Focus on Mammalian Embryogenomics

Biological interpretations of transcriptomic profiles in mammalian oocytes and embryosS L Rodriguez-Zas^{1,2,3}, K Schellander⁴ and H A Lewin^{1,2}

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Abstract

The characterization of gene-expression profiles in oocytes and embryos is critical to understand the influence of genetic and environmental factors on preimplantation and fetal development. Numerous gene-expression microarray studies using different platforms and species are offering insights into the biological processes extensively represented among the genes exhibiting differential expression. Major advances on understanding the direct relationship between gene expression and developmental competence are being reported. Integration of information across studies using meta-analysis techniques can increase the precision and accuracy to identify expression profiles associated with embryo development. Gene network and pathway analyses are offering insights into gene interactions and expression profiles of embryos. All these advances are cementing the way toward a comparative and systems approach to understanding the complex processes underlying vertebrate development.

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Introduction

Embryo development in mammals is marked by distinctive biological processes that occur during the preimplantation and early postimplantation periods. The systematic study of genes and pathways during this period can reveal insights into the biological processes and underlying molecular mechanisms involved in embryonic development, embryonic stem cell differentiation, and somatic cell nuclear transfer (SCNT). The nature and regulation of gene expression during the preimplantation stages are likely to be critical for later development of the conceptus (Brison & Schultz 1997). Even a defect in a single gene is sufficient to cause implantation failure (Copp 1995). Thus, identification and characterization of differentially regulated genes in oocytes and embryos are vital to

understand the critical events occurring during the peri-implantation period (Khurana & Niemann 2000, Cui & Kim 2006) and to develop precise criteria for assessing the health of early embryos (Watson *et al.* 1999).

Transcriptome profiling using microarray technology is a widely used approach to examine the expression of multiple (hundreds to thousands) of genes across stages and conditions. One of the drawbacks of microarray analysis to study embryo development is the lack of connection between the expression profile of genes at one stage and the developmental phenotype of the embryo in the next stage. Efforts have been made to establish this connection using transcriptional analysis of biopsied embryos (El-Sayed *et al.* 2006), meta-analysis of multiple experiments (Adams *et al.* 2007), or by gene network reconstruction using techniques described by Ko *et al.* (2007a, 2007b).

Statistical approaches with extensive theoretical foundation have been extended to accurately model gene-expression data, providing both a biological and statistically sound hypothesis testing framework (Wolfinger *et al.* 2001, Cui *et al.* 2003, Kerr 2003, Bolstad *et al.* 2004, Rodriguez-Zas *et al.* 2006). The challenge of microarray analysis has moved from a 'how to' implementation phase to a 'biological interpretation'

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phase. A systems biology approach that integrates information across genes and/or studies can offer a comprehensive and accurate characterization of gene–gene and gene–environment interactions influencing embryo development.

Individual embryogenomic studies

Analysis of individual embryo microarray experiments follows a standard approach applicable to microarray studies in general. This approach involves experimental design, performing the actual microarray experiment from sample collection to obtaining the fluorescence intensity measurements of gene expression, and statistical analysis. Various tools and resources are used to elucidate biological interpretations of the results from the analysis. Public resources such as Gene Ontology (<http://geneontology.org>), NCBI Entrez Gene (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>), KEGG pathway database (<http://www.genome.jp/kegg/pathway.html>), and commercial tools including Ingenuity Pathway Analysis (<http://www.ingenuity.com>) integrate information and facilitate the interpretation of results within a biological context. A detailed review of microarray analysis strategies with an application to endometrial studies was compiled by White & Salamonsen (2005). The following sections review selected the literature that has provided insights into the transcriptome of oocytes and embryos, with primary focus on cattle.

Mammalian oocyte transcriptomics

Vallée *et al.* (2006) identified conserved genes in the oocytes of three distant species that can help understand the unique role of maternal transcripts in early embryonic development. These transcripts have specific functions either in oogenesis, oocyte maturation, fertilization, and/or the early phase of preimplantation development. Maternal mRNAs are stored in an inactive, masked form, and recruited for translation in a stage-specific manner during oocyte maturation or early embryogenesis (Bachvarova 1992). The relative abundance of molecules stored in an oocyte differs between species and accounts for the differences in time post-insemination and stage of preimplantation development when zygotic gene expression is triggered and directs early development and differentiation. Global gene-expression analysis using microarray allows the establishment of a molecular transcriptome blueprint of oocytes during maturation (Fair *et al.* 2007) to better understand oogenesis, folliculogenesis, and the critical events occurring during preimplantation period (Yao *et al.* 2004, Misirlioglu *et al.* 2006).

