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Genes of the transforming growth factor-beta signalling pathway are associated with pre-implantation embryonic development in cattle

Geng Li[1,2], Karam Khateeb[2], Erin Schaeffer[2], Bao Zhang[3] and Hasan Khatib[2]*

[1] College of Animal Science and Technology, China Agricultural University, Beijing 100193, China
[2] Department of Animal Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA
[3] College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China

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One of the main factors affecting cattle fertility is pre-implantation development of the bovine embryo, which is a complex process regulated by various signal-transduction pathways. The transforming growth factor-β (TGF-β) signalling system, which is responsible for many biological processes including cell proliferation, differentiation and apoptosis, also is involved in embryo development. We hypothesized that altered expression of TGF-β genes in pre-implantation bovine embryos is associated with morphological abnormalities of these embryos. To test this hypothesis, we produced embryos *in vitro* and classified them at the blastocyst stage as either normally developed blastocysts or degenerates (growth-arrested embryos). The expression patterns of 25 genes from the TGF-β pathway were assessed using quantitative real time PCR. Ten genes showed differential expression between the two embryo groups, four genes displayed similar expressional profiles, and 11 genes had no detectable expression. An altered expression profile was statistically significant for 10 of the 14 expressed genes, and all were up-regulated in degenerate embryos vs. blastocysts. Furthermore, genomic association analysis of the cows from which embryos were produced revealed a significant association of ID3 and BMP4 polymorphisms—two of the most significant differentially expressed genes—with fertilization rate and blastocyst rate, respectively. Taken together, we conclude that TGF-β pathway genes, especially BMP4 and ID3 play a vital function in the regulation of pre-implantation embryo development at both embryo and maternal levels. Hence, these genes may be suitable as genetic markers for embryo development and fertility in cattle.

Keywords: Transforming growth factor-β, bovine, pre-implantation embryo, embryo survival.

Reproductive deterioration in high-producing dairy cows has caused substantial economic loss to the dairy cattle industry (Lucy, 2007). Two of the key factors decreasing fertility of dairy cow are low fertilization rate and early embryonic mortality (Royal et al. 2000; Sheldon et al. 2006), two abnormalities that occur during pre-implantation embryo development. Although genetic factors affect reproductive performance (Shook, 2006), identification of specific genes has been a challenge, probably due to the low accuracy of fertility data collected in the field and to the low heritability of these traits (VanRaden et al. 2004). Thus, understanding the genetic regulation of bovine pre-implantation development and identifying associated biomarkers are becoming progressively essential to improve dairy cattle fertility.

To investigate the relationship between genetic factors and pre-implantation embryo development in cattle, an *in-vitro* fertilization (IVF) system was created in our laboratory. This system enables us to identify genetic factors affecting fertilization and early embryonic development at both the maternal and embryo levels. At the maternal level, our IVF system has been used to uncover associations of cows' genotypes with fertilization success and blastocyst rate of embryos produced from these cows (Khatib et al. 2008a, b, 2009a, b; Driver et al. 2009; Wang et al. 2009; Huang et al. 2010a). At the embryo level, differential gene expression between normal blastocysts and degenerate embryos has been investigated by applying RNA-Seq

*For correspondence; e-mail: hkhatib@wisc.edu

(Huang & Khatib, 2010), microarray expression (Huang et al. 2010b) and candidate gene and pathway analyses (Laporta et al. 2011; Zhang et al. 2011).

The transforming growth factor-β (TGF-β) signalling pathway has long been acknowledged for signal transduction and other intracellular activities, such as cell division, differentiation, migration, apoptosis and transformation (Santibanez et al. 2011). In addition, several studies have suggested involvement of the TGF-β signalling cascade and its components in pre-implantation embryo development as well as ovarian function (Shimasaki et al. 2004; Zhang et al. 2007). For example, Assou et al. (2010) recently reported that known members of TGF-β pathway showed dynamic changes in gene expression profiles during the three early stages of human embryonic development, including oocytes, day-3 embryos, and human embryonic stem cells on day 7. Also, results from the mouse showed that multiple bone morphogenetic proteins (BMPs) and SMAD6 in the TGF-β pathway were expressed in a stage-specific pattern and were developmentally regulated in oocytes and pre-implantation embryos (Wang et al. 2004). BMPs and GDF9, also members of TGF-β, have been reported to be crucial regulators of folliculogenesis in mouse models (Trombly et al. 2009; Otsuka et al. 2011).

