Original Article

Expression Patterns of Cell Adhesion Molecules in Bovine Preimplantation Embryos Cultured *in vitro*

Anucha Sathanawongs[1*] Korakot Nganvongpanit[1] Supamit Mekchay[2]

Abstract

This study aimed to investigate gene expression patterns throughout preimplantation embryos and their comparative expression in morphological quality blastocysts using real-time RT-PCR. Bovine oocytes underwent maturation process, fertilized and were cultured *in vitro*. Degree of fragmentation and differentiation were the criteria to categorize day-7 blastocysts as of good and poor quality. All of the embryos from each developmental stage were used to quantify transcript abundance. It was found that E-cadherin was highly expressed in immature and mature oocytes, then it decreased at 2- to 16-cell stages, and later increased in morula and blastocyst stages. The β-catenin transcript was highly abundant up to the 4-cell stage and further down regulated in the later stages. However, the transcript abundance of desmocollin 2 (DSC2) was high only at morula and blastocyst stages. The relative abundance of E-cadherin, β-catenin and DSC2 mRNA in good quality blastocysts were statistically significantly higher than in poor quality blastocysts. Moreover, only the protein expression levels of E-cadherin and β-catenin were significantly higher in good than in poor blastocysts. In conclusion, E-cadherin and β-catenin are highly expressed in good quality bovine blastocysts. Therefore, they can be used as biomarker for blastocyst quality.

Keywords: bovine, β-catenin, desmocollin 2, E-cadherin, preimplantation embryo

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บทคัดย่อ

แบบแผนการแสดงออกทางโมเลกุลของเซลล์ยึดติดในตัวอ่อนโค ระยะก่อนการฝังตัวที่เลี้ยงภายนอกร่างกาย

อนุชา สธนวงศ์[1] กรกฎ งานวงศ์พาณิชย์[1] ศุภมิตร เมฆฉาย [2]*

การทดลองนี้ศึกษาคุณลักษณะการแสดงออกของจีนเซลล์ยึดติดภายในตัวอ่อนระยะก่อนการฝังตัว และเปรียบเทียบการแสดงออกของจีนจากตัวอ่อนที่มีคุณภาพต่างกันด้วยเทคนิค real-time RT-PCR ทำการเลี้ยงไข่อ่อนโคให้สุก ปฏิสนธิและเลี้ยงตัวอ่อนภายนอกร่างกาย ทำการศึกษาการแตกของเซลล์ (fragmentation) และการเปลี่ยนแปลงรูปร่างของตัวอ่อนระยะบลาสโตซีสที่อายุ 7 วันเพื่อประเมินคุณภาพตัวอ่อนเป็นคุณภาพดีและไม่ดี จากการทดลองพบว่า มีการแสดงออกของจีนอีแคดฮีรินมากในไข่อ่อนและไข่ที่ทำให้สุก แล้วลดต่ำลงในตัวอ่อนระยะ 2 เซลล์จนถึงระยะ 16 เซลล์ จากนั้นมีการแสดงออกมากขึ้นในตัวอ่อนระยะมอรูลาและบลาสโตซีส การแสดงออกของจีนเบต้า แคทีนินมีมากในตัวอ่อนระยะ 4 เซลล์ และลดต่ำลงในตัวอ่อนทุกระยะถัดไป อย่างไรก็ตามการแสดงออกของจีนเดสโมโคลลิน 2 แสดงออกเฉพาะตัวอ่อนระยะมอรูลาและบลาสโตซีสเท่านั้น การแสดงออกของจีนอีแคดฮีริน เบต้าแคทีนินและจีนเดสโมโคลลิน 2 ในตัวอ่อนบลาสโตซีสที่มีคุณภาพดีมีมากกว่าในตัวอ่อนที่มีคุณภาพไม่ดีอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ระดับการแสดงออกของโปรตีนอีแคดฮีรินและเบต้าแคทีนินในตัวอ่อนคุณภาพดีมีมากกว่าตัวอ่อนที่มีคุณภาพไม่ดีอย่างมีนัยสำคัญทางสถิติ สรุปได้ว่าการแสดงออกของจีนอีแคดฮีรินและเบต้า แคทีนินจะมีการแสดงออกมากในตัวอ่อนโคที่มีคุณภาพดี ส่งผลให้สามารถใช้จีนทั้งสองนี้เป็นชีวดัชนีสำหรับการประเมินคุณภาพของตัวอ่อนได้

คำสำคัญ: โค จีนเบต้าแคทีนิน จีนเดสโมโคลลิน 2 จีนอีแคดฮีริน ตัวอ่อนระยะก่อนการฝังตัว

