Preimplantation Genetic Screening (PGS)

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The human embryo has a low capacity to implant in vivo as well as in vitro.

Assisted hatching, co-culture, blastocyst transfer, sequential culture media, cytoplasm transfer and improved controlled ovarian stimulation protocols have tried to improve the outcome of assisted reproductive technologies (ART). Despite all these attempts, implantation rates remain low, especially in older patients. A clear relationship between maternal age and the frequency of numerical chromosomal abnormalities in oocytes retrieved after controlled ovarian stimulation has been observed (Dailey et al., 1996).

Increased aneuploidy in embryos from older patients (Munné et al., 1995a, b) generated the concept that numerical chromosomal aberrations might be the cause of the reproductive failure in older patients. No difference in frequency of aneuploidy in IVF-embryos and ICSI-embryos was observed (Munné et al., 1998a). A clear relation between embryo morphology and chromosomal abnormalities has been demonstrated: the review paper of Pellestor (1995) shows 36.6% and 86.6% chromosomal abnormalities in good-quality and poor-quality embryos respectively. Furthermore increased abortion rates are observed in older patients. Since rates of aneuploidy are higher in preimplantation embryos than in spontaneous aborti (Munné et al., 1995a), it is assumed that at least a portion of chromosomal abnormal embryos will be lost before implantation.

It is conceivable that exclusion of all aneuploid embryos before transfer might improve implantation and reduce abortion rates after ART.

Developments within the fields of assisted reproductive technologies (ART), embryo micromanipulation and molecular genetics have resulted in the development of preimplantation genetic diagnosis (PGD) (Handyside et al., 1990, 1998). PGD was primarily designed to prevent the birth of genetically affected children in couples at risk (Liebaers et al., 1998). Blastomeres are removed from early cleavage embryos (day 3) or blastocysts by an opening made in the zona pellucida. Subsequently the blastomere can be tested by fluorescence in-situ hybridization (FISH) or by the polymerase chain reaction (PCR). PGD has also been carried out on oocytes by analysing the first and/or second polar body before IVF or ICSI (Verlinsky et al., 1996).

The possibility to detect simultaneously various chromosomes in preimplantation embryos resulted in the development of preimplantation genetic screening or PGS. PGD is carried out in couples at risk while this is not the case for PGS. The aim of PGS is to increase the ongoing pregnancy rate after ART by improving the implantation and reducing abortion rates. Implantation rates after IVF or ICSI between 10% and 20% reveal that our current selection criteria for embryo transfer are inadequate.

Besides the morphological quality and the developmental stage of the in-vitro embryos, PGS might be an additional criterion to select the right embryo for transfer. Which couples should undergo PGS? So far it is clear

from the literature that PGS should be reserved for selected groups only: patients >37 years of age, patients with repeated implantation failures after transfer of morphologically normal embryos, patients with unexplained recurrent abortions and patients where the partner has a very low sperm count. Prospective randomized trials are necessary to analyse the value of PGS in every ART cycle. If PGS could detect the "super-embryo", it might solve the problem of multiple pregnancies by transferring a single embryo.

The multicolour FISH analysis is based on the detection of chromosome-specific signals in the interphase nuclei of the blastomeres. The colour of each signal is chromosome specific, so that a limited number of chromosomes can be simultaneously tested. Since we are still far from PGS analyzing the 23 autosomes and sex chromosomes of the embryo, currently available FISH methods allow us to screen only for a limited number of chromosomes. So far most studies have been carried out by mixtures of FISH probes for 5 chromosomes (13, 18, 21, X and Y), but mixtures of 6 chromosomes have been described (13, 16, 18, 21, X and Y) (Munné et al., 1998b). Re-hybridization of the same blastomere in a second round can extend the screening chromosomes to 10.