Decreased oocyte competence with maternal aging is a major factor in human infertility. Hamatani *et al.* (2004) demonstrated that genes involved in mitochondrial

function and oxidative stress were differentially expressed in oocytes pertaining to mice of different ages. Moreover, alteration in the expression of genes involved in chromatin structure, DNA methylation, genome stability, and RNA helicases suggested the existence of additional mechanisms in oocyte aging in mice. In a similar study in human oocytes, a variety of major functional categories, including cell cycle regulation, cytoskeletal structure, energy pathways, transcription control, and stress responses, were found to be influenced by maternal age (Steuerwald *et al.* 2007).

Global activation of the embryonic genome is the most critical event at early stages of mammalian development. After fertilization, a rich supply of maternal proteins and RNAs support development, whereas a number of zygotic and embryonic genes are expressed in a stage-specific manner leading to embryonic genome activation. In cattle prior to the major genome activation at 8- to 16-cell stage, the so-called minor genome activation is initiated as early as the 1-cell zygotic stage (Memili & First 2000). A comparative analysis of matured bovine oocytes and 8-cell stage embryos treated with α -amanitin using the bovine affymetrix array identified changes in gene-expression profiles related to the transcriptional machinery, chromatin structure, and cellular functions that are expected to result in a unique chromatin structure capable of maintaining totipotency during embryogenesis and leading to differentiation during postimplantation development (Misirlioglu *et al.* 2006). Recently, gene-expression analysis of bovine oocytes pre- and post-resumption of meiotic maturation showed that ~54% of the probe sets representing 23 000 transcripts were detected in bovine oocytes. Many genes related to cytoplasmic polyadenylation element-dependent polyadenylation complex machinery were found to be differentially expressed (Fair *et al.* 2007). Genes specifically upregulated in mature oocytes were more likely to be involved in DNA replication, amino acid metabolism, G protein-coupled receptors, and signaling molecules (Cui *et al.* 2007b).

Mammalian embryo transcriptomics

Embryo preimplantation stages in mammals are marked by major events including transition from maternal to embryonic genome activation, cell-to-cell adhesion or compaction, and differentiation of cells to inner cell mass and trophectoderm at blastocyst stage. These events are controlled or accompanied by orchestrated expression of thousands of developmentally important genes. Stanton *et al.* (2003) estimated that ~15 700 genes are expressed during preimplantation development in the mouse. A review by Ruddock-D'Cruz *et al.* (2007) identified more than 100 genes associated with important biological processes during preimplantation in the cattle

embryos. Such processes included compaction/cavitation, metabolism, transcription/translation, DNA methylation and histone modification, oxidative stress, response to or production of growth factors, cytokine signaling, cell cycle regulation, and apoptosis. [Ruddock-D'Cruz et al. \(2007\)](#) also summarized the expression profile of 68 of these genes in cattle at the stages of follicle, oocyte, zygote, 2-, 4-, 8-, 16-cell, morula, and blastocyst.

Early embryonic mortality is a recognized cause of reproductive failure in cattle leading to the loss of a large number of potential calves, retarded genetic progress, and significant loss of money and time in rebreeding cows ([Khurana & Niemann 2000](#), [Morris et al. 2001](#)). Embryo mortality is a result of intrinsic defects within the embryo, an inadequate maternal environment, asynchrony between embryo and mother, or failure of the mother to respond appropriately to embryonic signals ([Hansen 2002](#)). With the advent of reproductive technologies, this developmental failure becomes more evident.