It is worth mentioning that most reported data on TGF-β pathway genes have been generated in the mouse model, and there is little information on other species such as cattle. Interestingly, in a recent transcriptomic study of the bovine IVF system, significance analysis of microarrays (SAM) identified 67 transcripts differentially expressed between blastocysts and degenerative embryos, of which 33 showed at least a two-fold difference (Huang et al. 2010b). To further identify signalling pathways associated with embryonic development, Gene Set Enrichment Analysis (GSEA) and gene ontology (GO) enrichment analysis were carried out. The TGF-β pathway was found to be up-regulated in degenerate embryos compared with blastocysts using microarrays (Huang et al. 2010b). However, several genes from this pathway were not included in the microarray analysis. Given that the TGF-β signalling pathway has a crucial role in ovarian and embryonic development, we hypothesized that correct balance of expression of genes from this pathway is needed for proper pre-implantation development of IVF embryos. Also, there is limited information on the extent of contributions of maternal and embryonic genomes to the survival of the developing embryo. Therefore, the present study aimed to investigate the expression profiles of TGF-β genes in degenerate embryos and normal blastocysts and to evaluate the association between maternal genotypes of these genes and fertility traits such as fertilization success and blastocyst rate.

Materials and Methods

Two experiments were performed to assess involvement of the TGF-β pathway genes in embryo development and fertility traits. In the first experiment, we compared the expression profiles of the TGF-β pathway genes in two populations of embryos differing in their morphology and development. The most significant differentially expressed genes between the embryo groups were tested in the second experiment for genomic association with fertility traits in a cow population.

Expression profiles of TGF-β pathway genes in cattle embryos

Embryo production and morphological classification. Oocytes were aspirated from ovaries obtained from a local slaughterhouse and underwent maturation until they were combined with frozen-thawed semen. The procedures of *in-vitro* fertilization and subsequent embryo culture were as described in Khatib et al. (2008a, b). Embryos that showed signs of cellular compaction by day 5 of culture (morula stage) were further cultured until day 8. Embryos failing to show signs of compaction were excluded from further analysis. Embryos that exhibited a clear inner cell mass and a fluid-filled cavity (blastocoele) on day 8 were classified as blastocysts, and those with abnormal blastocyst formation and morula-phenotype were classified as degenerates. Embryos randomly collected from each morphological group ($n=20$) were pooled and preserved in RNA later (Ambion, Austin, TX). Three sets (blastocysts and degenerates) of embryo pools were used in the RNA expression analysis, in which two sets of biological replicate pools were produced from one sire, and one set of embryos was produced from a second sire.

Real-time RT-PCR quantification. Total RNA was extracted from pools of embryos using RNaqueous Micro (Ambion) and quality controlled by a RNA6000 PicoChip (Agilent Technologies, Santa Clara CA, USA). The mRNA amplified by MessageAmp II (Ambion) was converted to cDNA using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA). Dilution of cDNA was used as template in qRT-PCR with the iQ SYBR Green Supermix kit (Bio-Rad Laboratories). The reference gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was selected as an endogenous control as described by Huang et al. (2010b). In brief, the housekeeping genes ribosomal protein large P0 (*RPLPO*), actin, beta (*ACTB*) and *GAPDH* were tested for expression stability across embryo samples examined for quantitative gene expression. The expression of *GAPDH* embryo was markedly invariable across embryo samples. Primers for 25 genes from the TGF-β pathway (Table S1) [Table S1–S3 are available online as Supplementary Material on Cambridge Journals Online (http://journals.cambridge.org)] were designed to amplify fragments that span at least one intron to avoid genomic DNA contamination using the Beacon Designer software (Premier Biosoft International, Palo Alto, CA, USA). Each sample was tested in quadruplicate. The relative quantification of gene

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expression was performed using $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

Gene selection. In a previous study, the GO analysis revealed up-regulation of the TGF-β pathway in degenerate embryos compared with blastocysts using microarray expression analysis (Huang et al. 2010b). The dataset is available at GEO with the accession number GSE24936. The genes *INHBA, DCN, ID3, THBS2, TGFBR2, PPP2R1A, THBS4, BMP2, BMP4, PITX2, SMAD2, RPS6KB2, ACVR1, BMPR1A, BMPR1B and TGFBR1* were found to be present in the microarray. Although not represented in the array, the genes *BMP3, SMAD1, SMAD6, LEFTY, ACVR1B*, and *ACVR1C* were selected for qRT-PCR because they are part of the TGF-β pathway. The genes *GDF9, BMP15*, and *BMPER* were selected because they are members of the TGF-β family.

