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Gene expression profiling of human oocytes following *in vivo* or *in vitro* maturation

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BACKGROUND: Immature human oocytes matured *in vitro*, particularly those from gonadotrophin stimulated ovaries, are developmentally incompetent when compared with oocytes matured *in vivo*. This developmental incompetence has been explained as poor oocyte cytoplasmic maturation without any determination of the likely molecular basis of this observation. METHODS: Replicate whole human genome arrays were generated for immature and mature oocytes (matured *in vivo* and *in vitro*, prior to exposure to sperm) recovered from women undertaking gonadotrophin treatment for assisted reproduction. RESULTS: More than 2000 genes were identified as expressed at more than 2-fold higher levels in oocytes matured *in vitro* than those matured *in vivo* ($P < 0.05$, range 4.98×10^{-2} – 2.22×10^{-4}) and 162 of these are expressed at 10-fold or greater levels ($P < 0.05$, range 4.98×10^{-2}–1.38×10^{-3}). Many of these genes are involved in transcription, the cell cycle and its regulation, transport and cellular protein metabolism. CONCLUSIONS: Global gene expression profiling using microarrays and bioinformatics analysis has provided a molecular basis for differences in the developmental competence of oocytes matured *in vitro* compared with *in vivo*. The over-abundance of transcripts identified in immature germinal vesicle stage oocytes recovered from gonadotrophin stimulated cycles and matured *in vitro* is probably due to dysregulation in either gene transcription or post-transcriptional modification of genes. Either mechanism would result in an incorrect temporal utilization of genes which may culminate in developmental incompetence of any embryos derived from these oocytes.

Keywords: oocyte; gene expression; *in vitro* maturation; microarray

Introduction

Mammalian ovarian folliculogenesis is characterized by growth of follicles and the associated oocytes. The growing oocyte remains arrested in Prophase I of the first meiotic division and is highly transcriptionally and translationally active (Bachvarova, 1985). During the growth phase, the oocyte accumulates both RNA and proteins required for completion of the meiotic cell cycle, events such as chromatin remodeling and cell cycle activation associated with fertilization, the first mitotic cell cycles, the establishment of an embryonic genome and normal metabolic and homeostatic processes (Zheng *et al*., 2005). Oocytes that fail to complete the growth phase or fail to accumulate and regulate these molecules resulting in an incorrect temporal utilization would be expected to exhibit delays or failure in progression through preimplantation development following fertilization. The ability to complete preimplantation development to the blastocyst stage and successfully implant in a receptive uterus is generally referred to as developmental competence and this may be completely independent of meiotic competence (Trounson *et al*., 2001). Human oocytes acquire the ability to complete nuclear maturation when the follicle reaches ~5 mm in diameter (Wynn *et al*., 1998) and the oocytes are ~90 μm in diameter (Durinzi *et al*., 1995) but complete developmental competence is probably not achieved until the follicle reaches a diameter of 10–12 mm (Trounson *et al*., 2001). Transcripts accumulated throughout the human oocyte growth phase control development until a transcriptionally active embryonic genome is established at the 4- to 8-cell stage of embryo development (Braude *et al*., 1988).

Much of our knowledge of transcription and translation in oocytes has been acquired from the study of gametes from laboratory animal species. Little is known about the corresponding molecular events in the human oocyte due to the limited number of oocytes available for study. Understanding of the molecular events that result in a developmentally competent human oocyte and the extent to which gene expression profiles can be altered by pathologies that underlie infertility or the

cellular manipulations that are used for assisted reproduction procedures, are critical for understanding and addressing improvements in fertility. The advent of commercial microarrays and high-fidelity RNA amplification techniques now makes it possible to profile the polyadenylated [poly(A)] RNA transcripts in the rarely available human oocyte.

