Gene networks in the embryo and endometrium related to embryo survival

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Summary

Low fertility is one of the major problems limiting the efficiency of the dairy industry. Successful pregnancy establishment and development to term depend on the suitability of the endometrial environment and the intrinsic quality of the embryo. Accurate assessment of both, however, is challenging. Therefore, the present survey reviews current knowledge of the molecular networks in the endometrium related to pregnancy establishment and maintenance to term. Accordingly, distinct molecular signatures of the bovine endometrium, elucidated by innovative approaches, could be correlated with pregnancy success. However, not only must the reproductive tract provide a suitable environment but the embryo itself must be competent to express its developmental program. Numerous studies have correlated molecular networks of bovine embryos with their developmental capacity. However, most studies were, by their nature, invasive and the findings were therefore difficult to extrapolate beyond the developmental stages examined. Hence, a second aim is to present new strategies employing embryo biopsies for a direct connection between molecular signatures and embryo developmental capacity. Large scale differences, at the molecular level, were reported for blastomeres whose counterparts developed to the blastocyst stage compared to those with lower developmental competence, thus unraveling distinct molecular fingerprints related to ability to develop to the blastocyst stage. Similarly, distinct molecular signatures at the blastocyst stage were associated with embryo developmental competence to term. In summary, further understanding of molecular signatures related to endometrial receptivity and embryo developmental capacity has been gained from novel innovative strategies including embryo microdissection as well as by examining endometrial samples collected in the cycle preceding the one in which conception occurs.

Introduction

Low fertility is one of the major problems limiting the efficiency of the dairy industry with the majority of embryonic losses occurring between days 8 and 16 after mating (Diskin & Sreenan 1980, Diskin *et al.* 2006). In the bovine, fertility is dependent upon the following: 1) the ability of a cow to cycle and 2) development of appropriate endocrine conditions within the reproductive tract to ensure a healthy and receptive environment capable of supporting embryonic/fetal development culminating in a viable offspring. Imbalance of embryo-maternal communication is believed to be a major cause of pregnancy failure in large part as a consequence of molecular dysregulation (Glencross *et al.* 1973, Tabibzadeh & Babaknia 1995, Spencer *et al.* 1996,

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Tabibzadeh 1998, Paria *et al.* 2000, Paria *et al.* 2001, Paria *et al.* 2002, Riesewijk *et al.* 2003, Dey *et al.* 2004, Giudice 2004, Bauersachs *et al.* 2006, Wang & Dey 2006, Spencer *et al.* 2007, Spencer *et al.* 2008, Bauersachs *et al.* 2009, Boomsma *et al.* 2009). Thus, there is a large amount of evidence that the properties of the maternal environment are major determinants of pregnancy establishment and maintenance. Predictive criteria to classify the suitability of a given endometrial environment, however, remain elusive.

Additionally, the embryo must be competent to successfully undergo preimplantation development, elongation, implantation and term development. Embryo quality is related to the embryo's intrinsic properties and environmental consequences established by the dam or during assisted reproductive techniques. Indeed, in vitro produced bovine embryos remain inferior in terms of viability compared to embryos developed in vivo (Farin & Farin 1995, Numabe *et al.* 2000). Likewise, various studies have shown that bovine embryo production under specific culture conditions resulted in not only an altered embryonic and foetal development following transfer, but also altered gene expression of transcripts related to metabolism and growth (Khosla *et al.* 2001, Lazzari *et al.* 2002). Consequently, deviant expression of developmentally important genes has been implicated as a causative factor of embryonic death during preimplantation development (Lonergan *et al.* 2006) and several studies have reported candidate genes related to developmental competence (Lonergan *et al.* 1999, Brevini *et al.* 2002, Rief *et al.* 2002, Knijn *et al.* 2005, Dode *et al.* 2006, Corcoran *et al.* 2007). Direct connections between embryonic gene expression profiles and subsequent developmental capacity, however, remain elusive and the underlying molecular mechanisms regulating developmental competence are unknown.

Consequently, this review summarizes knowledge with respect to endometrial networks affecting bovine receptivity taking into account direct relationships between endometrial gene expression profiles and later pregnancy success. Secondly, this review focuses on embryonic molecular signatures related to embryo developmental competence and novel strategies which enable concomitant assessment of molecular signatures and developmental competence of individual bovine embryos.

