 the trophectoderm and blastomere samples from 12 couples were tested for PGD of chromosomal rearrangements. Results were obtained from 64 samples (88,9%), 8 samples were not properly amplified by WGA. 35 samples from analysed samples (54,6%) carried unbalanced translocation, either alone or in combination with other chromo somal imbalancies. A further 15 samples (23, 4%) were normal/balanced for the rearranged chromosomes, but affected by aneuploidy of other chromosomes. Only 9 samples (14, 1 %) were with normal number of chromosomes. Of the 12 patients who completed their t reatment cycles, 2 became pregnant. In the first case, the miscarriage occurred. In the second case, the pregnancy is continuing. In one case, couple with complex rearrangement decided to use donor gametes after 4 unsuccessful IVF cycles and patient became pregnant.