Focus on Mammalian Embryogenomics

Preimplantation embryo programming: transcription, epigenetics, and culture environmentVeronique Duranthon, Andrew J Watson¹ and Patrick Lonergan²*UMR Biologie du développement et de la Reproduction, INRA, 78352 Jouy en Josas Cedex, France,*¹*Departments of Obstetrics and Gynaecology, Physiology and Pharmacology, The University of Western Ontario and Children's Health Research Institute-Victoria Research Laboratories, London, Ontario, N6A 4G5 Canada and*²*School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland**Correspondence should be addressed to P Lonergan; Email: pat.lonergan@ucd.ie***Abstract**

Preimplantation development directs the formation of an implantation- or attachment-competent embryo so that metabolic interactions with the uterus can occur, pregnancy can be initiated, and fetal development can be sustained. The preimplantation embryo exhibits a form of autonomous development fueled by products provided by the oocyte and also from activation of the embryo's genome. Despite this autonomy, the preimplantation embryo is highly influenced by factors in the external environment and in extreme situations, such as those presented by embryo culture or nuclear transfer, the ability of the embryo to adapt to the changing environmental conditions or chromatin to become reprogrammed can exceed its own adaptive capacity, resulting in aberrant embryonic development. Nuclear transfer or embryo culture-induced influences not only affect implantation and establishment of pregnancy but also can extend to fetal and postnatal development and affect susceptibility to disease in later life. It is therefore critical to define the basic program controlling preimplantation development, and also to utilize nuclear transfer and embryo culture models so that we may design healthier environments for preimplantation embryos to thrive in and also minimize the potential for negative consequences during pregnancy and post-gestational life. In addition, it is necessary to couple gene expression analysis with the investigation of gene function so that effects on gene expression can be fully understood. The purpose of this short review is to highlight our knowledge of the mechanisms controlling preimplantation development and report how those mechanisms may be influenced by nuclear transfer and embryo culture.

*Reproduction (2008) 135 141–150***Introduction**

Preimplantation development directs the formation of an implantation- or attachment-competent embryo so that metabolic interactions with the uterus can occur, pregnancy can be initiated, and fetal development can be sustained (Watson 1992, Watson & Barcroft 2001). The preimplantation embryo exhibits a form of autonomous development fueled by products provided by the oocyte and also from activation of the embryo's genome

(Schultz 2005). Despite this autonomy, the preimplantation embryo is highly influenced by factors in the external environment and in extreme situations, such as those presented by embryo culture or nuclear transfer, the ability of the embryo to adapt to changing environmental conditions or chromatin to become reprogrammed can exceed its own adaptive capacity, resulting in aberrant embryonic development (Niemann & Wrenzycki 2000, Schultz & Williams 2002, Gao *et al.* 2003). Nuclear transfer or culture-induced influences not only affect implantation and establishment of pregnancy but also can extend to fetal and postnatal development and affect susceptibility to disease in later life (Barker 2003, Ecker *et al.* 2004, Yang *et al.* 2007). It is therefore critical to define the basic program controlling preimplantation development, and also to utilize nuclear transfer and embryo culture models so that we may design healthier environments for preimplantation embryos to thrive in and also minimize the potential for

This article was presented at the 2nd International Meeting on Mammalian Embryogenomics, 17–20 October 2007. The Co-operative Research Programme: Biological Resource Management for Sustainable Agricultural Systems of The Organisation for Economic Co-operation and Development (OECD) has supported the publication of this article. The meeting was also sponsored by Le conseil Régional Ile-de-France, the Institut National de la Recherche Agronomique (INRA), Cogenics-Genome Express, Eurogentec, Proteigene, Sigma-Aldrich France and Diagenode sa.

negative consequences during pregnancy and post-gestational life. In addition, it is necessary to couple gene expression analysis with the investigation of gene function so that effects on gene expression can be fully understood. The purpose of this short review is to highlight our knowledge of the mechanisms controlling preimplantation development and report how those mechanisms may be influenced by nuclear transfer and embryo culture.

Preimplantation development: blastocyst formation and embryonic genome activation (EGA)

Preimplantation development is characterized by a series of cleavage divisions that subdivide the oocyte into smaller and smaller compartments, activation of the embryonic genome, compaction, cavitation (blastocyst formation), and finally zona hatching and implantation to the uterine wall (Watson 1992, Watson & Barcroft 2001). The principal achievement of preimplantation development is the formation of a fluid-filled structure called the blastocyst which is composed of an outer epithelial trophectoderm (TE), encircling a small group of cells called the inner cell mass (ICM) and a large fluid-filled cavity (Watson & Barcroft 2001). The TE, the first differentiated cell type of development, is a specialized tissue that initiates implantation or attachment and is the progenitor of the placenta. The ICM is the pluripotent progenitor of the embryo proper (Rossant 2004, Yamanaka *et al.* 2006). The program of preimplantation development is therefore directed at the formation of the TE and the specification of these distinct cell lineages. This process begins with the onset of compaction, which also establishes cell polarity in the outer cells of the early embryo (Rossant 2004, Yamanaka *et al.* 2006). Compaction follows a major event in establishing the embryo's gene expression program called EGA. EGA has long been thought of as a global and promiscuous activation of genes whose regulated repression was then necessary to establish the preimplantation developmental program. Thanks to large-scale transcriptomic analyses, it now appears that a highly regulated gene expression program is initiated as soon as embryonic genome expression begins (Hamatani *et al.* 2004, Wang *et al.* 2004, Zeng *et al.* 2004). Because early perturbations of this 'embryonic program' have long-term significant effects on reproductive performance, we will discuss recent data concerning the regulation of EGA and its importance for long-term development.

Gene reprogramming at embryonic genome activation: passage through totipotency

Reprogramming reflects the ability of a nucleus to modify its gene expression pattern when placed in a new environment. Fertilization brings together the haploid genomes of two highly differentiated cells, the

gametes, into the oocyte cytoplasm. One of the first functions of the fertilized embryo is to reprogram the newly formed embryonic genome to a totipotent state. Totipotency is a rare and transient property, characterized by the ability of an individual embryonic cell to give rise to a whole, normal, and fertile individual. It is displayed only by fertilized eggs and early embryos in mammals, spans over few cell cycles, and is already lost at the blastocyst stage. Such reprogramming relies on extensive epigenetic modifications of the genome that coordinate nucleo-cytoplasmic interactions. During fertilization gametic genomes are initially transcriptionally silent; gene reprogramming is thus concomitant with embryonic genome transcriptional activation. Over the last decade, the interest in early embryonic genome reprogramming has significantly increased with the awareness that mammalian oocyte cytoplasm is able to reprogram not only gametic genomes but also somatic cell genomes, although with a lower efficiency. Both genetic alterations of the oocyte cytoplasmic content and nuclear transfer experiments, primarily applied to mice and cows, have provided new approaches to understand this unique property of the oocyte. While they are compatible with high rates of preimplantation development, these manipulations induce long-term effects; only a small percentage of the somatic cell nuclear transfer embryos develop to birth, for example, and depletion of the oocyte cytoplasm in maternal Ezh2, involved in epigenetic remodeling of the embryonic genome provokes reduction of birth weight in mice (Erhardt *et al.* 2003). These long-term developmental effects are attributed to faults in genome reprogramming pointing to the crucial role of early epigenetic events for long-term development.