Endometrial gene networks for embryo survival

While the oviduct is the critical environment for fertilization, initial embryo development and transport of the embryo into the uterus (Austin 1951, Austin 1952, Fazeli *et al.* 2004), the uterus hosts the bovine embryo from days 4-5 of development to parturition. Consequently, the embryo-endometrial dialogue requires several endogenous molecules produced in the endometrium and/or the embryo (Glencross *et al.* 1973, Tabibzadeh & Babaknia 1995, Spencer *et al.* 1996, Tabibzadeh 1998, Paria *et al.* 2000, Paria *et al.* 2001, Paria *et al.* 2002, Riesewijk *et al.* 2003, Dey *et al.* 2004, Giudice 2004, Bauersachs *et al.* 2006, Wang & Dey 2006, Spencer *et al.* 2007, Spencer *et al.* 2008, Bauersachs *et al.* 2009, Boomsma *et al.* 2009). Among these molecules are adhesion molecules, namely integrins, cadherins, and selectins (Lessey 1997, Tabibzadeh 1998), cytokines (Salamonsen *et al.* 2000, Achache & Revel 2006, Ledee *et al.* 2007), growth factors/ growth factor receptors (Cross *et al.* 1994, Carson *et al.* 2000, Norwitz *et al.* 2001, Paria *et al.* 2001, Lim *et al.* 2002) as well as chemokines (Salamonsen *et al.* 2007). Integrins, for example, are involved in cell–cell and cell–substrate interactions (Albelda & Buck 1990) and appropriate endometrial expression is necessary for receptivity in humans (Lessey 1997). Among the cytokine family the importance of LIF for embryo implantation in different species has been reported (Stewart *et al.* 1992, Chen *et al.* 1995, Danielsson *et al.* 1997, Salamonsen *et al.* 2000, Song *et al.* 2000, Lim *et al.* 2002, Li *et al.* 2010). In addition to cytokines, the important role of growth factors and their receptors has been reported for embryo growth and uterine receptivity

in several studies (Cross *et al.* 1994, Carson *et al.* 2000, Norwitz *et al.* 2001, Paria *et al.* 2001, Lim *et al.* 2002). In particular, hepatocyte growth factor (HGF), macrophage colony stimulating factor (MCSF), colony stimulating factor 1 (CSF1), insulin-like growth factors (IGF1 and IGF2) and especially granulocyte-macrophage colony stimulating factor (GMCSF) have been reported to affect conceptus survival (Kauma 2000, Salamonsen *et al.* 2000, Ledee *et al.* 2007).

Pregnancy maintenance is under the control of progesterone (Savouret *et al.* 1990) with inadequate circulating concentrations of progesterone causing pregnancy failure (Ghosh & Sengupta 2004, Lonergan 2011). Whereas a delayed progesterone increase after ovulation is associated with lower conception rate in cattle (Henricks *et al.* 1971), low concentrations of progesterone caused by inadequate luteal function are associated with reduced embryo development (Rizos *et al.* 2010). Alternatively, progesterone supplementation before embryo transfer is discussed to speed up blastocyst formation, blastocyst expansion (Ferguson *et al.* 2011) and promotes conceptus elongation (Garrett *et al.* 1988, Geisert *et al.* 1991, de Ziegler *et al.* 1998). Overall, it is generally accepted that these effects of progesterone are mediated through nuclear receptors, which in turn alter the endometrial gene expression profile (Suzuki *et al.* 2008). Indeed, addition of exogenous progesterone resulted in altered endometrial expression of fatty acid binding protein (*FABP*), diacylglycerol O-acyltransferase 2 (*DGAT2*), myostatin (*MSTN*), crystalline gamma S (*CRYGS*) and other genes in the bovine (Forde *et al.* 2009a, Forde *et al.* 2009b). Furthermore, progesterone treatment was found to affect the expression of genes involved in endometrial WNT signaling (Satterfield *et al.* 2008), the expression of fibroblast growth factor 7 (*FGF7*), fibroblast growth factor 10 (*GF10*), insulin like growth factor binding proteins (*IGFBP1, -3, -4*), hepatocyte growth factor (*HGF*) and others in sheep endometrial epithelia (Spencer & Bazer 2002). Thus, progesterone affected the expression of endometrial genes as well as uterine receptivity in parallel.

Endometrial gene expression as a molecular cue for pregnancy establishment

Aberrant endometrial gene expression during the critical period of pregnancy establishment was correlated with implantation failure and infertility (Tabibzadeh & Babaknia 1995, Tapia *et al.* 2008). Thus, selection of recipients based on endometrial gene expression pattern might be useful for predicting subsequent endometrial receptivity before transferring valuable bovine embryos. Studies in mice with delayed implantation (Reese *et al.* 2001) and in ewes in which uterine glands were knocked out (Spencer *et al.* 1999, Gray *et al.* 2002) have contributed substantial information about potential markers for uterine receptivity and embryo implantation. The results of these retrospective studies, however, do not address whether pre-conception properties of the endometrium are related to subsequent pregnancy outcome. To fill this gap, we have conducted studies in which endometrial samples were collected by the cytobrush approach at days 3 and 7 (day 0 = day of oestrus) of the cycle preceding the one in which bovine embryos were transferred to recipients (Figure 1). These samples were categorized retrospectively according to pregnancy outcome of the recipients and were analysed with respect to the global transcriptome profile (Salilew-Wondim *et al.* 2010, Ponsuksili *et al.* 2012).

