

1 **Dynamic changes in gene expression during human early**
2 **embryo development: from fundamental aspects to clinical**
3 **applications**

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32 **Abstract**

33 **BACKGROUND:** The first week of human embryonic development comprises a series of
34 events that change highly specialized germ cells into undifferentiated human embryonic stem
35 cells (hESCs) that display an extraordinarily broad developmental potential. The
36 understanding of these events is crucial to the improvement of the success rate of *in vitro*
37 fertilization (IVF). With the emergence of new technologies such as Omics, the gene
38 expression profiling of human oocytes, embryos, and hESCs has been performed and
39 generated a flood of data related to the molecular signature of early embryo development.

40 **METHODS:** In order to understand the complex genetic network that controls the first week
41 of embryo development, we performed a systematic review and study of this issue. We
42 performed a literature search using PubMed and EMBASE to identify all relevant studies
43 published as original articles in English up to March 2010 (n=165). We also analyzed the
44 transcriptome of human oocytes, embryos and hESCs.

45 **RESULTS:** Distinct sets of genes were revealed by comparing the expression profiles of
46 oocytes, embryos on day-3 and hESCs, that are associated with totipotency, pluripotency and
47 reprogramming properties, respectively. Known components of two signalling pathways
48 (WNT and transforming growth factor-beta) were linked to oocyte maturation and early
49 embryonic development.

50 **CONCLUSION:** Omics analysis provides tools for understanding the molecular mechanisms
51 and signalling pathways controlling early embryonic development. Furthermore, we discuss
52 the clinical relevance of using a non-invasive molecular approach to embryo selection for the
53 single embryo transfer (SET) program.

54

55 **Introduction**

56 In humans, early embryo development is a complex process that consists of sequential
57 maturation events of the oocyte, fertilization and embryo growth (4-cell, 8-cell, morula and
58 blastocyst). Indeed, oocytes and spermatozoa are atypical, highly specialized cell types
59 compared to somatic cells. Yet, after fertilization, a zygote is formed, the ultimate totipotent
60 cell that can be considered to be the ultimate undifferentiated cell type, as it gives rise to all
61 cell types and live offspring. Totipotency persists for the very first cell doublings, from the
62 single cell and zygote to at least the 4-cell pre-embryo. Initiation of transcription in the newly-
63 formed embryonic genome reportedly occurs at the 4- and 8-cell stage (Braude et al., 1988).
64 A few studies have documented key events that follow fertilization in humans such as
65 decreases in abundance of individual mRNAs (Taylor et al., 2001), overall patterns of gene
66 expression in individual human oocyte and preimplantation embryos stage (Dobson et al.,
67 2004), and more generalizable transcription profiles of pooled morphologically normal human
68 oocytes and embryos (Zhang et al., 2009). These data provide fundamental resources for
69 understanding the genetic network controlling the early stages of human embryo
70 development. At the morula stage, the human pre-embryo undergoes compaction, with the
71 loss of the cellular distinction between the blastomeres. This is followed by the high
72 expression of genes involved in tight intercellular junctions. Then, when the human embryo
73 has reached the 35- to 65-cell stage, the trophoblast cells pump nutrients and water into the
74 interior of the cell-sphere, forming a blastocyst, within which the inner cell mass (ICM) cells
75 continue to proliferate. It is at the blastocyst stage that ICM cells are harvested for the
76 derivation of embryonic stem (ES) cells (Reubinoff et al., 2000; Thomson et al., 1998).
77 Pluripotent cells can be isolated, adapted and propagated indefinitely *in vitro* in an
78 undifferentiated state as human embryonic stem cells (hESCs). hESCs are remarkable in their

79 ability to generate virtually any cell type; hence they carry many hopes for cell therapy.
80 Human mature MII oocytes, as well as hESCs, are able to achieve the feat of cell
81 reprogramming towards pluripotency, either by somatic cell nuclear transfer or by cell fusion,
82 respectively (Cowan et al., 2005; Hochedlinger et al., 2004; Saito et al., 2008; Sung et al.,
83 2006). Knowledge gathered from the field of ESCs was at the heart of the groundbreaking
84 discovery that both mouse and human somatic cells can be reprogrammed into a pluripotent
85 state by defined factors. The field of induced pluripotent stem cells (iPSCs) has marked a new
86 era in stem cell research, and has also provided data pertinent to improving the understanding
87 of pluripotency (Takahashi et al., 2007; Yu et al., 2007). Since totipotency and pluripotency
88 are at the center of early embryonic development, comprehending their molecular
89 mechanisms is crucial to our understanding of reproductive biology and to regenerative
90 medicine.

91 DNA microarray technology is one of the most widely used and potentially revolutionary
92 research tools derived from the human genome project (Venter et al., 2001). This technology
93 provides a unique tool for the determination of gene expression at the level of messenger
94 RNA (mRNA) on a genomic scale. Its capacity has opened new paths for biological
95 investigation and generated a large number of applications (Stoughton, 2005), including the
96 analysis of the transcriptomic profiles from early embryo development and the identification
97 of new prognostic biomarkers for use in the *in vitro* fertilization (IVF) program (Hamatani et
98 al., 2004a; Assou et al., 2008). The application of microarray technology to the analysis of
99 human oocyte and early embryo cleavage poses specific challenges associated with the
100 picogram levels of mRNA in a single oocyte and embryo, the plasticity of the embryonic
101 transcriptome, the scarcity of the material and ethical considerations.

102 In this review we analyzed data from published reports and include our own data to define the
103 genomic profile during early embryonic development. Once the molecular signature is

104 established, biomarkers can be identified on a large scale, validated and tested prior to clinical
105 applications for embryo selection to improve single embryo transfer (SET) programs. Such a
106 research workflow should provide an understanding of the molecular and cellular mechanisms
107 of oocyte and embryo function, as well as important insights for the development of
108 diagnostic tests for oocyte quality and embryo competence.

109 **Methods**

110 A search of English-language publications from 4 computerized databases (PubMed,
111 EMBASE, Science Direct and Ingenta Connect) was undertaken. We built an expression
112 compendium by combining U133 Plus 2.0 (Affymetrix, Santa Clara, USA) microarray data
113 from the US National Center for Biotechnology Information, from the Gene Expression
114 Omnibus (GEO) through the provisional accession numbers (the GSE7234, GSE7896,
115 GSE11450, and GSE7896 series), and from 15 samples from our own laboratory (5 pooled
116 oocytes, 2 embryos on day-3, and 8 hESC lines). This study received institutional review
117 board (IRB) approval, as well as French authority: Agence de la Biomédecine (ABM). The
118 information gathered was analyzed through the GCOS 1.2 software (Affymetrix), using the
119 default analysis settings and global scaling as the first normalization method, with a trimmed
120 mean target intensity value (TGT) for each array arbitrarily set to 100, in agreement with the
121 MIAME recommendations (Brazma et al., 2001). Principle component analysis (PCA) was
122 performed using GeneSpring[®] software to provide a global view of how the various sample
123 groups were related. Hierarchical clustering was carried out with CLUSTER and TREEVIEW
124 software (Eisen et al., 1998). To uncover functional biological networks, we imported gene
125 expression signatures into the Ingenuity Pathways Analysis (IPA) Software (Ingenuity
126 Systems, Redwood City, CA, USA).

127

128 **Results**

129 **Global analysis of the human oocytes gene expression profile**

130 The human oocyte is equipped with an extraordinary biological competence. It can be
131 fertilized and, at the same time, is able to reprogram the sperm chromatin, giving rise to
132 totipotency and driving towards early embryonic development. However, the molecular
133 mechanisms underlying oocyte competence are still largely unknown. Throughout follicle
134 growth, the nuclear maturation of oocytes is arrested at meiotic prophase I. However, the
135 cytoplasm progresses through a series of maturational stages, accumulating mRNAs and
136 proteins that enable the oocyte to be fertilized and to progress through the first cleavage
137 divisions until embryonic genes begin to be expressed. To improve the understanding of
138 oocyte maturation and competence, microarray technology permits the assessment of changes
139 of the global gene expression profiles in human and mouse oocytes (see Table I).

140 **Gene expression of the oocyte during nuclear maturation**

141 Nuclear maturation is a fundamental developmental event that has been extensively studied
142 for several years. However, the molecular mechanisms underlying this critical process remain
143 incompletely understood. Many exciting biological questions surrounding this event persist.
144 Differences in the global mRNA transcript profile have been reported between mature oocytes
145 (metaphase II (MII)) and immature oocytes (germinal vesicle (GV) and metaphase I (MI)) in
146 both mice and human (Assou et al., 2006; Cui et al., 2007; Wang, Q. T. et al., 2004; Yoon et
147 al., 2006; Zhang et al., 2007). The genome-wide gene expression of human immature and
148 mature oocytes revealed minor modifications in the transcript profiles between GV and MI
149 oocytes, while MII oocytes showed overexpression of more than 400 genes and a striking

150 underexpression of more than 800 genes in comparison with less mature oocytes (Assou et al.,
151 2006). The major modifications of the transcript profile appear to occur during the final stage
152 of oocyte maturation. Components of the metaphase-promoting factor, the anaphase-
153 promoting complex, and a number of oocyte specific genes were overexpressed in MII oocyte
154 (Assou et al., 2006). Similarly, a global transcriptome comparison of GV-stage and MII-stage
155 mouse oocytes revealed the expression of more than 12,000 genes by murine oocytes, more
156 than 1,600 of which were upregulated in GV and 2,000 in MII oocytes (Cui et al., 2007). The
157 authors reported a higher representation of genes associated with protein metabolism, the
158 mitotic cell cycle, electron transport, fertilization, microtubule/cytoskeletal protein family,
159 DNA replication, G protein-coupled receptors, and expression signalling in mature MII
160 oocytes. The focus of our previously published study was to apply a microarray approach to
161 identify new potential regulators and candidate genes involved in human oocyte maturation.
162 In addition, (Assou et al., 2006) observed that genes involved in the proteasome pathway were
163 over-represented during human oocyte maturation (Fig. 1), suggesting an important role in
164 oogenesis for this gene family. This observation is of great interest in light of the recent
165 findings on the role of the proteasome in transcription (Szutorisz et al., 2006). The proteasome
166 is known to interact with chromatin during multiple stages of transcription, through both
167 proteolytic and non-proteolytic activities (Collins and Tansey. 2006). Proteolytic activities of
168 the proteasome are important for promoting the exchange of transcription factors on
169 chromatin, and possibly for facilitating multiple rounds of transcription initiation. Non-
170 proteolytic activities of the proteasome are important for transcriptional elongation checkpoint
171 and histone modification (Collins and Tansey. 2006). Previous studies described the *BARD1*-
172 *BRCA1* heterodimer as an E3 ubiquitin ligase (Lorick et al., 1999; Ruffner et al., 2001). Gasca
173 et al. (2007) observed that multiprotein E3 ubiquitin ligase complex containing *BARD1*,
174 *BRCA1* and *BRCA2*, termed *BRCC* are involved in human oocyte maturation. These proteins

175 function predominantly in the nucleus to regulate cell cycle progression, DNA repair and gene
176 transcription (Henderson. 2005). *BARD1* is an important regulator of *BRCA1* activity: the
177 binding of *BARD1* with *BRCA1* maintains both proteins in the nucleus, consequently
178 preventing apoptosis (Fabbro et al., 2004). Guillemin et al. (2009) reported that the apoptosis
179 inhibitor protein *BCL2L10* undergoes dynamic sub-cellular redistribution during human
180 oocyte maturation. Knockdown of mouse *Bcl2l10* using RNA interference (RNAi) in cultured
181 oocytes resulted in maturation arrest at the MI stage accompanied by abnormal spindle and
182 chromosome organizations (Yoon et al., 2009). These findings indicate that *BCL2L10* may
183 play a role in oocyte maturation.

