Review

# Molecular methods for selection of the ideal oocyte

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## Abstract

Some recent strategies for identifying the ideal oocyte for insemination in assisted reproduction techniques are reviewed. Established methods of assessing the female gamete, such as morphological evaluation of oocytes and cytogenetic analysis of polar bodies using fluorescence in-situ hybridization, will soon be joined by more advanced cytogenetic methods such as the use of comparative genomic hybridization to improve understanding of oocyte genetics. It seems likely, however, that the greatest advances will originate from the evolution of molecular genetic technologies. The application of microarray technology to individual oocytes and their associated cumulus cells has recently been accomplished, providing a simultaneous assessment of activity for thousands of genes and revealing potential viability markers. Furthermore, improved equipment and optimized methods of mass spectrometry have provided sufficient sensitivity to allow proteomic profiles to be generated from single oocytes and embryos, while metabolomic investigations have searched for indicators of oocyte/embryo quality in spent culture medium. Techniques of this type may ultimately lead to non-invasive tests for oocyte quality revealing previously hidden information concerning both oocyte and embryo developmental competence. Once fully validated, these new approaches are expected to revolutionize oocyte and embryo selection, leading to improved implantation rates and higher probabilities of success using elective single embryo transfer.

***Keywords:*** *aneuploidy, CGH, cumulus cells, gene expression, in-vitro fertilization, microarray, polar body*

## A problem for assisted reproduction: high embryo wastage and low implantation rates per oocyte retrieved

Despite remarkable progress in both the clinical and embryological aspects of assisted reproductive technologies, the take-home baby rate, or infants delivered per cycle started, is still disappointingly low. Two recent studies have clarified this point, shedding light on the efficiency of assisted reproduction by attempting to quantify accurately the proportion of oocytes and embryos that ultimately generate a pregnancy. In the first study it was demonstrated that 85% of embryos produced *in vitro* and transferred into the uterus fail to develop into an infant, leaving only a small fraction (15%) destined to become a live birth (Kovalevsky and Patrizio, 2005). Low implantation and live birth rates were also obtained in a second study, which evaluated the biological wastage in assisted reproduction by taking into account the number of oocytes inseminated, the number of euploid embryos produced and transferred, and the resulting live births in patients undergoing preimplantation genetic screening (PGS). In this particular cohort of patients, it was found that only 8% of the oocytes inseminated produced euploid embryos for transfer when analysed for nine chromosomes (13, 15, 16, 17, 18, 21, 22, X, Y). Of these, 1.5% implanted and only 1% of the initial oocytes inseminated eventually resulted in live births (Patrizio *et al*., 2007).

Another recent paper (Inge *et al.*, 2005) reported that the majority of oocytes collected after ovarian stimulation are unable to provide viable embryos, inferring that many may be abnormal. These clinical observations strongly suggest that new areas of research are needed to answer the question: how can the few oocytes with the potential to produce a child be identified from among the cohort of those retrieved? Although cytogenetic anomalies are a major cause of implantation failure, many other factors may contribute to this problem. The current morphological criteria and the standard cytogenetic methods used to select and classify oocytes are not sufficient for choosing the ideal oocyte for fertilization and the resulting embryo for transfer. Research into new molecular and cytogenetic methods for the identification of competent oocytes is underway in a number of the leading research and clinical laboratories, and has begun to yield promising results.

## Aneuploidy in human oocytes

Although the prevalence of aneuploidy is expected to be greater than average in patients with indications for PGS, there can be little doubt that a high frequency of chromosomal abnormality is a general feature of human embryos produced *in vitro*. This has been demonstrated by a large number of studies, both clinical [e.g. preimplantation genetic diagnosis (PGD) and PGS] and scientific, using various cytogenetic techniques. Most studies suggest that at least two-thirds of human preimplantation embryos contain aneuploid cells (Delhanty *et al.*, 1997; Munné and Cohen, 1998; Wells and Delhanty, 2000; Voullaire *et al.*, 2000, 2002; Coonen *et al.*, 2004; Baart *et al.*, 2007). Chromosomal anomaly is a prominent example of a defect causing implantation failure, which is invisible to morphological analyses typically employed in order to guide the decision of which embryo(s) to transfer.

