

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

chromosome morphology (Verlinsky *et al.*, 1998; Kuliev *et al.*, 2003; Pujol *et al.*, 2003). More recently, comparative genomic hybridization (CGH) has been used to assess the copy number of chromosomes in polar bodies and oocytes, although to date most analyses have been performed in a research context (Wells *et al.*, 2002; Gutierrez-Mateo *et al.*, 2004; Fragouli *et al.*, 2006a,b). Comparative genomic hybridization has the major advantage that every chromosome is tested, rather than the limited subset assessed using fluorescence in-situ hybridization (FISH). Additionally, the technique avoids the need for fixation and spreading, techniques that are difficult to successfully apply to single cells.

The principal drawback of CGH is that it is a time-consuming method, making it difficult to perform within the narrow window available for preimplantation testing. Although recent advances in oocyte cryopreservation have largely overcome the problems associated with timing (Borini *et al.*, 2006; Bianchi *et al.*, 2007), the method remains labour intensive. The best hope for overcoming this limitation is the introduction of microarray-based CGH, an approach that is relatively easily automated, dramatically reducing the amount of time required for analysis. Significant progress has been made in the development of microarrays for preimplantation testing, and array-CGH protocols are currently undergoing preclinical testing in a number of PGD laboratories.

Despite the clinical successes of PGS, the fact remains that even transfer of embryos derived from euploid oocytes cannot guarantee a viable pregnancy. Clearly, there are many factors, both intrinsic and extrinsic, that influence oocyte competence. Most of these factors remain unknown at the current time. However, recent advances in the study of gene expression are shedding light on the underlying processes that determine the quality of an oocyte.

Analysis of gene expression

The investigation of gene expression has the potential to provide a detailed insight into pathways active in oocytes and their intimately associated cumulus cells. Almost all cellular processes display some degree of regulation at the transcriptional level, and consequently fluctuations in gene activity are usually indicative of an alteration in the utilization of a particular cellular pathway. It has been postulated that elevated expression of genes involved in detecting and responding to cellular stresses (e.g. DNA damage, hypoxia) may serve as an indicator of reduced competence.

A number of research groups, including that of the authors, have been interested in assessing gene expression in human oocytes, embryos and cumulus cells, aiming to improve understanding of the poorly defined events occurring during oogenesis and preimplantation development. It is also hoped that such research will define the gene expression profiles displayed by healthy oocytes, assisting efforts to identify genes and cellular pathways that provide an insight into oocyte/embryo quality.

Several investigations using reverse transcription, followed by real-time polymerase chain reaction (PCR), have found that specific genes display alterations in activity apparently related to oocyte or embryo quality (Wells *et al.*, 2005; Dode

et al., 2006; Russell *et al.*, 2006). Changes in oocyte mRNA associated with advancing maternal age have been reported, potentially linked to the well documented age-related increase in oocyte aneuploidy (Steuerwald *et al.*, 2001). The application of the same technology has also revealed that morphologically abnormal preimplantation embryos frequently display atypical patterns of gene expression (Wells *et al.*, 2005). These findings suggest that the analysis of gene expression is a worthwhile approach for the identification of new viability markers.

Despite the accuracy and sensitivity of real-time PCR, it is limited by the restricted number of genes that can be assessed per single oocyte or embryo (generally fewer than 10 genes). An alternative technology for the study of gene expression, which avoids this limitation, is the microarray. Utilization of microarray technology permits the simultaneous analysis of tens of thousands of genes and offers great promise for the identification of novel targets and pathways relevant to oogenesis.

The problem faced in applying microarrays to oocytes, cumulus cells and embryos is the minute amount of material available for testing. Microarrays generally require several orders of magnitude more RNA than can be recovered from a single cell. To overcome this problem, some studies have pooled large numbers of samples, a possibility for research involving model organisms, but rarely achievable in humans. The only alternative to pooling is RNA amplification.