Early development relies on maternal transcripts and proteins that are progressively degraded while embryonic genome transcription progressively increases. This maternal to embryonic transition (MET) provides the embryo with the opportunity to restrict the maternally encoded genetic program and to set up an embryonic program of gene expression (Schultz 2005). It has been now extensively characterized by large-scale transcriptomic analyses in mice, where three groups of genes have been identified: genes encoding oocyte-specific transcripts that are definitively eliminated during MET, genes encoding embryonic transcripts whose expression begins at EGA, and genes whose transcripts are first inherited from the oocyte then synthesized from the embryonic genome (representing only about 40% of the genes; Hamatani *et al.* 2004). The first two categories of genes are responsible for the global change in the program of gene expression during the period of EGA. In the case of fertilization, reprogramming at EGA thus corresponds to both a change in the genetic origin of the transcripts (maternal or embryonic) and a change in the program of gene expression; it occurs without any significant change in embryo morphology. Functional

changes are in fact more progressive in the embryo; both the rates of maternal transcript degradation and the stability of the maternally encoded proteins vary so that some maternal information may still contribute to embryonic development after EGA.

EGA in the mouse embryo

In the mouse embryo, EGA occurs at the two-cell stage (although transcription is first detected in the male pronucleus prior to pronuclear fusion), that is, early during the cleavage period and long before the first differentiation at the blastocyst stage (Schultz 2005). Among genes transcribed at EGA, genes involved in basic cellular function, ion transport, ribonucleotide metabolism, and also ribosome biogenesis, protein synthesis, RNA metabolism, and transcription are overrepresented (Hamatani *et al.* 2004, 2006, Zeng & Schultz 2005). A second transition in gene expression has been reported between the four- and eight-cell stages in the mouse, corresponding to the activation of genes which may be key regulators of TE differentiation (Hamatani *et al.* 2004). A subgroup of genes are transiently expressed at each cleavage stage (Hamatani *et al.* 2004, Zeng *et al.* 2004). In particular, expression of long terminal repeat retrotransposons is reported to occur at EGA (Evsikov *et al.* 2004), and specific transposable elements act at that stage as alternative promoters and first exons for a subset of host genes transcribed as chimeric transcripts (Peaston *et al.* 2004). The expression of such repetitive elements may be regulated by RNA interference mechanisms (Svoboda *et al.* 2004).

In mice, EGA is concomitant with extensive epigenetic remodeling of the parental genomes into the newly formed embryo (Morgan *et al.* 2005). Epigenesis involves all the factors modifying gene expression in a cell-division heritable way, without any alteration of DNA sequence (Holliday 1994). It is responsible for the acquisition of different gene expression programs in different cells during the development of multicellular organisms. Epigenetic marks involve posttranslational modifications (methylation, acetylation, phosphorylation, and ubiquitination) of nucleosomal histones, DNA methylation, and non-histone proteins that bind to chromatin. Briefly, transcriptionally inactive heterochromatin is characterized by deacetylated histones, methylation of histone H3 lysine 9, and DNA methylation, whereas acetylation of H3 and H4 histones, methylation of histone H3 lysine 4, and low level of DNA methylation are associated with active euchromatin regions. These modifications of nucleosomal histones alter the higher-order chromatin structure to render the DNA accessible to the regulatory and transcriptional machinery. These different levels of epigenetic marks tightly interact: proteins displaying high affinity for methylated DNA, for example, associate with histone deacetylase and methyltransferase.

In the mouse, at fertilization, the metaphase 2 arrested maternal genome is packaged with histones already displaying various modifications (acetylation or methylation) in different regions of the genome and exhibits a relatively high level of DNA methylation. In the paternal genome, protamines are first replaced with histones which are more acetylated than those inherited by the maternal genome but evidence of early histone methylation appears soon after this incorporation. An active demethylation of the paternal DNA then occurs before DNA replication and only some specific regions of heterochromatin around centromeres, intercisternal A-particle retrotransposons, and paternally methylated imprinted genes escape it (Morgan *et al.* 2005). Both parental genomes are thus epigenetically asymmetric, which is likely responsible for the precocious transcriptional activation of the paternal genome observed in the mouse. During the first cleavages, a passive DNA demethylation of the whole embryonic genome progressively occurs due to maternally inherited Dnmt1 exclusion from the nuclei, resulting in a low methylation level at the morula stage (Morgan *et al.* 2005). Whether histone modifications are also reprogrammed during this passive phase of DNA demethylation remains unclear. Later on, differential *de novo* remethylation occurs in the ICM due to preferential localization of Dnmt3b in these cells rather than TE cells.

Is the mouse embryo a representative model for EGA reprogramming?

Neither the abrupt kinetics of EGA occurring at the two-cell stage nor the extent of associated epigenetic remodeling events are shared by non-murine embryos. In all non-murine embryos, EGA spans over several cell cycles with a weak transcriptional activity from the end of the 1-cell stage, but a major transcriptional activation at the 4- (pig, human) or 8- to 16-cell stage (sheep, cow, rabbit; Telford *et al.* 1990). This implies a longer reliance on maternally inherited information and a shortened delay between EGA and cell differentiation. Whether this affects the nature of genes preferentially expressed at EGA, or the number of transcription waves, remains unknown since very few large-scale analyses of gene reprogramming at EGA have been published in these species (Whitworth *et al.* 2004, Misirlioglu *et al.* 2006). In cattle, early genes that are transcribed include genes involved in transcription regulation, cell adhesion, signal transduction, transporters, and metabolism (Misirlioglu *et al.* 2006). Transient expression of genes at EGA has also been reported in the rabbit (Pacheco-Trigon *et al.* 2002) but large-scale comparisons of early encoded functions in species with different EGA kinetics remain to be done.