Association study of TGF-β pathway genes with fertilization and blastocyst rates

To further evaluate effects of the differentially expressed genes on fertilization rate and blastocyst rate, maternal genotypes were tested for association with these traits. The genes *BMP4, THBS2*, and *ID3* were selected because they showed the most significant fold differences in expression between blastocysts and degenerates.

Phenotypic data. Oocytes were collected from a total of 496 ovaries obtained from 496 Holstein cows and fertilized by semen from 12 Holstein bulls. For each cow, fertilization rate was defined as the number of cleaved embryos at day 2 post-fertilization divided by the total number of fertilized oocytes collected from one ovary. Blastocyst rate was defined as the number of embryos that reached the blastocyst stage (day 8) out of the total number of cultured embryos. A total of 7865 fertilizations were performed and a total of 5270 embryos were produced to generate fertilization and blastocyst rate data.

Polymorphism identification and genotyping. DNA was isolated from ovaries ($n=496$) using standard phenol/chloroform protocols. DNA concentrations were measured using an Ultraspec 2100 spectrophotometer (Amersham Biosciences, Piscataway, NJ, USA). For single nucleotide polymorphism (SNP) identification, one DNA pool was constructed from 20 random ovary samples containing equal amounts of DNA from each (25 ng/μl). The DNA pool was amplified by 12 sets of primers designed from the exons of the three candidate genes (Table S1). Amplification was performed in a 25-μl reaction volume, which included 25 ng genomic DNA, 50 ng each primer, 200 μM each dNTP, 2·5 μl 10 × PCR buffer (Promega, Madison, WI, USA), and 0·5 μ Taq DNA polymerase (Promega). The PCR products of the pooled DNA samples were sequenced using BigDye terminator (Applied Biosystems, Foster City, CA, USA) and SNPs were identified by visually inspecting sequence traces. For genotyping, three sets of primers (Table S1) were designed in *ID3, BMP4* and *THBS2*. The PCR products of SNP rs109818980 (*ID3*), rs109778173 (*BMP4*), and rs110619673 (*THBS2*) were digested with the restriction enzymes *Fau*I, *Hin*fI and *Tai*I, respectively and electrophoresed on a 2·0% agarose gel. The patterns of genotypes obtained for each restriction enzyme are presented in Table S2.

Statistical analysis

For expression analysis, normalized gene expression values (ΔCt) were analysed using a general linear model as follows:

$$y_{ijk} = \mu + b_i + p_j + \text{embryo}_{ijk} + e_{ijk} \quad (1)$$

where y_{ijk} is the normalized gene expression value (ΔCt) of sample k from pool j fertilized by bull i; μ represents an overall mean for the trait considered; p_j is the random effect of pool j; b_i is the fixed effect of bull i; embryo_{ijk} is the fixed effect of the embryo type; and e_{ijk} represents the residual. Association between the normalized gene expression and the type of embryo was tested using a likelihood ratio test by comparing model (1) to a reduced model without the embryo effect. The mean and the range of the fold change for each gene were calculated as $2^{-\Delta\Delta Ct}$ using the estimated $\Delta\Delta Ct$ value ± SE. The analysis was performed by the LME4 package in R software.

The association of SNP genotypes with fertilization or blastocyst rate was tested using the following mixed linear model,

$$y_{ijk} = \mu + o_i + b_j + \text{SNP}_{ijk} + e_{ijk} \quad (2)$$

where y_{ijk} represents the fertilization rate or embryo survival rate of oocyte k from ovary i fertilized by bull j; μ represents an overall mean for the trait considered; o_i is the random effect of the ith ovary from which oocytes were harvested; b_j represents the random effect of the sire used in the fertilization; SNP_{ijk} represents the fixed effect of the genotype for the SNP considered; and e_{ijk} represents the residuals. Ovary and bull variables were assumed to be uncorrelated. Association between fertilization rate or embryo survival rate and SNP genotype was analysed by the MIXED procedure of SAS (9.0).