Amplification of the RNA can be achieved using linear amplification (in-vitro transcription-based protocols) or exponential amplification (PCR-based strategies) (Bermúdez *et al.*, 2004; Kocabas *et al.*, 2006a; Jones *et al.*, 2007; Wood *et al.*, 2007; Wells *et al.*, unpublished data). Although both methods can generate sufficient material for subsequent microarray analysis, most groups have focused on linear amplification via a two-round in-vitro transcription method (**Figure 1**). In the authors' hands, this approach generates ~10 µg of material from single oocytes, sufficient for testing using a microarray (Applied Biosystems, USA). The main advantage of linear amplification is that it is less prone to amplification artefacts (e.g. preferential amplification of small mRNA species) than PCR-based methods.

Preliminary microarray studies in oocytes

Currently, gene expression analyses (microarray or real-time PCR) are being utilized for research purposes, with the aim of identifying novel markers of oocyte competence by assessing oocytes or cumulus cells. Oocytes, cumulus cells, follicular fluid and culture media are also being assessed using metabolomic and proteomic approaches for the same purpose. **Figure 2** summarizes the methods used for assessing different components of the follicular unit.

Comparison of microarray findings with oocyte characteristics, such as stage of maturity, morphology, patient aetiology and aneuploidy, has begun to yield a wealth of information concerning cellular activities occurring within the oocyte. A pilot study, assessing single high quality MII oocytes and also oocytes pooled in groups of five or 10 revealed over 8237 genes expressed

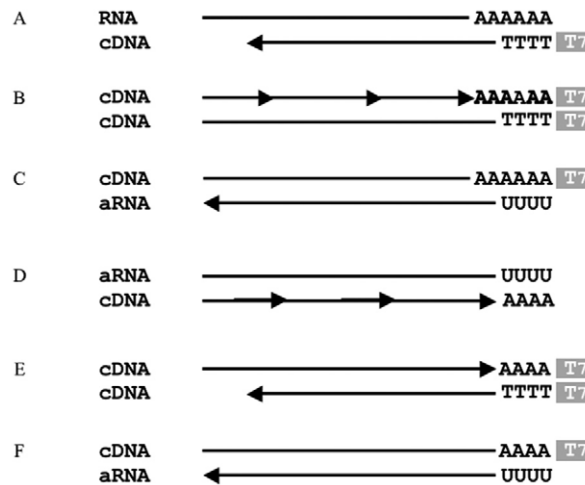


Figure 1. RNA amplification for the analysis of single oocytes using microarrays. (A) RNA is extracted from oocytes or cumulus cells and converted to cDNA. This is accomplished by annealing an oligo-dT primer to the polyA tail of the mRNA and using reverse transcriptase. The oligo-dT primer also contains a T7-bacteriophage promoter sequence. (B) The original mRNA template is degraded, creating multiple short fragments of mRNA, which serve as primers for second-strand synthesis using a DNA polymerase, resulting in the production of double-stranded cDNA. (C) A T7-bacteriophage derived RNA polymerase is introduced and generates new RNA molecules, directed by the T7-bacteriophage promoter at the end of each cDNA fragment. (D) A second round of reverse transcription is undertaken using random primers and reverse transcriptase. (E) Double-stranded cDNA is generated using DNA polymerase and the oligo-dT primer containing the T7-bacteriophage promoter sequence. (F) Finally, a second round of amplification is performed by once again adding a T7-bacteriophage-derived RNA polymerase. Labelled nucleotides are incorporated into the RNA during amplification, permitting subsequent detection after hybridization to a microarray.