Interrelationship between pregnancy success and endometrial gene expression at day 3 of the preceding cycle

It has been shown that recipients of contrasting receptivity differ at day 3 of the preceding cycle with regard to many biological processes and molecular functions (Ponsuksili *et al.*
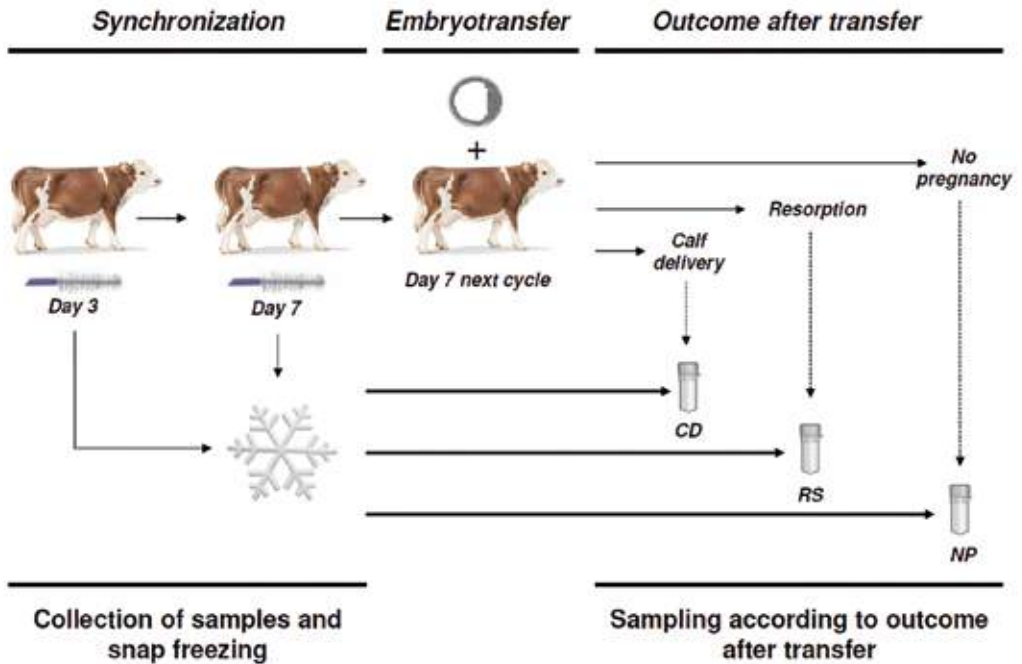
Figure 1. Overall experimental strategy to correlate the endometrial gene expression profile with recipient receptivity considering the next cycle. To accomplish that, Simmental heifers were synchronized and endometrial samples were collected at cycle days 3 and 7 by cytobrush followed by transfer of an embryo at day 7 of the next cycle. Subsequently, endometrial samples were categorized according to the later pregnancy status namely into Calf delivery (CD), initial pregnancy being terminated by day 42 termed Resorption (RP) and No pregnancy (NP), for global gene expression analysis (Salilew-Wondim *et al.* 2010, Ponsuksili *et al.* 2012).

2012). More specifically, molecular signaling pathways in the endometrium were differentially expressed between high and low fertility groups prior to implantation and molecules involved in integrin signaling, cytoskeleton signalling, and Rac signaling were enriched in the receptive endometrium. Previously, transcripts in these pathways were found to increase during the window of implantation and therefore have been proposed as markers for implantation (Aplin 2000, Bowen & Hunt 2000, Thomas *et al.* 2003, Revel *et al.* 2005, Achache & Revel 2006). Integrins for instance, participate in cell-matrix and cell-cell adhesion in many physiologically important processes and the acquisition of adhesion ligands contributes to endometrial receptivity (Aplin 2000, Bowen & Hunt 2000). Alternatively, many transcripts that belong to functions relevant to energy supply, including mitochondrial pathways, showed lower abundance at day 3 of the preceding oestrous cycle in cows having a less receptive endometrium (Ponsuksili *et al.* 2012). This indicates that defects in these pathways may negatively affect uterine receptivity. Interestingly, gene-set enrichment pathway analysis has identified mitochondrial dysfunction as a biological process related to timing of menopause in humans, coinciding with a reduction in circulating oestrogen concentrations (Stolk *et al.* 2012). Similarily, the observed expression differences related to mitochondrial function were prominent at day 3 post oestrus, when oestrogen was still the dominant ovarian hormone in the bovine. Thus, level of ovarian hormones at day 3 might affect endometrial regulation of mitochondrial biogenesis and activity and, in turn, affect receptivity of the endometrium.