Most mortality among *in vitro*-produced embryos is sustained within the first 2–3 weeks after fertilization ([Farin et al. 2001](#), [Morris et al. 2001](#), [Sreenan et al. 2001](#)). The explanation for this high rate of developmental failure with respect to the defect within the embryo (intrinsic errors) remains unclear. However, the extent and regulation of altered gene expression during preimplantation development is likely to be critical for later development of the conceptus ([Brison & Schultz 1997](#)). The gene-expression profiles of *in vitro* and *in vivo* bovine blastocysts were characterized using a cDNA microarray platform including 932 bovine expressed sequence tags (ESTs) from a normalized bovine total leukocyte cDNA library and 459 amplicons ([Corcoran et al. 2006](#)). In this study, 384 genes were differentially expressed out of which 85% were downregulated *in vitro*- compared with *in vivo*-derived blastocysts. Analysis of the gene-expression profiles of mouse 4-cell, morula, and blastocyst stage embryos revealed differential regulation of genes implicated in the process of compaction and blastocoel formation ([Cui et al. 2007a](#)).

Early embryonic development, implantation, and maintenance of pregnancy are critically dependent on an intact embryo-maternal communication ([Giudice 2003](#), [Wolf et al. 2003](#)). The gene-expression profiles of individual uterine samples from pregnant and nonpregnant (control) heifers were studied using 3072 cDNA clones derived from suppression subtractive hybridization of endometrium samples from pregnant and nonpregnant cows and spotted on a nylon membrane ([Bauersachs et al. 2006](#)). In this study, 41 genes induced by interferon or candidates to be involved in the regulation of transcription, cell adhesion, modulation of maternal immune system, and endometrial remodeling were expressed at higher levels in pregnant heifers compared with controls.

SCNT provides a powerful experimental tool to explore mammalian development. The technique combines several methodologies, all of which contribute to the outcome of the procedure. For example, in cattle, the origin of the donor cell line, quality and genetics of recipient oocytes, and embryo culture conditions can all have a significant impact on the overall success rate of the SCNT process (reviewed by [Obach & Wells 2007](#)). All of the above-mentioned experimental variables appear to affect the reprogramming of the donor nucleus, which in turn dictates whether the SCNT pregnancies are carried to term. Reprogramming errors affect downstream development of the placenta and the fetus, which may cause severe diseases such as hydrops and large offspring syndrome ([Constant et al. 2006](#)). Recently, it has become apparent that defects in cloned embryos are most seriously manifested in the trophoblast, affecting its normal development ([Arnold et al. 2006](#), [Fletcher et al. 2007](#)). A current hypothesis is that the abnormal development of the trophoblast, leading to abnormal development of the placenta, is the major cause of wholesale losses of cloned embryos during the peri-implantation period ([Hill et al. 2000](#), [De Sousa et al. 2001](#), [Arnold et al. 2006](#), [Yang et al. 2007](#)). Thus, SCNT, although affecting a large number of genes and developmental processes, is a potent perturbation method for exploring the systems biology of the peri-implantation embryonic and placental development.

In order to address the question of the extent of reprogramming of donor cells and the affects of SCNT on gene-expression patterns in blastocyst, [Smith et al. \(2005\)](#) compared gene-expression profiles in donor cells and day-7 blastocysts produced by artificial insemination (AI), *in vitro* fertilization (IVF), and SCNT using a 7872 element cDNA microarray representing ~6300 unique genes. The source of cDNA clones was predominantly from a term placenta cDNA library, which contained a broad representation of placenta-specific, highly divergent, and novel transcripts ([Everts et al. 2005](#), [Larson et al. 2006](#), [Kumar et al. 2007](#)).

The vast majority (84.2%) of genes represented on the array were differentially expressed in the fibroblast donor cells when compared with the SCNT embryos, with more than 1500 genes exhibiting more than twofold difference. Twenty-three genes associated with 'stemness' were overexpressed in the SCNT embryos when compared with the donor cells. This was the first dramatic demonstration of the extent of nuclear reprogramming that occurs during the SCNT process. With so many genes requiring reprogramming, it is safe to assume that the reprogramming process, although appearing robust, may be prone to error. Failure to reprogram the donor cell nucleus completely and correctly may affect downstream development and redifferentiation ([Yang et al. 2007](#)) resulting in placental abnormalities, and in extreme cases, lethality.