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Introduction

There are clear differences between *in vivo* and *in vitro* derived embryos of domestic animals. After the transfer to recipients, embryos from both sources show similar implantation rates whereas embryonic and foetal losses appear to be significantly higher in embryos cultured *in vitro* (Reichenbach et al., 1992). Moreover, about 30% of newborn ruminants show increased body size accompanied by several abnormalities described as large offspring syndrome (Young et al., 1998). Therefore, it has been postulated that conditions provided for *in vitro* culture are rather suboptimal and much effort has currently been concentrated on improving the quality of *in vitro* embryos. The fact that a variety of culture media are used during *in vitro* embryo production makes it very difficult to compare embryo quality and viability. Remarkable differences have also been reported in the pregnancy rate after the transfer of morphologically good and poor quality blastocysts (Balaban et al., 2000). The observed differences in quality between bovine blastocysts are almost certainly related to differences in gene expression in the embryos. Moreover, it is well understood that the culture environment influences the expression of developmentally important genes in the embryo (Wrenzycki et al., 2001; Lonergan et al., 2003).

During embryogenesis, the morphogenetic events of compaction and cavitations are dependent on molecules mediating cell adhesion. Although families of associated molecules are believed to mediate these processes, many gene families have been investigated in the early bovine embryos such as E-cadherin (Aghion et al., 1994; De Vries et al., 2004), β-catenin (Ohsugi et al., 1999; De Vries et al., 2004) and desmocollin family (Collins et al., 1995). E-cadherin is a transmembrane cell surface molecule that involves Ca^{2+}-dependent cell to cell adhesion of cell (Takeichi, 1988). E-cadherin mediated cell to cell adhesion is related to compaction (Fleming et al., 1991) and transcript and protein are reported to be found in the inner cell mass (ICM) and trophectoderm (TE) cells of expanded bovine blastocysts (Shehu et al., 1996; Barcroft et al., 1998). β-catenin, in addition to having a role in cell to cell adhesion, functions as intracellular signalling molecule from the cytoplasm to the nucleus (Willert and Nusse, 1998). Anti-sense mRNA technique, used to show the function on xenopus embryos, has shown that β-catenin also plays

a pivotal role for cell polarization (Heasman et al., 2000). DSC2 is one of the members of the cadherin superfamily, the desmosomal cadherin (Buxton and Magee, 1992; Buxton et al., 1993). The desmosome junction is small and plays a role in the integrity and signalling activity of the tissue layer (Green and Gaudry, 2000). In mouse, desmosomes are formed by 32-cell stage when blastocoel cavitation begins (Fleming et al., 2001).

In current clinical practice, *in vitro* embryos are graded morphologically, based on the synchrony of cleavage, blastomere size, homogeneity of the cytoplasm and presence of fragmented nuclei (Van Soom et al., 2001). The blastocyst formation is dependent on the differentiation of ICM and TE, which are regulated by the embryonic expression and cell adhesion genes of E-cadherin, β-catenin or desmocollin family. The low implantation rate following IVF potentially results in high order multiple pregnancies associated with significantly elevated risks. This can be overcome by selecting the most viable embryos and transferring in a fewer number. The objective of this study was to examine the relative transcripts expression of cell adhesion genes including E-cadherin, β-catenin and DSC2 throughout the developmental stages and their comparative gene expression in good or poor morphology embryos using real-time RT-PCR. Moreover, the protein expression of those genes in good or poor morphology embryos was studied by western blot analysis.

Materials and Methods

Oocyte collection: Bovine ovaries were obtained from a local slaughter house and transported to the laboratory in a thermo flask containing 0.9% saline solution supplemented with Benzylpenicillin/ Dihydrostreptomycin (Streptocombin®, Germany). The cumulus-oocyte complexes (COCs) were aspirated from follicles (2-8 mm in diameter) with an 18-gauge needle and COCs were selected for *in vitro* maturation. The selected oocytes were washed in the maturation medium (Modified Parker medium) supplemented with 15% fetal calf serum (FCS), 0.5 mM L-glutamine, 0.2 mM pyruvate, 50 µg/ml gentamycin sulphate and 10 µl/ml FSH (Folltropin®, Canada). The COCs were cultured in groups of 50 oocytes in 600 µl of maturation medium in four-well dishes (Nunc®, Denmark). Maturation was performed for 24 hours at 39^0C under a humidified atmosphere of 5% CO_2 in air.