Several groups have used microarrays to profile the transcriptome of the human oocyte (Bermudez *et al*., 2004; Dobson *et al*., 2004; Assou *et al*., 2006; Kocabas *et al*., 2006; Li *et al*., 2006a; Zhang *et al*., 2006; Gasca *et al*., 2007). Although much of the information is useful and concurs with previous findings in the oocytes of laboratory animal species, interpretation of gene expression patterns is nonetheless restricted because of the dependence on a number of critical factors that themselves influence expression, e.g. oocytes aged *in vitro* were used to represent the mature metaphase II (MII) oocyte in some or all instances (Bermudez *et al*., 2004; Dobson *et al*., 2004; Assou *et al*., 2006; Gasca *et al*., 2007), a limited coverage of the human transcriptome on the microarray (Bermudez *et al*., 2004; Dobson *et al*., 2004; Li *et al*., 2006a), a lack of sufficient biological replication to produce statistically meaningful results for all samples studied (Bermudez *et al*., 2004; Dobson *et al*., 2004; Assou *et al*., 2006; Li *et al*., 2006a; Zhang *et al*., 2006; Gasca *et al*., 2007) and the use of single oocytes for some arrays (Bermudez *et al*., 2004; Dobson *et al*., 2004; Li *et al*., 2006a). It has been demonstrated previously that the amplification and microarray methodologies employed in the present study fails to produce a fully representative gene expression profile from a single oocyte because of the bias introduced when amplifying from low template RNA samples (Jones *et al*., 2007). There is no published data to suggest that other amplification and microarray methodologies are not similarly limited. Furthermore, the majority of the studies of the oocyte transcriptome in the medical literature report on an isolated developmental stage and make comparisons with unrelated, or loosely related, material such as preimplantation embryos (Dobson *et al*., 2004; Li *et al*., 2006a), cumulus cells (Assou *et al*., 2006), somatic tissues and embryonic stem cells (Kocabas *et al*., 2006; Zhang *et al*., 2006) and as such, it is difficult to derive meaningful data about the gene expression profile of a developmentally competent human oocyte.

The present study was undertaken to comprehensively identify genes associated with maturing human oocytes and biological processes or regulation of the transcriptome that are associated with the gain or loss of developmental competence. The majority of oocytes recovered from infertile women following superovulation are at the mature meiotic metaphase II (MII) stage and 70–80% of these are capable of fertilization with ~50% of zygotes able to complete development to the blastocyst stage (Jones, 2000). Approximately 35% of transferred embryos/blastocysts derived from these oocytes develop to term (Blake *et al*., 2005). In contrast, when oocytes with an immature nucleus are recovered following superovulation and are matured *in vitro*, only 12% of the resulting zygotes develop to the blastocyst stage (Chen *et al*., 2000) and only 14% of these embryos when transferred develop to term (Veeck *et al*., 1983; Nagy *et al*., 1996; Edirisinghe *et al*., 1997; Tucker *et al*., 1998; De Vos *et al*., 1999; Chen *et al*., 2000; Vanhoutte *et al*., 2005). The majority of the reported pregnancies have been isolated case reports resulting from *in vitro* maturation of partly mature metaphase I (MI) oocytes.

The present study compared the transcriptome of oocytes with relatively high developmental competence (*in vivo* matured oocytes following superovulation) with the transcriptome of oocytes with very low developmental competence (*in vitro* matured oocytes following superovulation for IVF) in an attempt to identify a molecular basis for the difference in developmental competence. Identification of the molecular basis for oocyte developmental competence may result in improvements to maturation protocols for immature oocytes recovered not only following superovulation but also for immature oocytes recovered in the absence of gonadotrophin stimulation. The former would be important for the few patients for whom the majority of oocytes recovered following superovulation is immature and therefore not suitable for microinjection and also for human somatic cell nuclear transfer, as these oocytes presently represent the largest pool of donor oocytes available for research and autologous stem cell therapy.