Smith *et al.* (2005) compared the gene-expression profiles of AI, IVF, and SCNT 7-day blastocysts. Surprisingly, the SCNT embryos had a gene-expression pattern that was more similar to the AI embryos than the AI were to IVF embryos. One interpretation of this result is that the SCNT process results in robust nuclear reprogramming. An alternative explanation is that the SCNT embryos were not completely reprogrammed and thus unable to respond appropriately to their environment because they should be more similar to IVF based on similar (but not identical) culture conditions prior to embryo transfer. One essential difference between the IVF and SCNT embryos is that the IVF embryos were fertilized by sperm *in vitro*, whereas SCNT embryos received a diploid nucleus from cultured donor cells. The effect of culture conditions on gene-expression patterns has been widely recognized and may be responsible for epigenetic modifications that are detrimental to the developing embryo and fetus (Bourc'his *et al.* 2001, Kang *et al.* 2001).

Various studies in mice and cattle have shown that the production of embryos under specific culture environments resulted in not only altered gene expression of transcripts related to metabolic and growth but also altered conceptus and fetal development following transfer (Khosla *et al.* 2001, Lazzari *et al.* 2002). Recent experiments designed to distinguish the effects of IVF and embryo culture and maturation on gene expression have shown strong effects of embryo culture on transcripts associated with RNA processing and protein synthesis (Smith S L, Everts R E, Sung L-Y, Du F, Page R, Henderson B, Rodriguez-Zas S L, Nedambale T, Renard J-P, Lewin H A, Yang X & Tian X C unpublished data) and other metabolic/cellular functions (reviewed by Rud-dock-D'Cruz *et al.* 2007). Thus, the greater similarity between 7-day AI and SCNT when compared with 7-day IVF embryos may represent a lack of full developmental competence due to the differential effects of embryo culture on SCNT embryos rather than a desirable phenotype of these embryos. In that case, the 25 genes differentially expressed in SCNT when compared with AI and IVF embryos may be distinctive markers for early aberrancies associated with reprogramming errors. Several of these genes (i.e. *MITF*, *DUSP6*, *FOLR1*, *COL4A1*, *MIES2*) are important players in growth and development, including that of the placenta. Expression of *CD81*, an imprinted gene in mouse placenta, was expressed at 2.5-fold greater levels in AI when compared with SCNT embryos. All other imprinted genes detected by the microarray were expressed at normal levels in SCNT embryos, although it could not be determined if imprinting was affected because the microarray is unable to distinguish maternal from paternal transcripts. Analysis of other functions of the differentially expressed genes in SCNT embryos, such as those for DNA methylation and chromatin remodeling, showed no significant differences, supporting the view that the

differentially expressed genes are not involved in epigenetic modifications that lead to reprogramming errors, at least at the 7-day stage.

Regardless of the number of genes whose expression in blastocysts is affected by SCNT, it is clear that nuclei reprogrammed by SCNT can carry embryonic development to the blastocyst stage with a high degree of efficiency. It is noteworthy that 60–80% of cells in the blastocyst are committed to the extraembryonic tissues (Koo *et al.* 2002). Thus, aberrantly reprogrammed genes expressed in early trophoblast may not be detected at 7 days of development if the resulting differential expression is localized to a fractional population of cells fated to form the extraembryonic tissues. This raises the important question as to whether gene expression differences in cloned embryos are essentially restricted to the extraembryonic tissues during early preimplantation development. This question was addressed recently by comparing gene-expression profiles in unamplified RNA collected from microdissected embryonic disk and extraembryonic tissues from day-25 embryos created by AI and SCNT (Everts R E, Sommers A, Green C A, Oliveira R, Rodriguez-Zas S L, Sung L-Y, Du F, Evans A C O, Boland M, Fair T, Lonergan P, Renard J P, Yang X, Tian X & Lewin H A unpublished data) using a 13 257 oligonucleotide microarray platform. This microarray was designed primarily from mRNAs expressed in placenta, spleen, developing embryos, and extraembryonic tissues (Everts *et al.* 2005, Loo *et al.* 2007). Surprisingly, only ten genes were differentially expressed in SCNT embryonic disks when compared with AI embryonic disks, whereas 188 genes were differentially expressed in extraembryonic tissues from SCNT embryos when compared with AI embryos. Functional categories that were significantly affected included cellular development, molecular transport, lipid metabolism, gene expression, cell death and cell growth, and proliferation. These results suggest that reprogramming errors disproportionately affect the development and differentiation of extraembryonic tissues. As a consequence, the timing of implantation and early development of the placenta may be affected due to multiple aberrancies. These results thus suggest that the aberrant reprogramming of genes expressed in embryonic trophoblast may be the major cause of losses of cloned embryos observed during the peri-implantation period. It is therefore critically important to determine the role that genes differentially expressed in SCNT trophoblast play in embryo elongation, implantation, and placental development. A similar study using *in situ* synthesized oligo microarrays revealed that SCNT and IVF blastocysts exhibited similar gene-expression profiles (Beyhan *et al.* 2007). The similarity of profiles was observed in two donor cell lines with different gene-expression profiles, suggesting the complete reprogramming in SCNT embryos at the blastocyst stage.