In vitro fertilization: After maturation, the oocytes were transferred into 600 µl of fertilization medium (TALP medium) supplemented with 2 µg/ml of heparin (Sigma, USA), 0.2 mM pyruvate (Sigma, USA) and 25 µl/ml penicillinamine, hypotaurine and epinephrine (Sigma, USA). Swim-up technique has been applied to obtain motile sperm cell from frozen-thawed semen (Parrish et al., 1988). Briefly, frozen-thawed sperm cells were incubated in a tube which contained 1.5 ml of sperm-TALP supplemented with 6 mg/ml BSA (Sigma, USA) and 10 mM pyruvate in CO_2 incubator for 60 min. After that, the supernatant

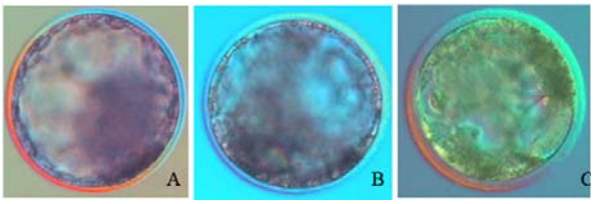

Figure 1 Representative photos of embryos in good quality (A): blastocysts were characterized by early cavitation, resulting in the formation of an eccentric and then expanded cavity lined with a distinct ICM and TE cells; poor quality embryos: blastocysts exhibited multiple vacuoles (B) and several degenerative foci in the inner cell mass (C) with cells appearing dark and necrotic (400x magnification).

was recovered and centrifuged at 250 g for 10 min to recover motile sperm cells as pellet. *In vitro* fertilization was performed using a final concentration of $2x10^6$ spermatozoa/ml per 50 oocytes for 20 hours in the same condition with maturation.

In vitro embryo culture: Following insemination, zygotes were stripped off from residual cumulus cells and adhesive spermatozoa by vortexing for 90 sec in Charles Rosenkrans (CR1) culture medium. The zygotes were washed once in fresh culture medium and cultured in groups of up to 50 zygotes in 600 µl CR1 medium (Wrenzycki et al., 2001). The CR1 medium was supplemented with 10% FCS, 20 µl/ml beta-mercaptoethanol and 10 µl/ml MEM-NEAA (Sigma, USA). The culture was performed in a humidified atmosphere with 5% CO_2 at 39°C for 7 days and the medium was changed every 2 days.

Embryo grading: To assess the expression of mRNA with respect to quality, bovine embryos were classified as good or poor embryo quality (Robertson and Nelson, 1998). The time of post insemination at which the embryo reached specific developmental stages was considered as developmental criteria to classify the quality of embryos. The morphological features were assessed under stereomicroscope including the presence or absence of cellular fragments, symmetry of blastomeres and characteristics of ICM and TE cells. The good quality include; 1) with the expected stage of development, 2) without or with very minimal fragmentation, 3) symmetrical in shape and intact zona pellucida, 4) uniform in size and distribution of blastomeres and 5) clearly differentiated ICM and TE cells. The poor quality included; 1) inconsistent with the expected stage of development after fertilization; 2) non-symmetrical in shape, 3) high degree of fragmentation, 4) non-uniform and irregularly distributed blastomores and 5) without distinct ICM and TE cells (Fig 1).

Embryo storage: Prior to freezing, all embryos were washed two times with PBS and treated with acidic Tyrode (Sigma, USA) pH 2.5-3 to dissolve the zona pellucida. The zona-free embryos were further washed two times in PBS and frozen in cryotubes containing minimal amounts of lysis buffer [0.8% Igepal (sigma, USA), 40 U/µl RNasin (Promega, USA), 5mM dithiothreitol (DTT) (Promega, USA)]. Embryos for western blot analysis were additionally

treated with protease inhibitor (Sigma, USA). Until used for RNA isolation or western blotting all frozen embryos were stored at -80^{0}C.

RNA isolation and reverse transcription: A total of three pools, each of which contained 10 embryos were used for mRNA isolation using oligo(dT)$_{26}$ attached to magnetic beads (Dynal®, Norway). The embryos in lysis buffer were mixed with 40 µl binding buffer (20 mM Tris HCl pH 7.5, 1 M LiCl, 2 mM EDTA pH 8.0) and incubated at 65^{0}C for 5 min to obtain complete lysis of the embryo and release of RNA. Ten µl of oligo(dT)$_{25}$ magnetic bead suspension was added to the samples, and incubated at room temperature for 30 min. The hybridized mRNA and Oligo (dT) magnetic beads were washed three times with washing buffer (10 mM Tris HCl, 0.15mM LiCl, 1mM EDTA). Finally, mRNA samples were eluted in 12 µl DEPC-treated water and reversely transcribed in 20 µl reaction volume containing 2.5 µM oligo (dT) 12N (where: N= G, A or C) primer, 4 µl of 5x first stand buffer (375 mM KCl, 15 mM $MgCl_2$, 250 mM Tris–HCl pH 8.3), 2.5 mM of each dNTP, 10 U RNase inhibitor (Promega, USA) and 100 U of SuperScript II reverse transcriptase (Invitrogen, Germany). In terms of the order of adding reaction components, mRNA and oligo(dT) primer were mixed first, heated to 70^{0}C for 3 min, and placed on ice until remaining reaction components were added. The reaction was incubated at 42^{0}C for 90 min, and terminated by heat inactivation at 70^{0}C for 15 min.