Materials and Methods

Human oocyte collection

Human oocytes in excess to the requirements of treatment for assisted reproduction were donated to research following informed consent from patients undertaking a long down-regulation superovulation protocol with GnRH agonists and gonadotrophin for treatment of infertility at Monash IVF Pty Ltd, Melbourne, Australia, and SISMER, Bologna, Italy. The oocytes were obtained under ethics approval from Monash Private Surgical Hospital Human Research Ethics Committee (#02044), SISMER Institutional Review Board and Monash University Standing Committee on Ethics in Research involving Humans (#2004/469MC and #2005/272ED). Oocyte–cumulus complexes were evaluated for maturity in the embryology laboratories within 1–4 h of collection following removal of cumulus cells using hyaluronidase (25 IU/ml Hyalase; CP Pharmaceuticals, Wrexham, UK, or 40 IU/ml; Medicult, Jyllinge, Denmark). Immature, germinal vesicle (GV) and GV breakdown/MI oocytes and mature MII oocytes in excess to requirements were donated to research within 4–6 h of collection. Oocytes donated by Monash IVF patients were transported to Monash Immunology and Stem Cell Laboratories (MISCL) in HEPES buffered culture medium (Sydney IVF oocyte wash buffer; William A. Cook Australia, Eight Mile Plains, Qld or Quinns Advantage® medium; Sage, Cooper Surgical Co., Trumbull, CT, USA) at 37°C and were processed to preserve RNA immediately. Oocytes donated by SISMER patients were processed to preserve RNA on site, stored in liquid nitrogen and transferred to MISCL by air freight on dry ice.

Human oocyte development in vitro

A proportion of oocytes at the GV stage showing clear cytoplasm and no obvious inclusions were not processed immediately for RNA but were placed in pre-equilibrated *in vitro* maturation medium (Medicult) supplemented with 0.075 IU/ml human recombinant FSH (Gonal-F; Serono Australia, Frenchs Forest, NSW, Australia), 0.1 IU/ml hCG (Profasi; Serono Australia) and 5 mg/ml human serum albumin (HSA; Sage, Cooper Surgical Co.) and incubated at 5% CO_2 for

24 h at 37°C. At the end of the incubation period, oocytes were assessed for maturity and oocytes with a visible polar body in the perivitelline space were processed for RNA as *in vitro* matured MII oocytes (IVM).

Oocyte processing for RNA preservation

Oocytes were re-evaluated to ensure the maturational status and morphological quality prior to processing to preserve RNA. The zona pellucida was removed chemically by exposure to 0.2% Pronase (Sigma Chemical Company, St Louis, MO, USA) in HEPES buffered culture medium or acidified Tyrode's solution and the naked oocyte immediately washed 3× in HEPES buffered culture medium supplemented with 5 mg/ml HSA (Sage, Cooper Surgical Co.). Each naked oocyte was then washed 4× in Dulbecco's phosphate buffered saline (Gibco BRL, Invitrogen Corporation, Grand Island, NY, USA) without protein and transferred in a minimal volume of medium to an RNA–DNA-free PCR tube containing 5 μl of Picopure extraction buffer (Arcturus Bioscience Inc., Mountain View, CA, USA). Tubes were snap frozen in liquid nitrogen and stored at a minimum temperature of −80°C until required.

RNA extraction, labeling and hybridization on Codelink whole human genome microarrays

Prior to RNA extraction, oocyte lysates were pooled in groups of five according to the oocyte maturational stages: GV, MI, MII and IVM, as it has been demonstrated that pooling in these numbers generates a representative gene expression profile with high fidelity and sensitivity (Jones *et al*., 2007). In order to remove any bias associated with the donor's age or etiology of infertility and generate a gene expression profile typical of the maturation conditions and/or developmental stage, oocytes from different donors were pooled wherever possible.

Total RNA from the pooled lysates in Picopure extraction buffer was isolated using the Picopure RNA Isolation Kit (Arcturus Bioscience Inc.) according to the manufacturer's protocol. Conversion of the T7 tagged complementary DNA (cDNA) was achieved in two rounds of synthesis using the RiboAmp HS RNA Amplification Kit (Arcturus Bioscience Inc.). The T7 tagged cDNAs were purified through Micro-Spin S-400HR columns (GE Healthcare Biosciences, Little Chalfont, England) and biotinylated cRNA generated by *in vitro* transcription using the Codelink Expression Assay Reagent Kit (GE Healthcare Biosciences, Piscataway, NJ, USA). Biotin-labeled cRNA was purified using an RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) and the quantity and purity evaluated by UV spectrophotometry at 260 and 280 nm. The total yield of biotinylated cRNA was 30–68 μg for GV, 35–46 μg for MI, 10–72 μg for MII and 26–32 μg for IVM oocytes. The final cRNA concentrations were adjusted to 1 μg/μl.