Information from multiple transcriptional analyses of transferable blastocysts from various origins is starting to

Figure 1 Hierarchical clustering for the differentially expressed genes between biopsies from blastocysts that resulted in no pregnancy and calf delivery (A) and resorption and calf delivery (B).

accumulate (Rizos *et al.* 2003, de A Carmargo *et al.* 2005, Smith *et al.* 2005, Wrenzycki *et al.* 2005). Recently, El-Sayed *et al.* (2006) addressed the relationship between transcriptional profile of embryos and pregnancy success by measuring the gene expression of blastocyst biopsies taken prior to transfer to recipients. This technique allowed the collection of cells from embryos prior to transfer without lethal effects on the embryo at later stages of development. In that study,

several clusters of genes were found to be differentially expressed between biopsies derived from blastocysts that resulted in no pregnancy, resorption, or calf delivery (Fig. 1). Ontological classification of the genes revealed that biopsies resulting in calf delivery were enriched for transcripts necessary for implantation (*COX2* and *CDX2*), carbohydrate metabolism (*ALOX15*), growth factor (*BMP15*), signal transduction (*PLAU*), and placenta-specific transcripts (*PLAC8*). Biopsies from embryos

that were resorbed are enriched with transcripts involved protein phosphorylation (*KRT8*), plasma membrane (*OCLN*), and glucose metabolism (*PGK1*, *AKR1B1*). Biopsies from embryos that resulted in no pregnancy were enriched with transcripts involved in inflammatory cytokines (tumour necrosis factor), protein amino acid binding (*EEF1A1*), transcription factors (*MSX1*, *PTTG1*), glucose metabolism (*PGK1*, *AKR1B1*), and *CD9*, which is an inhibitor of implantation.

Cross-species transcriptome profiling

Cross-species gene-expression comparison is a powerful tool for the discovery of evolutionarily conserved mechanisms and pathways in early mammalian development. Immature and mature bovine oocytes were hybridized to a human cDNA array containing 1176 fragments involved in tumor suppression, cell cycle regulation, signal transduction, transcription factors, cell adhesion, apoptosis, and stress response (Dalbiès-Tran & Mermillod 2003). Subsequent analysis revealed the expression of about 300 human genes in bovine oocytes, the majority of which had never been investigated in oocytes before. Similarly, array analyses have been performed using RNA samples from monkey, pig, mouse, and salmon on human high-density oligonucleotide arrays to identify conserved genes or gene networks between these species (Chismar *et al.* 2002, Moody *et al.* 2002, Tsoi *et al.* 2003). With increasing accumulation of species-specific ESTs in public databases, especially for those whose genes are not well characterized, cross-species hybridization enables the identification of conserved genes, and assignment of gene names to previously uncharacterized ESTs. For example, Adjaye *et al.* (2004) used RNA derived from human and bovine fetal brains as targets for hybridization onto human cDNA microarray with 349 characterized genes.