Quantitative real-time PCR: Quantitative analysis of E-cadherin, β-catenin, DSC2, and Histone 2a (H2a) mRNA in the embryos of each stage and group was assessed by real-time quantitative RT-PCR. The ABI Prism® 7000 apparatus (Applied Biosystems, USA) was used to perform the quantification using SYBR® Green JumpStart™ *Taq* ReadyMix™ (Sigma, USA) incorporation for dsDNA-specific fluorescent detection dye. Quantitative analysis of E-cadherin, β-catenin and DSC2 cDNA in embryos were performed and compared with H2a as an endogenous control. PCR was performed by using 2 µl of each sample cDNA and specific primers. The primer sequences were designed for PCR amplification according to the bovine cDNA sequence (Table 1) using Primer Express® Software v2.0 (Applied Biosystems, USA). Standard curves were generated for both target and endogenous control genes using serial dilution of plasmid DNA (10^1-10^8 molecules). The PCRs were performed in 20 µl reaction volume containing of 10.2 µl SYBR® Green universal master mix (Sigma, USA) optimal levels of forward and reverse primers and 2 µl of embryonic cDNA. During each PCR reaction, samples from the same cDNA source were run in duplicate to control the reproducibility of the results. A universal thermal cycling parameter (initial denaturation step at 95^{0}C for 10 min, 45 cycles of denaturation at 95^{0}C for 15 sec and 60^{0}C for 60 sec) was used to quantify each gene. After the end of the last cycle, dissociation curve was generated by starting the fluorescence acquisition at 60^{0}C and taking measurements every 7 sec interval until the temperature reached 95^{0}C. Final quantitative analysis was done using the relative standard curve method as used in Tesfaye et al. (2004) and results were reported as the relative expression level compared to the calibrator cDNA after normalization of the transcript amount to the endogenous control.

Western blot analysis: A group of 20 blastocysts were used for each quality group, which included good and poor embryo quality. The proteins were extracted from the embryo in loading buffer (26% of 1M Tris, 12% of SDS, 20% of 2-mercaptoethanol and 40% of Glycerol). After being boiled for 5 min, the proteins were separated on 10% SDS-PAGE gel and were then transferred onto nitrocellulose transfer membrane, pore size 0.45 µm (Protran®, Germany) using Trans-Blot® SD; Semi-Dry transfer Cell (Bio-Rad, USA). The membrane was stained with Ponceau S to evaluate the transfer quality and blocked for 1 hour in Tris-buffered saline (20 mM Tris, 150 mM NaCl) containing 0.05% Tween-20 (TBS-T) and 1% Polyvinylpyrolidone (PVP) (Sigma, USA). The membrane was then incubated at 40C overnight with primary antibody which was diluted 1: 500 in TBS-T containing 0.1% PVP prior to use (anti-rabbit E-cadherin primary antibody from Dunn Labotechnik, Germany and anti-mouse β-catenin primary antibody from Clontech Laboratories, USA). After incubation, the membrane was washed 6 times for 10 min in TBS-T and the hybridization with the secondary antibody was performed at room temperature for 1 hour. The secondary antibody, horseradish-peroxidase-conjugated donkey anti-rabbit or anti-mouse secondary antibody (Amersham Bioscience, UK), was diluted 1:50,000 in TBS-T containing 0.1% PVP. The membrane was finally washed 6 times for 10 min in TBS-T. The peroxidase activity was detected using the ECL Plus Western blotting detection system (Amersham Biosciencs, UK) following the manufacturer's instructions and visualized using Kodak BioMax XAR film (Kodak, Japan).