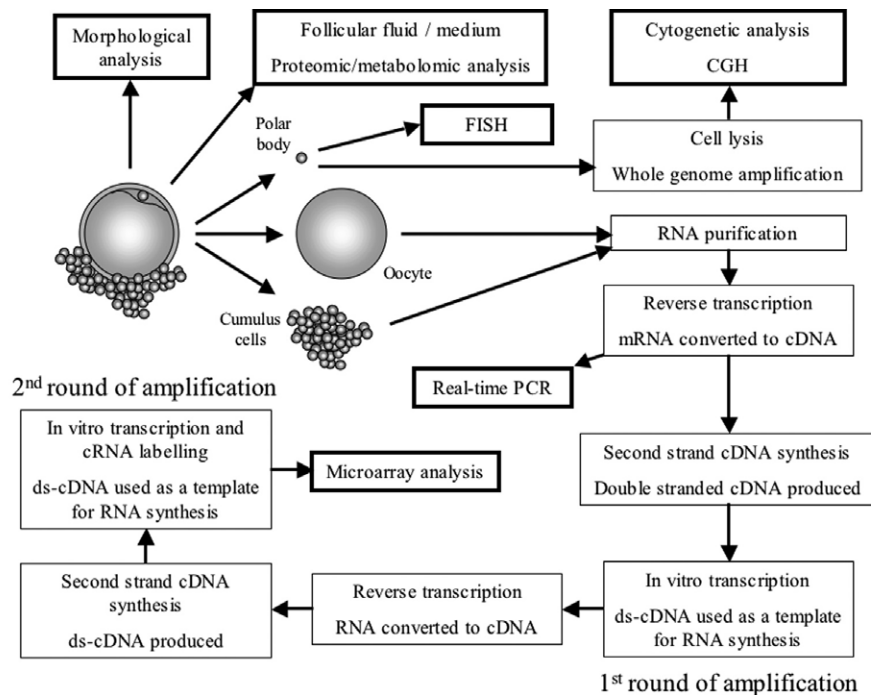


Figure 2. Summary of the methods used for assessing different components of the follicular unit. Currently gene expression analyses [microarray or real-time polymerase chain reaction (PCR)] are utilized for research purposes, with the aim of identifying novel markers of oocyte competence by assessing oocytes or cumulus cells. Oocytes, cumulus cells, follicular fluid and culture media are also being assessed using metabolomic and proteomic approaches for the same purpose. Cytogenetic testing of polar bodies using fluorescence in-situ hybridization (FISH) is already well established in a clinical context, although only employed by a small minority of IVF laboratories. Comparative genomic hybridization (CGH) has provided a more detailed cytogenetic analysis for several research studies and there is renewed interest in employing this technique clinically. ds = double stranded.

in all of the samples tested (Wells *et al.*, 2006). In addition to these core 'oocyte' genes, transcripts from a large number of additional genes (7750) were detected in individual oocytes, but did not display universal expression. These differentially expressed genes may explain some of the underlying variation in competence between individual oocytes.

Analysis of the transcriptome of aneuploid and normal oocytes, as determined by CGH analysis of the corresponding polar body, has identified several differentially expressed genes with roles in chromosome segregation during meiosis, including genes affecting cell cycle checkpoints, spindle dynamics and chromosomal movement. The remarkably high rate of aneuploidy seen in human oocytes has been without a satisfactory explanation for decades. However, genes consistently displaying abnormal expression in aneuploid oocytes may provide an insight into this problem, highlighting underlying problems in the meiotic machinery that lead to the generation of abnormal eggs. Another interesting observation is that some genes displaying altered expression in aneuploid oocytes code for secreted proteins, suggesting that non-invasive methods of assessing oocyte aneuploidy could be developed in the future.

Other studies have also provided a valuable insight into oocyte gene expression via their use of microarray technology. The changes in levels of specific mRNA transcripts that occur as an oocyte matures from the germinal vesicle stage to MII have been catalogued in the mouse (Cui *et al.*, 2007; Su *et al.*, 2007) potentially revealing genes of relevance to oocyte maturation. Comparison of human MII oocytes that were mature at the time of retrieval with those matured from the germinal vesicle stage *in vitro* (IVM oocytes) revealed significant differences in gene expression. Genes associated with nuclear maturity (e.g. DNA synthesis, cell cycle, chromosome segregation) were generally expressed at appropriate levels in IVM oocytes, whereas genes involved in certain cytoplasmic functions (e.g. protein synthesis and modification) continued to display immature patterns of expression. This suggests that although IVM oocytes successfully progress to meiosis II cytoplasmic deficiencies may ultimately prevent them from forming a viable embryo. Abnormal expression of specific proteins related to homeostasis was also observed in IVM oocytes, highlighting specific problems with current IVM protocols, such as deficiency of certain metal ions (Wells and Patrizio, unpublished data).