Prior to hybridization, 10 μg biotinylated cRNA was fragmented in 25 μl of 1× fragmentation buffer (Codelink Expression Assay Reagent Kit) at 95°C for 20 min. Hybridization reaction buffer (260 μl) was prepared by mixing 25 μl of fragmented cRNA, 78 μl of hybridization buffer component A, 130 μl of hybridization component B and 27 μl of nuclease-free water (Codelink Expression Assay Reagent Kit). The mixture was incubated at 90°C for 5 min, chilled on ice and injected into the inlet port of the hybridization chamber of Codelink Whole Human Genome Bioarrays printed with 54 840 discovery probes representing 18 055 human genes and an additional 29 378 human expressed sequence tags (EST) (GE Healthcare Biosciences). The chamber ports were sealed with 1 cm sealing strips and hybridized for 18 h at 37°C on an orbital shaker (Innova 4080; New Brunswick Scientific Co., Edison, NJ, USA) at 300 rpm. After hybridization, the hybridization chamber was removed from each slide and arrays were briefly rinsed with 0.75× TNT buffer (0.1 M Tris–HCl, pH 7.6, 0.15 M NaCl, 0.05% Tween-20; Sigma Aldrich Corporation, St. Louis, MO, USA) at room temperature, followed by an hour at 46°C in 0.75× TNT buffer. The signal was developed by incubating the arrays in a 1:5000 Cy5-streptavidin working solution (GE Healthcare Biosciences) at room temperature for 30 min followed by four successive 5 min washes in 1× TNT buffer. Arrays were dried by centrifugation and scanned on an Axon GenePix Array Scanner (Molecular Devices Co., Sunnyvale, CA, USA) with the laser set at 635 nm, the photomultiplier tube at 600 V and the scan resolution at 5 μm and images captured as TIFF files. Codelink Expression Analysis version 4.2 software (GE Healthcare Biosciences) was used to analyze images for each slide. Spots with intensities below that of the negative control (absence of an oligonucleotide probe) were excluded, as were those with irregular shapes or near-background intensity or oligonucleotides masked as part of the quality control process during manufacture. Spot quality and signal intensities were exported to Genespring compatible report format.

Microarray data analysis

The Codelink Expression Analysis output was loaded into Genespring GX 7.3.1 (Agilent Technologies, Santa Clara, CA, USA) and values below 0.01 were set to 0.01 with per chip normalization to the 50th percentile and per gene normalization to the median.

A principal components analysis based on all genes was applied to all microarrays in the experiment prior to any detailed statistical analysis to identify any samples that showed significant variability and to identify whether there was discrimination between the different developmental stages. Prior to statistical analysis of MII oocytes matured *in vivo* versus *in vitro* filtering was conducted to eliminate data of poor quality. Data were progressively filtered: less precise measurements based on control strength were removed, measurements for each condition with <80% confidence were removed, probes recorded as absent in all samples were removed and the measurements for probes representing the positive and fiducial controls rather than probes representing the 54 840 Codelink discovery probes were removed. A Welch *t*-test with a false discovery rate of 0.05 followed by a Benjamini and Hochberg multiple testing correction was applied to the quality data to identify probes that were expressed significantly differently at the 5% level between oocytes matured *in vivo* and *in vitro*. Genespring GX 7.3.1 Bioscript Library 2.2 Biological Pathways analysis was used to identify the probes associated with particular gene ontology (GO) biological processes represented on the microarrays that were significantly over-represented at the 5% level within the list of probes identified as having significantly different expression levels between *in vivo* and *in vitro* matured oocytes.

