THE UTILITY OF PREIMPLANTATION GENETIC TEST IN COUPLES WITH HISTORY OF INFERTILITY AND PREGNANCY LOSS

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Abstract

Preimplantation genetic test (PGT) is cutting-edge technology for early detection of genetic abnormalities in embryos prior to their implantation in the uterus. PGT prevents the transfer of affected embryos during in vitro fertilization (IVF) procedures and termination of pregnancy. In our study we report the results from 185 couples with history of infertility and pregnancy loss that had undergone IVF procedures with PGT. Trophectoderm biopsy was carried out on 497 blastocyst stage embryos originating from 231 oocyte retrieval cycles. Array-based comparative genomic hybridization (aCGH) and next-generation sequencing (NGS) were subsequently performed. One hundred and ninety-six embryos had balanced profile (40.16%) and 292 embryos showed unbalanced profile (59.84%). Embryo transfer was performed in 109 cases (58.92%) and biochemical pregnancy was reported in 33.03%. Live birth rate was 29.36% and pregnancy loss occurred in 3.67% of cases. Our results show that PGT reduces the number of meaningless transfers, eliminates the trauma of termination of desired pregnancy and possible medical complications. In couples with reproductive failure PGT decreases the risk for pregnancy loss, increases the chance of conceiving with a chromosomally balanced embryo and live birth of a healthy offspring per transfer.

Key words: PGT, IVF, aCGH, NGS
**Introduction.** PGT is cutting-edge technology for early detection of genetic abnormalities in embryos prior to implantation in the uterus. PGT prevents the transfer of affected embryos during IVF procedures and termination of pregnancy in pathological cases [1,2]. PGT consists of the following steps: biopsy of polar body, blastomere or trophodermal cells, DNA isolation and genetic analysis. PGT is justified in cases of life-threatening or chronic diseases with severe, multiple, often progressive physical and/or mental disabilities, and in cases that are treated by ineffective and expensive therapies [3].

There are three main groups of PGT – PGT for monogenic disorders (PGT-M), PGT for structural rearrangements (PGT-SR) and PGT for aneuploidy (PGT-A) [4]. PGT-M is applied in families in which one or both parents are carriers of known monogenic defect; thus ensuring selection of unaffected embryos and prevention of the transmission of specific genetic mutations to the offspring. PGT-SR identifies embryos carrying unbalanced chromosomal rearrangements in families where one or both parents have a structural chromosomal abnormality [5,6]. PGT-A detects embryos with de novo aneuploidies, deletions or duplications in couples with advanced maternal age, recurrent pregnancy losses, previous unsuccessful IVF procedures or severe male infertility [7].

**Materials and methods.** The aim of our study was to assess the utility of PGT in couples with infertility; to demonstrate its ability in reducing the risk of a birth of chromosomally unbalanced offspring and pregnancy loss.

One hundred and eighty-five couples with reproductive failure were included in the study. Maternal age ranged from 26 to 45 years. Patients were divided in four age groups – 26–30 (17.30%), 31–35 (18.38%), 36–40 (28.11%) and 41–45 years old (36.22%), respectively. All couples underwent IVF-PGT cycles after extensive genetic counseling. Informed consent was obtained from each participant in the study.

All procedures performed were in accordance with the ethical standards of the institutional and national research committee and with the 1975 Helsinki declaration and its later amendments or comparable ethical standards.

On average three to five cells were retrieved for genetic analysis of day 5–6 embryos. SurePlex Amplification kit (Illumina) was used for cell lysis and WGA. Quality and quantity of the amplified DNA was assessed by horizontal low-voltage agarose gel electrophoresis. Chromosome analysis was performed by array-based comparative genomic hybridization (aCGH) or next-generation sequencing (NGS).

Array CGH was performed according to manufacturer’s protocol on 24sure+ or 24sure v3 microarray platforms (Illumina). The microarrays were processed on Agilent G2502C scanner. The library preparation for PGT-NGS was made according to the VeriSeq PGS protocol (Illumina) and sequenced on MiSeq. The software analysis of aCGH and NGS data was performed with BlueFuse Multi version 4.3 (Illumina).
Results and discussion. Results were obtained for a total of 497 blastocysts originating from 231 oocyte retrieval cycles from 185 couples.