Recently, a multi-species cDNA microarray containing 3456 transcripts from three distinct oocyte libraries from bovine, mouse, and *Xenopus laevis* oocytes was used to analyze genes preferentially or commonly expressed between the oocytes of the three species (Vallée *et al.* 2005, 2006). From 1541 clones conserved in all three species, 268 clones were exclusively expressed in the oocytes of all species. This approach has contributed to increased efficiency in identifying novel oocyte-specific transcripts and elucidating important evolutionarily conserved mechanisms of gene expression in different species. Furthermore, this approach may facilitate the identification of new genes not previously identified due to low expression levels in a given species. A similar investigation profiled the transcriptome of fully grown mouse oocytes and the eggs of *X. laevis* and *Ciona intestinalis* using 19 000 ESTs derived from a fully grown mouse oocytes cDNA library (Evsikov *et al.* 2006). This study revealed that 2090 mouse gene homologs were

transcribed in eggs of *X. laevis* and *C. intestinalis*. In addition, a cohort of functional homologs was observed in the three model organisms highlighting the conserved biological mechanisms operating in the eggs of these species.

Multiple embryogenomic studies

Systems biology approaches that consider multiple studies using meta-analysis and dissection of gene networks can exploit the extensive collection of embryogenomic expression data available and offer additional insights into the molecular regulation of embryo development. Implementation of microarray data meta-analysis ranges from comparison of lists of genes with differential expression or simultaneous consideration of gene-expression data across treatments and experiments (Singh *et al.* 2005), to combination of *P* values (Rhodes *et al.* 2002) or estimates (Choi *et al.* 2003). The insights gained from the integration of multiple studies can be used for accurate reconstruction of gene pathways, characterization of previously unknown relationships between genes, and biomarker identification. Application of formal meta-analytical approaches and gene network analysis to embryogenomic data holds great promise. In the following sections, we demonstrate the successful application of meta-study and gene network approaches to embryonic gene-expression data. Exhaustive description of the statistical approaches and data used and detailed discussion of results is presented in Rodriguez-Zas *et al.* (2008).

Example

Gene-expression data from nine microarray experiments designed to assess the expression profile of mouse embryos exposed to teratogenic agents, that can cause birth defects, were combined. Gene-expression data were obtained from the NCBI Gene Expression Omnibus GEO (<http://www.ncbi.nlm.nih.gov/geo>) repository and included the GEO series (GSE) 1068, 1069, 1070, 1072, 1074, 1075, 1076, 1077, and 1079. Briefly, gene-expression measurements were obtained from the headfold of embryos exposed to either ethanol, methylmercury, low oxygen, the metabolic toxin 2-chloro analog of 2'-deoxyadenosine, or mitochondrial peripheral benzodiazepine receptor site ligands (Nemeth *et al.* 2005, Singh *et al.* 2005, Green *et al.* 2007). All treatment levels were present in at least two microarrays following a dye-swap design and a total of 90 microarrays were analyzed. All studies used the same cDNA platform including 2382 sequence-verified human gene elements that effectively hybridized to mouse, human, and rat target mRNA. Microarray elements with weak intensity were filtered and a log-transformation, LOESS normalization, and global adjustment for dye and array effects were implemented (Wolfinger *et al.* 2001, Cui *et al.* 2003,

Kerr 2003). The adjusted fluorescence intensities were analyzed within and across studies by microarray element.

Meta-analyses

Six meta-analytic approaches were compared (Adams *et al.* 2007). The approaches were simple comparison of lists of genes differentially expressed within study (EXP), Fisher's meta-test (FIS), all treatments sample-level meta-analysis (ALL), two treatments sample-level meta-analysis (TWO), standardized estimate study-level meta-analysis (STU_std), and non-standardized estimate study-level meta-analysis (STU_non). Briefly, the FIS approach implements Fisher's test that aggregates the individual studies significance probability P values (Hedges & Olkin 1995). The ALL and TWO sample-level meta-analyses combine the adjusted gene-expression data from all studies and the effects of study and microarray were modeled as blocking factors. In the ALL sample-level meta-analysis, all treatment levels across the nine studies were compared and of interest was the P value that indicates differential expression of at least one of the treatment level from the overall expression. In the TWO sample-level meta-analysis, the original treatment levels from five studies were assigned to one of two treatment levels (treated or control) and of interest were both the estimate of differential expression and the P value. These five studies were also analyzed using two complementary study-level meta-analyses. The standardized study-level meta-analysis (STU_std) combines the standardized estimates of the differential expression between treated and control and the non-standardized study-level meta-analysis (STU_non) combines the raw, non-standardized estimates. The study-level meta-analyses provide additional insights into the consistency of the magnitude and directionality of the treatment effect on gene expression across studies and into the impact of the standardization of estimates on the results. Consequently, results from FIS were compared with ALL and results from TWO were compared with STU_std and STU_non.