Which chromosomes should be tested is still a matter of discussion. Munné et al. (1999) analyse for trisomies with potential of arriving to term (X, Y, 13, 18 and 21) and for trisomies commonly found in abortions (14, 15, 16 and 22). The combination of chromosomes X, Y, 13, 16, 18, 21 and 22 might detect up to 65% of the embryos destined to abort (Sasabe et al., 1999). On the other hand the chromosomes most involved in spontaneous abortions are not necessarily the ones impairing implantation in older patients. Bahçe et al. (1999) demonstrated that the chromosomes most involved in aneuploidies in older patients were 1, 15, 17 and 22.

The clinical application of PGS has been tested in several comparative studies. Three classes of patients were the subject of these studies: older patients, patients with repeated implantation failure and patients with recurrent abortion. Gianaroli et al. reported in 1997 the effect of PGS on implantation in patients >38 years of age and/or in patients with >3 previous IVF failures. Probe mixtures of chromosomes X, Y, 13, 18 and 21 were used. Eleven patients underwent PGS, while 17 controls had assisted zona hatching. On a total of 61 analysed embryos 55% were chromosomally abnormal. In 10 cycles at least 1 normal embryo was transferred. Four clinical pregnancies ensued, resulting in an implantation rate of 28.0%.

In the control group also 4 clinical pregnancies ensued, while an implantation rate of 11.9% was observed. In 1998 (unpublished data) they presented a larger series at a FISH workshop. They compared the aneuploidy screening in 42 patients >36 years of age, 22 patients with >3 unsuccessful previous cycles and 21 patients with balanced translocations or gonosomal mosaicism. The percentages abnormal embryos were 63%, 54% and 58% (NS) in the 3 groups respectively, while the implantation rates were 25.6%, 19.6% and 21.7% (NS) respectively. The clinical application of aneuploidy testing on polar bodies of oocytes was reported by Verlinsky et al. (1999). They tested the oocytes of 425 patients >35 years of age for the chromosomes 13, 18 and 21 in 659 IVF cycles. FISH results were obtained in 3217 (81.6%) of 3943 biopsied oocytes. Aneuploidy was present in 1388 (43.1%) oocytes. In 614 cycles embryo transfer was done, resulting 131 clinical pregnancies and 88 healthy children born after confirmation of the PGS.

A recent paper of Munné et al. (1999) describes in a multi-centre comparative study the value of aneuploidy testing in patients aged >35 years undergoing IVF. In 36 cycles embryos were tested for chromosomes 13, 18, 21 X and Y. Chromosome 16 was added in another 50 cycles, while in 31 cycles chromosomes 13, 14, 15, 16, 18, 21,

22, X and Y were analysed in 2 rounds.

No difference was observed in implantation rates (17.6% in test group vs. 13.7% in control group). On the other hand the abortion rate decreased significantly after PGS (15.0% vs. 33.8%; $p<0.05$), resulting in a significantly increased ongoing pregnancy rate (15.9% vs. 10.6%; $p<0.05$). The issue of PGS and recurrent miscarriage has been addressed by Pellicer et al. (1999).

The mixture of FISH probes contained probes for chromosomes 13, 16, 18, 21, 22, X and Y. Sixty-six embryos of 9 patients with recurrent miscarriage were compared with 62 embryos from 10 women <36 years of age and 41 embryos of 6 women >36 years of age. The rates of chromosomal aberrations in the three groups were 53.0% a, 19.3% a, b and 46.3% b respectively (pa=0.001; pb=0.045). In the group with recurrent miscarriage as well as in the younger control group 2 clinical pregnancies ensued. In both groups 1 of the pregnancies resulted in a miscarriage. No pregnancies were achieved in the older group.

So far PGS seems to be a powerful tool to select the right embryo for transfer in selected cases. Where a significant improvement of implantation is not immediately observed, it is obvious that the outcome of ART might be improved by a reduction of the abortion rate with consequently higher ongoing pregnancy rates. More and larger multi-centre comparative studies are needed to evaluate which chromosomes are most likely to improve implantation.

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