Preimplantation genetic screening: a reappraisal
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In vitro fertilization (IVF) has revolutionized reproductive medicine and it has established itself firmly in modern day society. The International Committee for Monitoring Assisted Reproductive Technology reported 601,243 performed cycles of IVF in 2002 worldwide, with an estimated 219,000 to 246,000 babies born (de Mouzon et al., 2009). In the Netherlands, 16,769 cycles were performed in 2009, resulting in 4,862 babies, which means that one out of 38 of all children born in the Netherlands stems from IVF (Nederlandse Vereniging voor Obstetrie en Gynaecologie, 2010).

A long period of preclinical research preceded the introduction of IVF (Edwards and Craft, 1990). Research showing in vitro fertilisation of human oocytes (Rock and Menkin, 1944; Edwards et al., 1966; Jones, 2003), culturing of human embryos to the morula stage (Shettles, 1955), and later to the blastocyst stage (Steptoe et al., 1971; Shettles, 1971) formed the foundation for the clinical use of human in vitro fertilisation. This resulted in 1973 in the first reported pregnancy after an IVF procedure (De Kretzer et al., 1973), and in 1978 in the first child being born after IVF (Steptoe and Edwards, 1978).

Since then, the IVF procedure has been subject to many changes. In the early days of IVF oocyte retrieval was carried out in a natural cycle. Later, clomiphene citrate and hCG were introduced to time the oocyte retrieval, followed by controlled ovarian hyperstimulation (COS) by gonadotrophins to obtain more oocytes, and GnRH analogues to reduce the risk of premature ovulation (Edwards, 1996).

The change of IVF treatment in a natural cycle to treatment in a stimulated cycle resulted in more oocytes and thus more embryos available for transfer. This allowed the transfer of multiple embryos as more pregnancies were reported when more embryos were transferred (Jones et al., 1982). With improving clinical and laboratory protocols, transfer of multiple embryos not only led to improved pregnancy rates, but also to an increased number of multiple pregnancies (Steptoe et al., 1986; Kingsland et al., 1990; Seoud et al., 1992). Due to the risks to mother and child, strong pleas for the transfer of only one or two embryos per transfer were made (ESHRE Capri Workshop, 2000).

This increased pressure to transfer only one or two embryos increased the necessity for optimal embryo selection. The only embryo selection methodology available for many years, was morphological assessment of the embryo at a single time-point before transfer (Veeck, 1990; Ceyhan et al., 2009). This assessment involved the number and shape of the blastomeres and the relative amount of fragmentation per embryo (Puissant et al., 1987; Veeck, 1990). Later on, cumulative embryo scoring systems were developed that involved morphological assessment at multiple time points or stages of development (Steer et al., 1992). Apart from cell number, shape and regularity of blastomeres, other morphological characteristics were suggested to relate to successful IVF outcome, such as the observation of the polar bodies, the presence of granular cytoplasm at the oocyte stage, the distribution and number of nucleoli at the pronuclear stage, the occurrence of early cleavage, the presence of multinucleation at cleavage stage, the cleavage rate of the embryo or the ability to develop in good quality blastocysts in extended culture (Ebner et al., 2003). The added clinical value
of these characteristics has not been properly studied and hence they are not being used in every IVF-laboratory.

The inherent problem with all embryo scoring systems is that no system is able to identify the embryo that is going to implant and lead to a successful pregnancy with 100% certainty. Not all morphologically perfect embryos implant and not all embryos with suboptimal morphology fail to lead to the birth of a healthy child. As a consequence implantation rates in general do not exceed 35 percent after embryo selection based on morphological evaluation (Gerris, 2005; Centers for Disease Control and Prevention et al., 2010). Therefore, new parameters that predict whether an embryo will result in the birth of a healthy child after transfer have been searched for. Such parameters included assays that use follicular fluid or culture medium for amino acid profiling, proteomic profiling or respiration-rate measurement to determine metabolic activity and viability of the embryo or microscopical techniques such as birefringence imaging which uses polarization light microscopy to assess the meiotic spindle or the zona pellucida (Nagy, 2008).

Thus far, none of these selection methods have been proven to be a better predictor for embryo viability than morphology. One of the reasons for this is that proper studies into the efficacy of embryo selection techniques are rare. Flaws in these studies are manifold.