Results

Global characteristics of human oocyte gene expression according to maturational stage and maturation conditions

A total number of 5 GV, 3 MI, 11 MII and 3 IVM microarrays, representing five pooled oocytes for each array, were completed. The total number of probes detected varied from 22 532–31 837 probes for GV oocytes (10 017–12 734 genes), 27 789–28 843 probes for MI oocytes (12 011–12 697 genes), 12 238–29 593 probes for MII oocytes (4801–12 031 genes) and 20 522–22 624 probes for IVM oocytes (9237–9933 genes). Principal components analysis of gene expression based on all genes on the arrays identified

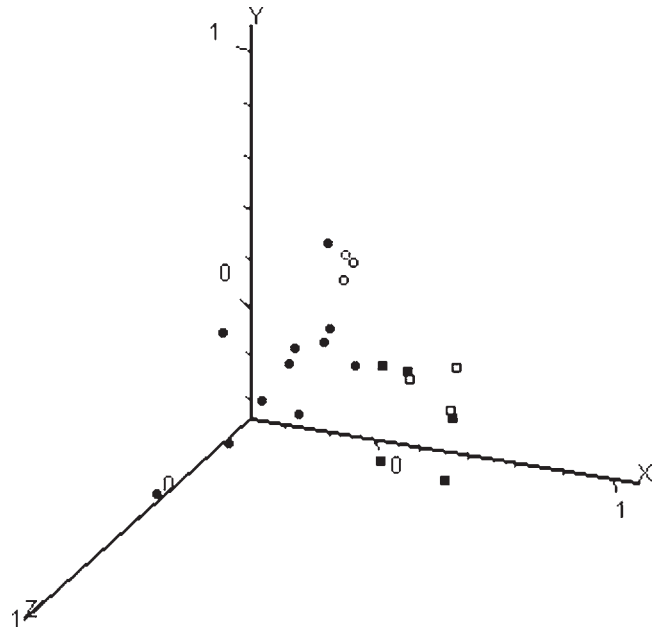

Figure 1: Principal components analysis of the 22 human stage-specific oocyte microarrays based on all genes: GV stage oocytes (closed squares), GV breakdown or metaphase I oocytes (open squares), *in vitro* matured metaphase II oocytes (open circles) and *in vivo* matured metaphase II oocytes (closed circles).

variability in gene expression among the *in vivo* matured MII oocytes (Fig. 1). Nevertheless the developmental stages and maturation conditions for the most part clustered together with immature, GV and MI oocytes showing similar gene expression profiles and *in vivo* and *in vitro* matured oocytes showing distinctly different gene expression profiles with very little overlap between the two maturation conditions. To minimize false positives, only those probes detected in the majority of replicates for each developmental stage/maturation condition were taken as expressed. In the majority of GV oocytes 24 562 probes (10 962 genes) were detected, and 27 980 probes (12 329 genes) were detected in the majority of MI oocytes. Fewer probes were detected in the majority of MII oocytes regardless of maturation condition (17 650 and 21 336 probes representing 7546 and 9479 genes for *in vivo* and *in vitro* matured oocytes, respectively; Table I).

Comparative gene expression for human oocytes matured in vivo or in vitro

When the data for *in vivo* and *in vitro* matured oocytes were filtered for quality data and the data further limited to probes represented in the majority of MII (6/11) or the majority of IVM (2/3) replicates, there was a significant difference in gene expression ($P < 0.05$) between *in vivo* and *in vitro* matured oocytes for 4226 probes (2766 genes). When this data were further limited to those probes that were statistically significantly different at the 2-fold or higher level, 3250 probes (2390 genes) were identified (Table II). The majority of probes (3166 probes representing 2348 genes; Table II) were expressed at greater than 2-fold higher levels in oocytes matured *in vitro*. This list was subjected to further analysis to determine which of the functional categories assigned by GO terms (Gene Ontology Consortium, 2001) were over-represented at the 95% confidence level with a minimum overlap set to two genes. Over-representation does not evaluate gene expression levels but instead groups genes according to processes that occur more often in the gene list of interest than could be predicted by the distribution among all genes for a particular process represented on the array. One hundred and ninety-eight GO biological processes were identified to be significant at the 95% level which were re-categorized into 15 main groups (Table III) including in order of significance probes involved in transcription, the cell cycle, transport and cellular protein metabolism.

