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ARTICLE

Chromosome analysis in embryos from young patients with previous parity

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Abstract This study included 173 young couples of proven fertility who had previously undergone preimplantation genetic screening for chromosomes X and Y for family balancing. Several months later, when the outcome of the pregnancies was already known, the blastomeres from the corresponding embryos transferred were reanalysed by fluorescence in-situ hybridization (FISH) for chromosomes 13, 16, 18, 21, 22 with the aim of investigating correlation with embryo viability and the level of FISH sensitivity (embryos confirmed to be euploid). According to the results, informative in 152 couples, the proportion of euploid embryos was significantly lower in 53 nonpregnant women when compared with 99 women with term pregnancy (49% versus 75% respectively, $P < 0.001$). In addition, in 21 nonpregnant patients, all embryos transferred were found to be chromosomally abnormal. The level of FISH sensitivity was calculated in the group of term pregnancies where the number of euploid embryos was expected to exceed or match with the number of babies born. The resulting false-negative rate was 4.0% per patient and 1.9% per embryo. These findings confirmed the limited prediction power of embryo morphology on implantation but also the relevance of chromosomal abnormalities in causing embryo demise.

KEYWORDS: aneuploidy, chromosomes, embryo mosaicism, fluorescence in-situ hybridization, implantation, preimplantation genetic screening

Introduction

For several years, fluorescence in-situ hybridization (FISH) has represented the method of choice for the enumeration of chromosomes in human oocytes and embryos. Although very likely destined to be replaced by more modern and efficient techniques, FISH still offers a series of benefits, including its full validation and the definition of accuracy rates, besides a substantially lower cost compared with other innovations (Munné et al., 2010b).

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The assessment of the chromosomal constitution of in-vitro-generated embryos has demonstrated that chromosomal abnormalities are very frequent, with some arising from meiotic errors and others during fertilization and the first mitotic divisions (Gianaroli et al., 2010; Munné et al., 2007). Although a correlation was found between embryonic morphology and chromosomal defects such as chaotic mosaicism, polyploidy and multinucleation, it was shown that even in top-quality embryos and blastocysts the incidence of abnormalities can be as high as 50%, confirming the limited prediction power of embryo morphology on implantation (Magli et al., 2007).

When analysing the type of anomalies, especially frequent is the occurrence of mosaicism, a phenomenon whereby not all cells in an embryo have the same chromosomal content, as described for the first time 20 years ago (Delhanty et al., 1993). The high rate of chromosomal mosaicism might indicate that mitotic errors are a common feature of human preimplantation development, but since the first cleavage divisions are fully dependent on maternally derived gene transcripts and proteins stored in the oocyte, full competence of the oocyte is required to prevent aneuploidy formation by the efficient activation of the cell-cycle checkpoints that control cell division (Ambartsumyan and Clark, 2008). For this reason, reduced oocyte quality, which is inversely related to maternal age, may lead to chromosomal segregation errors during meiosis and/or the first embryo cleavages, with the consequent occurrence of mosaicism (Delhanty, 2005; Munné et al., 2007). However, the detection of high rates of numerical chromosomal abnormalities in embryos from young women suggests that these abnormalities are not exclusively related to maternal age (Munné et al., 2006b; Vanneste et al., 2009).

As the majority of data on aneuploidy on oocytes and embryos comes from assisted reproduction treatment, the possibility exists that the tendency to develop meiotic and mitotic errors might also be affected by the infertile condition as well as by the treatment itself, namely ovarian stimulation and/or the in-vitro culture systems (Baart et al., 2007; Bean et al., 2002; Santos et al., 2010). In other words, the question whether chromosomal mosaicism is a physiological or a pathological event in humans is still unanswered, but it is clear that it is highly relevant for preimplantation genetic screening (PGS), in which selection of embryos for transfer is often based on analysis of a single cell (Van Echten-Arends et al., 2011). The accuracy of FISH in single cells is currently determined by reanalysing all the cells of the diagnosed embryos that are not transferred or cryopreserved. The resulting error rate (false-positives + false-negatives) is centre-dependent, but in large datasets ranges from 5 to 7% (Colls et al., 2007; Magli et al., 2007).

The present study adopted a different approach to verify FISH accuracy by retesting the blastomeres from embryos that had already been transferred following PGS for chromosomes X and Y in couples seeking a pregnancy of a specific gender. The reanalysis included couples for which the clinical outcome was known (no implantation, full-term implantation, implantation ending in spontaneous abortion). The same blastomeres belonging to the embryos transferred that had been tested for chromosomes X and Y in the ICSI cycle were analysed for chromosomes 13, 16, 18, 21 and 22. This was done with two aims. The first was to evaluate the incidence of aneuploidy in the embryos transferred in young, fertile patients and to verify whether the chromosomal results for the five autosomes correlated with the corresponding embryo viability. For this reason, the results obtained from the chromosome testing were analysed in relation to the transfer outcome (pregnant and nonpregnant patients). The second aim was to estimate the level of FISH accuracy considering that, in patients with term pregnancies, the number of euploid embryos were expected to exceed or match with the number of healthy babies born. This approach only permitted to estimate the sensitivity of the technique (embryos confirmed to be euploid) but not the specificity (embryos confirmed to be aneuploid).

Materials and methods

Patients

A total of 173 couples (age, mean ± SD, 33.5 ± 4.3 years) undergoing an assisted cycle in combination with PGS for chromosomes X and Y at the Farah Hospital assisted reproduction unit in Amman between 2008 and 2010 were retrospectively selected to enter this study.

The reason for these patients to undergo intracytoplasmic sperm injection (ICSI) in combination with PGS for X and Y was sex-selection for family balancing (Kilani and Haj Hassan, 2001). As presented in **Table 1**, all couples already had children, on average 2.9 children of the same sex, they were looking for another child with a preference for one gender over the other. Despite having had previous pregnancies, 52 couples had secondary infertility due to tubal factor or polycystic ovaries ($n = 9$), male factor ($n = 39$) or both female and male factors ($n = 4$). Fourteen couples had both male and female children. Altogether there were 121 couples who were fertile,

Table 1 Couples' reproductive condition and parity.

	Female factor (n = 9)	*Male factor (n = 39)*	*Female and male factor (n = 4)*	*Fertile (n = 121)*	*Total (n = 173)*
Maternal age (years)	33.9 ± 3.1	31.6 ± 4.2	31.2 ± 4.0	34.2 ± 4.2	33.5 ± 4.3
Children	17	81	6	401	505
Females	14	68	6	367	455
Males	3	13	0	34	50

Values are mean ± SD or *n*.