In addition, the extent of epigenetic changes associated with EGA varies between species (Beaujean *et al.* 2004). DNA methylation has been mainly investigated.

Active paternal DNA demethylation is less pronounced in cattle than in the mouse, it is undetectable in sheep and rabbits, and a partial asymmetrical demethylation has been reported in only half of human embryos (Fulka *et al.* 2004). Subsequent passive demethylation also differs among species, being reduced in sheep and barely detectable in the rabbit. The differential remethylation of the ICM also varies since both in the rabbit (Shi *et al.* 2004) and in the human (Fulka *et al.* 2004), DNA methylation is higher in the TE than in ICM. Variation in levels of histone deacetylases and histone acetyltransferases throughout bovine embryonic development have been reported (McGraw *et al.* 2003, 2007). McGraw *et al.* (2003, 2007) described the temporal expression profile, during preimplantation embryo development, of 15 key regulators involved in RNA, DNA or histone methylation, chromatin modification or silencing, and transcription regulation; all were present to different degrees in the developmental stages tested, and they can be divided into three different groups depending on their respective mRNA profile. More detailed comparative analysis of specific regions of the genome have yet to be carried out in order to understand the developmental consequences of these epigenetic reprogramming events, their consequences on gene expression reprogramming at EGA and on totipotency reprogramming.

Long-term consequences of reprogramming at EGA: somatic cell nuclear transfer embryos as an experimental model

Although the birth of normal, fertile cloned animals from many species proves that a sufficient reprogramming may be obtained after somatic cell nuclear transfer (SCNT), long-term developmental effects likely related to early reprogramming faults frequently occur (Yang *et al.* 2007). Large variations in full-term developmental potential of various donor cell types are now well described but still not understood (Panarace *et al.* 2007). Molecular studies are aimed at characterizing the extent of reprogramming faults and their functional consequences. Reprogramming here refers to both extinction of genes expressed by the differentiated donor cell, and transcriptional activation of embryonically expressed genes, which includes extensive epigenetic reprogramming. Comparing early SCNT embryos to control embryos revealed their abnormal epigenetic status. In cattle, for example, passive demethylation is delayed and weakened in SCNT embryos; in addition, histone acetylation and methylation patterns are altered (Santos *et al.* 2003, Beaujean *et al.* 2004). Faults in gene expression are also observed at various developmental stages. During the preimplantation period in the mouse, alterations in imprinted gene expression (Mann *et al.* 2004), persistent expression of genes specific to the donor nucleus (Gao *et al.* 2003), and deficient expression of genes involved in pluripotency

maintenance (Boiani *et al.* 2002) are reported. These candidate gene analyses remain, however, unsatisfactory to understand reprogramming faults since results highly depend on the gene, the stage of analysis, the technique used for nuclear transfer, and the donor cell type.

Resorting large-scale transcriptional studies should help to determine general trends of early reprogramming and to identify relevant candidate genes, if any. Most studies have reported that a global reprogramming has already occurred by the blastocyst stage since the SCNT blastocyst gene expression program is closer to that of control blastocysts than to that of the donor cell (Pfister-Genskow *et al.* 2005, Smith *et al.* 2005, Beyhan *et al.* 2007). This reprogramming appears, however, incomplete since some genes are differentially expressed between control and SCNT blastocysts. At that stage, however, the relationship between gene expression profiles and embryo full-term development is probably not direct (Smith *et al.* 2005). Tracking earlier gene reprogramming faults should provide information about initial events. Global reprogramming already occurred in cattle SCNT morulae (Duranthon *et al.* unpublished results) and genes involved in transcription and its regulation are mis-regulated at EGA in mouse cloned embryos, probably leading to further gene expression abnormalities (Vassena *et al.* 2007). Further analyses are required to better integrate such early events and their long-term consequences and these should also take into account interspecific variations.

Preimplantation development: from totipotency to the first differentiative events

Compaction and cell polarity

The first step toward differentiation is to establish intercellular communication. Compaction is signaled by an increase in cell-to-cell contact between embryonic blastomeres. It is driven by the establishment of adherens junctions consisting of E-cadherin and catenin complexes (Fleming *et al.* 2000, Johnson & McConnell 2004). Compaction is initiated at the eight-cell stage in the mouse but the timing varies across species (Telford *et al.* 1990). In all cases, it results in the formation of a morula by which the 16-cell stage in the mouse creates a topology that forms outer and inner cells that are completely surrounded by the outer cells (Fleming *et al.* 2000, Johnson & McConnell 2004). These cell types are the progenitors of the TE and ICM.

Trophectoderm differentiation

The predominant models of TE differentiation include the 'inside-outside hypothesis' and 'the cell polarity model' (Tarkowski & Wroblewska 1967, Johnson & Ziomek 1981a, 1981b; Fig. 1). The inside-outside hypothesis states that lineage specification is defined by position and cell-to-cell contact so that inner cells are subject to

Figure 1 Cell polarization models during compaction. (A) The inside–outside hypothesis states that lineage specification is defined by position and cell-to-cell contact so that inner cells are subject to symmetrical contact while outer cells maintain contact on three sides and have a free apical surface which defines a polarity axis as reflected by formation of focal tight junctions and adherens junctions. (B) The cell polarity model states that cell fate is established at the eight-cell stage and propagated by symmetrical or asymmetrical cell divisions that either generate two polar cells by dividing a radial polarity axis or an outer polar and an inner apolar cell. Although very similar the models differ by suggesting cell position directs cell fate (inside–outside) versus cell fate driving cell position (polarity model; Johnson & McConnell 2004).

symmetrical contact, while outer cells maintain contact on three sides and have a free apical surface which defines a polarity axis as reflected by formation of focal tight junctions and adherens junctions (Johnson & Ziomek 1981a,1981b, Yamanaka *et al.* 2006). The cell polarity model predicts that cell fate is established at the eight-cell stage in the mouse and propagated by symmetrical or asymmetrical cell divisions that either generate two polar cells by dividing a radial polarity axis or an outer polar and an inner apolar cell (Johnson & Ziomek 1981a,1981b, Yamanaka *et al.* 2006). Although very similar, the models differ by suggesting that cell position directs cell fate (inside–outside) versus cell fate driving cell position (polarity model). Studies appear to favor the cell polarity model and have recorded polarized lectin binding, apical microvilli, cytoskeletal elements, and other organelles in eight-cell stage mouse embryos (Johnson & McConnell 2004, Yamanaka *et al.* 2006). In addition, studies have indicated that while polarity is established more efficiently in the presence of cell-to-cell adhesion, it is not required to maintain polarity once it has been established. Finally, polarization and compaction both occur in the presence of protein synthetic inhibitors indicating that these events are driven by posttranslational processes applied to a preexisting protein pool (Kidder & McLachlin 1985, Wiley *et al.* 1990). What are the key proteins directing polarity and compaction? Adherens junction components are among the most critical proteins involved in the establishment of polarity and compaction but others include the partitioning (PAR) complex (PAR 1, 3, and 6), atypical PKCs, and tight junction-associated