Table 1 Details of primers used for quantitative real-time PCR

Gene	GenBank accession number	Primer sequences	Annealing temperature (°C)	Product size (bp)
E-cadherin	AY508164	5′- GACACTGGAGGTATCAGCGCAC-3′ 5′- TGATCTGGACCAGCGACTTAGG -3′	60	194
β-catenin	BT020888	5′- ATTCAGCAGAAGGTCCGAGTGC -3′ 5′- GTTGAAGCTACTGCCTCCGGTC -3′	60	208
DSC2	M81190	5′- CGCAACAACTCCGGATGGATAT -3′ 5′- GGTGGGTAATGCTGGAAACTGC -3′	60	238
H2a	NM178409	5′-CTCGTCACTTGCAACTTGCTATTC-3′ 5′-CCAGGCATCCTTTAGACAGTCTTC-3′	60	148

Statistical analysis: The mRNA expression analysis for studied genes in all groups and the bovine preimplantation embryos was analysed based on the relative standard curve method. The relative expression data were analyzed using the SAS version 8.0 (SAS Institute Inc., USA) software package. Differences in mean between experimental groups or developmental stages were tested using ANOVA followed by a multiple pair wise comparisons using a t-test. A value of $p< 0.05$ was considered to be significantly different.

Results

The aim of this study was to determined the expression profile throughout bovine preimplantation embryos and comparing these transcript abundances between good and poor quality blastocysts of E-cadherin, β-catenin and DSC2 genes. The slope of the standard curve was close to -3.4 with a correlation coefficient (R^2) of approximately 0.99 in all cases, indicating maximal PCR efficiency. To correct any variation in both mRNA quality and quantity, quantitative results were normalized with the reference gene histone 2a as recommended by Robert et al. (2002).

Temporal expression profile of E-cadherin, β-catenin and DSC2 transcripts in bovine embryos: First, in order to fully understand temporal expression pattern of all transcripts namely: E-cadherin, β-catenin and DSC2, a real-time RT-PCR analysis was conducted throughout the preimplantation stages of *in vitro* produced bovine embryos (Fig 2). The 16-cell stage embryo has been used as a calibrator with relative expression level set to 1. The E-cadherin mRNA transcript was detected at a higher level in immature and mature oocytes, morula and blastocyts. However, this transcript abundance was lower at 2-cell to 16-cell developmental stages. The expression of β-catenin transcripts in developmental embryos was highly abundant up to the 4-cell stage and further down-regulated in the later stages and a slight increase was found at the blastocyst stage. On the contrary, the DSC2 transcripts were not detected in earlier developmental stages. This transcript was up-regulated at morula and highly expressed at the blastocyst.

Effect of blastocyst quality on mRNA expression: The embryos at blastocyst stage were quantified for the expression of those transcripts between good and poor quality. Figure 3 shows the relative expression of transcripts compared between good and poor embryos at blastocyst. The good quality embryos have been used as calibrators setting its relative expression levels to 100. The relative abundance of E-cadherin mRNA in poor quality blastocysts was 49% lower than good quality blastocysts, and β-catenin transcript in poor quality was 51% lower than good quality blastocyst. The DSC2 mRNAs was also found to reduce 38% in poor quality compared to good quality blastocysts ($p< 0.05$).

Effect of blastocyst quality on protein expression: For the expression of E-cadherin and β-catenin at the protein level between good and poor quality embryos, western blot analysis was carried out using proteins extracted from the blastocyst of the two quality groups (Fig 4). The protein expression of E-cadherin (119 kDa) decreased and that of β-catenin (92 kDa) slightly decreased in poor quality embryos compared to good quality embryos.

