Investigation of gene expression profiles before and after embryonic genome activation and assessment of functional pathways at the human metaphase II oocyte and blastocyst stage

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Objective: To compare the oocyte versus the blastocyst transcriptome and provide data on molecular pathways before and after embryonic genome activation.
Design: Prospective laboratory research study.
Setting: An IVF clinic and a specialist preimplantation genetics laboratory.
Patient(s): Couples undergoing or having completed IVF treatment donating surplus oocytes or cryopreserved blastocysts after patient consent.
Intervention(s): Sets of pooled metaphase II (MII) oocytes or blastocysts were processed for RNA extraction, RNA amplification, and analysis with the use of the Human Genome Survey Microarrays v2.0 (Applied Biosystems).
Main Outcome Measure(s): Association of cell type and gene expression profile.
Result(s): Totals of 1,909 and 3,122 genes were uniquely expressed in human MII oocytes and human blastocysts respectively, and 4,910 genes were differentially expressed between the two sample types. Expression levels of 560 housekeeping genes, genes involved in the microRNA processing pathway, as well as hormones and hormone receptors were also investigated.
Conclusion(s): The lists of genes identified may be of use for understanding the processes involved in early embryo development and blastocyst implantation, and for identifying any dysregulation leading to infertility. (Fertil Steril® 2013;99:803–14. ©2013 by American Society for Reproductive Medicine.)
Key Words: Preimplantation development, human embryo, human blastocyst, human MII oocyte, housekeeping genes, microRNA, hormones, hormone receptors

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Preimplantation embryo development is characterized by a series of events following fertilization of the mature human oocyte, including formation of the maternal and paternal pronuclei (within 3–10 hours after insemination), followed by formation of the zygote, which at ~20 hours after insemination starts undergoing mitotic divisions every 12–18 hours (cleavage stage), reaching the morula (compaction) and eventually the blastocyst stage (cavitation) of 150–200 blastomeres before implantation. Embryonic transcription is not required for cleavage to occur, because the initial stages of development are dependent on the proteins and transcripts that

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accumulated in the oocyte during its long developmental arrest in the prophase of meiosis I, before fertilization (1). In humans, embryonic genome activation (EGA) occurs at the 4–8-cell stage (48–72 hours after fertilization), at which point the genes that are required for growth and differentiation in the embryo are expressed for the first time (2). At the blastocyst stage, the cells have differentiated into the outer epithelial trophectoderm (TE), the surrounding cells that initiate implantation and form extraembryonic structures, such as the placenta, and a small group of cells called the inner cell mass (ICM), which has the capacity to form all of the tissues of the fetus (3).

Several studies have defined the gene expression profile for each of the stages of preimplantation development with the aim to provide an insight into the molecular pathways that control them. Assou et al. (4) recently reviewed studies on gene expression profiling of cumulus cells, oocytes at different stages of maturation (germinal vesicle [GV] to metaphase II [MII] stage) and from donors of varying age, and preimplantation embryos.

A comparison of the oocyte versus the blastocyst transcriptome provides an indication of the most important molecular pathways before and after embryonic genome activation (EGA) (2). Although the oocyte is one cell, and a typical human blastocyst consists of 150–200 cells, they each represent an entire organism. Despite the differences in RNA abundance between these two sample types, direct comparison of levels of gene expression and separate detailed analysis of the transcriptomic profile of each sample, would provide important information, because the oocyte is the only cell that can coordinate and manage the recruitment of large amounts of stored mRNA to support maturation, fertilization, and early development before EGA, and the blastocyst expresses those genes most vital for supporting hatching and implantation. Owing to the difficulty of obtaining human embryos for research and the challenge of working with such limited amounts of starting material, microarray studies of early embryo development up to the blastocyst stage have been limited (5–28) (Table 1). Our aims in the present study were: 1) to define and compare the gene expression profile of human MII oocytes and human blastocysts; and 2) to investigate the expression of genes maintaining basic cell functions and implicated in key molecular pathways not previously investigated at the human blastocyst stage.

MATERIALS AND METHODS

Sample Collection and Processing

Samples were donated from patients undergoing in vitro fertilization (IVF) treatment at the Centre for Reproductive and Genetic Health (CRGH), London, United Kingdom, and included immature oocytes (collected 40 hours after hCG injection) and surplus cryopreserved blastocyst embryos. Written patient consent was obtained from all patients (Human Fertilisation and Embryology Authority license no. RO113). Institutional Review Board approval was obtained by the Research Ethics Committee (REC3), North London (REC reference no. 10/H0709/26). Immature oocytes that had matured in G-IVF Plus medium (Vitrolife) within 4 hours from collection, as well as thawed blastocysts that had successfully recovered in culture, were selected for microarray analysis. All manipulations were conducted rapidly to minimize the overall handling time and prevent RNA degradation. RNA was extracted from pooled samples with the use of the Allprep DNA/RNA Micro kit (Qiagen), following the manufacturer's instructions. The purified RNA was then amplified in two rounds and digoxigenin-labeled with the use of the NanoAmp RT-IVT Labeling kit (Applied Biosystems). A total of three MII oocyte samples, consisting of three pooled human MII oocytes each, as has been generally recommended, and three blastocyst samples, consisting of three pooled human blastocysts each, were processed for microarray analysis (29) (array data available on request). Details of sample collection, RNA quality assessment, microarray processing, data filtering, and analysis with the use of Human Genome Survey Microarrays v2.0 (Applied Biosystems) were as previously described (12). The mean maternal age was 35.77 $\pm$ 4.05 years. The average age per group ranged from 34 years to 37 years (SD 1.36).

The samples pooled together were from different donors, aiming to generate an overall representative gene expression profile from each tested sample type, overcoming individual variation (30). The triplicate array experiments for blastocyst-oocyte comparison were all performed with the use of arrays from a single batch, and all arrays were processed at the same time.

Determination of Gene Lists for Expression Analysis

A list of genes that can be defined as housekeeping (HKGs) (ubiquitously and stably expressed in all tissue/cell types and responsible for the maintenance of basic cell functions) and a list of HKGs specifically defined for human embryonic stem cells (hESCs) (genes most stably expressed in undifferentiated and early differentiating hESCs, showing little overlap with results obtained from somatic cells and tissues) were obtained from Zhu et al. (31) and Synnergren et al. (32), respectively. Genes involved in microRNA processing, as listed by Mtango et al. (33), were also investigated.

A list of genes coding for peptide hormones and hormone receptors (HRs) was created by searching the HMR Base database (http://crdd.osdd.net/raghava/hmrbase/, data retrieved December 2009) (34).

Functional Annotations and Investigation of Gene Expression Levels

Functional annotations of genes of interest were obtained from the Panther database (www.pantherdb.org) and investigated on the microarray expression list to identify whether these were detected in each sample type, what their level of expression was (high, medium, low) and whether their expression differed significantly between blastocysts and oocytes. The Panther database was used to assign expressed genes to different categories based on biologic or molecular function (35).

The grouping of genes by level of expression was based on the signal values from each microarray as described in Jaroudi