Interrelationship between pregnancy success and endometrial gene expression at day 7 of the preceding cycle

The results of our studies revealed distinct differences in the endometrial gene expression profile on day 7 of the oestrous cycle between females categorized as having high and low endometrial receptivity to an embryo in the following cycle (Salilew-Wondim *et al.* 2010, Ponsuksili *et al.* 2012). Several biological processes including macromolecular localization and protein metabolism were overrepresented in genes elevated in biopsies associated with calf delivery (Figure 2A). In contrast, biological processes like response to stimulus, immune system process, developmental process, cellular metabolic process and regulation of transcription were found to be overrepresented in genes elevated in samples that did not result in a pregnancy (Figure 2B). Moreover, endometrial activation of distinct molecular pathways including tight junction formation, integrin signalling, focal adhesion, apoptosis, cell cycle, peroxisome proliferator activated receptors, GnRH signaling pathway, insulin signaling pathway, inositol phosphate metabolism and glycan biosynthesis was observed in heifers that delivered a calf compared to those that failed to become pregnant (Salilew-Wondim *et al.* 2010). Likewise, endometrial transcripts of genes of the solute carrier family, protein kinases and phosphatases and transmembrane proteins were found to be more abundant in heifers that resulted in a successful pregnancy. Importantly, genes believed to play a role within the immune system, including a cluster of differentiation molecules and chemokine molecules, were down regulated in endometrial samples correlated with pregnancy establishment and calf delivery (Salilew-Wondim *et al.* 2010). This is in agreement with our recent study in which several inflammatory and immune mediators playing a role in T- and B cell signalling as well as NF-kB signalling were more highly expressed at day 7 in the receptive endometrium (Ponsuksili *et al.* 2012). That might indicate that a rather moderate endometrial immune response may be a prerequisite for later embryo implantation. Furthermore, in humans the implantation window is characterized as an inflammatory event associated with increased expression of inflammatory mediators and immune cell infiltration (van Mourik *et al.* 2009) with extensive alterations of endometrial genes involved in cell-mediated immune response reported to be associated with female infertility (Ledee *et al.* 2011).
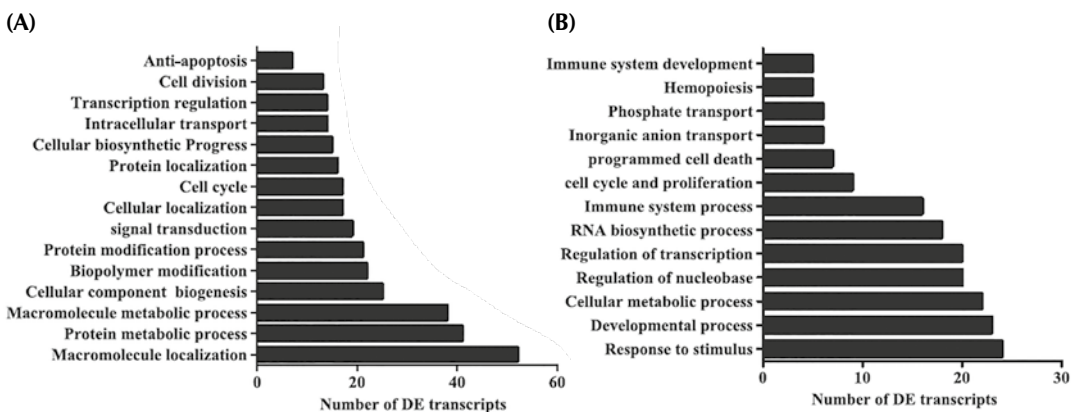
Figure 2. Overrepresentation of gene sets defined by the biological process of Gene Ontology for differential expressed genes whose transcript level was increased in endometrial samples associated with calf delivery (A). Overrepresentation of gene sets defined by the biological process of GO for genes whose transcript level was decreased in endometrial samples associated with calf delivery compared to those that did not result in a pregnancy (B) as published previously (Salilew-Wondim *et al.* 2010)

Moreover, samples collected on day 7 of the oestrous cycle, which corresponded with low uterine receptivity, showed an increase in expression of transcripts associated with inhibition of angiogenesis and NRF2-mediated oxidative stress (Ponsuksili *et al.* 2012). Endometrial angiogenesis, however, has been previously reported to play an important role in implantation and maintenance of pregnancy (Saino *et al.* 2004)(Sugino *et al.* 2002). Thus, contrasting expression of genes involved in angiogenesis among the high and low receptive groups might reflect a poor preparation of the endometrium to receive and support an embryo. Correspondingly, higher expression of oxidative stress response genes was found in the low receptive group at day 7 post oestrus (Ponsuksili *et al.* 2012). With respect to transcripts involved in oxidative stress, however, several lines of evidence indicate that oxidative stress is associated with pregnancy loss or complications of various pathological backgrounds (Liu *et al.* 2011, Loset *et al.* 2011).