Results

Two separate and complementary experiments were done in this study to investigate the roles of TGF-β genes in fertility traits in cattle. In the first experiment, we assessed and compared expression profiles of these genes in degenerate embryos that do not make a complete transition to blastocysts vs. embryos that reach the blastocyst stage in a timely manner. In the second experiment, we tested the

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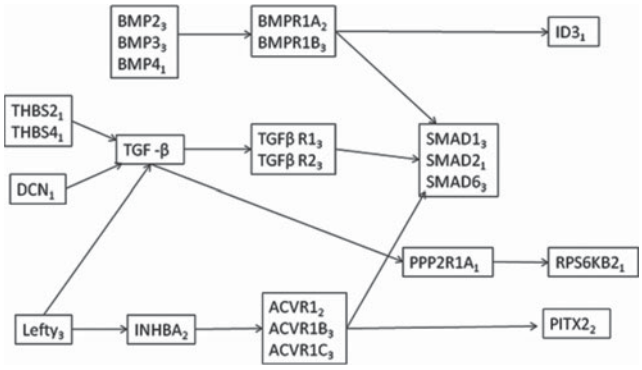

Fig. 1. Genes of TGF-β pathway investigated in the current study. [1]Genes that showed differential expression between the embryo groups. [2]Genes expressed in both blastocysts and degenerate embryos, but did not show significant differential expression. [3]Genes not detectable in pre-implantation embryos using qRT-PCR.

effects of the dams' genotypes on the fertilization and blastocyst rates of their embryos.

Differential expression of the TGF-β pathway genes

A total of 25 genes from the TGF-β pathway were evaluated for their expression patterns in blastocysts and degenerate embryos; 14 genes were expressed and 11 genes were not detectable in embryos examined (Fig. 1). Figure 2 shows differential expression of the 14 expressed genes in embryos. Expression of all examined genes, except for *ACVR1*, was higher in degenerate embryos than in blastocysts. The mRNA expression levels of the following genes were significantly increased in degenerate embryos: DNA-binding protein inhibitor 3 (*ID3*), thrombospondin-2 (*THBS2*), bone morphogenetic protein 4 (*BMP4*), growth differentiation factor-9 (*GDF9*), BMP binding endothelial regulator (*BMPER*), and decorin (*DCN*) (Table S3). Moreover, lesser fold differences in expression, but still statistically significant, were observed for SMAD family member 2 (*SMAD2*), thrombospondin-4 (*THBS4*), protein phosphatase 2, regulatory subunit A, alpha (*PPP2R1A*) and ribosomal protein S6 kinase, 70-kDa polypeptide 2 (*RPS6KB2*). However, the effect of embryo group was not statistically significant for the expression levels of BMP receptor, type 1A (*BMPR1A*), paired-like homeodomain 2 (*PITX2*), inhibin, beta A (*INHBA*) and activin A receptor, type 1 (*ACVR1*) with fold change ranging from 1·16 to 2·64. Expression of *BMP2, BMP3, BMP15, BMPR1B, SMAD1, SMAD6, TGF-βR2, TGF-βR1, ACVR1B, ACVR1C*, and *LEFTY2* was not detected in cattle embryos.

Association of differentially expressed genes with fertilization rate and blastocyst rate