184 **Gene expression of oocytes according to female age**

185 Oocyte quality is a main factor in human reproductive failure and has been strongly
186 associated with advanced reproductive age. Recent studies have indicated differential gene
187 expressions between younger and older oocytes in human (Steuerwald et al., 2007; Grondahl
188 et al., 2010) and mouse (Hamatani et al., 2004b; Pan et al., 2008). Age affects the expression
189 of human oocyte genes in a variety of major functional categories, including cell cycle
190 regulation, cytoskeletal structure, energy pathways, transcription control, and stress responses
191 (Steuerwald et al., 2007). Recently, Grondahl et al. (2010) performed full-genome microarray
192 analysis on individual oocytes of women with advanced age in comparison with a younger
193 patient. They observed a substantial difference between younger and older oocytes at the
194 transcriptional level of genes involved in central biological functions, from establishment and
195 organization of the meiotic spindle checkpoint, through protein metabolism and DNA repair
196 to the regulation of meiotic division and cell cycle. In the mouse, gene profiles involved in
197 oxidative stress, mitochondrial function, chromatin structure, DNA methylation and genome
198 stability are altered with old age (Hamatani et al., 2004b). In addition, the expression profiling

199 of young and old mouse oocytes revealed changes in the expression of several kinetochore
200 components of the spindle assembly checkpoint (SAC); including protein kinases (e.g., *Bub1*,
201 *BubR1*, Aurora kinase) and *Cdc20*, a critical activator of the Anaphase Promoting Complex
202 (Pan et al., 2008).

203 **Gene expression of oocytes under *in vitro* conditions**

204 Intrinsic oocyte developmental competence, as assessed by development up to the blastocyst
205 stage, has been positively associated with the site of oocyte maturation (Rizos et al., 2002).
206 The site of oocyte maturation has a profound effect on oocyte quality; oocytes matured in
207 vivo are of superior competence compared to those matured in vitro (Rizos et al., 2002).
208 Microarray technologies were applied to humans and to mice to identify the differences
209 between development in vitro versus in vivo matured oocytes (Jones et al., 2008; Pan et al.,
210 2005; Wells and Patrizio. 2008). Jones et al. (2008) compared the transcriptome of oocytes
211 matured in vivo (with relatively high developmental competence) with the transcriptome of
212 oocytes under in vitro maturations (IVM) conditions (with low developmental competence)
213 and identified an over-abundance of a large number of genes in human oocytes matured in
214 vitro compared with in vivo. This study suggested, that the increase in gene expression
215 detected in vitro could be due to either a deregulation in gene transcription or the post-
216 transcriptional modification of genes, causing an inadequate temporal utilization of transcripts
217 which could be translated as a developmental incompetence of any embryos resulting from
218 these oocytes. Similarly, Zheng et al. (2005) indicated that the reduced developmental
219 competence of non-human primate oocytes under IVM was a result of a failure of these
220 oocytes to undergo the normal pattern of transcript silencing. Wells and Patrizio (2008)
221 elegantly differentiated the mRNA expression in human oocytes at different maturational
222 stages under IVM. They observed that high levels of mRNAs, proteins, substrates and

223 nutrients are accumulated in the oocyte during maturation and are associated with oocyte
224 developmental competence (Song et al., 2005; Watson. 2007). In addition, IVM seems to alter
225 the abundance of certain MII oocyte mRNAs compared with in vivo MII oocytes (Lee et al.,
226 2008; Zheng et al., 2005). The differences in gene expression detected may assist in the
227 design of optimized protocols for ovarian stimulation and in further refining media
228 formulations for IVM.

229 **Gene expression of oocytes in PCOS patients**

230 Polycystic ovary syndrome (PCOS) is a good model for studying the loss of oocyte quality.
231 Studies comparing gene expression arrays in tissues from patients with PCOS compared with
232 normal responders have reported similar pathways for differentially expressed genes in whole
233 ovaries (Jansen et al., 2004; Oksjoki et al., 2005) and oocytes (Wood et al., 2007). Wood et al
234 (2007) identified 374 genes with different mRNA transcript levels when analyzing
235 morphologically indistinguishable oocytes from normal responders and PCOS patients
236 (Wood et al., 2007). Many of the genes found to be differentially expressed in PCOS are
237 involved in signal transduction, transcription, DNA and RNA processing, and the cell cycle.
238 Of these, 68 are involved in chromosome alignment and segregation; other genes contain
239 putative androgen receptors (Wood et al., 2007). These differences provide a partial
240 explanation for the reduction of oocyte capacity observed in PCOS patients. Cumulus cells
241 (CCs) play a major role in the control of oocyte metabolism, and therefore it is likely that
242 dysfunction of these cells could play a role in PCOS.

243 **Human oocyte-cumulus cells relationships**

244 The oocyte is surrounded by several cell layers, CCs, which are tightly connected to each
245 other and to the oocyte through gap junctions such as *GJA1* (Cx43) and *GJA4* (Cx37), that

246 facilitate the bi-directional traffic between the oocyte and CCs (Tanghe et al., 2002). The
247 oocytes create and control their microenvironment by promoting differentiation of the
248 cumulus cell phenotype through secretion of paracrine signalling factors, such as growth and
249 differentiation factor 9 (*GDF9*) and bone morphogenic protein-15 (*BMP15*), which are
250 members of the transforming growth factor β (*TGF β*) family (Eppig et al., 1997). Absence of
251 these two oocyte-secreted factors has been shown to cause infertility (Dong et al., 1996;
252 Galloway et al., 2000). *GDF9* show increased cumulus production of hyaluronic acid
253 synthetase (*HAS2*), an enzyme responsible for the hyaluronic acid synthesis which is the
254 major structural backbone of the cumulus extracellular matrix (Elvin et al., 1999), as well as
255 the proteoglycan *versican*, another important component of the cumulus matrix (Russell et al.,
256 2006). Other genes were induced and regulated by *GDF-9* such as Peroxiredoxin 2 (*PRDX2*)
257 (Leyens et al., 2004) and *SMAD* family member 2 and 3 (*SMAD2/3*) (Dragovic et al., 2007).
258 Studies on *SMAD2/3* conditional knockout mice indicate that *SMAD2/3* are indispensable for
259 normal cumulus expansion (Li, Q. et al., 2008). Furthermore, CCs play an essential role,
260 particularly for normal oocyte growth, ovulation, fertilization and embryo development
261 (Chang et al., 2002). Active components of the cumulus matrix come from several sources:
262 they are synthesized directly by CCs under the control of endocrine and oocyte-derived
263 factors, secreted by mural granulosa cells (GCs), or enter the follicle from blood plasma
264 (Eppig. 1982, 1991; Tanghe et al., 2002; Vanderhyden et al., 2003). In addition, CCs and
265 oocytes have different metabolic needs. Oocytes themselves are unable to synthesize
266 cholesterol and poorly metabolize glucose for energy production. Inversely, the CCs
267 metabolize alternative substrates, such as cholesterol and glucose, which are essential for the
268 development and function of oocyte (Eppig et al. 2005). The exact nature and diversity of
269 oocyte-CC signalling molecules are complex and dynamic. Errors in the regulatory cumulus-
270 oocyte complex (COC) may result in the production of oocytes unable to undergo embryo and

271 pregnancy outcomes. The study of the CC gene expression profile offers the opportunity by a
272 non-invasive method, to predict oocyte competence, because bidirectional communication
273 between the CC and the oocyte are necessary for acquisition of this competence.

274 **Global analysis of human embryo gene expression profile**

275 Embryo survival requires that the oocyte be sufficiently equipped with stored maternal
276 products and that embryonic gene expression start at the correct time. Human early embryos
277 are highly sensitive to the environment under *in vitro* culture conditions. The culture
278 conditions can alter the gene expression pattern (Lonergan et al., 2006). Several studies have
279 explored global patterns of embryos gene expression in both mouse (Hamatani et al., 2004a;
280 Rinaudo et al., 2004; Wang, Q. T. et al., 2004; Zeng et al., 2004) and human (Dobson et al.,
281 2004; Li, S. S. et al., 2006; Wells et al., 2005; Zhang et al., 2009) embryos (Table I). Gene
282 expression profiling of early mouse embryos showed characteristic patterns of maternal RNA
283 depletion and revealed that embryonic genome activation (EGA) happens in two phases: the
284 first phase is zygotic genome activation (ZGA), the second mid-preimplantation gene
285 activation (MGA), which precedes the dynamic morphological and functional changes from
286 the morula to the blastocyst stage (Hamatani et al., 2006). Two major groups of gene activity
287 can be defined: firstly in oocytes and first early cleavage stages and the second consisting of
288 4-cell to blastocyst stages, which correlates with the transition from maternal to zygotic
289 expression. Several transcripts of molecules involved in *WNT* or *BMP* signalling pathways
290 were identified in the embryonic transcriptome, indicating that these critical regulators of cell
291 fate and patterning are conserved and functional at these stages of pre-implantation
292 development. Dobson et al. (2004) reported the global patterns of gene expression in human
293 embryos on day 2 and 3 (n=8) by using DNA microarray analysis. This study revealed that the
294 first few days of oocyte maturation and embryo development were characterized by a

295 significant decrease in transcript levels, suggesting that decay of RNAs associated with
296 gamete identity is integral to embryo development (Dobson et al., 2004). Furthermore, this
297 study revealed that developmental arrest and activation of the embryonic genome are
298 unrelated events. Wells et al. (2005) used reverse transcription and real-time fluorescent PCR
299 to quantify the expression patterns of nine known genes implicated in DNA damage and cell
300 division (*BRCA1*, *BRCA2*, *ATM*, *TP53*, *RBI*, *MAD2*, *BUB1*, *APC*, and *beta-Actin*) at the early
301 stages of human embryonic development. The data suggest that embryos with patterns of gene
302 expression appropriate for their developmental stage may have superior viability to those
303 displaying atypical gene activities (Wells et al., 2005). Li et al. (2006) used a cDNA
304 microarray containing 9,600 transcripts to investigate 631 differently expressed genes in
305 oocytes, as well as the 4-cell and 8-cell human embryonic stages. Numerous genes were found
306 to be expressed at a value double that of the median value of all genes expressed; 184 genes in
307 oocytes, 29 in 4-cell embryos, and 65 genes in 8-cell embryos. These results indicate that the
308 expression of some zygotic genes had already occurred at the 4-cell embryonic stage (Li et al.,
309 2006). In addition, Chen et al. (2005) observed global gene expression changes during the
310 hatching of mouse blastocysts, an essential process for implantation. This study observed that
311 there were 85 genes which were upregulated in blastocysts at the hatching stage. These genes
312 included cell adhesion molecules, epigenetic regulators, stress response regulators, and
313 immunoresponse regulators (Chen et al., 2005). Furthermore, Adjaye et al. (2005) identified
314 biomarker transcripts specific to inner cell mass (ICM) such as (*OCT4/POU5F1*, *NANOG*,
315 *HMGB1*, and *DPPA5*) when comparing gene expression profiles of ICM and trophoectoderm
316 cells (TE) from human blastocysts. This data indicates that the emergence of pluripotent ICM
317 lineages from the morula is controlled by metabolic and signalling pathways including *WNT*,
318 mitogen-activated protein kinase (*MAPK*), transforming growth factor- β (*TGF- β*), *NOTCH*,
319 integrin-mediated cell adhesion and apoptosis-signalling pathways (Adjaye et al., 2005).