One important flaw is the chosen outcome measure. Most studies on embryo selection in IVF use embryo parameters, such as embryo development or implantation rate per embryo as the primary outcome. By using implantation rate as the main outcome, a unit of analysis error is introduced. Implantation rate is an inappropriate measure since the denominator (number of embryos transferred) depends on the strategy, not on the design (Mastenbroek et al., 2005). In the same way, embryos cannot be the basis for sample size calculations, as it will -by definition- lead to underpowered studies.

In some studies selection for transfer is not based on the criterion studied but on traditional morphological observations (Gerris, 2005). Reported studies on embryo selection are often retrospective cohort studies or non-randomized comparisons, whereas the optimal design is a rigorously designed, well powered, double-blind randomized controlled study. In this thesis, we used this design to evaluate one of the most promising embryo selection alternatives to morphological evaluation of the last decade: preimplantation genetic screening (PGS).

In PGS, a single blastomere is aspirated from each embryo, and the copy number of a set of chromosomes is determined. Embryos that are identified as abnormal are then discarded, and embryos with a normal genetic constitution are selected for transfer. The underlying rationale for this screening was an expected increase in live birth rates after IVF, because numerical chromosomal abnormalities were known to exist in human preimplantation embryos (Angell et al., 1983), and embryos containing these abnormalities were thought not to implant or develop to term and hence to contribute to low live birth rates in specific groups of patients (Wilton, 2002).
The beneficial effect of PGS was expected to be greatest in women of advanced maternal age, since aneuploidies in clinically recognized pregnancies occur more frequently when a woman passes 35 years of age (Hassold and Hunt, 2001) and it is in these women that pregnancy chances decline sharply both in normal conception and after IVF (Lintsen et al., 2007). Next to women of advanced maternal age, PGS has been offered to women with a history of recurrent miscarriage, women with a history of repeated implantation failure (i.e. several failed IVF cycles), and women with a partner with low sperm quality (severe male factor), mainly since high percentages of aneuploidies have been found in the embryos of these women (Munne et al., 1995; Marquez et al., 2000; Werlin et al., 2003; Silber et al., 2003; Munne et al., 2004; Kahraman et al., 2004; Wilding et al., 2004; Platteau et al., 2005; Rubio et al., 2005; Baart et al., 2006). More recently, PGS has also been offered to younger women (under 35 years of age), as high aneuploidy rates were found in their embryos as well (Baart et al., 2006; Goossens et al., 2009).

**Background and outline of the thesis**

At the time of starting the studies described in this thesis, the use of PGS had become increasingly common, in particular among women of advanced maternal age (Verlinsky et al., 2004; Sermon et al., 2007). It was even suggested that PGS would become a standard procedure for women undergoing IVF (Verlinsky et al., 2004). Evidence supporting the use of PGS was limited. Observational studies comparing IVF with and without PGS had shown that PGS was associated with higher implantation rates for transferred embryos but not with an increase in the rate of ongoing pregnancies per initiated cycle or per follicular aspiration (Gianaroli et al., 1999; Munne et al., 1999; Obasaju et al., 2001; Munne et al., 2003; Montag et al., 2004).

Therefore, we decided to conduct a multicenter, double-blind, randomized controlled trial comparing ongoing-pregnancy rates after IVF with and without PGS in women of advanced maternal age (**Chapter 2**).

This was followed by the full analysis of 360 human preimplantation embryos from our randomized controlled trial on PGS to investigate whether mosaicism, the phenomenon that not all blastomeres from a preimplantation embryo contain the same chromosomal constitution, would hamper the efficacy of PGS (**Chapter 3**).

To validate the mosaicism rates we found, we subsequently performed a systematic review of the literature on mosaicism in human preimplantation embryos (**Chapter 4**).

After the report of our randomized controlled trial on the efficacy of PGS, multiple other trials were published. Still there was enormous controversy and a call for more randomized controlled trials (Cohen and Grifo, 2007; Munne et al., 2007; Simpson, 2008). We critically assessed whether this call was ethically sound (**Chapter 5**).

To underpin our position, we systematically reviewed the literature and performed meta-
analyses on the reported effectiveness of all available randomized controlled trials on PGS (Chapter 6).

Finally, we discussed whether there is still a role for embryo selection in human IVF at all (Chapter 7).

The results of the work presented in this thesis are summarized in Chapter 8.

References


Centers for Disease Control and Prevention, American Society for Reproductive Medicine, and Society for Assisted Reproductive Technology (2010) 2008 Assisted Reproductive Technology Success Rates: National Summary and Fertility Clinic Reports.


INTRODUCTION