Genes with the most significant differential expression between embryo types (*ID3, GDF9, BMP4*, and *THBS2*) were further investigated for association analysis of cows' genotype with fertilization and blastocyst rates. Using the pooled DNA sequencing method in the ovary/cow population, SNPs were identified in *ID3, BMP4*, and *THBS2*. No SNPs were detected in *GDF9*. The SNPs, rs109818980, rs109778173 and rs110619673, are located in the 3′-untranslated region (3′-UTR) of *ID3*, the coding region (CDS) of *BMP4*, and the 3′-UTR of *THBS2*, respectively. SNPs were in Hardy–Weinberg equilibrium (Table 1). Estimates of the three genotypic classes in each SNP for blastocyst and fertilization rate and relevant P values are given in Table 1. Analysis of SNP rs109818980 in *ID3* revealed a significant association with fertilization rate ($P=0{\cdot}029$). Oocytes from genotypes TT ovaries had 5·2 and 5·3% lower fertilization rates than those from TC and CC ovaries, respectively (Table 1). Blastocyst rate was significantly associated with SNP rs109778173 of *BMP4* ($P=0{\cdot}006$), whereas the association with fertilization rate was not statistically significant ($P=0{\cdot}095$). Embryos produced from genotype TT cows showed 10·5 and 16·1% higher blastocyst rates than GG and GT cows, respectively (Table 1). For SNP rs110619673 of *THBS2*, no significant associations were found with the examined traits (Table 1).

Discussion

In the current study, we explored involvement of the TGF-β pathway in development of pre-implantation bovine embryos at both the embryonic and maternal levels. At the embryo level, we found that some genes of the TGF-β pathway were up-regulated in the growth-arrested embryos compared with normal blastocysts. At the maternal level, two genes, found differentially-expressed in embryos, showed significant association with fertilization and blastocyst rates.

Little is known about the expression pattern of the TGF-β genes and their functions in the developing bovine embryo. Interestingly, the 14 genes that were expressed in pre-implantation embryos represent all components of the signalling cascade of TGF-β pathway including ligands (*BMP4, GDF9*, and *INHBA*); receptors (*BMPR1A* and *ACVR1*); SMAD proteins (*SMAD2*); upstream regulators (*THBS2, THBS4, DCN*); and downstream regulators (*ID3, BMPER, PPP2R1A, RPS6KB2, PITX2*). The signalling process of this pathway necessitates coordination of gene regulation among the different members of the pathway. For example, the activation of latent TGF-β requires binding of the thrombospondin-1 (*THBS1*) to the TGF-β precursor complex (Murphy-Ullrich & Poczatek, 2000). Protein phosphatase 2 (PPP2) and ribosomal protein S6 kinase (RPS6K) are key regulators implicated in the phosphorylation of receptor and SMADs in the TGF-β signalling cascade (Zolnierowicz, 2000; Fenton & Gout, 2010). ID proteins are direct targets of BMP and TGF-β signalling, which serve as essential mediators in biological responses in the downstream pathway (Miyazono & Miyazawa, 2002). This cell distribution and coordination of gene expression among the TGF-β genes

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Table 1. *P*-values of the Hardy-Weinberg equilibrium (HWE) test, estimate of blastocyst rate (± SE), and estimate of fertilization rate (± SE) for the ovary SNP genotypes in ID3, BMP4, and THBS2

			Blastocyst rate		Fertilization rate	
Gene/SNP	Genotype†	HWE *P*-value	Estimate ± SE	*P*-value	Estimate ± SE	*P*-value
ID3/ rs109818980	TT(237)	0·089	0·338 ± 0·022	0·247	0·631 ± 0·028	0·029
	TC(154)		0·304 ± 0·024		0·683 ± 0·029	
	CC(38)		0·371 ± 0·044		0·684 ± 0·041	
BMP4/rs109778173	GG(243)	0·607	0·349 ± 0·019	0·006	0·675 ± 0·027	0·095
	GT(162)		0·293 ± 0·022		0·635 ± 0·028	
	TT(23)		0·454 ± 0·053		0·625 ± 0·046	
THBS2/rs110619673	CC(251)	0·424	0·332 ± 0·022	0·371	0·652 ± 0·028	0·957
	TC(147)		0·304 ± 0·026		0·657 ± 0·030	
	TT(26)		0·369 ± 0·054		0·660 ± 0·046	