320 The molecular mechanism of early embryonic development is still unclear. To identify genes
321 that are involved in early embryonic development, we have conducted an analysis related to
322 the expression profiles of five pooled oocytes, two embryos on day-3 and eight human
323 embryonic stem cell (hESC) lines. In order to ascertain whether there is a specific molecular
324 signature corresponding to each stage of early embryonic development, we performed
325 principal component analysis (PCA) and hierarchical clustering (Figure 2). All of the oocyte,
326 embryo and hESC samples clustered as homogeneous groups (Figure 2A) in agreement with
327 the robustness of the Affymetrix microarrays (Irizarry et al., 2005). Hierarchical clustering on
328 10,000 genes delineated five major clusters of genes (Figure 2B). The hierarchical clustering
329 dendrogram showed that hESCs are distantly related to oocytes and embryos on day-3 (Figure
330 2C), revealing the distinctive patterns of maternal RNA degradation and embryonic gene
331 activation, which contribute to the dramatic morphological changes during early embryonic
332 development. We identified genes whose expression gradually increased or decreased during
333 early embryonic development (Figure 3A-B). Taken together, these results seem to indicate
334 that early embryonic development, particularly totipotent cells and highly pluripotent cells
335 (hESCs), have quite distinct genetic programs. This supports the notion of a gradual decrease
336 of developmental potential from the pre-implantation embryo stage to stem cells.

337 DNA damage can have severe cellular consequences, especially in cells which are the origin
338 of all cells of the future human being. Hence, DNA repair systems play a crucial role in early
339 embryonic development. The pathways and strategies for DNA repair in early embryos have
340 been reviewed elsewhere (Jaroudi et al., 2007). The importance of DNA repair gene products
341 during development is highlighted by the phenotypes observed for human genetic disorders
342 associated with DNA damage response (Hales. 2005). Synthesis and repair of DNA, as well
343 as methylation of DNA, is crucial in gametogenesis, fertilization and pregnancy. Recently,

344 DNA repair gene expression was investigated in human oocytes and blastocysts to identify the
345 pathways involved at these stages and to detect potential differences in DNA repair
346 mechanisms of pre- and post-embryonic genome activation (Jaroudi et al., 2009). Large
347 numbers of repair genes were detected indicating that all DNA repair pathways are potentially
348 functional in human oocytes and blastocysts. Expression levels of DNA repair genes at the
349 pre- and post-EGA transcriptional level suggest differences in DNA repair mechanisms at
350 these developmental stages (Jaroudi et al., 2009).

351 **Epigenetic regulation of human early embryonic development**

352 Epigenetics refers to a collection of mechanisms that can cause a change in the phenotype of a
353 cell without altering its DNA sequence (Goldberg et al., 2007). Early embryonic development
354 is regulated epigenetically. During the development process, the epigenetic signature changes
355 as the cell enters fertilization and/or differentiation. DNA methylation is an important
356 epigenetic event, regulating gene expression and chromatin structure in any developmental
357 processes including gene imprinting and embryogenesis (Kafri et al., 1992). To maintain
358 methylated DNA, DNA methyltransferases (*DNMTs*) are necessary (Vassena et al., 2005).
359 This family of enzymes is divided into two major classes: *DNMT1* and *DNMT3* (including
360 *DNMT3A* and *DNMT3B*). *DNMT1* enzyme is the main methyltransferase by far and its
361 exceptional preference for hemimethylated DNA indicates its role in the maintenance of
362 methylated status during DNA replication. Genetic studies have suggested that *DNMT1* may
363 play a role in the methylation of repeat elements in growing oocytes (Gaudet et al., 2004). Our
364 genes expression data indicate that the *DNMT1* mRNA was overexpressed in mature MII
365 oocytes and decreased progressively in embryos on day-3 and in hESCs (Figure 3B),
366 revealing that the oocyte-specific *DNMT1* transcript is expressed in human oogenesis and
367 persists in embryos at day-3. *DNMT3B* transcripts were detected in mature MII oocytes and

368 hESCs (Assou et al., 2009), and the expression of *DNMT3A* was enriched in undifferentiated
369 hESCs (Assou et al., 2007; Huntriss et al., 2004; Richards et al., 2004), indicating that the
370 *DNMT3* family methyltransferases are candidate epigenetic regulators during preimplantation
371 development. Both *DNMT3A* and *DNMT3B* enzymes are involved in *de novo* methylation
372 processes with different substrate preferences. *DNMT3A* acts preferentially on unmethylated
373 DNA while *DNMT3B* can act on both hemimethylated and unmethylated DNA (Vassena et
374 al., 2005). These enzymes are responsible for the establishment of DNA methylation during
375 development (Okano et al., 1999; Watanabe et al., 2002).

376 As imprinting is closely related to embryogenesis, we analyzed the expression of imprinted
377 genes in oocytes, embryos on day-3 and hESCs. We looked at the expression of forty
378 imprinted human genes (Morison et al., 2001) in our data (the list of imprinted genes is
379 available at <http://www.otago.ac.nz/IGC>). The analysis of the expression levels of these
380 imprinted genes compared with their expression levels in mature MII oocytes, embryos on
381 day-3, and hESCs reveals their reprogramming time points in humans. Several imprinted
382 genes (see Table II) were expressed to high levels in the oocytes, embryos, and hESCs. The
383 study of the methylome and the epigenome is important because minor defects can lead to
384 serious human diseases. Indeed, several syndromes are associated with imprinting defects
385 during preimplantation development (van der Maarel. 2008; Yamazawa et al., 2008). Beyond
386 transcription and epigenetics, the discovery of hundreds of microRNAs (miRNAs) has
387 defined yet another level of mRNA regulation that could have a major impact on defining the
388 mechanisms controlling early embryonic development (Blakaj et al., 2008; Kedde et al.,
389 2008). These short mRNAs have the ability to target and regulate the translation and stability
390 of target mRNAs, and thus are likely to have profound effects on embryonic gene expression
391 patterns.

392 Expression of known components of two pathways in human early embryo development

393 Known components of two signalling pathways involved in human early embryonic
394 development, (i) the *WNT* signalling pathway and (ii) the *TGF- β* superfamily pathway, have
395 been observed.

396 (i) *WNT* signalling

397 The *WNT* gene family consists of numerous conserved glycoproteins that regulate pattern
398 formation during embryogenesis in a wide variety of tissues (Morkel et al., 2003). Limited
399 data is available about the role of the *WNT* pathway in the reproduction process. It has been
400 shown that this pathway is operational during human and mouse pre-implantation
401 development stages (Adjaye et al., 1999). Furthermore, it has also been reported that
402 activation of the canonical *WNT* pathway is sufficient to maintain self-renewal of both human
403 and mouse ESCs (Sato et al., 2003). We observed that the main components of the *WNT*
404 pathway such as *DVL2*, *CTNNB1*, *CTNNA2*, *CTNND1*, *LEF1*, *CSNK1G3*, and *DAAMI* were
405 overexpressed in MII oocytes; *FZD4*, *GSK3B*, *CTNND1*, *APC*, *SENP5*, *SENP8*, *CSNK2A2*,
406 and *CSNK1E* to be overexpressed in embryos on day-3; *FZD3*, as well as *FZD7*, *SNP6*,
407 *CXXCI*, *AXIN*, and *SFRP1* in hESCs. These findings imply that the *WNT* pathway may
408 participate in early embryonic development. On the other hand, we observed that expression
409 of glycogen synthase 3 kinase (*GSK-3 β*) is downregulated in the hESCs, corroborating the
410 reported inactivation of *GSK-3 β* , which leads to the activation of the *WNT* pathway and the
411 maintaining of the undifferentiated state of ESCs (Sato et al., 2004). In the absence of a *WNT*
412 signal, the *GSK-3 β* kinase phosphorylates β -catenin and targets it for ubiquitin-mediated
413 destruction. Activation of the *WNT* pathway by a *WNT* ligand inhibits *GSK-3 β* activity,
414 resulting in the accumulation of β -catenin. Stable β -catenin can interact with DNA-binding

415 *TCF* factor, at the site where the complex activates transcription of target genes. Treating
416 ESCs with *WNT* or inhibiting *GSK-3* activity can stimulate ESC self-renewal and support
417 pluripotency (Sato et al., 2004). Recently, two studies (Lluis et al., 2008; Marson et al., 2008)
418 show that *WNT*- β -catenin signalling stimulates nuclear reprogramming. These studies, using
419 distinct reprogramming methods, offer insights into the mechanisms underlying the
420 acquisition and maintenance of pluripotency.

421 (ii) *TGF- β* superfamily

422 *TGF- β* and its family members, including *BMPs*, *NODAL*, and *ACTIVINS*, have been
423 implicated in the development of oocytes and embryos (Chang et al., 2002). Kocabas et al.
424 (2006) confirmed the presence of *TGF- β* pathways in human oocytes, previously described
425 only in mice (Mummery. 2001). *TGF- β* family signals play important roles in the
426 maintenance of self-renewal and pluripotency in both human and mouse ESCs (Watabe et al.,
427 2009). Inhibition of *TGF- β /ACTIVIN/NODAL* signalling by SB-431542, a chemical inhibitor
428 of the kinases of type 1 receptors for *TGF- β /ACTIVIN/NODAL* (Inman et al., 2002), resulted
429 in decreased expression of the markers of undifferentiated states (James et al., 2005). Using
430 Ingenuity Software Knowledge Base (Redwood City, CA; <http://www.ingenuity.com>), we
431 observed the expression of most members of the *TGF- β* superfamily in human oocytes,
432 embryos on day-3, and hESCs (Figure 3C). However, some components of the *TGF- β*
433 pathway, such as *ACTIVIN/INHIBIN*, are absent in oocytes but present in both embryos on
434 day-3 and hESCs, whereas *NODAL* is present only in hESCs. It was recently shown that
435 human iPSCs rely on *ACTIVIN/NODAL* signalling to control *NANOG* expression and thereby
436 maintain pluripotency (Vallier et al., 2009). The transcription factors *SMAD4* and *SMAD7* are
437 significantly overexpressed in hESCs but are underexpressed in both oocytes and embryos on
438 day-3. In addition, it was shown that mouse embryos deficient in *SMAD4* display defective

439 epiblast proliferation and delayed outgrowth of the ICM (Sirard et al., 1998). Expression of
440 the signalling receptors for *TGF- β* in human oocytes and embryo at blastocysts stage, suggests
441 a role for *TGF- β* in early pre-implantation development.