proteins (Yamanaka *et al.* 2006). Since these proteins contribute to the formation of an apical protein complex their position satisfies the conditions of the cell polarity model. Thus, the foundation for TE formation lies in compaction and establishment of cell polarity in outer embryonic blastomeres. But which factors control the decision to become TE or ICM? Recent research applied to the mouse has established that TE and ICM differentially express several lineage-specific transcription factors. *Cdx2* becomes restricted to the TE and is required for TE formation (Yamanaka *et al.* 2006; Fig. 2). In contrast, *Oct4* and *Nanog* become restricted to and influence ICM fate (Yamanaka *et al.* 2006; Fig. 2). The current understanding of their roles has led to a model that predicts mutual antagonism between *Oct4* and *Cdx2* in supporting the formation of TE and ICM fates in the blastocyst (Yamanaka *et al.* 2006). It will be interesting to see whether this model extends to other mammalian species.

Blastocyst formation

Blastocyst formation or cavitation is dependent upon TE differentiation as it is the ion and water transport functions of the TE that mediate the fluid dynamics that control blastocyst formation (Watson 1992, Watson & Barcroft 2001). Na/K-ATPase, aquaporins (AQP; water channels), and tight junctions have established roles in coordinating blastocyst formation (Watson 1992, Watson & Barcroft 2001). The model that has been tested is that blastocyst formation is dependent upon the polarized distribution of the Na/K-ATPase confined to the

Figure 2 Cell lineage and transcription factors. Trophoblast and inner cell mass differentially express lineage-specific transcription factors. *Cdx2* becomes restricted to the trophoblast and is required for trophoblast formation. In contrast, *Oct4* and *Nanog* become restricted to and control inner cell mass fate. The model predicts mutual antagonism between *Oct4* and *Cdx2* in supporting the formation of trophoblast and inner cell mass fates in the blastocyst (Yamanaka *et al.* 2006).

basolateral membrane domains of the TE. This establishes a trans-trophoblast ion gradient that facilitates movement of water across the epithelium facilitated by the presence of both apical and basolateral AQPs (Fig. 3). These events combined with the establishment of a TE tight junctional seal to prevent the loss of fluid out of the embryo through paracellular routes results in the expansion of the embryo and the formation of the blastocyst (Watson 1992, Watson & Barcroft 2001). Over the years, considerable evidence has been collected that supports this hypothesis.

Na/K-ATPase is confined to the basolateral membrane domain of the mural TE (Watson & Barcroft 2001). In addition, enzyme activity increases just prior to blastocyst formation in all mammalian species examined to date (Watson 1992, Watson & Barcroft 2001). The expression of all the principal Na/K-ATPase isoforms has been defined as well as the functions of Na/K-ATPase

$\alpha 1$ and $\beta 1$ subunits in supporting blastocyst formation in the mouse (Watson *et al.* 1990, MacPhee *et al.* 2000, Barcroft *et al.* 2004, Madan *et al.* 2007). In addition, aquaporins and their role in facilitating blastocyst formation in the mouse have been investigated (Barcroft *et al.* 2003, Offenberger & Thomsen 2005). Treatment of mouse blastocysts with pCMPs (mercuric AQP blocker) results in the attenuation of the fluid transport that accompanies exposure of mouse blastocysts to hyperosmotic media (Barcroft *et al.* 2003; Fig. 3). In addition to these critical gene products, recent studies have applied subtractive hybridization and gene array screening methods to identify a growing list of genes that are implicated in regulating compaction and blastocyst formation (Ko *et al.* 2000, Hamatani *et al.* 2006, Goossens *et al.* 2007). These studies are invaluable for identifying and directing studies to new gene targets that are implicated in the basic program that governs preimplantation development. They ensure that our understanding of the basic program controlling preimplantation development will remain a very rich area of research well into the future.

What is next for these studies? Over the past few years, four different endogenous cardiotonic steroids (CTS) have been isolated from human plasma, bovine adrenals, hypothalamus, and amniotic fluid. These compounds include ouabain (identical to the plant-derived steroid), digoxin, marinobufagenin, and 19-nobufalin (Schoner 2002). The adrenal is the likely source of their production as levels decline dramatically following adrenalectomy in dogs and bovine adrenal-cortical cells secrete high levels of ouabain *in vitro* (Schoner 2002). Research is just beginning to define their physiological roles. The presence of these compounds in plasma is certain although their appearance in the reproductive tract has not been determined. They may, however, represent a novel hormonal signaling pathway for regulating blastocyst formation *in vivo*. In addition, one of the most exciting discoveries in recent years is that following cardiotonic steroid binding to the

Figure 3 Blastocyst formation model. Blastocyst formation is dependent upon trophoblast differentiation as the ion and water transport functions of the trophoblast mediate the fluid dynamics that control blastocyst formation. The hypothesis we have tested is that blastocyst formation is dependent upon the polarized distribution of the Na/K-ATPase confined to the basolateral membrane domains of the trophoblast. This establishes a trans-trophoblast ion gradient that facilitates movement of water across the epithelium facilitated by the presence of both apical and basolateral AQPs. These events combined with the establishment of a trophoblast tight junctional seal to prevent the loss of fluid out of the embryo through paracellular routes results in the expansion of the embryo and the formation of the blastocyst (Watson & Barcroft 2001).

Na/K-ATPase c-SRC tyrosine kinase forms a binary receptor which phosphorylates and assembles additional proteins into signaling modules which activate MAP kinase pathways (MAPK) and protein kinase C isozymes in a cell-specific way. We therefore speculate that in addition to its better recognized role of regulating ion transport, the Na/K-ATPase is also an important signaling molecule that activates c-SRC-mediated MAPK pathways that regulate cell junction formation during preimplantation development. The future will tell.