† In parenthesis is the number of cows genotyped

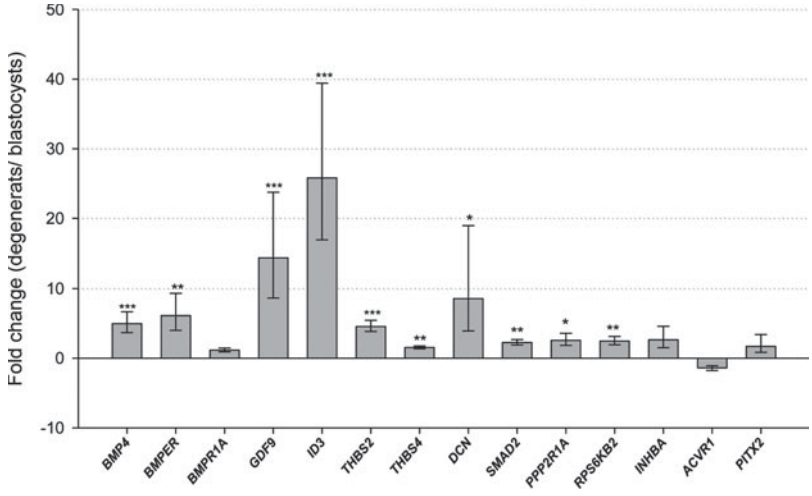

Fig. 2. Differential expression of genes in the TGF-β pathway is represented as mean ± maximum and minimum fold changes in degenerate embryos vs. blastocysts using qRT-PCR. Up-regulation in degenerative embryos or blastocysts is shown by bars above or below the *x*-axis, respectively. The qRT-PCR was performed in three sets of biological replicates of blastocysts and degenerate embryos. *$P \leqslant 0{\cdot}05$; **$P \leqslant 0{\cdot}01$; ***$P \leqslant 0{\cdot}001$.

testifies to the significance of this pathway in embryo development.

Furthermore, expression of many members of the TGF-β superfamily in the endometrium suggests a pivotal role for these genes in differentiation of the endometrium and the implantation process (Jones et al. 2006). Consequently, expression of TGF-β genes in pre-implantation bovine embryos suggests an important role for these genes in the embryo-uterus connection. Indeed, it has been reported that blastocysts express TGF-β proteins that induce apoptosis of endometrial epithelial cells during implantation (Jones et al. 2006). In addition, Chow et al. reported the presence of mRNAs and proteins of TGF-β receptors in the oviduct and uterus from day 1 to day 4 of pregnancy, which in turn suggests important roles of TGF-β signalling in the interaction between pre-implantation embryos and the reproductive tract (Chow et al. 2001). Collectively, expression of TGF-β signalling genes in the bovine embryo suggests an important role for the TGF-β pathway in the pre-implantation stage of bovine embryo.

In a previous study, we reported a significant differential expression of the TGFβR3 gene, a member of the TGF-β signalling pathway, in pre-implantation embryos (Huang et al. 2010b). To further investigate the role in blastocyst formation of the TGF-β genes, we examined their expression profiles in normally developed blastocysts compared with growth-arrested embryos produced from the same parents and cultured at the same laboratory conditions. Interestingly, of the 14 expressed genes, 10 showed statistically significant expression differences between the embryo types, of which all were found to be up-regulated in the degenerate embryos. Although it is unknown at this point whether the differential expression is causative to the morphological degeneration or a result of this degeneration, there is accumulating evidence that balanced gene expression is likely to be essential for early embryogenesis (Rodriguez-Zas et al. 2008).

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Members of BMP signalling subfamily, such as *BMP4, BMPER, BMPR1A* and *ID3* were found to be highly expressed in degenerate embryos compared with blastocysts (Fig. 2). These results are consistent with earlier studies that reported their function in maintaining pluripotency in the inner cell mass of bovine blastocysts (Pant & Keefer, 2009). Of particular interest are the findings of Koide et al. (2009) who demonstrated that over-activity of BMP4 signalling led to excessive apoptosis in early mammalian embryo development. Also, La Rosa et al. (2011) reported that supplementation of BMP4 to culture medium of IVF embryos decreased blastocyst production, and concluded that a balanced BMP signalling activity is required for proper pre-implantation development of cattle embryos.

The ID proteins function as key regulators of development by stimulating and maintaining proliferation and preventing premature differentiation (Yokota & Mori, 2002). Given that ID expression can be regulated by other members of the TGF-β pathway such as BMPs (Hogg et al. 2010), the up-regulation of *ID3* in degenerate embryos (Fig. 2) provides strong evidence of the role of these genes in early embryonic development. BMPER is a BMP-binding endothelial regulator and has been reported to modulate BMP4 signalling in endothelial cell differentiation and angiogenesis (Moser et al. 2003; Heinke et al. 2008). Taken together, we conclude that altered expression of the BMP signalling subfamily in pre-implantation bovine embryos can lead to or be used as a marker for abnormal embryonic development.