Embryo gene networks for embryo survival

In addition to properties of the endometrium, the embryo has to acquire a minimum intrinsic quality to enable elongation, implantation and development to term. Viable bovine embryos, that persist until days 16 and 19 post conception, activate certain genes that are related to implantation and embryo–maternal communication (Mamo *et al.* 2011). In cattle, the elongated day 14-17 embryo produces interferon-tau (IFNT) capable of binding to endometrial type-I IFN receptors (Li & Roberts 1994, Naivar *et al.* 1995, Bazer *et al.* 1997, Spencer *et al.* 2008), thereby stimulating the expression of endometrial genes necessary for embryo implantation (Spencer *et al.* 2008). Embryo development beyond the blastocyst stage and subsequent implantation, however, depends on proper activation of the embryonic genome (Betts & King 2001); whereas deviant expression of developmentally important genes has been implicated as a causative factor of embryonic death during preimplantation development (Lonergan *et al.* 2006). Thus the mRNA makeup of the early embryo and/or blastocyst is suggested to reflect an embryo's intrinsic quality and might predict its future developmental capacity.

Molecular signatures presumably associated with embryo developmental competence

Embryos having different morphologies, contrasting maternal/in vitro environments, different developmental kinetics and different origins (vivo, vitro, nuclear transfer), not only exhibit contrasting degrees of developmental competence, but also display differences with respect to mRNA pattern. Thus, it is reasonable to consider these mRNA patterns as molecular signatures of presumed embryo developmental capacity. Several reviews have summarized relationships between candidate gene expression patterns and embryo quality (Wrenzycki *et al.* 2005, Lonergan *et al.* 2006, Wrenzycki *et al.* 2007, Duranthon *et al.* 2008). Bearing in mind that in vitro produced bovine embryos are of lower viability compared to in vivo produced embryos (Farin & Farin 1995, Numabe *et al.* 2000), qualitative differences in mRNA expression of connexin 43 (*CX43*) and genes from the leukemia inhibitory factor (*LIF*) family exist between in vivo and in vitro derived bovine blastocysts (Wrenzycki *et al.* 1996, Eckert & Niemann 1998). Later, various studies reported aberrations from the normal mRNA pattern for in vitro produced embryos compared to in vivo derived and nuclear transfer derived embryos (Eckert & Niemann 1998, Wrenzycki *et al.* 1999, Doherty *et al.* 2000, Lee *et al.* 2001, Minami *et al.* 2001, Wrenzycki *et al.* 2001, Rief *et al.* 2002, Rizos *et al.* 2002a, Rizos *et al.* 2002b, Rizos *et al.* 2003, Smith *et al.* 2005, Kato *et al.* 2007). However, although the relationship between bovine

blastocyst quality and gene expression has been the focus of numerous studies (Wrenzycki *et al.* 2005, Lonergan *et al.* 2006, Wrenzycki *et al.* 2007, Duranthon *et al.* 2008), and these studies indeed provided valuable information about bovine embryonic gene expression, the conclusions that could be drawn from a small number of candidate genes, was rather limited.

Subsequently, cDNA-arrays allowed simultaneous quantification of a set of transcripts, even in single embryos (Brambrink *et al.* 2002). These cDNA microarrays were used to compare differential expression between in vivo and in vitro derived bovine blastocysts (Corcoran *et al.* 2007) and between bovine blastocysts that had developed in different culture systems (Hoelker *et al.* 2009, Cote *et al.* 2011). Accordingly, we have conducted a study to examine the effect of alternative in vivo and in vitro culture conditions at the time of major embryonic genome activation on developmental rates and transcriptome profiles of bovine blastocysts (Gad *et al.* 2012). Furthermore, cDNA microarrays have been employed to contrast gene expression in embryos derived from somatic cell nuclear transfer compared to "normal" fertilization (Smith *et al.* 2005). Interestingly, that study reported that the global transcriptome of clones was more similar to in vitro derived blastocysts than to in vivo derived blastocysts, whereas the global transcriptome was shown to differ largely between ex vivo and nuclear transfer derived elongated bovine embryos (Betsha *et al.* 2013) as well as in Day 50 placentas (Salilew-Wondim *et al.* 2013).