Impact of culture on preimplantation development

In addition to nuclear transfer, measuring the influences of culture on embryonic gene expression has emerged as an important experimental paradigm for investigating epigenetic and environmental influences on preimplantation development and their longer terms effects on fetal and postpartum development. We have presented that precise control of gene expression during preimplantation development is particularly important as several developmental events occur during this period including: (i) the first mitotic division, the timing of which has been associated with developmental competence in a variety of mammalian species (Loneragan *et al.* 1999, 2006); (ii) embryonic genome activation, when the embryo transfers from a reliance on maternal RNA derived from the oocyte to expression of its own genome (Telford *et al.* 1990); (iii) morula compaction, and (iv) blastocyst formation, as described above, and in ruminants, subsequent elongation prior to implantation.

While few studies have examined the temporal pattern of transcript abundance from zygote to blastocyst stage, the large majority of reports describe relative transcript abundance at the blastocyst stage only. While formation of the blastocyst is undoubtedly an important checkpoint/landmark on the developmental axis, it is important to remember that the blastocyst is the product of a sequence of events that precede it, as outlined above. Although not the main subject of this review, there is evidence to demonstrate that the environment to which the oocyte is exposed during maturation can influence the pattern of transcripts in the matured oocyte (Watson *et al.* 2000, Lonergan *et al.* 2003) and in the resulting blastocyst (Russell *et al.* 2006). However, most evidence suggests that the pattern of mRNA abundance in the blastocyst, and the quality of the blastocyst in terms of establishing and maintaining a pregnancy, is dictated by the post-fertilization conditions of culture (Lonergan *et al.* 2006). For example, Knijn *et al.* (2002) examined transcript abundance in cattle blastocysts derived from oocytes matured either *in vitro* or *in vivo* and found no differences for the small panel of transcripts examined, suggesting that blastocysts produced in a common post-fertilization culture environment have a similar transcript profile irrespective of the origin of the oocyte. In addition,

several groups have reported that culture of *in vitro* produced zygotes *in vivo* in the sheep (Rizos *et al.* 2002) or cow (Tesfaye *et al.* 2007) oviduct results in embryos with a morphology, pattern of mRNA expression and an ability to withstand cryopreservation, similar to that of true *in vivo* derived embryos. There is a large and continually increasing body of evidence demonstrating that the culture environment to which embryos are exposed *in vitro* can perturb gene expression in the developing embryo. While this applies mainly to the culture medium used and its inclusions (Wrenzycki *et al.* 2005, Lonergan *et al.* 2006), the conditions of incubation are also important; for example, the relative abundance of specific transcripts in cow *in vitro* produced embryos alters in response to changes in the oxygen environment post-compaction (Harvey *et al.* 2004).

Culture of *in vitro* produced zygotes *in vivo*

Heterologous versus homologous culture

The oviductal environment can support embryonic growth up to the blastocyst stage across a wide range of species following trans-species transfer (Rizos *et al.* 2007). Culture of cow embryos in the oviduct of the ewe is suitable for the development of embryos from the zygote to blastocyst stage and even through the early stages of elongation. Though not perfect, one advantage of this *in vivo* culture system is the ability to culture large numbers of embryos in a 'near *in vivo*' environment and in a cost-effective manner. While the yield of blastocysts following such *in vivo* culture is not superior to that following culture *in vitro*, the quality of the blastocysts is significantly improved (Rizos *et al.* 2002). However, heterologous transfer and culture of embryos is never totally satisfactory from an experimental design viewpoint. Recently, endoscopy has been successfully used to access the oviducts of cattle for the *in vivo* culture of *in vitro* matured or fertilized embryos in the homologous oviduct (Besenfelder *et al.* 2001, Tesfaye *et al.* 2007). While this technique requires a significant level of skill and experience (currently only practiced routinely by one group worldwide for the tubal transfer of embryos) it offers much promise for comparative studies of embryo development and gene expression *in vivo* and *in vitro*.

Effect of *in vivo* embryo environment on embryo gene expression

Ruminants experience relatively high rates of embryonic and early fetal mortality (about 40%). Published estimates indicate a fertilization rate of 90% and an average calving rate of about 55%, suggesting an embryonic/fetal mortality of about 35%; it is estimated that 70–80% of the total embryonic loss occurs between days 8 and 16 after insemination (day 16 corresponding to the period of maternal recognition of pregnancy;

Sreenan *et al.* 2001). The importance of progesterone in the establishment and maintenance of pregnancy in ruminants is well-known. While there is much evidence showing the importance of progesterone levels in the immediate postconception period (days 4–7) on subsequent pregnancy maintenance (McNeill *et al.* 2006) and increasing data on progesterone-induced changes in gene expression in the uterus (Spencer *et al.* 2004), there is little known about the gene expression changes induced in the embryo at this time. Advancement of conceptus development following administration of early exogenous progesterone has been described in both cattle (Garrett *et al.* 1988) and sheep (Satterfield *et al.* 2006). Despite the presence of mRNA for the progesterone receptor on cow embryos (Fair *et al.*, unpublished observations), evidence for a direct effect of progesterone on embryo development is lacking. Addition of progesterone to culture medium is reported to have no effect or only to have an effect in the presence of coculture cells (Lavranos & Seamark 1989). The mechanisms through which preimplantation concentrations of progesterone regulate embryo survival and growth are not well investigated but are thought to be mediated by secretions from the endometrium. In sheep, progesterone acts on the endometrium to induce a number of genes that encode for proteins secreted into the uterine lumen, including galectin 15 (LGALS15) and secreted phosphoprotein one (SPP1 or osteopontin). The advanced development of blastocysts in progesterone-treated ewes is hypothesized to involve early induction of specific genes in the endometrial epithelia, such as LGALS15 and components of uterine histotroph (Satterfield *et al.* 2006) and it is likely that a similar mechanism operates in cattle although the precise details have not yet been elucidated.

Understanding the implications of culture-induced changes in mRNA abundance

Just what does it mean for the embryo when the relative abundance of certain transcripts alters in response to a changing environment? The consequences of differential mRNA abundance for subsequent development (i.e., the functional significance of such changes) are difficult to interpret. Few studies have attempted to correlate the differences in mRNA abundance observed at the blastocyst stage, such as those outlined above, with the ability of the embryo to establish a pregnancy. One such study (El-Sayed *et al.* 2006) addressed the relationship between transcriptional profile of embryos and the pregnancy success based on gene expression analysis of blastocyst biopsies taken prior to transfer to recipients. Microarray data analysis revealed a total of 52 differentially regulated genes between embryos resulting in a calf delivery versus those not resulting in a pregnancy. Biopsies resulting in calf delivery were enriched with genes necessary for implantation,

carbohydrate metabolism, growth factors, signal transduction, and placental development, while those failing to establish a pregnancy were enriched with transcripts for inflammatory cytokines, protein amino acid binding, transcription factors, glucose metabolism, and inhibition of implantation. Another approach toward testing functionality is to examine genes found to be differentially expressed in one model in a second model of competence (Mourot *et al.* 2006, Patel *et al.* 2007). A more direct approach is to alter the levels of mRNA (either under express or overexpress) and look for a phenotypic effect. RNA interference (RNAi) has become a well-established technique to study gene function in several species (Madan *et al.* 2007). In domestic species, however, the use of RNA interference technology in domestic species is still in its infancy with only a handful of papers published on the subject (Tesfaye *et al.* 2007). It will be critical to define gene function so that a full interpretation of culture-induced changes in gene expression can be arrived at.