Within the meta-analytical approaches that considered statistical significance of differential expression across all nine studies and multiple treatment levels, there were 539 genes differentially expressed (raw $P < 0.001$, false discovery rate-adjusted $P < 0.06$) in at least one analysis (EXP, FIS, or ALL) of all nine studies. The number of genes differentially expressed within EXP ranged from 1 to 257. The minimum fold change between any two treatment levels among the genes differentially expressed was 1.8. The FIS and ALL approaches identified 216 and 257 genes differentially expressed respectively with 74 genes in common. For 183 genes, samples that were assigned to the same treatment level across studies have the same expression patterns thus resulting in more precise estimates. Conversely, for 142 genes, samples that apparently received the same treatment level did not have similar expression patterns, thus augmenting the uncertainty of the estimates.

Within the meta-analytical approaches that considered direction and statistical significance of differential expression across five studies and two treatment levels (treated versus control), the TWO and STU_std approaches identified 20 and 9 genes differentially expressed ($P < 0.01$) respectively. The relative power of the sample versus study-level meta-analyses depends on the similarity of the samples assigned to the same treatment level across studies and number of studies available. The lower number of genes differentially expressed found with the TWO and STU approaches compared with ALL and FIS approaches can be attributed to the fewer studies considered and the grouping of treatment levels into two major levels.

A funnel plot comparing results from EXP, TWO, STU_std, and STU_non for gene sterol-C4-methyloxidase-like (*SC4MOL*, U93162) is presented in Fig. 2. The funnel plot demonstrates how for *SC4MOL*, individual study analyses failed to identify differential expression; however, the TWO and STU_std meta-analyses were able to combine the consistent patterns, gain power, and detect differential expression. The apparent departure of the STU_std result from the other analyses is an artifact of the standardization of estimates. The overlap of the TWO and STU_non estimates confirms that the estimates from both meta-analyses approaches are consistent. For *SC4MOL*, TWO gave a slightly more significant result than STU_std because TWO combines ~ 40 gene-expression measurements meanwhile STU_std combines five observations, the estimates of differential expression between treatment levels from the five studies.

Among the genes detected by the meta-analysis approaches there was extensive representation of genes pertaining to glycolysis, cell communication, and proteasome pathways previously reported by Singh *et al.* (2005). Additional biological processes including cellular development were also uncovered.

Figure 2 Comparison of the *SC4MOL* gene expression estimates (central markers) of differential expression between control and treated samples and 95% confidence limits (whiskers) from the analyses within study (1068, 1069, 1074, 1075, and 1076) and across studies using the sample-level meta-analysis (TWO), the standardized estimate study-level meta-analysis (STU_std), and the non-standardized estimate study-level meta-analysis, with '*' denoting $P < 0.01$.

Gene network analysis

Gene pathway prediction is challenging because the networks underlying complex biological processes like embryonic development usually include tens of genes meanwhile the computational demands of most gene network building algorithms grow exponentially with the number of genes. Typical pathway reconstruction algorithms impose limits on the networks explored and gene-expression measurements are dichotomized, potentially jeopardizing the discovery of weak yet critical gene interactions (Li & Zhan 2006, Wang *et al.* 2006).

The Bayesian network is a flexible and frequently sought approach to infer gene pathways. Relationships between genes are represented using directed acyclic graphs and within this framework, the probability distributions of genes conditional on precedent genes in the network are independent (Pe'er 2005). Ko *et al.* (2007a, 2007b) implemented a novel Bayesian network approach that uses data-driven weighted mixture of Gaussian models to describe the continuous gene-expression data. The mixture Bayesian network approach overcomes the

limitations of other network implementations and was successful in the reconstruction of gene pathways.