The indication for IVF-PGT was the female factor in 55.14%, with tubal factor infertility being the leading cause for rejection – 39.22%, followed by constitutional chromosomal rearrangements – 10.78%. Twenty percent of the couples performed PGT due to male factor – genetic abnormalities accounted for 13.51%. In 3.78% of couples both female and male factor were present. In the remaining 21.08% of cases the underlying infertility remains unknown.

Of all 497 embryos tested 9 were non-interpretable (1.81%) and were excluded from further statistical analysis. Of the 488 embryos for which result was obtained 196 had balanced chromosomal profile (40.16%) and 292 showed unbalanced profile (59.84%). Of all unbalanced embryos 11.99% belonged to women in the 26–30-year group, 22.95% to women in the 31–35-year group, 30.48% to the 36–40-year group and 34.59% – to 41–45-year group. In the first three age groups the percentage of unbalanced embryos was similar (53.85%, 53.17% and 55.97%, respectively). The fourth age group (41–45-year group) showed the highest incidence of unbalanced embryos – 73.19% (Table 1). As shown in previous studies [8,9] the percentage of abnormal embryos increased with maternal age. Our data supported previous findings that the proportion of aneuploid embryos is estimated to be around 50% [10,11].

Table 1

<table>
<thead>
<tr>
<th>Age group 26–30</th>
<th>31–35</th>
<th>36–40</th>
<th>41–45</th>
<th>All age groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of embryos</td>
<td>65</td>
<td>126</td>
<td>159</td>
<td>138</td>
</tr>
<tr>
<td>Balanced embryos (%)</td>
<td>30 (46.15)</td>
<td>59 (46.83)</td>
<td>70 (44.03)</td>
<td>37 (26.81)</td>
</tr>
<tr>
<td>Unbalanced embryos (%)</td>
<td>35 (53.85)</td>
<td>67 (53.17)</td>
<td>89 (55.97)</td>
<td>101 (73.19)</td>
</tr>
</tbody>
</table>

Detected chromosomal abnormalities were divided into four groups: 1) only whole chromosome aneuploidies; 2) only segmental aneuploidies; 3) combined (whole chromosome + segmental aneuploidies); 4) mosaic aneuploidies. Whole chromosome aneuploidies constituted 70.2% of all cases in the first group. The second group constituted 18.84% of all cases. The third and fourth group represented 8.22% and 2.74%, respectively. Detailed distribution of the detected chromosomal aberration types is presented in Fig. 1. Nearly one third of all aneuploidies (27.35%) involved chromosomes 16, 21 and 22, which corresponds to previous findings [12,13]. Sex chromosome aneuploidies were quite rare: XXY and monosomy X represented 1.41% and 2.82% of all aneuploid chromosomes.

Live birth rate, biochemical pregnancy rate and miscarriage rate in the different age groups of our cohort are presented in Fig. 2. The most significant increase in live birth rate was observed in the most advanced age group. The average live
birth rate in women aged 41–45 undergoing IVF without PGT was 3.6% \(^{14}\). The live birth rate in this age group after IVF-PGT was significantly higher (22.22%). In summary, we reported live birth rate of 29.36% across all age groups, who underwent embryo transfer after IVF-PGT.

Approximately 25% of all pregnancies are lost before they are detected \(^{15}\). IVF-PGT has the potential to significantly reduce miscarriage rate. Our data
showed miscarriage rate of 3.67% in all age groups, which is markedly lower than what can be expected for untested embryos.

**Conclusion.** PGT decreases the number of meaningless transfers and reduces: the number of cases that require termination of desired pregnancy, the possible medical complications, and the associated psychological trauma. In couples with reproductive problems PGT increases the chance of conceiving with chromosomally balanced embryo, improves live birth rate per transfer and decreases the risk for pregnancy loss.

**REFERENCES**


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