442 **The expression program of human embryonic stem cells**

443 hESCs do not exist in nature but are derived from the inner cell mass (ICM) of the human
444 blastocyst (Thomson et al., 1998) and are considered equivalent to ICM cells, having captured
445 the phenotype of a cell type that exists only transiently *in vivo*. hESCs have the capacity to
446 differentiate into the derivatives of all three germ layers: endoderm, mesoderm and ectoderm
447 (Carpenter et al., 2003; Lu et al., 2007); thus hESCs have great potential in the field of
448 regenerative medicine. Although artificial, hESCs represent an excellent model for
449 investigating fundamental aspects of the early embryo development and the pluripotency.
450 Numerous genomic studies have used microarray techniques to capture a detailed view of
451 ESC gene expression (Abeyta et al., 2004; Assou et al., 2007; Beqqali et al., 2006;
452 Bhattacharya et al., 2004; Cowan et al., 2005; Forsyth et al., 2008; Golan-Mashiach et al.,
453 2005). These transcriptome data can generate molecular signatures of ESCs that define the
454 individual components, and the pathways, that regulate pluripotency and maintain the
455 undifferentiated state. We performed a meta-analysis of 38 papers on the topic (Assou et al.,
456 2007). We determined that 1,076 genes were found to be upregulated in hESCs by at least
457 three studies when compared to differentiated cell types. In fact, only a single gene, *OCT3/4*,
458 was found to be in common to all 38 lists of hESC-specific genes. Our analysis shows that
459 gene expression analyses are sensitive to experimental conditions, therefore requiring extreme
460 caution during data interpretation. A cross-comparison of the gene lists generated by these
461 efforts shows that *OCT3/4*, *NANOG*, *SOX2*, *DNMT3B*, *LIN28*, and *CD24* are commonly
462 expressed in the majority of hESCs (Assou et al., 2007). A "core" set of transcription factors,

463 *OCT3/4*, *SOX2* and *NANOG*, is common between the hESC lines and is considered to be a
464 general criteria for hESC characterization and pluripotency (Hoffman et al., 2005; Zaehres et
465 al., 2005). A list of *OCT3/4*, *SOX2*, and *NANOG* target genes has been determined by cross-
466 species genomic analysis followed by chromatin immunoprecipitation and gene expression
467 analysis (Boyer et al., 2005). Genes that are activated by *OCT3/4*, *SOX2*, and *NANOG* in
468 ESCs are mostly upregulated and important for ESC self-renewal. Cross-species comparisons
469 between human and mouse ESC transcriptomes revealed both distinct and similar features
470 (Rao. 2004; Sato et al., 2003; Wei et al., 2005). Wei et al. (2005) compared gene expression
471 profiles between hESC and mESC, and showed that *LIFR* was overexpressed in mice, but not
472 in humans; *FGF2* was overexpressed in humans, while *FGF4* was abundant in mice. Epiblast
473 derived mouse ESCs also maintain self-renewal by the dependence on FGF2, hence activated
474 FGF signalling (Brons et al., 2007; Tesar et al., 2007). The accumulating ESC transcriptome
475 data is now being aimed at identifying molecular markers that will be useful for quality
476 control of ESCs for clinical applications.

477 **Common molecular signatures between human metaphase II oocytes and ICM**

478 ESCs and mature MII oocytes are able to reprogram the nuclei of fully differentiated human
479 somatic cells and render these cells pluripotent (Cowan et al., 2005; Rideout et al., 2001).
480 Through the comparative analysis of the transcriptome of such cells, it may be possible to
481 achieve a better understanding of the mechanism of nuclear reprogramming, the molecular
482 events which occur during the first week of human embryo development, and the
483 identification of the genes involved in pluripotency initiation (Kocabas et al., 2006; Zhang et
484 al., 2007; Assou et al., 2009). Kocabas et al. (2006) compared the transcriptome of human
485 MII oocytes and hESCs with a reference sample consisting of a mixture of total RNA from
486 different normal somatic cells. In this study, the authors identified 1,626 transcripts that were

487 significantly upregulated in the hESs relative to somatic cells. Intersection of these 1,626
488 transcripts enriched in hESCs with the 5,331 transcripts enriched in human MII oocytes,
489 identified 388 common genes that are hypothesized to play an important role in oocyte
490 reprogramming and early events associated with human early embryo development (Kocabas
491 et al., 2006). Zhang et al. (2007) obtained a large amount of information on gene expression
492 in human oocytes at GV stage as compared with hESCs. In total, 1,629 genes were
493 upregulated in GV oocytes when compared with hESCs and human foreskin fibroblasts
494 (hFIBs). This list of genes, including *MATER*, *ZAR1*, *NPM2*, *FIGLA*, some imprinted genes,
495 and some components of four signalling pathways such as *MOS-MPF*, *NOTCH*, *WNT* and
496 *TGF-beta* (Zhang et al., 2007), may provide good candidate genes for understanding the
497 oocyte maturation and the early embryonic development. On the other hand, Assou et al.
498 (2009) compared the gene expression profiles of mature MII oocytes with hESCs, and then
499 both to somatic tissues. There was a common oocyte/hESC gene expression profile, which
500 included genes associated with pluripotency, such as *LIN28* and *TDGF1*, and a large
501 chromatin remodeling network (*TOP2A*, *DNMT3B*, *JARID2*, *SMARCA5*, *CBX1*, *CBX5*).
502 Interestingly, a large set of genes was also found to code for proteins involved in the
503 ubiquitination and proteasome pathway (Assou et al., 2009). This list provides reliable
504 candidate genes for future studies on molecular mechanisms of pluripotency and nuclear
505 reprogramming. The zinc finger motif is a DNA binding domain dependent on a zinc ion
506 frequently found in transcription factors. In seeking to identify genes encoding zinc finger
507 domain proteins expressed in both mature MII oocytes and hESCs, we observed that the
508 distribution of genes encoding these proteins on the genome is not random: they are
509 significantly enriched in chromosomes 19 and 20 in mature MII oocytes, and in 16 and 17 in
510 hESCs (Assou et al., 2009). As can be seen in Figure 4, seven zinc finger proteins were

511 common to mature MII oocytes and hESCs, three were found in oocytes only (e.g. *ZNF528*,
512 *ZNF468*, *ZNF313*), and three in hESCs only (e.g. *ZNF268*, *ZNF232*, *ZNF423*).

513 **Human early embryo development transcriptome data visualization through an internet** 514 **interface**

515 *Amazonia!* (<http://www.amazonia.transcriptome.eu>) is a freely accessible web site dedicated
516 to the visualization of large, publicly available human transcriptome data, including data on
517 stem cells, human embryo and germinal cells, for facilitating the free exchange of information
518 on genomics. Data is visualized as expression bar plots with a color code enabling the
519 recognition of cell type (Assou et al., 2007; Le Carrouer et al., 2010). Genes are accessed either
520 by keywords or through lists. Most interestingly, when data is obtained using the same
521 platform format, sample labeling, and data normalization, it is possible to combine different
522 experiments in a single virtual experiment. *Amazonia!* generate bar plots that combine
523 expression in oocytes, embryos and hESCs with more than 200 germinal and somatic tissues.

524 **Omics: tools for the identification of reliable biomarkers for** 525 **oocyte and embryo selection**

526 Since the birth of Louise Brown, the first baby conceived by IVF in the UK in 1978, more
527 than 2 million children in the world have been born as a result of assisted reproductive
528 technology (ART). Since then, these techniques have advanced considerably. ART protocols
529 continue to evolve with the aim of achieving higher pregnancy rates, reducing multiple
530 pregnancies and obtaining healthier babies. However, despite the incontestable advances, the
531 pregnancy rate is still relatively low and has not increased significantly in the last decade
532 (Andersen et al., 2007). The selection of embryos with higher implantation potential is one of
533 the major challenges in ART. Initially, multiple embryo transfer was used to maximize

534 pregnancy rates. However, improved embryo quality and rising multiple pregnancy rates has
535 resulted in the decrease in number of embryos for replacement. Therefore, selection of the
536 “best” embryo has become crucial, particularly with elective SET being strongly
537 recommended. There is therefore a need to develop new objective approaches for embryo
538 selection. The classical methods to select healthy embryos under IVF and ICSI conditions are
539 based on morphological criteria such as early embryonic cleavage, the number and size of
540 blastomeres, fragmentation degree, and the presence of multi-nucleation at the 4- or 8-cell
541 stages (Fenwick et al., 2002). However, most studies suggest, that embryos with proper
542 morphological appearance alone are not sufficient to predict a successful implantation.
543 Beyond the criteria of embryo selection, defining oocyte quality remains one of the most
544 difficult challenges (Wang, Q. et al., 2007). Many morphologically normal embryos do not
545 achieve implantation or spontaneously abort during pregnancy. Considering the limitation of
546 morphologic evaluation and cytogenetic screening methods, there is now a movement towards
547 more sophisticated, high-performance technologies and the emerging “omics” science, such
548 as genomics, transcriptomics, proteomics, and metabolomics (Hillier. 2008). These
549 approaches may contribute to the design of a non-invasive viability assay to assist in the
550 embryos selection in IVF or ICSI programs. It is necessary to distinguish the invasive
551 approaches, which are based on direct analysis of the oocyte and embryo, from non-invasive
552 approaches (Figure 5). Can omics approaches help shape the future of ART?

553 *Transcriptomic approach*

554 Several investigations using reverse transcription followed by real-time PCR have found that
555 specific genes display alterations in activity apparently related to oocyte or embryo quality
556 (Dode et al., 2006; Fair et al., 2004). Despite the accuracy and sensitivity of real-time PCR, it
557 is still limited by the restricted number of genes that can be assessed per single oocyte or

558 embryo. Microarray technology permits the simultaneous analysis of tens of thousands of
559 genes, and provides greater hope for the identification of new biomarkers. Transcriptomics
560 represent a valuable approach to biomarkers development. Both technical and statistical
561 advances are currently facilitating the integration of this approach to IVF programs. The
562 transcriptomic approach has been used experimentally in humans on throphoblastic biopsies
563 of blastocysts (Jones et al., 2008a). Analysis of the transcriptome of aneuploid and normal
564 oocytes, as determined by comparative genomic hybridization (CGH) analysis of the
565 corresponding polar bodies, has identified several differentially expressed genes with roles in
566 chromosome segregation during meiosis, including genes affecting cell cycle checkpoints,
567 spindle dynamics, and chromosomal movement (Fragouli et al., 2006b; Fragouli et al., 2006a;
568 Fragouli et al., 2009; Gutierrez-Mateo et al., 2004). An indirect and attractive approach for
569 predicting embryo and pregnancy outcomes has been recently reported by using
570 transcriptomic data of CCs gene expression (Assou et al., 2008). The CCs are abundant and
571 easily accessible, which makes them an ideal material to use for the potential assessment of
572 oocyte and embryo quality. Data concerning biomarker expression in these cells should yield
573 more precise information, improving the embryologists' capacity to select competent embryos
574 either for fresh replacement or for embryo freezing. By studying genes expression profile of
575 CCs or GCs using microarrays or reverse transcription-polymerase chain reaction (RT-PCR)
576 and quantitative RT-PCR some studies have identified candidate biomarkers for (i) oocyte
577 quality and competence (Zhang et al., 2005; Cillo et al., 2007; Hamel et al., 2008;
578 Adriaenssens et al., 2010), (ii) early embryo development (Anderson et al., 2009; McKenzie
579 et al., 2004; van Montfoort et al., 2008) and (iii) embryo quality and pregnancy outcome
580 (Assou et al., 2008 ; Hamel et al., 2010) (Table III). Zhang et al. (2005) reported that the
581 expression of pentraxin-3 (*PTX3*) in CCs was positively correlated with oocyte competence.
582 *PTX3* was initially studied for its possible role in inflammatory reaction processes (Gewurz et

583 al., 1995). Fertilization *in vivo* is very low in *PTX3* knockout mice (Varani et al., 2002).
584 Feuerstein et al (2007) reported that a number of genes including prostaglandin-endoperoxide
585 synthase-2 (*PTGS2*) and steroidogenic acute regulatory protein (*STAR*) were also negatively
586 associated with oocyte competence. In addition, the expression of gremlin 1 (*GREM1*) is also
587 indicative of embryo quality (McKenzie et al., 2004; Cillo et al., 2007; Anderson et al., 2009,
588 Adriaenssens et al., 2010). *GREM1* is an antagonist of *BMPs* and *TGF- β* signalling (Hsu et
589 al., 1998). It has been proposed that *GREM1* regulates the *GDF9* and *BMP15* balance (Pangas
590 et al., 2004). By using microarrays, our group reported a specific transcriptomic signature in
591 CCs including 630 genes correlated with embryo and pregnancy outcomes. Among this gene
592 list, we identified *BCL2L11*, *PCK1*, and *NFIB* (Assou et al., 2008). *BCL2L11* is involved in
593 triggering cell death in response to abnormalities, *PCK1* is associated with energy production,
594 and *NFIB* regulates some of the earliest processes in embryonic development. Other studies
595 associated the gene expression profile in follicular cells (CCs or GCs) with pregnancy (Hamel
596 et al., 2010). They found that variation of *PGKI*, a transferase enzyme involved in the process
597 of glycolysis and regulation of G-protein signalling 2 (*RGS2*) in follicular cells are likely
598 candidate genes that could have a utility in the prediction of pregnancy. All these studies
599 provide supporting evidence to the concept that CCs are a promising source of reliable
600 biomarkers for predicting oocyte quality and/or embryo and/or pregnancy outcome (Table
601 III). These efforts will ultimately lead to improved efficiency outcomes of ART.