Concluding statements

Preimplantation development is characterized by extensive epigenetic modifications of the newly formed embryonic genome that permits the onset of a highly regulated gene expression program. While this program is directed at the formation of a functional implantation-competent blastocyst, alterations of its initial steps affect embryo development far beyond this stage. Current research is focused upon understanding the epigenetic mechanisms that enable the early embryo to initiate its normal developmental program and also adjust that program to respond to environmental perturbations. Paradoxically, the ability to support early embryo development *in vitro*, which of course has been of great benefit to understanding the mechanisms controlling early development and to also providing new ways to propagate animal species and treat human infertility, has now come under greater scrutiny due to its capacity to affect the embryonic developmental program and thus influence fetal and postpartum development. To alleviate these concerns it is vital that research focuses once again on *in vivo* development. The functional analysis of the molecular basis of these culture or nuclear transfer-based alterations will reveal new aspects of early developmental regulation. It should help to better define the limits of the early mammalian embryo developmental autonomy and is necessary to design healthier *in vitro* conditions, and perhaps to the development of new strategies to modify individual phenotypes.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Funding

This article is based on research presented at the 2nd International Meeting on Mammalian Embryogenomics, which was sponsored by the Organisation for Economic Co-operation and Development (OECD), Le conseil Régional Ile-de-France, the Institut National de la Recherche Agronomique (INRA), Cogenics-Genome Express, Eurogentac, Proteigene, Sigma-Aldrich France and Diagenode sa. All authors declare that they have no relationship with any of the meeting sponsors. All authors received funding from the OECD to attend the meeting. V D is a Senior Researcher at an INRA-funded institute; this is a non-commercial national research institute.

References

- Barcroft LC, Offenberger H, Thomsen P & Watson AJ 2003 Aquaporin proteins in murine trophectoderm mediate transepithelial water movements during cavitation. *Developmental Biology* **256** 342–354.
- Barcroft LC, Moseley AE, Lingrel JB & Watson AJ 2004 Deletion of the Na/K-ATPase alpha1-subunit gene (Atp1a1) does not prevent cavitation of the preimplantation mouse embryo. *Mechanisms of Development* **121** 417–426.
- Barker DJ 2003 The developmental origins of adult disease. *European Journal of Epidemiology* **18** 733–736.
- Beaujean N, Hartshorne G, Cavilla J, Taylor J, Gardner J, Wilmut I, Meehan R & Young L 2004 Non-conservation of mammalian preimplantation methylation dynamics. *Current Biology* **14** R266–R267.
- Besenfelder U, Havlicek V, Mossbacher G & Brem G 2001 Collection of tubal stage bovine embryos by means of endoscopy. A technique report. *Theriogenology* **55** 837–845.
- Beyhan Z, Ross PJ, Lager AE, Kocabas AM, Cunniff K, Rosa GJ & Cibelli JB 2007 Transcriptional reprogramming of somatic cell nuclei during preimplantation development of cloned bovine embryos. *Developmental Biology* **305** 637–649.
- Boiani M, Eckardt S, Scholer HR & McLaughlin KJ 2002 Oct4 distribution and level in mouse clones: consequences for pluripotency. *Genes and Development* **16** 1209–1219.
- Ecker DJ, Stein P, Xu Z, Williams CJ, Kopf GS, Bilker WB, Abel T & Schultz RM 2004 Long-term effects of culture of preimplantation mouse embryos on behavior. *PNAS* **101** 1595–1600.
- Erhardt S, Su IH, Schneider R, Barton S, Bannister AJ, Perez-Burgos L, Jenuwein T, Kouzarides T, Tarakhovsky A & Surani MA 2003 Consequences of the depletion of zygotic and embryonic enhancer of zeste 2 during preimplantation mouse development. *Development* **130** 4235–4248.
- Evsikov AV, de Vries WN, Peaston AE, Radford EE, Fancher KS, Chen FH, Blake JA, Bult CJ, Latham KE, Solter D *et al.* 2004 Systems biology of the 2-cell mouse embryo. *Cytogenetic and Genome Research* **105** 240–250.
- Fleming TP, Ghassemifar MR & Sheth B 2000 Junctional complexes in the early mammalian embryo. *Seminars in Reproductive Medicine* **18** 185–193.
- Fulka H, Mrazek M, Tepla O & Fulka J Jr 2004 DNA methylation pattern in human zygotes and developing embryos. *Reproduction* **128** 703–708.
- Gao S, Chung YG, Williams JW, Riley J, Moley K & Latham KE 2003 Somatic cell-like features of cloned mouse embryos prepared with cultured myoblast nuclei. *Biology of Reproduction* **69** 48–56.