Table I. The number of probes and genes detected in the majority of microarrays for immature (GV and MI) oocytes and oocytes matured *in vivo* (MII) and *in vitro* (IVM).

Developmental stage	Number of independent samples	Number of probes detected	Number of genes
GV	5	24 562	10 962
MI	3	27 980	12 329
MII	11	17 650	7546
IVM	3	21 336	9479

GV, germinal vesicle; MI, metaphase I; MII, metaphase II; IVM, *in vitro* matured MII oocytes.

Only a small number of probes were identified at greater than 2-fold higher levels in oocytes matured *in vivo* (84 probes representing 42 genes; Table II). This list was also subjected to analysis to determine the GO biological processes most over-represented at the 95% confidence level with a minimum overlap set to two genes. Four GO biological processes were identified to be significant at the 95% level which were re-categorized into three main groups (Table III) including, in ranked order, transport, cell growth and signal transduction.

Differentially expressed genes 10-fold or more different in oocytes matured in vitro or in vivo

In order to clarify a gene list that might better reflect differences between oocytes matured *in vivo* and *in vitro* a cut-off of 10-fold different was applied to the list of 4226 probes (2766 genes) that were demonstrated to be statistically different. The subset of these probes expressed at 10-fold or greater levels in oocytes matured *in vivo* or *in vitro* together with the National Center for Biotechnology Information accession number for all known genes, EST and corenucleotide sequences are provided in Supplementary Table 1. Note that some probes on the Codelink microarrays represent more than one nucleotide sequence and occasionally represent more than one gene. Supplementary Table 1 also includes the official gene name and gene symbol for each of the genes represented, the chromosomal location, the known associated biological processes, the mean normalized expression value from the microarrays for oocytes matured *in vitro* and *in vivo*, the *P*-value and the relative ratio of expression differences.

For oocytes matured *in vitro*, 197 probes (162 genes) were expressed at 10-fold greater levels than in oocytes matured

Table II. Fold change differences in gene expression for the majority of *in vivo* (MII) and *in vitro* matured (IVM) oocyte microarrays (*$P < 0.05$).

Trend	Gene expression	Significantly* different	>2-fold	>5-fold	>10-fold	>25-fold	>50-fold
IVM>MII	Genes	2627	2348	1025	162	7	3
	Probes	3776	3166	1280	197	12	3
IVM< MII	Genes	139	42	15	3	0	0
	Probes	450	84	23	6	0	0

Table III. Functional characterization of transcripts more than 2-fold higher in *in vitro* matured oocytes and *in vivo* matured oocytes.

Rank	Category	Number of overlapping probes in gene list
In vitro matured oocytes		
1	Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	469
	A. Transcription	324
	B. DNA metabolism	71
	C. RNA metabolism	109
2	Cell cycle	135
3	Transport	254
4	Cell division	49
5	Cellular protein metabolism	382
6	Response to stress	74
	A. Response to DNA damage stimulus	57
	B. Response to oxidative stress	2
7	Cell death	78
8	Signal transduction	259
9	Cell proliferation	61
10	Generation of precursor metabolites and energy	22
11	Cell organization and biogenesis	60
	A. Cytoskeleton organization and biogenesis	22
	B. Chromosome organization and biogenesis	10
12	Biological process unknown	67
13	Reproduction	28
14	Cellular lipid metabolism	35
15	Development	72
In vivo matured oocytes		
1	Transport	6
2	Cell growth	2
3	Signal transduction	2

The most frequent categories of GO biological processes were identified using GeneSpring GX 7.3.1 Bioscript Library 2.2 Biological Pathways Analysis. Only GO biological processes where two or more probes overlapped with a likelihood of a random overlap <5% are shown. These biological processes were further re-categorized so that the most frequent GO biological processes were grouped according to statistical significance.

in vivo. Genes from all 23 chromosomes were represented in this list. Additionally, the biological processes over-represented in the list of genes that were expressed at 2-fold higher levels in oocytes matured *in vitro* are similarly represented in the list of genes that are expressed at 10-fold higher levels. For oocytes matured *in vivo*, only six probes (three genes) were expressed at 10-fold greater levels than oocytes matured *in vitro*.