602 ***Proteomic approach***

603 Proteomics describes changes in the expression of proteins translated from a single genome.
604 The human proteome, estimated at over a million proteins, is highly diverse and dynamic. The
605 analysis of the protein composition of the embryos poses a particular challenge because of the
606 low concentration and wide dynamic range of the population of molecules which need to be

607 analyzed; unlike mRNA, proteins cannot be amplified for analysis. Recent advances in mass
608 spectrometry have led to the development of methods sensitive enough to allow the
609 examination of the secretome of single oocytes or embryos. The secretome has been defined
610 as the subset of secreted or consumed proteins that can be found in the environment where the
611 oocyte or embryo grows. It is now possible to obtain comprehensive protein profiles from
612 limited amounts of complex biological fluids including IVF spent culture mediums. The
613 secretome of an individual human embryo has been previously analyzed by surface-enhanced
614 laser desorption and ionization time-of-flight (SELDI-TOF) mass spectrometry (MS)
615 technology, and distinctive secretome signatures were observed at each embryonic
616 developmental stage from fertilization to the blastocyst stage (Katz-Jaffe et al., 2006; Katz-
617 Jaffe et al., 2009). However, no clear biomarkers of embryo viability and implantation have
618 yet been reported. Dominguez et al. (2010) compared blastocyst conditioned media with
619 control medium using protein microarrays, thus revealing increased expression of interleukin
620 10 (*IL-10*) and soluble tumor necrosis factor (*TNF*) receptor 1 (*TNFR1*) and decreased
621 expression of stem cell factor (*SCF*) and chemokine ligand 13 (*CXCL13*) (Dominguez et al.,
622 2010). Other studies have reported a link between the level of cytokines in human follicular
623 fluid (hFF) and the implantation potential of the embryo derived from the oocyte of this
624 follicle. Using a bead-based multiplex sandwich immunoassay (Luminex), Ledee et al. (2008)
625 demonstrated that only granulocyte colony-stimulating factors (*G-CSF*) measured in
626 individual hFF samples together with 26 other cytokines was particularly elevated in the hFF
627 corresponding to the embryos with highest implantation potential (Ledee et al., 2008). In
628 addition, significantly higher levels of interleukin 10 (*IL10*) and interferon (*IFN- γ*) were
629 found in hFF from oocytes that generated early cleavage embryos. However, Luminex
630 technology is complex and expensive. Further studies will help to confirm the advantageous
631 applications of proteomics testing in the field of IVF.

632 *Metabolomic approach*

633 Metabolomics aims at the comprehensive and quantitative analysis of metabolites in
634 biological samples. This approach is presently being applied to human embryos (Botros et al.,
635 2008; Seli et al., 2008, 2010) and oocytes (Singh et al., 2007). Because of the chemical
636 diversity of cellular metabolites, no single analytical platform can measure the metabolome in
637 a cell. This is due to the absence of methods to amplify metabolites, the labile nature of many
638 metabolites, and their chemical heterogeneity and complexity. Thus, measuring the
639 metabolome is a considerable analytical challenge. Rapid technological advances in MS and
640 nuclear magnetic resonance (NMR) have enabled the simultaneous detection of a wide range
641 of small molecules. Recently, the development of a screening technology using Raman and
642 near infrared (NIR) spectroscopy were used to detect biomarkers in culture medium with
643 algorithms generated for positive, as compared to negative, IVF outcomes (Scott et al., 2008;
644 Seli et al., 2007). NIR analysis is not typically used for target metabolite identification, but is
645 used for overall spectral profile comparisons. Recent studies using a SET program on days-2
646 and -3 showed higher mean viability scores for embryos that resulted in a pregnancy with fetal
647 heart activity, compared with those without (Seli et al., 2008; Vergouw et al., 2008). They
648 showed that metabolomic profiling of culture media from embryos was independent of
649 morphology. Large-scale, prospective studies are necessary to relate the metabolic profile of
650 medium to the developmental potential of human oocytes and embryos. In addition, the ideal
651 metabolomic profile should be confirmed by several independent platforms.

652 **Conclusion**

653 This review highlights the recent advances in elucidating the molecular complexity in early
654 embryonic development. The knowledge of the global pattern of gene expression is important

655 for understanding critical regulatory pathways involved in the crucial steps of early
656 embryonic development. Certain gene expression profiles help to unravel the mystery of these
657 developmental stages. Further understanding of the biological role of these genes may expand
658 our knowledge of the oocyte maturation, fertilization, chromatin remodeling, totipotency,
659 pluripotency, and early morphogenesis steps. The practical implications of compiling gene
660 expression information on human oocytes and embryos would be enormous since it could
661 potentially help us to understand and solve problems related to infertility.

662 In recent years, the emergence of new technologies ‘Omics’ such as microarrays have already
663 started to be used in the IVF/ICSI program. The use of microarray technology in the analysis
664 of early embryonic development poses specific challenges associated with the picogram levels
665 of mRNA in a single cell/embryo. Another challenge for applying microarrays to early
666 embryonic development is the high degree of expression plasticity seen in early stage
667 embryos.

668 The ideal DNA microarrays would potentially give rise to diagnostics for assessing human
669 embryo quality in IVF program. The application of transcriptomic, proteomic and
670 metabolomic approaches have greatly broadened our understanding of early human
671 development. The challenge now is to correlate gene/protein/metabolite function and
672 regulation to specific events in early embryonic development. These tools may ultimately lead
673 to non-invasive tests for oocyte or embryo quality revealing previously hidden information
674 concerning both oocyte and embryonic developmental competence. Once fully validated,
675 these new approaches are expected to improve oocyte and embryo selection, leading to
676 increased implantation rates and higher success in elective SET. A multitude of advantages
677 may ensue from the use of a rapid, non-invasive and reliable technology as an adjunct to
678 embryo assessment for clinical applications. An improved understanding of embryo viability

679 should help to identify the healthy embryos that will most likely result in pregnancy and allow
680 more accurate decisions in selecting the best embryo for the SET program.

681

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689 **Figures**

690 **Figure 1:** Upregulation of proteasome genes during oocyte maturation. Histograms show
691 signal values of six proteasome genes (*PSMA2*, *PSMA5*, *PSMD2*, *PSMD9*, *PSMD11* and
692 *PSMG1*) in each stage of oocyte maturation. Gene expression is measured by pan-genomic
693 HG-U133 Plus 2.0 Affymetrix oligonucleotides microarrays, and the signal intensity for each
694 gene is shown on the Y-axis as arbitrary units determined by the GCOS 1.2 software
695 (Affymetrix). GV; germinal vesicle; MI, metaphase I; MII, metaphase II.

696 **Figure 2:** Principal component analysis (PCA) and hierarchical clustering of all samples from
697 different developmental stages. (A) PCA distributes samples into a three-dimensional space
698 based on the variances in gene expression; samples that have similar trends in their gene
699 expression profiles will cluster together in the PCA plot. This analysis, using GeneSpring®
700 software, resulted in a clear segregation of the fifteen replicated samples into three clusters,
701 corresponding to human mature oocytes, human embryos, and hESCs. Each colored point
702 represents a sample, characterized by the gene expression of all probe sets (54,675) on the
703 Affymetrix HG U133 Plus 2 array. The first, second, and third principal components are
704 displayed on the X-, Y- and Z-axes, respectively. (B) The expression signatures of samples
705 were visualized by hierarchical clustering on the 10,000 probe sets with the highest variation
706 coefficient. The colors indicate the relative expression levels of each gene, with red indicating
707 an expression above median, green indicating expression under median and black
708 representing median expression. Cluster (a) was a group of genes differentially overexpressed
709 in oocytes, including *DAZL*, *ZP1*, *ZP2*, *ZP3*, *ZP4*, *AURKA* and *HOXA7*. Cluster (b) featured
710 genes that were detected in both oocyte and embryo samples, such as the *NALP4*, *DPPA5*,
711 and *ACTL8*. Cluster (c) grouped genes that were detected in both embryo and hESC samples,
712 such as *NANOG*, *SALL4*, and *ANXA2*. Cluster (d) included genes differentially overexpressed

713 in hESCs, such as *POU5F1*, *CD24*, *SOX2*, *FZD7* and *ZIC3*. Cluster (e) assembled genes
714 overexpressed in day-3 embryos, such as *CCNA1*, *H3F3B* and *FGF9*. (C) The dendrogram
715 shows that all replicates are clustered by their appropriate stage. All the replicate samples of
716 the hESC group self-cluster into one branch. Both oocyte and embryo samples self-cluster into
717 another branch (dotted box) that further divides into two major sub-branches and into which
718 all the replicates from oocytes and embryos self-cluster.

719 **Figure 3:** Expression of selected genes in human oocytes, and embryos on day-3 and hESCs.
720 Figure show signal values of 18 genes that are gradually increased (A) or decreased (B)
721 during early embryonic development. (C) Characterization of *TGF- β* signalling pathway
722 during early embryonic development. Genes shown in red are upregulated in human oocytes
723 or in embryos on day-3 or in hESCs. Examples of genes overexpressed (red) in each stage are
724 indicated in boxes.

725 **Figure 4:** Chromosomal distribution of genes encoding zinc finger domain proteins were
726 expressed significantly differently in human mature oocytes (light violet) as compared to
727 hESCs (light pink) as well as to those commonly expressed in mature human oocytes and
728 hESCs (light yellow). The selected genes were retrieved in lists previously published in
729 (Assou et al., 2009).

730 **Figure 5:** Different direct or indirect approaches suggested for oocyte or embryo selection
731 (transcriptomic, proteomic and metabolomic approaches).