Although the roles in bovine embryo development of other differentially expressed genes are unknown, they have critical functions in the TGF-β signalling. For example, SMAD2 belongs to the SMAD family of proteins, which are transducers of TGF-β signalling from the cell surface to the nucleus and transcription factors mediating expression of target genes in the TGF-β cascade (Heldin et al. 1997; Massague et al. 2005). SMAD proteins are required for pluripotency maintenance of the inner cell mass in mouse blastocysts (James et al. 2005). Thus, considering the altered expression of these regulators in pre-implantation embryos observed in this study and their roles in the activation and signalling of TGF-β pathway components, we propose that they have significant functions in regulating proper development of bovine embryos.

To explore the impact of the TGF-β signalling pathway on early IVF embryo development and hence fertility traits, four genes that showed the most significant expression differences between embryo groups were tested for SNP association with fertilization and blastocyst rates. The association analysis was done using an IVF experimental system that has been recently developed to identify genes affecting fertilization and embryo development in cattle (Khatib et al. 2008a, b, 2009a, b; Driver et al. 2009; Wang et al. 2009; Huang et al. 2010a; Laporta et al. 2011). Allelic variants of *THBS2* did not show any significant association with fertility traits in our IVF system.

A significant association was observed between *ID3* maternal genotypes and fertilization rate. This result is consistent with a previous genome-wide association study, in which a SNP associated with fertilization rate was located within 50 kb distance of *ID3* (Huang et al. 2010a). Although the molecular regulation of fertilization success is not fully understood, maternal genome activity and oocyte quality appear to have critical roles in embryogenesis (Stitzel & Seydoux, 2007; Marteil et al. 2009). Recently, Hogg et al. (2010) observed that four ID isoforms (ID1-4) were expressed across ovine ovarian follicle development and possibly regulated by TGF-β signalling via SMADs. Consequently, the authors suggested mechanistic roles of the ID proteins in mammalian oocyte development (Hogg et al. 2010). Furthermore, ID proteins are key regulators for many cellular processes, such as cell proliferation, differentiation and cell cycle progression, which in turn are required for oocyte maturation, oocyte-to-embryo transition, and embryogenesis (Norton, 2000; Stitzel & Seydoux, 2007). Together, we propose that *ID3* affects fertilization rate through maternal genome effects that control oocyte quality and oocyte-to-embryo transition.

Maternal genotypes of *BMP4* were found to be significantly associated with blastocyst rate, suggesting its role in controlling intrinsic oocyte competence and development up to the blastocyst stage. Indeed, it has been acknowledged that blastocyst yield can be affected by intrinsic oocyte quality (Rizos et al. 2002), and the involvement of *BMP4* in ovarian function has been extensively reported (Shimasaki et al. 1999). The spatio-temporal expression of BMP4 signalling in follicle development has been broadly observed across different species including human, rat, bovine, swine and zebrafish (Nilsson & Skinner, 2003; Shimizu et al. 2004; Fatehi et al. 2005; Li & Ge, 2011; Tanwar & McFarlane, 2011). Functional studies have also shown that *BMP4* suppresses bovine granulose cell apoptosis and promotes follicle survival and development in rats (Nilsson & Skinner, 2003; Kayamori et al. 2009). Collectively, the differential expression of *BMP4* in embryos and the significant association of maternal genotypes with blastocyst rate indicate that this gene could regulate pre-implantation embryo development, not only through the embryo proper, but also through the maternal genome.

In summary, the findings of this study indicate that a proper gene expression level of the TGF-β genes is required for normal IVF blastocyst development and that TGF-β signalling is likely to affect pre-implantation development of IVF embryos. There is limited information on the contributions of the genomes of dams and embryos to the development and survival of pre-implantation embryos. Results of the present study indicate that, for some genes, both embryonic and maternal genomes are required to ensure proper development. Although the mechanism by which the TGF-β signalling pathway regulates early embryonic development remains unknown, potential genetic markers such as *ID3* and *BMP4* have been identified and can be used to improve reproductive performance of cattle.

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