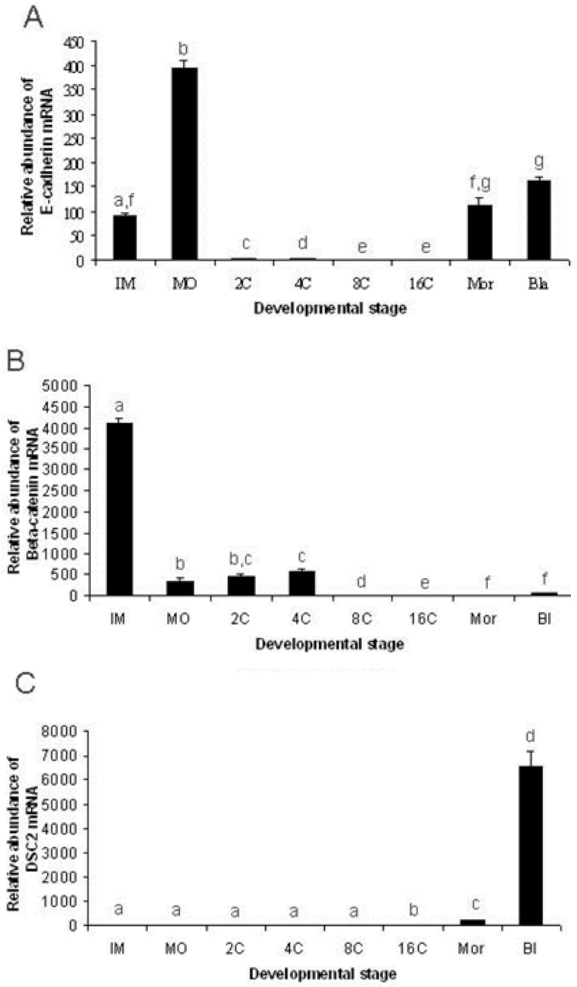

Figure 2 Relative abundance of E-cadherin, β-catenin and DSC2 transcripts in oocytes and *in vitro* bovine preimplantation stage embryos (immature oocyte IM: mature oocytes, MO: 2-cell 2C; 4-cell 4C; 8-cell 8C; 16-cell 16C; morula Mor; blastocyst Bla/Bl). Individual bars show the treatment mean±SD. Values with different superscripts (a, b, c, d, e, f, g) are significantly different ($p< 0.05$).

Discussion

In this study, the real-time RT-PCR was used to quantify the expression profile of adhesion genes namely E-cadherin, β-catenin and DSC2 throughout *in vitro* bovine preimplantation embryo development. Moreover, our study is the first to investigate the effects of blastocyst quality on the expression of those adhesion genes. The good quality of blastocyst yields very high implantation rates, while bad quality blastocyst implants at a much lower rate (Balaban et al., 2000). All gene candidates used in this study have been shown to be related to the compaction and formation blastocyst and the events taking place in the initial phase of embryo implantation (Haegel et

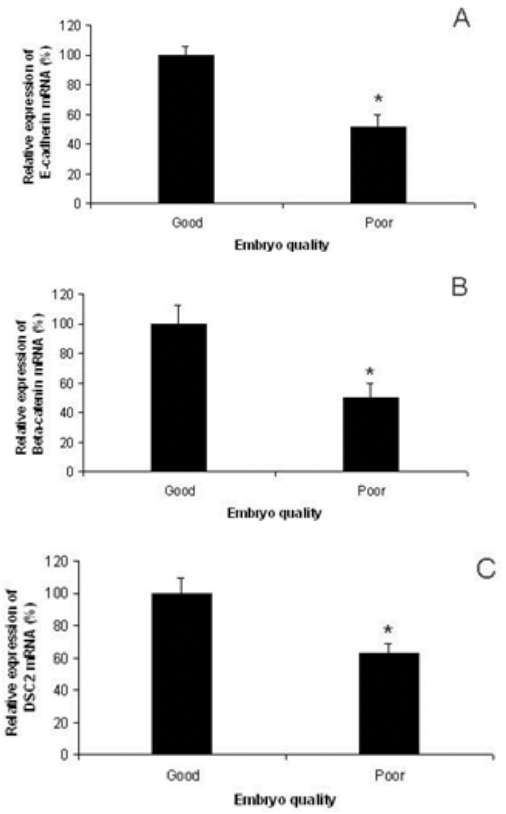

Figure 3 Relative expression of E-cadherin (A), β-catenin (B) and DSC2 (C) mRNA in bovine preimplantation embryos of good and poor quality. The symbol (*) means significantly different ($p< 0.05$).

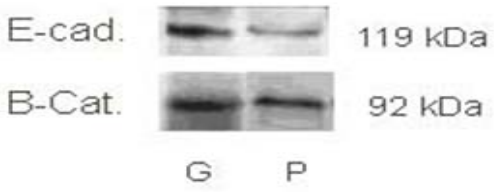

Figure 4 Western blot analysis for the presence of E-cadherin (E-cad.) and β-catenin (B-cat.) protein in good quality bovine embryos (G) compared to poor quality embryos (P).

al., 1995; Shehu et al., 1996; Barcroft et al., 1998; Green and Gaudry, 2000; Li et al., 2005).