- Garrett JE, Geisert RD, Zavy MT, Gries LK, Wettemann RP & Buchanan DS 1988 Effect of exogenous progesterone on prostaglandin F₂ alpha release and the interovulatory interval in the bovine. *Prostaglandins* **36** 85–96.
- Goossens K, Van Soom A, Van Poucke M, Vandaele L, Vandesompele J, Van Zeveren A & Peelman LJ 2007 Identification and expression analysis of genes associated with bovine blastocyst formation. *BMC Developmental Biology* **7** 64.
- Hamatani T, Carter MG, Sharov AA & Ko MS 2004 Dynamics of global gene expression changes during mouse preimplantation development. *Developmental Cell* **6** 117–131.
- Hamatani T, Ko M, Yamada M, Kuji N, Mizusawa Y, Shoji M, Hada T, Asada H, Maruyama T & Yoshimura Y 2006 Global gene expression profiling of preimplantation embryos. *Human Cell* **19** 98–117.
- Harvey AJ, Kind KL, Pantaleon M, Armstrong DT & Thompson JG 2004 Oxygen-regulated gene expression in bovine blastocysts. *Biology of Reproduction* **71** 1108–1119.
- Holliday R 1994 Epigenetics: an overview. *Developmental Genetics* **15** 453–457.
- Johnson MH & McConnell JM 2004 Lineage allocation and cell polarity during mouse embryogenesis. *Seminars in Cell and Developmental Biology* **15** 583–597.
- Johnson MH & Ziomek CA 1981a Induction of polarity in mouse 8-cell blastomeres: specificity, geometry, and stability. *Journal of Cell Biology* **91** 303–308.
- Johnson MH & Ziomek CA 1981b The foundation of two distinct cell lineages within the mouse morula. *Cell* **24** 71–80.
- Kidder GM & McLachlin JR 1985 Timing of transcription and protein synthesis underlying morphogenesis in preimplantation mouse embryos. *Developmental Biology* **112** 265–275.
- Knijin HM, Wrenzycki C, Hendriksen PJ, Vos PL, Herrmann D, van der Weijden GC, Niemann H & Dieleman SJ 2002 Effects of oocyte maturation regimen on the relative abundance of gene transcripts in bovine blastocysts derived *in vitro* or *in vivo*. *Reproduction* **124** 365–375.
- Ko MS, Kitchen JR, Wang X, Threat TA, Wang X, Hasegawa A, Sun T, Grahovac MJ, Kargul GJ, Lim MK *et al.* 2000 Large-scale cDNA analysis reveals phased gene expression patterns during preimplantation mouse development. *Development* **127** 1737–1749.
- Lavranos TC & Seamark RF 1989 Addition of steroids to embryo-uterine monolayer co-culture enhances embryo survival and implantation *in vitro*. *Reproduction, Fertility, and Development* **1** 41–46.
- Loneragan P, Khatir H, Piumi F, Rieger D, Humblot P & Boland MP 1999 Effect of time interval from insemination to first cleavage on the developmental characteristics, sex ratio and pregnancy rate after transfer of bovine embryos. *Journal of Reproduction and Fertility* **117** 159–167.
- Loneragan P, Gutierrez-Adan A, Rizo D, Pintado B, de la Fuente J & Boland MP 2003 Relative messenger RNA abundance in bovine oocytes collected *in vitro* or *in vivo* before and 20 h after the preovulatory luteinizing hormone surge. *Molecular Reproduction and Development* **66** 297–305.
- Loneragan P, Fair T, Corcoran D & Evans AC 2006 Effect of culture environment on gene expression and developmental characteristics in IVF-derived embryos. *Theriogenology* **65** 137–152.
- MacPhee DJ, Jones DH, Barr KJ, Betts DH, Watson AJ & Kidder GM 2000 Differential involvement of Na(+),K(+)-ATPase isozymes in preimplantation development of the mouse. *Developmental Biology* **222** 486–498.
- Madan P, Rose K & Watson AJ 2007 Na/K-ATPase beta 1 subunit expression is required for blastocyst formation and normal assembly of trophectoderm tight junction associated proteins. *Journal of Biological Chemistry* **282** 1212–1213.
- Mann MR, Lee SS, Doherty AS, Verona RI, Nolen LD, Schultz RM & Bartolomei MS 2004 Selective loss of imprinting in the placenta following preimplantation development in culture. *Development* **131** 3727–3735.
- McGraw S, Robert C, Massicotte L & Sirard MA 2003 Quantification of histone acetyltransferase and histone deacetylase transcripts during early bovine embryo development. *Biology of Reproduction* **68** 383–389.
- McGraw S, Vigneault C & Sirard MA 2007 Temporal expression of factors involved in chromatin remodeling and in gene regulation during early bovine *in vitro* embryo development. *Reproduction* **133** 597–608.
- McNeill RE, Sreenan JM, Diskin MG, Cairns MT, Fitzpatrick R, Smith TJ & Morris DG 2006 Effect of systemic progesterone concentration on the expression of progesterone-responsive genes in the bovine endometrium during the early luteal phase. *Reproduction, Fertility, and Development* **18** 573–583.
- Misirlioglu M, Page GP, Sagirkaya H, Kaya A, Parrish JJ, First NL & Memili E 2006 Dynamics of global transcriptome in bovine matured oocytes and preimplantation embryos. *PNAS* **103** 18905–18910.
- Morgan HD, Santos F, Green K, Dean W & Reik W 2005 Epigenetic reprogramming in mammals. *Human Molecular Genetics* **14** R47–R58.