The mixture Bayesian network approach of Ko *et al.* (2007a, 2007b) was applied to reconstruct the adherens junction pathway because of the identification of numerous differentially expressed genes corresponding to this pathway including Wiskott–Aldrich syndrome-like (WAS and WASP), RAS homolog gene family member A (*RhoA*), C3 botulinum toxin substrate 1 (*RAC*), catenin (cadherin associated), actin, and actinin. The mixture Bayesian network approach was able to predict 12 direct and indirect relationships between genes (Fig. 3) that were supported by the KEGG pathway database (<http://www.genome.jp/kegg/pathway.html>). Various subnetworks of this pathway have been described including the relationship between the nonreceptor tyrosine phosphatase *PTP1B* and cadherin (Xu *et al.* 2002), CDC42 and neural-WASP in rodent embryos (Shekarabi *et al.* 2005), and actinin and vinculin (Adey & Kay 1997, Rose *et al.* 1995). Only three gene relationships present in the KEGG adherens

Figure 3 Mixture Bayesian network reconstruction of the adherens junction pathway based on embryogenomic information with confirmed, indirect and unconfirmed relationships between genes denoted by continuous, dashed, and dotted lines respectively. Gene names are: *INSR*=insulin receptor; *RhoA*=RAS homolog gene family member A; *p120ctn*=Catenin (cadherin-associated protein) $\delta 1$; *PTP1B*=*PTPN1*=protein tyrosine phosphatase non-receptor type substrate 1; α -Catenin=catenin (cadherin-associated protein) $\alpha 1$; *Afadin*=*MLLT4*=myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*); translocated to 3; *ZO-1*=*TJP1*=tight junction protein 1 (zona occludens 1); *LAR*=protein tyrosine phosphatase receptor type F; α -Actinin=actinin $\alpha 2$ and 4; *LMWPTP*=*ACP1*=acid phosphatase 1 soluble protein 1; *Cdc42*=cell division cycle 42; *Fyn*=FYN oncogene related to SRC FGR; *WASP*=Wiskott–Aldrich syndrome-like (Was); *NWASP*=Wiskott–Aldrich syndrome-like (Was1). Blue continuous line edges denote direct gene relationships identified by the mixture Bayesian model and confirmed in the KEGG pathway. Green dash-dot edges denote indirect gene relationships identified by the mixture Bayesian model and confirmed in the KEGG pathway. Red dotted edges denote gene relationships present in the KEGG pathway and not identified by the mixture Bayesian model. Orange dashed lines denote gene relationships predicted by the mixture Bayesian approach and not present in the KEGG pathway.

junction pathway were not identified by the Bayesian network approach. Two of the unidentified relationships involved the same gene, *CDC42*, suggesting that the gene reporter present in the microarray platform may have not adequately captured the expected co-regulation of this gene. Five predicted relationships between genes were not present in the KEGG adherens junction. Some of these gene relationships may be real but not yet curated and entered into the KEGG database. For example, the KEGG adherens junction pathway does not include a relationship between *Fyn* and *NWASP*, however, reports that the phosphorylation of *NWASP* by *Fyn* induces cytosolic localization of nuclear *NWASP* (Badour *et al.* 2004, Park *et al.* 2005) support the predicted gene interaction. Likewise, Kabuyama *et al.* (2006) reported that the protein-tyrosine phosphatase 1B was a target of *RhoA* signaling.

Conclusions

Researchers in fields that encompass the study of embryo development have been in the forefront of using microarray technology. Characterization of gene-expression patterns in AI, IVF, and SCNT embryos is offering insights into the processes critical for embryo development. Further understanding of the relationship between the transcriptional profile of embryos and pregnancy success is being gained from state-of-the-art techniques including embryo biopsies and microdissection of embryonic and extraembryonic tissues. Although substantial progress has been made on uncovering genes influencing embryo development much work still remains to be done to completely characterize the association between gene-expression patterns and embryo phenotypes.

Embryogenomic information is already available for advanced analytical approaches that can offer additional insights. In particular, meta-analysis of multiple studies can offer more precise characterization of expression patterns than those typically obtained within a single study. Multiple complementary meta-analytical approaches should be considered as there is no optimal meta-analytical approach for all genes and sets of experiments considered. Likewise, advances in Bayesian networks permit the reconstruction of embryo-centered gene networks.

Declaration of interest

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