732

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1133 Tables

1134 Table I: Microarray studies of oocytes and embryos

human oocytes and embryos				
Techniques	Samples	Number of identified genes	Targets	Study
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	1,361 transcripts expressed in oocytes	Study oocyte transcriptomes	(Bermudez et al., 2004)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	1,514 overexpressed in oocytes compared with cumulus cells	Understanding of the mechanisms regulating oocyte maturation	(Assou et al., 2006)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	5,331 transcripts enriched in metaphase II oocytes relative to somatic cells	Comprehension of genes expressed in <i>in vivo</i> matured oocytes	(Kocabas et al., 2006)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	10,183 genes were expressed in germinal vesicle	Study of global gene expression in human oocytes at the later stages of folliculogenesis (germinal vesicle stage)	(Zhang et al., 2007)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	Of the 8123 transcripts expressed in the oocytes, 374 genes showed significant differences in mRNA abundance in PCOS oocytes	Understanding of PCOS	(Wood et al., 2007)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	---	Identify new potential regulators and marker genes which are involved in oocyte maturation.	(Gasca et al., 2007)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	283 genes found in the case	Identify molecular abnormalities in	(Gasca et al.,

array (Affymetix)		report sample	metaphase II (MII) oocytes	2008)
Whole Genome Bioarrays printed with 54,840 discovery probes representing 18,055 human genes and an additional 29,378 human expressed sequence tags (EST)	Oocytes	2,000 genes were identified as expressed at more than 2-fold higher levels in oocytes matured <i>in vitro</i> than those matured <i>in vivo</i>	Analysis of gene expression profile of oocytes following <i>in vivo</i> or <i>in vitro</i> maturation	(Jones et al., 2008b)
Applied Biosystems Human Genome Survey Microarray (32,878 60-mer oligonucleotide)	Oocytes	Germinal vesicle, <i>in vivo</i> -MII, and IVM-MII oocytes expressed 12,219; 9,735; and 8,510 genes, respectively	Characterized the patterns of gene expression in germinal vesicle stage and meiosis II oocytes matured <i>in vitro</i> or <i>in vivo</i>	(Wells and Patrizio, 2008)
HG-U133 Plus 2.0 array (Affymetix)	Oocytes	342 genes showed a significantly different expression level between the two age groups (women aged ,36 years (younger) and women aged 37–39 years (older))	Investigate the effect of age on gene expression profile in mature oocytes	Grondahl et al., 2010)
Two cDNA microarrays, each containing about 20,000 targets (representing in total ~29,778 independent genes according to Unigene Build 155).	Oocytes and embryos	1,896 significant changes in expression following fertilization through day 3 of development	Global analysis of the pre-implantation embryo transcriptome	(Dobson et al., 2004)

cDNA microarrays containing 9,600 cDNA spots	Oocytes and embryos	184; 29; and 65 genes were overexpressed in oocytes, 4- and 8-cell embryos, respectively	Identify differential expression profiles of genes in single oocytes, 4- and 8-cell preimplantation embryos	(Li et al., 2006)
Genome Survey Microarrays V2.0 (Applied Biosystems)	Oocytes and embryos	107 DNA repair genes were detected in oocytes	Identify the DNA repair pathways that may be active pre- and post-embryonic genome activation by investigating mRNA in human <i>in vitro</i> matured oocytes and blastocysts	(Jaroudi et al., 2009)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes and embryos	5,477 transcripts differentially expressed into transition from mature oocyte (MII) to 2-day embryo and 2,989 transcripts differentially expressed into transition from 2- to 3-day embryo	Study of global gene expression in human pre-implantation development	(Zhang et al., 2009)
Mouse oocytes and embryos				
NIA 60-mer oligo microarray (Agilent)	Oocytes	530 genes showed statistically significant expression changes between young and old oocytes	Comparison of transcriptional profile of oocytes obtained from aged mice with oocytes from young mice	(Hamatani et al., 2004b)
Genome Array MOE430 A and B chips (Affymetrix)	Oocytes	---	Identification of gene expression profiles of oocytes during the primordial follicle stage to the large antral follicle stage	(Pan et al., 2005)
7.4 K GenePlore TwinChip	Oocytes	214 genes that are upregulated between primordial and primary follicles. 396 upregulated genes between primary and secondary follicles	Study of global gene expression profiles of early folliculogenesis in primordial, primary, and secondary follicles	(Yoon et al., 2006)
Affymetrix 430 v2.0	Oocytes	3,002 genes changed	Investigate the relative changes of	(Su et al.,

GeneChips		significantly during GV-to-MII transition	transcripts present in GV-stage versus MII-stage oocytes	2007)
Applied Biosystems Mouse Genome survey Array (32,996 60-mer oligonucleotide)	Oocytes	1,682 genes with more highly expression in GV-stage oocytes than in MII-stage oocytes. 1,936 genes were more highly expressed in MII-stage oocytes	Comparison of gene expression profiles of germinal vesicle (GV) and metaphase II (MII)-stage oocytes	(Cui et al., 2007)
NIA 15k cDNA microarray	Embryos	428 genes upregulated and 748 downregulated in blastocyst compared to morula	Identification of genes expressed differentially between morula and blastocyst	(Tanaka et al., 2004)
NIA 60-mer oligo microarray (Agilent)	Embryos	---	Global gene expression profiling of all stages of pre-implantation embryos	(Hamatani et al., 2004a)
Affymetrix 25-mer DNA	Embryos	---	Global gene expression analysis has also been applied to examine the effects of oxygen atmosphere on mouse pre-implantation embryo gene expression patterns	(Rinaudo, P. F. et al., 2006)
GenePlorer TwinChip Mouse 7.4 K elements (containing 7,410 different genes)	Embryos	248 genes were differentially expressed between the two-cell embryos and two-cell block embryos	Analysis of differences in gene expression between two-cell and two-cell block embryos	(Jeong et al., 2006)
Affymetrix 430 2.0 GeneChips	Embryos	1,912 was statistically different in the IVF cohort when compared with the <i>in vivo</i> control embryos	Global gene expression analysis has also been applied to contrast gene expression between cultured and <i>in vivo</i> derived mouse embryos	(Giritharan et al., 2007)
Affymetrix 25-mer DNA	Oocytes and embryos	4,000 genes changed in expression over 5-fold	Analysis of gene activity during pre-implantation development	(Wang et al., 2004)

MG_U74Av2, MOE430A, and MOE430B GeneChips (Affymetrix)	Oocytes and embryos	9,414 genes differentially expressed in oocytes	Analysis of global patterns of gene expression during pre-implantation development	(Zeng et al., 2004)
NIA 60-mer oligo microarray (Agilent)	Oocytes and embryos	---	Identification of a large number of genes and multiple signaling pathways involved at each developmental stage of pre- implantation embryos	(Hamatani et al., 2006)

1135 Table II: Imprinted genes abundantly expressed in human oocytes, embryos on day-3 and
1136 hESCs

Oocytes		Embryos (Day-3)		hESCs	
paternally	Maternally	paternally	Maternally	paternally	Maternally
SNRPN, PLAGL1	TP73L, GRB10, H19, KCNQ1, CDKN1C, OSBPL5, UBE3, PRIM2A, MEG3	INPP5F, KCNQ1OT1, ZIM2, PEG3, PON1	TP73L, GRB10, CPA4, KCNK9, PHLDA2, OSBPL5, UBE3A, ZNF597	PEG10, SGCE, NDN, SNRPN, MAEGL2, DLK1, PEG3, SANG	H19, GRB10, PPP1R9A, CDKN1C, MEG3, SLC22A18

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1138 Table III: Potential follicular cells biomarkers correlated to oocyte quality, embryo
1139 competence or pregnancy outcome. CCs: cumulus cells, GCs: granulosa cells

Biomarkers	Name	Function	Samples (individual or pooled)	Approches	Outcome	Reference
PTX3	Pentraxin-3	involved in inflammatory reaction processes and acts to stabilize the expanded CCs	Pooled CCs from eggs	Microarrays (4 chips)	Positively correlated with oocyte competence	(Zhang, X. et al., 2005)

PCK1, BCL2L11, NFIB	Phosphoenolpyruvate carboxykinase 1, BCL2-like 11 (apoptosis facilitator), Nuclear factor I/B	PCK1: associated with energy production BCL2L11: involved in triggering cell death in response to abnormalities NFIB: regulates some of the earliest processes in embryonic development	CCs from individual eggs	Microarrays (50 chips)	Predict embryo and pregnancy outcomes	(Assou et al., 2008)
CYP19A1, SERPINE2, CDC42, FDX1	Cytochrome P450, family 19, subfamily A, polypeptide 1, Serpin peptidase inhibitor clade E member 2, Cell division cycle 42, Ferredoxin 1	CYP19A1: metabolizes androgen into estradiol-17 β SERPINE2: involved in apoptosis and chromatin condensation CDC42: involved in apoptosis, transcriptional activation, cell proliferation and cell polarity FDX1 and HSD3B1: responsible for progesterone synthesis	Mural GCs and CCs (pooled)	Microarrays (4 chips)	Positively associated with pregnancy	(Hamel et al., 2008)

CCND2, CXCR4, GPX3, HSPB1, DVL3, DHCR7, CTNND1, TRIM28	Cyclin D2, Chemokine (C-X- C motif) receptor 4, Glutathione peroxidase 3, Heat shock protein 1, Dishevelled, dsh homolog 3 7- dehydrocholester- ol reductase, Catenin, delta 1, Tripartite motif- containing 28	CCND2: plays an role in proliferation of cells (cell cycle regulator) CXCR4 and GPX3: have a role in relieve hypoxic stress HSPB1: act as co- repressor of estrogen signaling DVL3: plays a role in angiogenesis DHCR7: involved in progesterone and estrogen synthesis CTNND1: has a role in adhesion TRIM28: has a role in DNA repair	CCs from individual eggs	Microarrays (16 chips)	Negatively associated with oocyte competence	(van Montfoort et al., 2008)
HAS2, GREM1	Hyaluronan synthase 2, Gremlin 1	HAS2: involved in extracellular matrix (ECM) formation GREM1: antagonist of BMP and TGF- β	CCs from individual eggs	RT-PCR	Positively associated with oocyte developmental competence	(Cillo et al., 2007)

STAR, AREG, Cx43, PTGS2, SCD1 and SCD5	Steroidogenic acute regulatory protein, Amphiregulin, Connexin 43, Prostaglandin- endoperoxide synthase 2, Stearoyl-CoA desaturase 1 and 5	STAR: regulated the cholesterol transport into the inner mitochondrial AREG: act as mediators of LH Cx43: permit the transfer of metabolites for growth and development and maintenance of meiotic arrest of the oocyte PTGS2: involved in inflammation and mitogenesis SCD: involved in biosynthesis of monounsaturated fatty acids from saturated acids	CCs from individual eggs	RT-PCR	Negatively associated with oocyte competence	(Feuerstein et al., 2007)
HAS2, PTGS2, GREM1	Hyaluronan synthase 2, Prostaglandin- endoperoxide synthase 2, Gremlin 1		CCs from individual eggs	Quantitative RT-PCR	Positively associated with Oocyte competence and embryo development	(McKenzie et al., 2004)
BDNF, GREM1	Brain-derived neurotrophic factor, Gremlin 1	BDNF: neurotrophic factor playing a role in regulation of stress response	CCs from individual eggs	Quantitative RT-PCR	negative and positive predictors of embryo quality respectively	Anderson et al., 2009)
PGK1, RGS2 and RGS3, CDC42	Phosphoglycerate kinase 1, Regulator of G- protein signaling 2 and 3, Cell division cycle 42	PGK1: involved in glycolysis RGS: hydrolyzed GTP to GDP	Mural GCs and CCs (individual)	Quantitative RT-PCR	Associated with pregnancy	Hamel et al., 2010)