Other studies focused on human oocytes have catalogued the genes expressed and revealed genes that may serve as markers of oocyte competence (Gasca *et al.*, 2007). Further investigation of these genes may lead to new tests for oocyte maturity/quality and improvements to oocyte and embryo culture methods. Microarray studies, conducted in humans and mice, have also revealed age-related differences in oocyte gene expression (Hamatani *et al.*, 2004; Steuerwald *et al.*, 2007). Some of the genes highlighted by these studies may point to the underlying basis of the negative association of age and oocyte competence, a problem that seems to extend beyond a simple age-related increase in aneuploidy, and severely affects success rates of assisted reproduction for older women.

Comprehensive analyses of gene expression may also provide an improved understanding of the cause of specific infertile aetiologies. Wood and colleagues (Wood *et al.*, 2007), identified 374 genes with differences in mRNA transcripts when comparing

morphologically indistinguishable oocytes from ovulatory normal women and patients with polycystic ovarian syndrome (PCOS). Interestingly, 68 of the differentially expressed genes contained putative androgen receptor and/or peroxisome proliferating receptor γ binding sites, while other genes were involved with chromosome alignment and segregation (Wood *et al.*, 2007). These differences could provide a partial explanation for the reduced fecundity observed in PCOS.

Gene expression in cumulus cells

The investigation of gene expression in cumulus cells is also of great interest both scientifically and clinically. The cumulus cells have a critical role in supporting oocyte growth and maturation. Knowledge of the oocyte–cumulus interactions required for the generation of viable oocytes would be extremely useful, particularly for groups involved in the optimization of in-vitro maturation protocols.

Cumulus cells share the same follicular environment as the oocyte with which they are associated. Thus, analysis of gene expression in these cells may provide an indication of the microenvironment in which the oocyte matured. For example, if genes that are typically activated in response to hypoxia are found to be up-regulated in cumulus cells, it is likely that the corresponding oocyte has also experienced oxygen deprivation, potentially affecting its ability to form a viable embryo.

The fact that cumulus cells can be collected and analysed without manipulating or compromising the oocyte makes them attractive as targets for oocyte competence assays. Several studies have already linked differential gene expression in granulosa cells with subsequent embryonic developmental capacity (Robert *et al.*, 2003; McKenzie *et al.*, 2004). More recently microarray analysis has permitted the cataloguing of virtually all of the genes expressed in cumulus cells, a first step on the road towards identifying genes influencing or associated with oocyte competence (Hernandez-Gonzalez *et al.*, 2006).

Several groups, including that of the authors, are attempting to identify new markers of oocyte quality by correlating changes in cumulus cell gene expression with various measures of IVF outcome. For this purpose, RNA amplification and microarray analysis are applied to clumps of cumulus removed from individual oocytes. The oocytes are cultured separately and used for assisted reproductive treatment, with the outcome closely monitored. Results are preliminary, but show promise, suggesting that this approach may yield non-invasive tests for oocyte competence in the near future (Kocabas *et al.*, 2006b; Gasca *et al.*, 2007).

Analysis of protein expression and metabolomics

Although data concerning gene expression provide useful information regarding the genetic activity of oocytes and cumulus cells, a change in the number of mRNA transcripts derived from a given gene does not necessarily indicate altered utilization of the pathway in which it functions. Most genes experience some degree of regulation at the post-translational level, through protein modification, degradation or sequestration.

Consequently, there may be occasions when a change in the concentration of active protein is not mirrored by an alteration in gene activity. An additional consideration is that many genes produce more than one type of protein, accomplished by utilizing mechanisms such as alternative splicing and post-translational modification. Investigation of gene expression provides no information concerning post-translational events.

Recent advances in mass spectrometry have led to the development of methods sensitive enough to permit the proteomic examination of single oocytes and embryos. The use of surface-enhanced laser desorption and ionization time-of-flight mass spectrometry (SELDI-TOF MS) has allowed the generation of proteomic profiles for individual human blastocysts (Katz-Jaffe *et al.*, 2006a). Detection of various secreted proteins has also been successfully achieved (Katz-Jaffe *et al.*, 2006b). SELDI-TOF MS involves binding of proteins to a chip, followed by their release and ionization via laser activation. The mass: charge ratio of the liberated ions is determined by time-of-flight mass spectrometry. The mass: charge ratios of the many different protein fragments produced during this process results in a unique proteomic fingerprint for each sample assessed.