Discussion

The present study has defined the global gene expression profile of oocytes recovered at all maturational stages of development following gonadotrophin stimulation for assisted reproduction and prior to exposure to sperm. In addition, the global gene expression profile of immature GV stage oocytes recovered from gonadotrophin stimulated cycles and subsequently matured *in vitro* in culture medium supplemented with gonadotrophins has also been defined and this profile compared with the profile of more developmentally competent *in vivo* matured oocytes. The major difference between oocytes matured *in vivo* (MII) and *in vitro* (IVM) was that a large number of genes were more highly expressed in oocytes matured *in vitro* and many of these genes are involved in the biological processes of transcription, the cell cycle and its regulation, transport and cellular protein metabolism.

Immature GV stage oocytes recovered from stimulated cycles may be competent to resume meiosis and reach the MII stage of the meiotic cell cycle but have very limited developmental competence once fertilized (Veeck *et al*., 1983; Nagy *et al*., 1996; Edirisinghe *et al*., 1997; Tucker *et al*., 1998; De Vos *et al*., 1999; Chen *et al*., 2000; Vanhoutte *et al*., 2005). Human oocytes matured *in vitro* have an increased incidence of spindle abnormalities and chromosomal misalignments compared with human oocytes matured *in vivo* regardless of whether the immature oocytes are recovered from unstimulated (Racowsky and Kaufman, 1992; Li *et al*., 2006b) or hormonally stimulated ovaries (Cekleniak *et al*., 2001; Wang and Keefe, 2002). Contributing to the developmental incompetence, oocytes matured *in vitro* have a very high incidence of aneuploidy (Magli *et al*., 2006) and resulting embryos have a higher incidence of nuclear fragmentation, anuclear blastomeres (DeScisciolo *et al*., 2000), multinuclear blastomeres and aneuploidy compared with embryos resulting from oocytes matured *in vivo* (Nogueira *et al*., 2000). These physiological observations indicate a critical failure in cell cycle regulation, particularly spindle assembly and the cell cycle checkpoint and many of the genes involved in these processes were identified to be expressed at higher levels in oocytes matured *in vitro*.

Mammalian oocytes accumulate large amounts of RNA during the growth phase but transcription effectively ceases once the oocyte resumes the meiotic cell cycle (Bachvarova, 1985; Tomek *et al*., 2002). Many of the transcripts accumulated during the growth phase exist in a stable but translationally inactive form with short poly(A) tails (Bachvarova, 1992). Most stored messenger RNA (mRNA) is activated following the addition of several hundred adenine residues to the 3′ poly(A) tail at various times during maturation and early embryo development (Bachvarova, 1992). During meiotic maturation, some maternal mRNAs are adenylated whereas others are deadenylated or degraded (Paynton and Bachvarova,

1994). In fact, almost 50% of the accumulated maternal mRNA is degraded during meiotic maturation (De Leon *et al*., 1983; Lequarre *et al*., 2004) which correlates with the findings in the present study where significantly fewer transcripts were detected in mature MII oocytes regardless of the maturation conditions.