Nevertheless, it is still a challenge to interpret differences in global transcriptome profiles between pools of embryos classified according to their presumed developmental competence based on phenotype and/or origin. When considering these group-based findings, however, one has to consider that not all embryos of a given phenotype bear the same viability and individual embryos may respond differently to identical culture conditions (Lequarre *et al.* 1997, De Sousa *et al.* 1998, Krussel *et al.* 1998), which might be a reflection of their intrinsic quality. Hence, although the embryo gene expression blueprint may be indicative of subsequent developmental competence, current approaches based on presumed embryo developmental competence are suboptimal for identifying molecular markers that predict the ability of a blastocyst to establish a pregnancy. Moreover, molecular studies are, by their nature, invasive making it difficult to extrapolate the developmental competence beyond the developmental stages examined. As a consequence, new strategies have been designed to identify molecular networks related to future developmental competence of individual bovine embryos.

Molecular signatures for cleavage stage embryo survival to blastocyst stage

Recently, a strategy for unravelling molecular signatures indicative of developmental capacity of early bovine embryos to the blastocyst stage, without compromising the embryo's subsequent developmental competence, has been reported by our group (Held *et al.* 2012). Briefly, separated blastomeres in a two-cell embryo were classified according to their sister blastomere's development (Figure 3) and were analysed for their global gene expression profile. The results of that study revealed that developmental fates of sister blastomeres were highly correlated. Interestingly, other studies had shown that following blastocyst transfer at Day 7, different types of embryos (in vivo-derived, in vitro-derived, nuclear transfer-derived) elicit different responses from the endometrium around the time of initiation of implantation, and these responses are associated with different developmental outcomes (Madaschi *et al.* 2009, Mansouri-Attia *et al.* 2009). In other words, each sister blastomere represents one side of the same coin being also master of its own destiny even after isolation. Microarray analysis revealed a total of 632 genes that were differentially regulated between blastomeres, whose sister blastomeres reached the blastocyst stage and those that did not cleave following separation. The primary pathways that differed were related to oxidative phosphorylation and NRF2-mediated stress
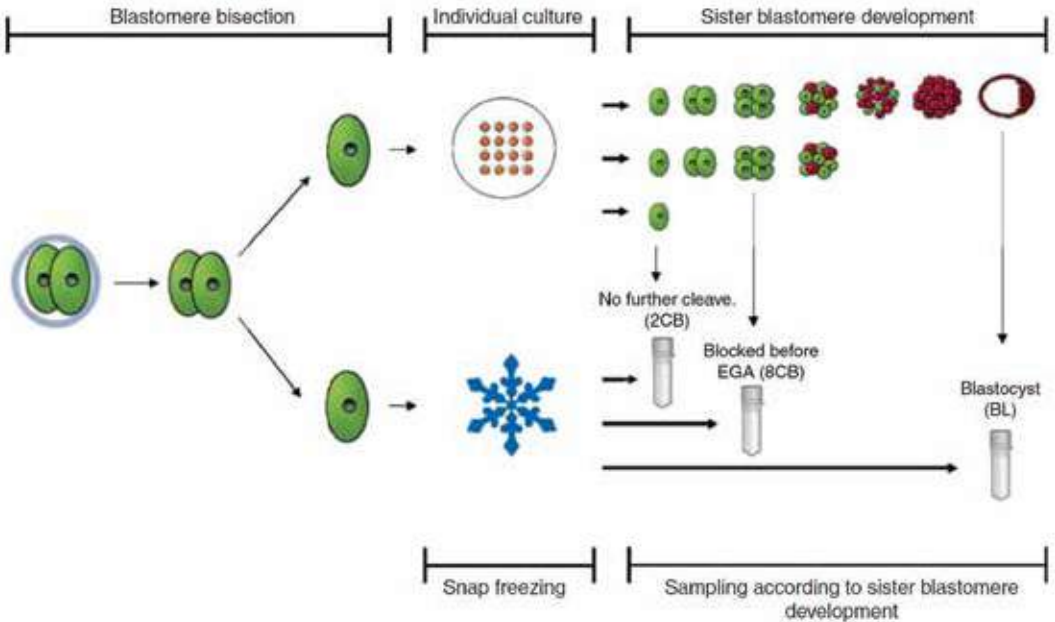
Figure 3: Single blastomeres derived from bisected 2-cell stage embryos were cultured individually, whereas the corresponding sister blastomeres were frozen and stored individually. Frozen stored blastomeres were pooled according to the subsequent development of their corresponding sister blastomeres into three groups: (1) corresponding sister blastomeres that did not cleave anymore after bisection (2-cell block, 2CB); (2) corresponding sister blastomeres that stopped development after two additional rounds of cell cleavage as 4-cell embryos following bisection (8-cell block, 8CB); and (3) corresponding sister blastomeres that developed to the blastocyst (BL) stage (Held *et al.* 2012; reproduced with permission).