The real-time assay can detect as little as 1000 copies of mRNA or even 400 copies (Gibson et al., 1996). The quantification of transcripts of targeted gene was performed using cDNA obtained from 10 embryos or oocytes pool of each development stage. However, this pool may cause bias by increasing transcript abundance during development, which is consistent with an increase in cell number, as observed in previous studies (Taylor and Piko, 1990). To prevent this bias, the bovine H2a was used as an endogenous control during real-time RT-PCR quantification of target transcripts. The bovine H2a has been shown to be expressed at a constant level during different stages of bovine preimplantation embryo (Robert et al., 2002; Tesfaye et al., 2004). E-cadherin performed by mediated cell to cell adhesion is associated with compaction at morula (Fleming et al., 1984). Moreover, E-cadherin transcript protein was found in the ICM and TE of expanded bovine blastocysts (Shehu et al., 1996; Barcroft et al., 1998). In this study, E-cadherin was expressed throughout all developmental stages of bovine preimplantation embryos. Our results are similar to Bloor et al. (2002). They studied the expression profile in human preimplantation embryos and the E-cadherin transcript was detected throughout the preimplantation developmental stage. In our study, the β-catenin transcript was also found to be at highest level at morula and blastocyst stage because β-catenin involves in morula and blastocyst formation (Li et al., 2005). Results of both E-cadherin and β-catenin protein levels at blastocyst were also in agreement with transcripts. For example, E-cadherin protein level significantly decreased in poor quality embryos compared with good quality embryos. However, the expression of β-catenin protein slightly decreased in poor quality embryos compared to good quality embryos. The analysis of gene expression in preimplantation embryos demonstrated that the components of the E-cadherin and β-catenin were derived from both maternal and zygotic gene activity similar to previous works done in mouse (Ohsugi et al., 1999) and human (Bloor et al., 2002). The expression of DSC2 transcript was detected highest level at blastocyst (Bloor et al., 2002). Additionally, the DSC2 transcript was detected in cumulus cells, unfertilized egg, 2- and 4-cell stage, but this was negative at 8-cell stage of mouse embryos (Collins et al., 1995). It was concluded that DSC2 protein was derived predominantly from the TE cells of the mouse (Fleming et al., 1991) and bovine embryos (Wrenzycki et al., 2003). It involved in the formation of desmosomal junctions rather than functioned in the stabilization of the TE during blastocyst expansion (Fleming et al., 1991; Collin et al., 1995).

In conclusion, this study indicated the expression profile of adhesion genes namely E-cadherin, β-catenin and DSC2 throughout bovine preimplantation embryos. The expression of those genes significantly affected blastocyst quality and thus might be used as markers for blastocyst quality from *in vitro* culture.

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References

Aghion, J., Gueth-Hallonet, C., Antony, C., Gros, D. and Maro, B. 1994. Cell adhesion and gap junction formation in the early mouse embryo are induced prematurely by 6-DMAP in the absence of E-cadherin phosphorylation. J Cell Sci. 107: 1369-1379.

Balaban, B., Urman, B., Sertac, A., Alatas, C., Aksoy, S. and Mercan, R, 2000. Blastocyst quality affects

the success of blastocyst-stage embryo transfer. Fertil Steril. 74: 282-287.

Barcroft, L.C., Hay-Schmidt, A., Caverney, A., Gilfoyle, E., Overstrom, E.W., Hyttel, P. and Watson, A.J. 1998. Trophectoderm differentiation in the bovine embryo: characterization of a polarized epithelium. J Reprod Fertil. 114: 327-339.

Bloor, D.J., Metcalfe, A.D., Rutherford, A., Brison, D.R. and Kimber, S.J. 2002. Expression of cell adhesion molecules during human preimplantation embryo development. Mol Hum Reprod. 8: 237-245.

Buxton, R.S. and Magee, A.I. 1992. Structure and interactions of desmosomal and other cadherins. Sem Cell Biol. 3: 157-167.

Buxton, R,S., Cowin, P., Franke, W.W., Garrod, D.R., Green, K.J., King, I.A., Koch, P.J., Magee, A.I., Rees, D.A., Stanley, J.R. and Steinberg, M.S. 1993. Nomenclature of the desmosomal cadherins. J Cell Biol. 121: 481-483.

Collins, J.E., Lorimer, J.E., Garrod, D.R., Pidsley, S.C., Buxton, R.S. and Fleming, T.P. 1995. Regulation of desmocollin transcription in mouse preimplantation embryos. Development. 121: 743-753.

De Vries, W.N., Evsikov, A.V., Haac, B.E., Fancher, K.S., Holbrook, A.E., Kemler, R., Solter, D. and Knowles, B.B. 2004. Maternal beta-catenin and E-cadherin in mouse development. Development. 131: 4435-4445.

Fleming, T.P., Warren, P.D., Chisholm, J.C. and Johnson, M.H. 1984. Trophectodermal processes regulate the expression of totipotency within the inner cell mass of the mouse expanding blastocyst. J Embryol Exp Morphol. 84: 63–90.

Fleming, T.P., Garrod, D.R. and Elsmore, A.J. 1991. Desmosome biogenesis in the mouse preimplantation embryo. Development. 112: 527-539.