In many cases, embryos are composed of a mixture of both aneuploid and euploid blastomeres (mosaic embryos). The potential of such embryos to implant and establish a viable pregnancy is not yet clear. On the contrary, there is little doubt that uniformly abnormal embryos, arising from chromosome malsegregation taking place during meiosis, are largely incapable of forming a sustainable pregnancy. Detailed analysis of human embryos using comprehensive cytogenetic techniques shows that approximately 30% of human cleavage stage embryos are affected by aneuploidy of meiotic origin (Voullaire *et al.*, 2000; Wells and Delhanty, 2000). In most cases these abnormalities are oocyte-derived. Such errors become increasingly common with advancing maternal age, arguing for chromosomal screening (i.e. PGS) to be applied to oocytes or embryos from patients of advanced maternal age.

Cytogenetic screening of oocytes or cleavage stage embryos, with chromosomally abnormal embryos excluded from transfer, has proven to be a successful strategy for reducing the risk of children affected by aneuploidy (e.g. Down's syndrome) and decreasing the rate of spontaneous abortion for several patient groups (Gianaroli *et al.*, 2001; Kuliev *et al.*, 2003; Munné *et al.*, 2005, 2006). A number of studies also indicate that preimplantation genetic screening (PGS) of embryos leads to an improvement in implantation rates, although this contention is not universally accepted (Munné *et al.*, 1999, 2003; Staessen *et al.*, 2004; Cohen *et al.*, 2007).

## Morphological assessment of oocytes

Morphological anomalies are frequently observed in human oocytes. After removal of cumulus cells prior to intracytoplasmic sperm injection (ICSI), oocyte abnormality rates of 60–70% have been reported in some studies. Several papers have provided evidence that certain morphological features can serve as useful indicators of oocyte quality (for review, see Balaban and Urman, 2006; Ebner *et al.*, 2006). The use of polarization light microscopy has shown that absence of the metaphase II (MII) spindle is associated with reduced rates of fertilization and blastocyst formation (Wang *et al.*, 2001; Moon *et al.* 2003; Rienzi *et al.*, 2003). Oocytes in which the spindle is shifted more than 90 degrees relative to the position of the first polar body also display reduced fertilization rates (Rienzi *et al.*, 2003). However, the question of whether or not the morphology of the polar body can be used to judge oocyte quality remains controversial.

A range of cytoplasmic defects, including variations in density, viscosity and texture, have also been related to alterations in the probability of a positive outcome following IVF treatment (Kahraman *et al.*, 2000; Meriano *et al.*, 2001; Ebner *et al.*, 2003). The presence of membrane-bound vacuoles in the cytoplasm appears to be associated with a reduced fertilization rate (de Sutter *et al.*, 1996; Ebner *et al.*, 2005), while aggregations of smooth endoplasmic reticulum have been linked to reduced blastocyst formation and pregnancy rate (Otsuki *et al.*, 2004).

While specific morphological abnormalities appear to be associated with oocyte quality, a precise quantification of the relative importance of different anomalies is currently lacking. In the absence of a comprehensive oocyte grading scheme, the power of morphological observations to aid oocyte/embryo selection is reduced. Furthermore, it is clear that only a minority of morphologically normal oocytes produce pregnancies, suggesting that most of the problems leading to poor embryonic development and implantation failure cannot be detected using standard microscopic evaluation.

## Cytogenetic assessment of oocytes

The potential for identifying viable oocytes by screening for chromosomal anomalies has been recognized for more than a decade (Verlinsky *et al.*, 1998). Oocytes can be tested for aneuploidy by biopsying the first and second polar bodies and subjecting them to cytogenetic analysis. The detection of extra or missing chromosomes in a polar body is indicative of a reciprocal loss or gain of chromosomes in the corresponding oocyte. Embryos derived from chromosomally normal oocytes can be given priority for transfer during assisted reproduction, potentially improving outcome by avoiding transfer of embryos carrying deleterious aneuploidies.

Classical cytogenetic techniques are difficult to apply to polar bodies, due to problems obtaining high quality chromosome spreads. For this reason, the vast majority of chromosomal tests performed on polar bodies have employed fluorescence in-situ hybridization (FISH). Using FISH, it is possible to assess 5–12 chromosomes in individual polar bodies/oocytes regardless of