- Mouroi M, Dufort I, Gravel C, Algriany O, Dieleman S & Sirard MA 2006 The influence of follicle size, FSH-enriched maturation medium, and early cleavage on bovine oocyte maternal mRNA levels. *Molecular Reproduction and Development* **73** 1367–1379.
- Niemann H & Wrenzycki C 2000 Alterations of expression of developmentally important genes in preimplantation bovine embryos by *in vitro* culture conditions: implications for subsequent development. *Theriogenology* **53** 21–34.
- Offenberg H & Thomsen PD 2005 Functional challenge affects aquaporin mRNA abundance in mouse blastocysts. *Molecular Reproduction and Development* **71** 422–430.
- Pacheco-Trigon S, Hennequet-Antier C, Oudin JF, Piumi F, Renard JP & Duranthon V 2002 Molecular characterization of genomic activities at the onset of zygotic transcription in mammals. *Biology of Reproduction* **67** 1907–1918.
- Panarace M, Aguero JL, Garrote M, Jauregui G, Segovia A, Cane L, Gutierrez J, Marfil M, Rigali F, Pugliese M *et al.* 2007 How healthy are clones and their progeny: 5 years of field experience. *Theriogenology* **67** 142–151.
- Patel OV, Bettgowda A, Ireland JJ, Coussens PM, Lonergan P & Smith GW 2007 Functional genomics studies of oocyte competence: evidence that reduced transcript abundance for follistatin is associated with poor developmental competence of bovine oocytes. *Reproduction* **133** 95–106.
- Peaston AE, Evsikov AV, Graber JH, de Vries WN, Holbrook AE, Solter D & Knowles BB 2004 Retrotransposons regulate host genes in mouse oocytes and preimplantation embryos. *Developmental Cell* **7** 597–606.
- Pfister-Genskow M, Myers C, Childs LA, Lacson JC, Patterson T, Betthausen JM, Goueleke PJ, Koppang RW, Lange G, Fisher P *et al.* 2005 Identification of differentially expressed genes in individual bovine preimplantation embryos produced by nuclear transfer: improper reprogramming of genes required for development. *Biology of Reproduction* **72** 546–555.
- Rizos D, Lonergan P, Boland MP, Arroyo-García R, Pintado B, de la Fuente J & Gutierrez-Adan A 2002 Analysis of differential messenger RNA expression between bovine blastocysts produced in different culture systems: implications for blastocyst quality. *Biology of Reproduction* **66** 589–595.
- Rizos D, Pintado B, de la Fuente J, Lonergan P & Gutierrez-Adan A 2007 Development and pattern of mRNA relative abundance of bovine embryos cultured in the isolated mouse oviduct in organ culture. *Molecular Reproduction and Development* **74** 716–723.
- Rossant J 2004 Lineage development and polar asymmetries in the preimplantation mouse blastocyst. *Seminars in Cell and Developmental Biology* **15** 573–581.
- Russell DF, Baqir S, Bordignon J & Betts DH 2006 The impact of oocyte maturation media on early bovine embryonic development. *Molecular Reproduction and Development* **73** 1255–1270.
- Santos F, Zakhartchenko V, Stojkovic M, Peters A, Jenuwein T, Wolf E, Reik W & Dean W 2003 Epigenetic marking correlates with developmental potential in cloned bovine preimplantation embryos. *Current Biology* **13** 1116–1121.
- Satterfield MC, Bazer FW & Spencer TE 2006 Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus. *Biology of Reproduction* **75** 289–296.
- El-Sayed A, Hoelker M, Rings F, Salilew D, Jennen D, Tholen E, Sirard MA, Schellander K & Tesfaye D 2006 Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnancy success after transfer to recipients. *Physiological Genomics* **28** 84–96.
- Schoner W 2002 Endogenous cardiac glycosides, a new class of steroid hormones. *European Journal of Biochemistry* **269** 2440–2448.
- Schultz RM 2005 From egg to embryo: a peripatetic journey. *Reproduction* **130** 825–828.
- Schultz RM & Williams CJ 2002 The science of ART. *Science* **296** 2188–2190.
- Shi W, Dirim F, Wolf E, Zakhartchenko V & Haaf T 2004 Methylation reprogramming and chromosomal aneuploidy in *in vivo* fertilized and cloned rabbit preimplantation embryos. *Biology of Reproduction* **71** 340–347.
- Smith SL, Everts RE, Tian XC, Du F, Sung LY, Rodriguez-Zas SL, Jeong BS, Renard JP, Lewin HA & Yang X 2005 Global gene expression profiles reveal significant nuclear reprogramming by the blastocyst stage after cloning. *PNAS* **102** 17582–17587.
- Spencer TE, Johnson GA, Bazer FW & Burghardt RC 2004 Implantation mechanisms: insights from the sheep. *Reproduction* **128** 657–668.
- Sreenan J, Diskin M & Morris D 2001 Embryo survival rate in cattle: a major limitation to the achievement of high fertility. In *Fertility in the High-Yielding Dairy Cow*, pp 93–104. BSAS Occasional Publication 26, Scotland, UK: British Society of Animal Science.
- Svoboda P, Stein P, Anger M, Bernstein E, Hannon GJ & Schultz RM 2004 RNAi and expression of retrotransposons MuERV-L and IAP in preimplantation mouse embryos. *Developmental Biology* **269** 276–285.
- Tarkowski AK & Wroblewska J 1967 Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. *Journal of Embryology and Experimental Morphology* **18** 155–180.
- Telford NA, Watson AJ & Schultz GA 1990 Transition from maternal to embryonic control in early mammalian development: a comparison of several species. *Molecular Reproduction and Development* **26** 90–100.
- Tesfaye D, Lonergan P, Hoelker M, Rings F, Nganvongpanit K, Havlicek V, Besenfelder U, Jennen D, Tholen E & Schellander K 2007 Suppression of connexin 43 and E-cadherin transcripts in *in vitro* derived bovine embryos following culture *in vitro* or *in vivo* in the homologous bovine oviduct. *Molecular Reproduction and Development* **74** 978–988.
- Vassena R, Han Z, Gao S, Baldwin DA, Schultz RM & Latham KE 2007 Tough beginnings: alterations in the transcriptome of cloned embryos during the first two cell cycles. *Developmental Biology* **304** 75–89.
- Wang Y, Wang F, Sun T, Trostinskaia A, Wygle D, Puscheck E & Rappolee DA 2004 Entire mitogen activated protein kinase (MAPK) pathway is present in preimplantation mouse embryos. *Developmental Dynamics* **231** 72–87.
- Watson AJ 1992 The cell biology of blastocyst development. *Molecular Reproduction and Development* **33** 492–504.
- Watson AJ & Barcroft LC 2001 Regulation of blastocyst formation. *Frontiers in Bioscience* **6** D708–D730.
- Watson AJ, Pape C, Emanuel JR, Levenson R & Kidder GM 1990 Expression of Na, K-ATPase alpha and beta subunit genes during preimplantation development of the mouse. *Developmental Genetics* **11** 41–48.
- Watson AJ, De Sousa P, Caveney A, Barcroft LC, Natale D, Urquhart J & Westhusin ME 2000 Impact of bovine oocyte maturation media on oocyte transcript levels, blastocyst development, cell number, and apoptosis. *Biology of Reproduction* **62** 355–364.
- Whitworth K, Springer GK, Forrester LJ, Spollen WG, Ries J, Lamberson WR, Bivens N, Murphy CN, Mathialagan N, Green JA *et al.* 2004 Developmental expression of 2489 gene clusters during pig embryogenesis: an expressed sequence tag project. *Biology of Reproduction* **71** 1230–1243.
- Wiley LM, Kidder GM & Watson AJ 1990 Cell polarity and development of the first epithelium. *BioEssays* **12** 67–73.
- Wrenzycki C, Herrmann D, Lucas-Hahn A, Korsawe K, Lemme E & Niemann H 2005 Messenger RNA expression patterns in bovine embryos derived from *in vitro* procedures and their implications for development. *Reproduction, Fertility, and Development* **17** 23–35.
- Yamanaka Y, Ralston A, Stephenson RO & Rossant J 2006 Cell and molecular regulation of the mouse blastocyst. *Developmental Dynamics* **235** 2301–2314.
- Yang X, Smith SL, Tian XC, Lewin HA, Renard JP & Wakayama T 2007 Nuclear reprogramming of cloned embryos and its implications for therapeutic cloning. *Nature Genetics* **39** 295–302.
- Zeng F & Schultz RM 2005 RNA transcript profiling during zygotic gene activation in the preimplantation mouse embryo. *Developmental Biology* **283** 40–57.
- Zeng F, Baldwin DA & Schultz RM 2004 Transcript profiling during preimplantation mouse development. *Developmental Biology* **272** 483–496.

Received 13 July 2007

First decision 24 August 2007

Accepted 5 October 2007