It could be argued that the dysregulation of gene expression observed in the oocyte matured *in vitro* originates in the GV oocyte that has failed to mature *in vivo* despite exposure to the ovulatory dose of hCG. GV oocytes recovered from the monkey following FSH/hCG stimulation (hCG; high developmental competence), FSH alone (FSH; moderate developmental competence) and no hormonal stimulation (NS; low developmental competence) showed remarkably little difference in the expression levels of 23 maternal mRNA's selected on the basis of high expression levels in oocytes and preimplantation embryos (Zheng *et al*., 2005). However, these same genes were expressed at significantly higher levels in oocytes matured *in vitro* than in oocytes matured *in vivo*, which is similar to the findings in the present study and suggests that the dysregulation occurs during maturation and does not arise in the GV oocyte. FSH oocytes and embryos showed a less severe alteration in gene expression than NS oocytes and embryos when compared with hCG oocytes and embryos (Zheng *et al*., 2005). For most of the genes examined in oocytes with low developmental competence, there is an over-abundance initially in the MII oocyte but this pattern is reversed with a substantial reduction in relative gene expression by the 2-cell stage (Zheng *et al*., 2005). For genes known to be required later in preimplantation development, there is parity in expression at the oocyte stage with a significant increase in relative expression levels by the pronucleate stage of development indicating that these genes are precociously up-regulated (Zheng *et al*., 2005). It is not ethically permissible to generate human embryos for the purpose of research so these observations cannot be directly confirmed in the human. Zheng *et al*. (2005) suggested that the reduced developmental competence of non-human primate oocytes matured *in vitro* was a result of a failure of these oocytes to undergo the normal pattern of transcript silencing. As we also identified over-abundance of a large number of genes in human oocytes matured *in vitro* compared with *in vivo*, we propose a failure in the normal post-transcriptional regulatory processes as an explanation of the relatively poor developmental competence of human oocytes matured *in vitro*.

What is the origin of the increased gene expression in oocytes matured *in vitro* compared with *in vivo*? An increase in gene expression detected by mRNA microarray can be due to either new transcription or polyadenylation of existing dormant transcripts. In the oocyte, differential mRNA stability is an important way to regulate the availability of transcripts for translation and is dependent on the presence in the 3′ untranslated region of the nuclear polyadenylation signal (AAUAAA) and the cytoplasmic polyadenylation signal (CPE) at a variable distance 5′ to the AAUAAA sequence (Bachvarova, 1992). Stored mRNAs are bound by protein complexes which include the cytoplasmic polyadenylation element binding protein (CPEB) which inhibit translation until CPEB is phosphorylated by Aurora A (Mendez *et al*., 2000). Phosphorylation of CPEB interacts with the cleavage and polyadenylation specific factor bound to the nuclear polyadenylation signal recruiting poly(A) polymerase to form the active polyadenylation complex thus initiating polyadenylation and translation (Mendez *et al*., 2000). Deadenylation on the other hand appears to happen as a default pathway on re-initiation of meiosis and occurs to transcripts lacking a CPE (Varnum and Wormington, 1990; Paynton and Bachvarova, 1994). Transcript abundance that was observed in oocytes matured *in vitro* could therefore be explained by a failure of the default deadenylation pathway that occurs in oocytes matured *in vivo*. Now that these genes have been identified it will be possible to follow the poly(A) tail length during the course of maturation and determine whether their transcripts arise by new transcription, precocious polyadenylation of existing transcripts that are normally dormant until required later in development or a failure of the normal default deadenylation pathway. Experiments are underway to determine the mechanism for the increased gene expression observed for oocytes matured *in vitro*. We are also proposing to examine the large number of genes expressed more than 2-fold higher in oocytes matured *in vitro* to determine if there is any sequence similarity in the promoter and non-translated regions that may help to explain why these transcripts are not silenced as in oocytes matured *in vivo*.

In conclusion, global gene expression profiling using whole human genome arrays and subsequent data mining has provided a molecular basis for the relative developmental incompetence of oocytes matured *in vitro* compared with *in vivo*. Immature GV stage oocytes recovered from gonadotrophin stimulated patients and matured *in vitro* have a large number of genes expressed at more than 2-fold higher levels than oocytes matured *in vivo*. This is probably due to dysregulation in gene transcription or post-transcriptional modification of genes resulting in an incorrect temporal utilization of transcripts which could manifest as developmental incompetence of any embryos resulting from these oocytes. Similar application of global gene expression profiling using microarrays may reveal the molecular basis for differences in developmental competences of oocytes recovered from women with different etiologies of infertility.

Supplementary Data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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