response. Similarly, 150 genes were differentially regulated between samples associated with blastocyst development and those whose sister blastomeres stopped cleaving before embryonic genome activation (Held *et al.* 2012), with the preceding genes primarily also related to the NRF2-mediated stress response pathway. Collectively, both comparisons showed an overlap of a total of 77 genes. Clustering of these differentially expressed genes according to molecular functions and pathways revealed antioxidant activity, NRF2-mediated oxidative stress response, and oxidative phosphorylation to be the main ontologies affected. Subsequently, expression of selected candidate genes playing a role in oxidative phosphorylation and oxidative stress response (*NDUFS1, MAPK14, CAT, PRDX1* and *PRDX6*) was validated in early and late cleaving two-cell embryos representing an independent model for developmental competence with early cleaving embryos presumed to be of higher developmental competence.

Molecular signatures for blastocyst survival to term

To correlate molecular signatures at the blastocyst stage with the ability of the embryo to establish a pregnancy, a novel model was established in which a biopsy was taken from a blastocyst, followed by transfer of the biopsied blastocyst to a recipient (El-Sayed *et al.* 2006). The preceding approach allowed analysis of gene expression profiles of in vitro-produced Day-

7 blastocyst biopsies based on the pregnancy outcome after transfer of the biopsied blastocyst to recipients (Figure 4). Biopsies from embryos resulting in calf delivery showed enrichment of 52 differentially expressed transcripts involved in pathways associated with implantation, carbohydrate metabolism, growth factors and signal transduction. In contrast, biopsies of embryos that did not result in pregnancy showed enrichment of transcripts involved in inflammatory cytokines, protein amino acid binding, transcription factors and glucose metabolism. Using biopsies of in vivo-derived Day 7 blastocysts (Ghanem *et al.* 2011), our studies revealed a total of 41 genes that were differentially expressed between biopsies associated with no pregnancy and those associated with calf delivery. Biopsies that were associated with failure to establish a pregnancy showed enrichment of transcripts related to tumor necrosis factor, protein binding, RNA binding, calcium ion binding, and oxidoreductase activity; whereas, transcripts related to protein binding, DNA binding, structural component of ribosome, growth factor activity, and signal transducer activity were found to be highly expressed in biopsies associated with calf delivery. Genes involved in oxidoreductase activity, structural component of ribosome, ATPase inhibitor activity, protein binding, calcium ion binding, and nucleotide binding were up regulated in embryo biopsies resulting in resorption; whereas, biopsies resulting in calf delivery were enriched with transcripts regulating signal transducer activity, cell redox homeostasis, DNA binding, growth factor activity, and protein binding. Interestingly, there was little variation in the expression profile between samples that resulted in no pregnancy and those that ended
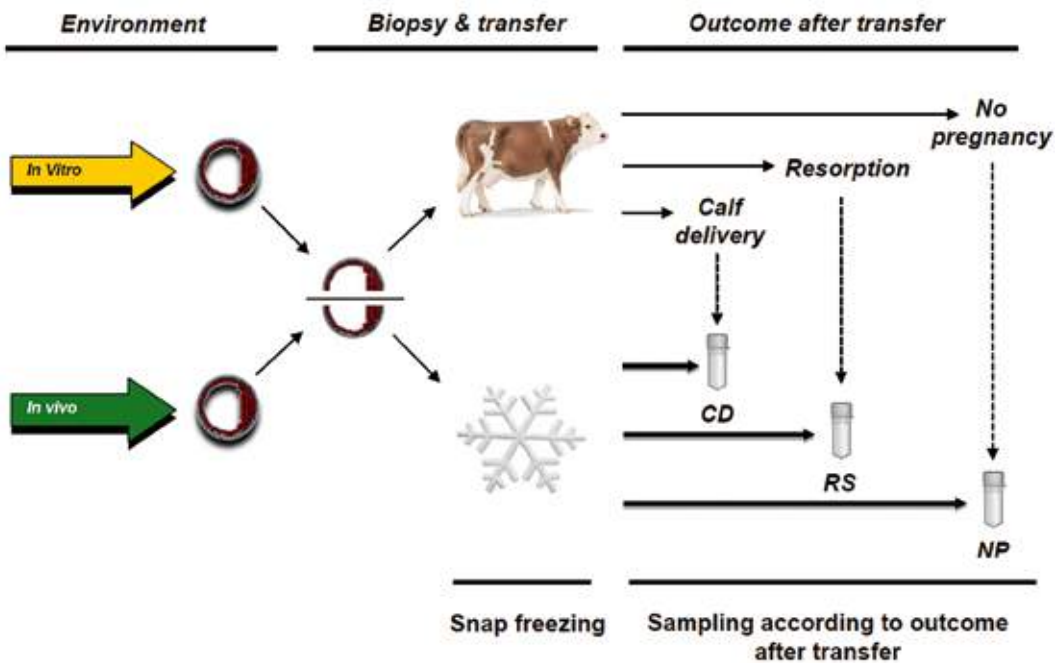
Figure 4. Overall experimental strategy to correlate blastocyst's molecular signature with its viability after transfer to recipient. To accomplish that, blastocysts (in vivo-derived and in vitro-derived) were split. One half was transferred to synchronized Simmental heifers followed by later pregnancy check, whereas the other proportion was immediately snap frozen. Later, frozen samples were categorized according to the pregnancy outcome of the transferred corresponding proportion, namely into Calf delivery (CD), initial pregnancy being terminated by day 42 termed Resorption (RP) and No pregnancy (NP), for global gene expression analysis (El-Sayed *et al.* 2006).