Although little proteomic data concerning oocytes is currently available, results obtained from preimplantation embryos suggest that research in this area will ultimately be fruitful in identifying new viability markers. Degenerating embryos have been shown to display numerous alterations in protein concentrations when compared with developing blastocysts (Katz-Jaffe *et al.*, 2006a). Of course, the real challenge for both gene expression and proteomic approaches is to find markers which are associated with viability, but are unrelated to morphology. Proteomic analysis of embryos has already detected distinct patterns of protein concentrations that were related neither to developmental stage nor morphology. In some cases, such changes may be related to viability and implantation potential, but these remain to be proven.

Additionally, measurement of metabolic by-products excreted into the media surrounding the oocyte could assist optimizing and/or developing new media for enhanced oocyte maturation as well as providing information concerning viability. Such studies have so far been carried out using mouse oocytes and embryos (Gardner and Leese, 1987; Lane and Gardner, 1996; Downs *et al.*, 2002; Roberts *et al.*, 2004; Preis *et al.*, 2005). In one of these investigations, it was shown that cumulus-oocyte complexes, which absorb larger amounts of glucose and produce more lactate, have the highest fertilization rates (Preis *et al.*, 2005).

Conclusions

Clinical data derived by calculating the live birth rates according to the number of embryos transferred or to the number of oocytes retrieved, are suggestive of very high wastage during assisted reproduction (Kovalevsky and Patrizio, 2005). There are several hypotheses that need to be validated to explain the low oocyte to baby rate during IVF procedures. If it is true that only a small fraction of the oocytes retrieved in a given assisted reproduction cycle have the potential to produce a viable embryo and a live birth, then the challenge is to correctly

identify the few competent oocytes for insemination and ensure that the resulting embryo(s) are prioritized for transfer.

The ability to reinitiate the meiotic process and undergo preimplantation development is progressively determined during the antral phase. It is well known that these changes involve the nuclear and cytoplasmic compartments, but the underlying cellular and molecular mechanisms are still poorly understood (Royere, 2006). In natural cycles, only one antral follicle generally becomes dominant, the remainder of the developing follicles undergoing atresia. This situation is in contrast to ovarian stimulation for assisted reproduction, in which these follicles are rescued from atresia at various stages of development, but their oocytes may be already intrinsically abnormal. Thus, when human chorionic gonadotrophin is administered to activate oocyte maturation prior to retrieval, oocytes still in earlier stages of development may be activated prematurely, thus leading to an increased risk of errors during the completion of meiosis and/or incomplete cytoplasmic maturation (Kovalevsky and Patrizio, 2005).

An alternative hypothesis is that the in-vitro culture system can affect the developmental competence of oocytes and embryos. However, cytogenetic analysis of polar bodies indicates that at least 22% of MII oocytes are already aneuploid when they are collected (Verlinsky *et al.*, 1998; Kuliev *et al.*, 2003; Pujol *et al.*, 2003; Gutierrez-Mateo *et al.*, 2004; Fragouli *et al.*, 2006a,b). Aneuploidy is remarkably common in human oocytes and is a major cause of spontaneous abortion, embryonic arrest, implantation failure, as well as failed IVF cycles. Despite the importance of aneuploidy in human reproduction the biological mechanisms underlying its origins remain poorly defined.

Comprehensive analysis of gene expression employing microarray technology has recently been achieved for minute samples, including single oocytes. The insight gained from such investigations promises to revolutionize the understanding of oocyte biology, revealing mechanisms giving rise to morphological and genetic anomalies, including aneuploidy. Research currently underway is likely to lead to improvements in in-vitro maturation media, as well as identification of new markers of oocyte competence.

The microarray approaches briefly described, involve the analysis of whole oocytes, rendering them useless for the purposes of clinical testing. More attractive prospects for clinical oocyte assessment may prove to be proteomic analysis of excreted proteins or analysis of cumulus cell gene expression, as these cells can be tested without jeopardizing the viability of the associated oocyte. Once fully validated, these approaches may turn out to be the ideal non-invasive methods for identifying oocytes destined to produce chromosomally normal, viable embryos with high likelihood for live birth, thus improving the overall efficiency of the IVF process.

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