VCAN, RPS6KA2, ALCAM, GREM1	Versican, Ribosomal protein S6 kinase polypeptide 2, Activated leukocyte cell adhesion molecule, Gremlin 1	VCAN: plays a central role in tissue morphogenesis and maintenance RPSK6KA2: involved in the EGF signaling cascade ALCAM: involved in immune response	Pooled CCs from eggs	Quantitative RT-PCR	Correlated with oocyte maturity, low fragmentation and embryo development	Adriaenssen s et al., 2010)
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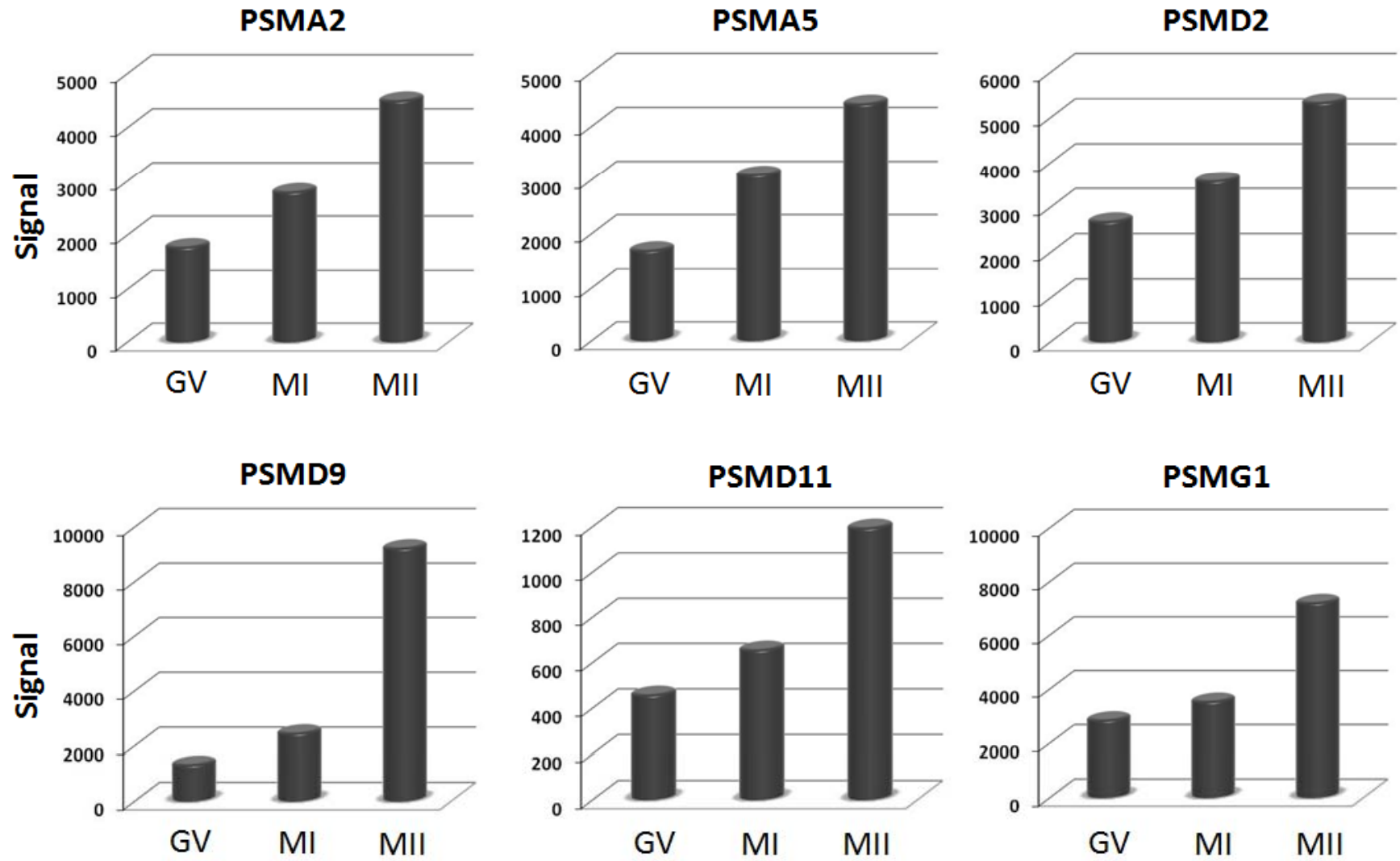
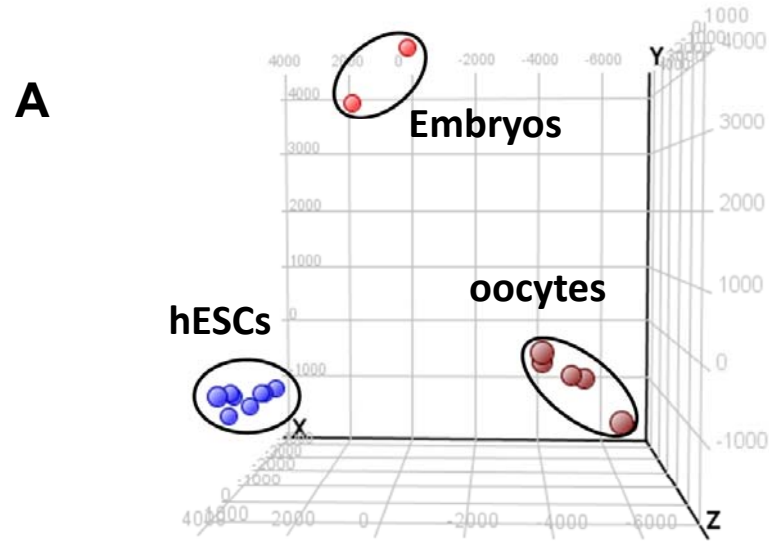


Figure 1

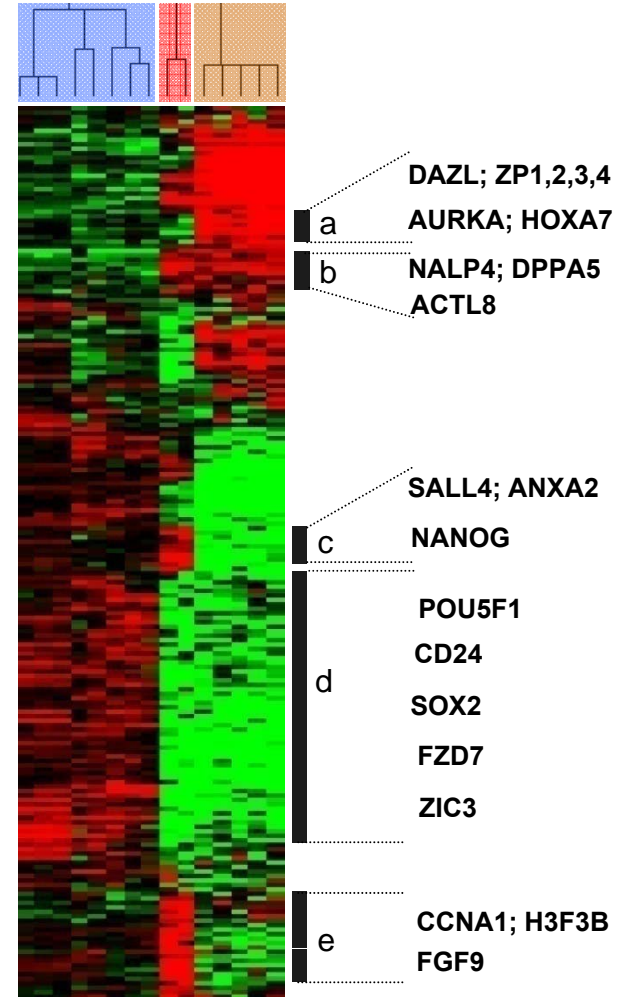


B

hESCs

Embryos (D3)

Oocytes



C

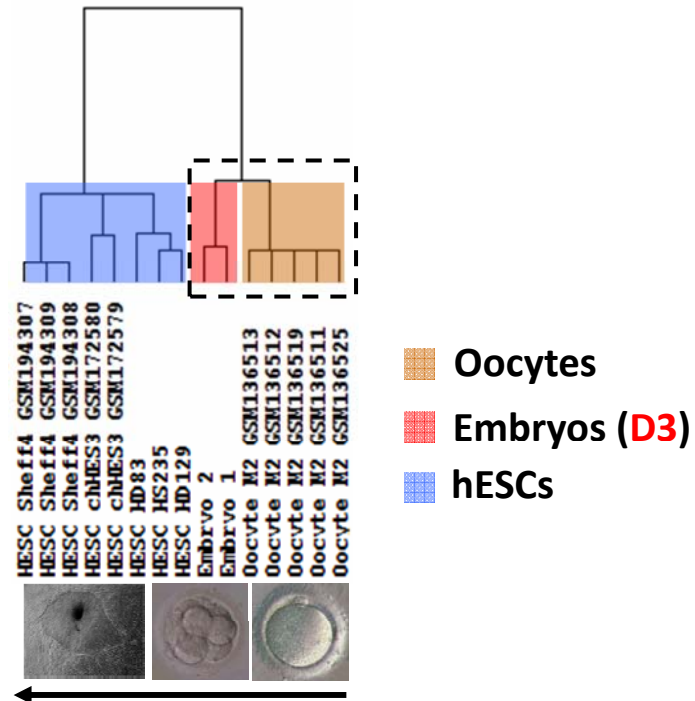
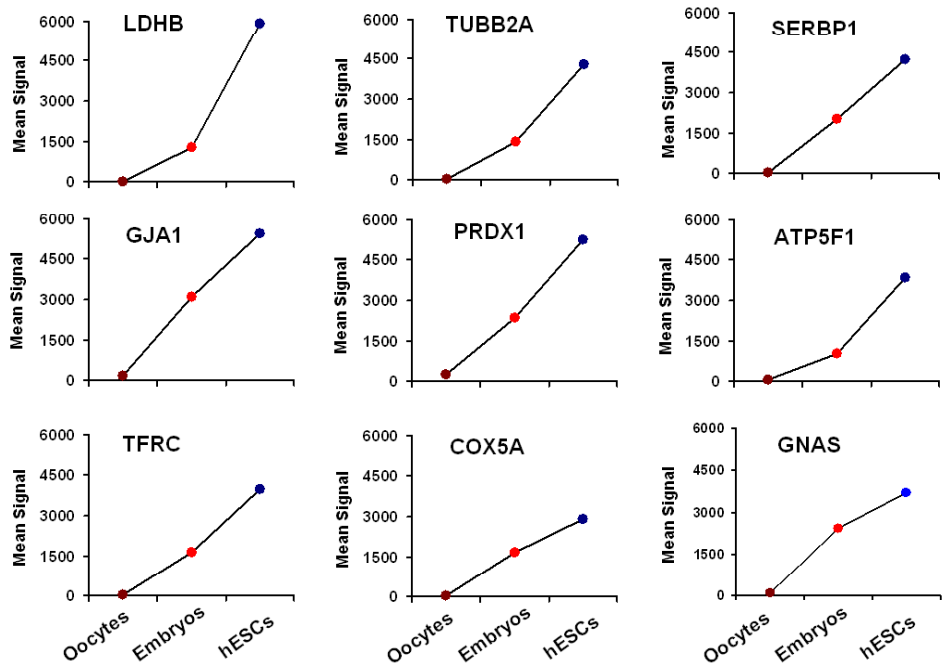
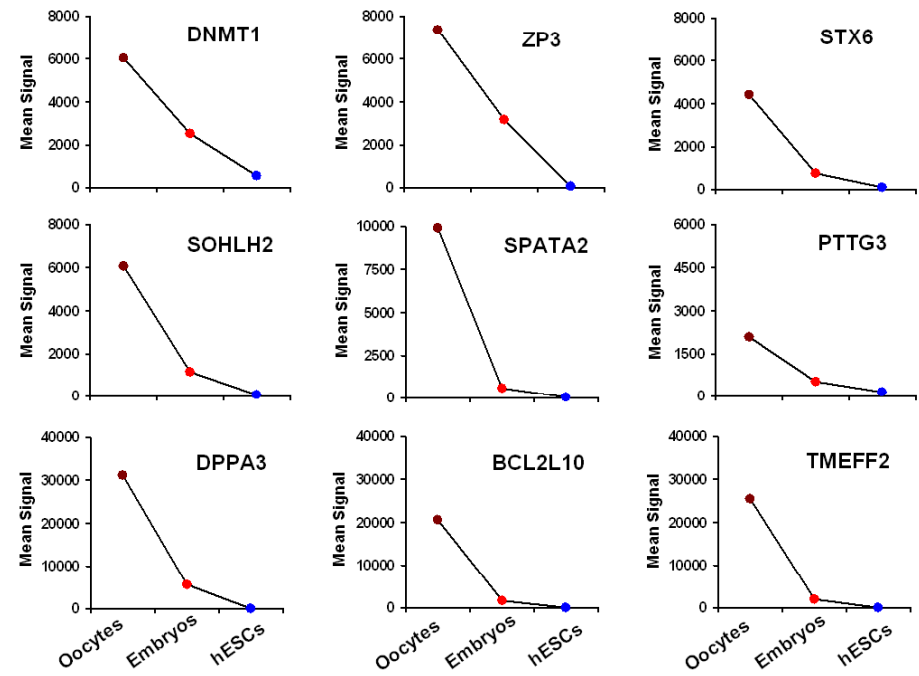