Fleming, T.P., Sheth, B. and Fesenko, I. 2001. Cell adhesion in the preimplantation mammalian embryo and its role in trophectoderm differentiation and blastocyst morphogenesis. Front Biosci. 6: 1000-1007.

Gibson, U.E.M., Heid, C.A. and Williams, P.M. 1996. A novel method for real time quantitative RT-PCR. Genome Res. 6: 995-1001.

Green, K.J. and Gaudry, C.A. 2000. Are desmosomes more than tethers for intermediate filaments? Nat Rev Mol Cell Biol. 1: 208-216.

Haegel, H., Larue, L., Ohsugi, M., Fedorov, L., Herrenknecht, K. and Kemler, R. 1995. Lack of beta-catenin affects mouse development at gastrulation. Development. 121: 3529-3537.

Heasman, J., Kofron, M. and Wylie, C. 2000. Beta-catenin signaling activity dissected in the early Xenopus embryo: a novel antisense approach. Dev Biol. 222: 124-134.

Li, J., Zhang, J.V., Cao, Y.J., Zhou, J.X., Liu, W.M., Fan, X.J. and Duan, E.K. 2005. Inhibition of the beta-catenin signaling pathway in blastocyst and uterus during the window of implantation in mice. Biol Reprod. 72: 700-706.

Lonergan, P., Rizos, D., Gutierrez-Adan, A., Moreira, P.M., Pintado, B., de La Fuente, J. and Boland, M.P. 2003. Temporal divergence in the pattern of messenger RNA expression in bovine embryos cultured from the zygote to blastocyst stage *in vitro* or *in vivo*. Biol Reprod. 69: 1424-1431.

Ohsugi, M., Butz, S. and Kemler, R. 1999. Beta-catenin is a major tyrosine-phosphorylated protein during mouse oocyte maturation and preimplantation development. Dev Dyn. 216: 168-176.

Reichenbach, H.D., Liebrich, J., Berg, V. and Brem, G. 1992. Pregnancy rates and births after unilateral transfer of bovine embryos produced in vitro. J Reprod Fertil. 95: 363-370.

Robert, C., McGraw, S., Massicotte, L., Pravetoni, M., Gandolfi, F. and Sirard, M.A. 2002. Quantification of housekeeping transcript levels during the development of bovine preimplantation embryo. Biol Reprod. 67: 1465-1472.

Robertson, I. and Nelson, R.E. 1998. Certification of the embryo. In: Manual of International Embryo Transfer Society. 3rd ed. D.A. Stringfellow and S.M. Seidel (eds.). Illinois: International Embryo Transfer Society. 103-134.

Shehu, D., Marsicano, G., Fle´chon, J.E. and Galli, C. 1996. Developmentally regulated markers of *in vitro*-produced preimplantation bovine embryos. Zygote. 4: 109-121.

Takeichi, M. 1988. The cadherins: Cell-cell adhesion molecules controlling animal morphogenesis. Development. 102: 639-655.

Taylor, K.D. and Piko, L. 1990. Quantitative changes in cytoskeletal beta- and gamma-actin mRNAs and apparent absence of sarcomeric actin gene transcripts in early mouse embryos. Mol Reprod Dev. 26: 111-121.

Tesfaye, D., Ponsuksili, S., Wimmers, K., Gilles, M. and Schellander, K. 2004. A comparative expression analysis of gene transcripts in post-fertilization developmental stages of bovine embryos produced *in vitro* or *in vivo*. Reprod Domest Anim. 39: 396-404.

Van Soom, A., Vanroose, G. and de Kruif, A. 2001. Blastocyst evaluation by means of differential staining: a practical approach. Reprod Domest Anim. 36: 29-35.

Willert, K. and Nusse, R. 1998. Beta-catenin: a key mediator of Wnt signaling. Curr Opin Cell Biol. 8: 95-102.

Wrenzycki, C., Herrmann, D., Keskintepe, L., Martins, A.Jr., Sirisathien, S., Brackett, B. and Niemann, H. 2001. Effects of culture system and protein supplementation on mRNA expression in pre-implantation bovine embryos. Hum Reprod 16: 893-901.

Wrenzycki, C., Herrmann, D. and Niemann, H. 2003. Timing of blastocyst expansion affects spatial messenger RNA expression patterns of genes in bovine blastocysts produced in vitro. Biol Reprod. 68: 2073-2080.

Young, L., Sinclair, K.D. and Wilmut, I. 1998. Large offspring syndrome in cattle and sheep. Rev Reprod. 3: 155-163.