up in resorption indicating high molecular similarity. Consequently, both sets of differentially expressed genes were combined to an aggregate signature of a blastocyst which failed to result in a calf. Importantly, comparison of this aggregate signature related to in vivo derived blastocysts with a similar signature derived from to in vitro derived embryos (El-Sayed *et al.* 2006) identified an overlap of 21 genes. Of these, 18 genes displayed an identical direction of expression (Table 1) and functional annotation analysis revealed involvement in biological functions related to protein binding & synthesis, oxidative stress & energy metabolism and mitochondrial function (Ghanem *et al.* 2011). Thus in vivo and in vitro derived blastocysts with term developmental competence share a similar molecular signature although derived from different environments.

Conclusion

With respect to endometrial receptivity, the present survey provides a comprehensive inventory of functional networks and genes expressed in the bovine endometrium representing the maternal part of the embryo-maternal cross-talk. Distinct molecular signatures of the bovine endometrium could be correlated with pregnancy success in the next ovulatory cycle. For

Table 1: Molecular signatures of bovine blastocysts related to pregnancy success

Genes	Gene function	*In vivo* biopsies			*In vitro* biopsies		
		No Pregnancy	Resorption	No Calf	No Calf	No Pregnancy	Resorption
PTTG1	Transcription factors		↑	↑	↑	↑	
KRT8	Protein binding and synth.	↓	↓	↓	↑↓	↓	↑
RPLP0	Protein binding and synth.	↓	↓	↓	↑↓	↓	↑
RPL3	Protein binding and synth.		↑	↑	↑		↑
RPS15A	Protein binding and synth.		↑	↑	↑		↑
COQ7	Protein binding and synth.		↓	↓	↑	↑	↑
EEF1A1	Protein binding and synth.	↑	↑	↑	↑	↑	
CD9	Protein binding and synth.		↑	↑	↑	↑	
HSPD1	Protein binding and synth.	↑	↑	↑	↑		↑
H2FAZ	DNA binding	↓	↓	↓	↓		↓
BMP15	Growth factor	↓	↓	↓	↓		↓
S100A14	Ion binding	↑	↑	↑	↑	↑	
TXN	Metabolism/Oxidative stress		↓	↓	↓	↓	↓
AKR1B1	Metabolism/Oxidative stress	↑		↑	↑	↑	↑
PGHS-2	Metabolism/Oxidative stress	↓	↓	↓	↓		↓
PLAC8	Tissue specific		↓	↓	↓		↓
TNF-α	Immune response	↑		↑	↑	↑	
FL405	Mitochondrial transcripts	↑	↑	↑	↑	↑	
Fl396	Mitochondrial transcripts		↑	↑	↑		↑
NADH	Mitochondrial transcripts	↑		↑	↑		↑
B2M	unknown	↓		↓	↓		↓

Up- (↑) and down-regulated (↓) genes in No pregnancy, Resorption and aggregate No Calf groups relative to calf delivery considering both in vivo- and in vitro-derived blastocysts
(El-Sayed *et al.* 2006, Ghanem *et al.* 2011)

instance, molecular networks related to cell-to-cell communication, mitochondrial metabolic homoeostasis, tissue expansion as well as inflammatory immune mediators have been shown to contribute to improved receptivity and pregnancy success.

With respect to embryo quality, new strategies to connect molecular signatures with developmental capacities have been developed. Firstly, large scale differences at the molecular level were identified for blastomeres whose counterparts developed to the blastocyst stage compared to those with lower developmental competence thus unraveling distinct molecular fingerprints related to developmental potential. Secondly, molecular signatures of in vivo and in vitro derived blastocysts that were correlated with embryo developmental competence to term were identified, revealing a common genetic signature. Finally, further understanding of molecular networks related to embryo developmental capacity and endometrial receptivity has been gained from novel innovative strategies including endometrial samples as well as embryo microdissection and blastocyst biopsies.

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