Figure 2

A**B****Figure 3A-B**

C

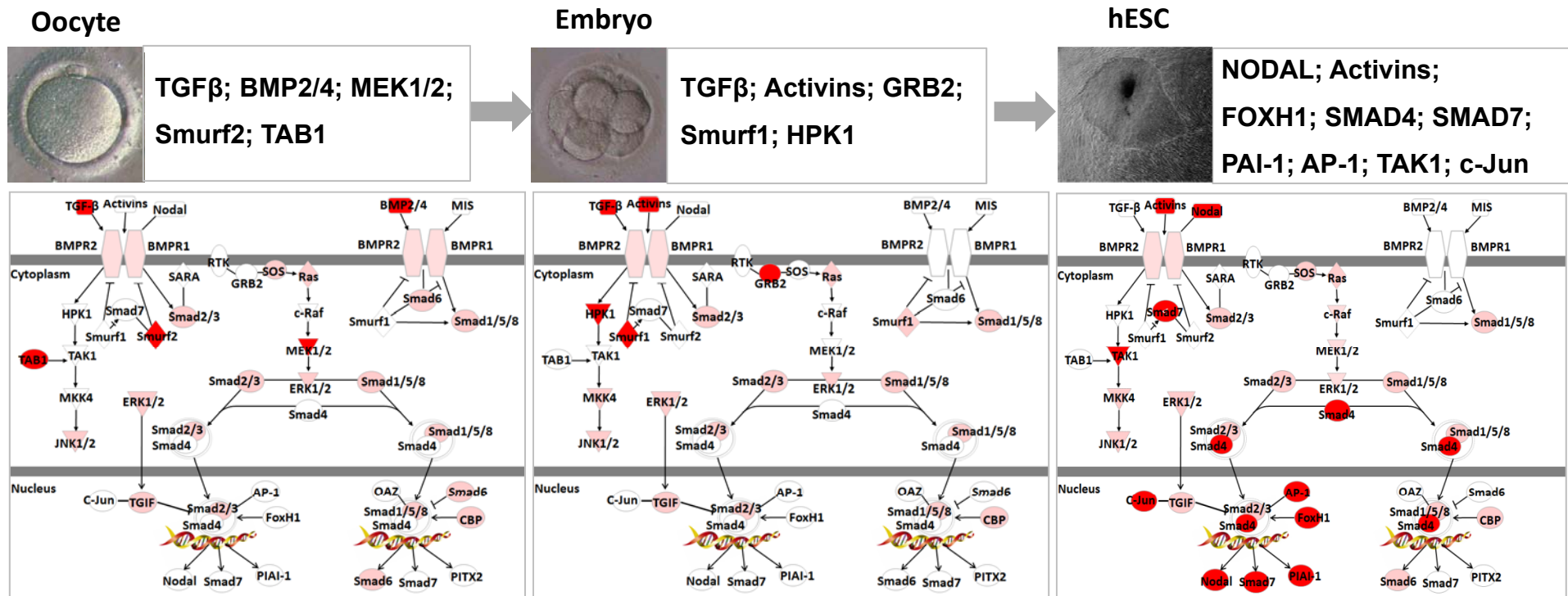


Figure 3C

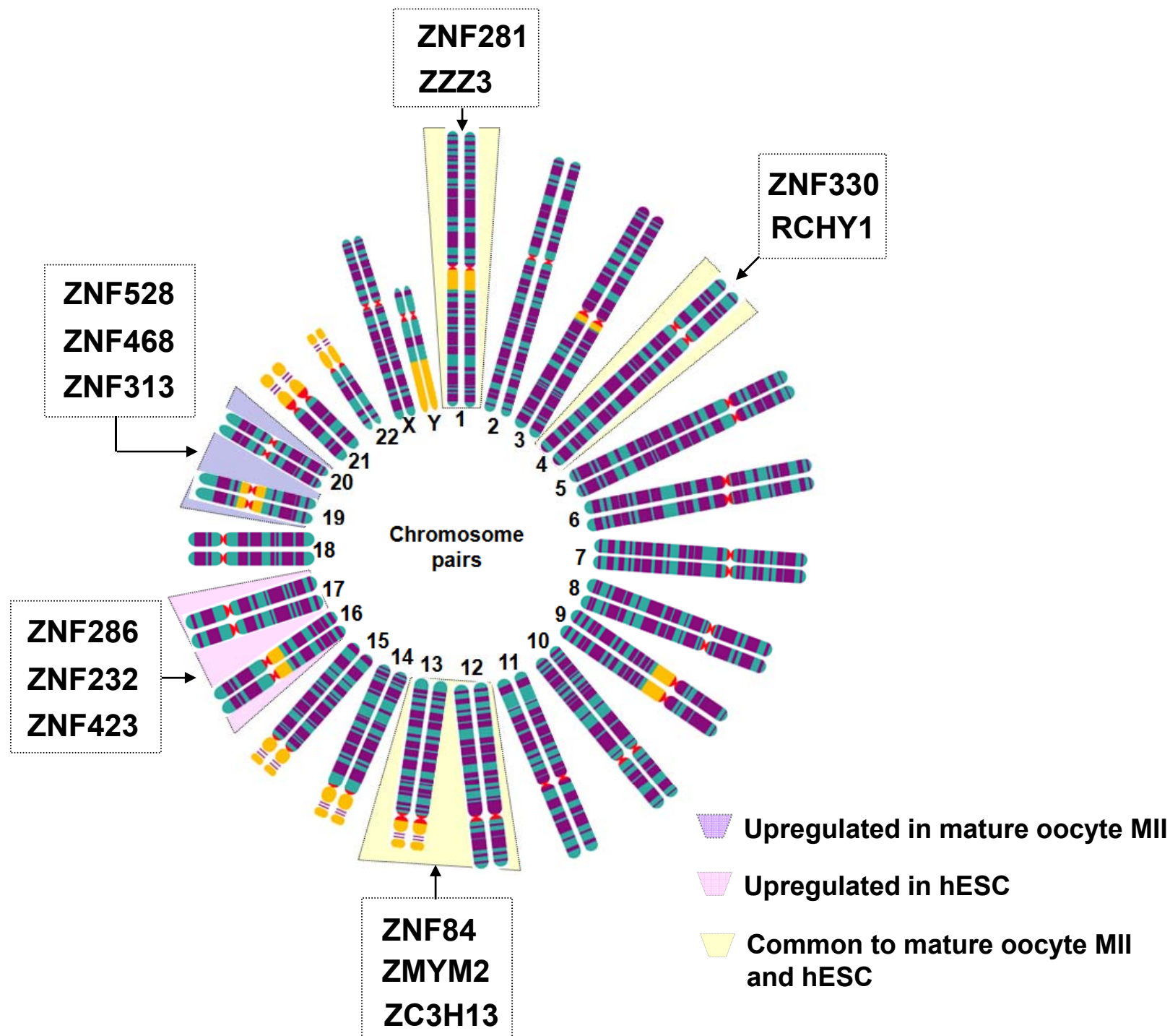


Figure 4

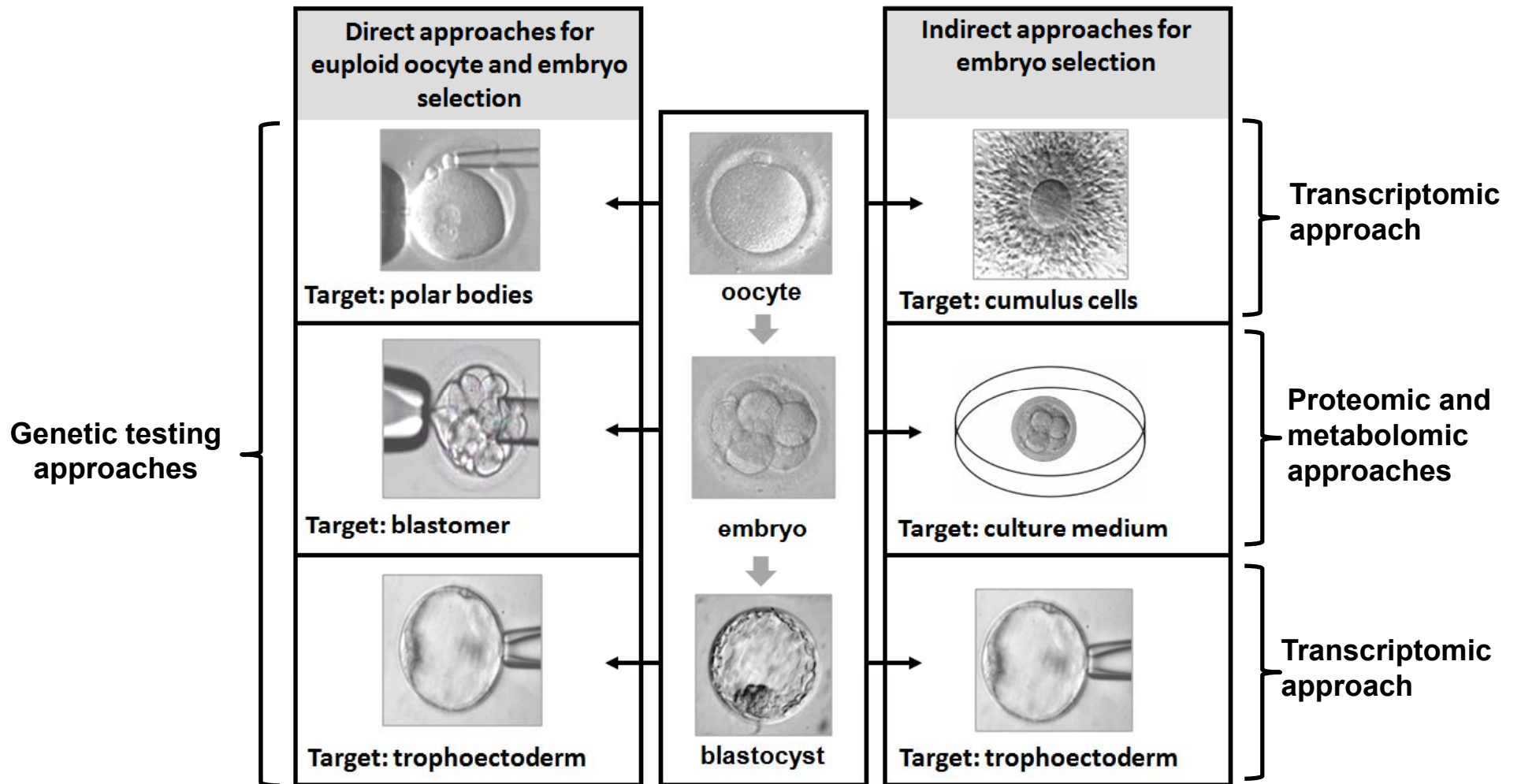


Figure 5