# Characterization of Placental Proteins in Bovine Somatic Cell Clone Fetuses*

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## ABSTRACT

**Somatic cell nuclear transfer in cattle has limited efficiency in terms of production of live offspring due to high incidence of fetal failure after embryo transfer to recipients. Such low efficiency of cloning could possibly arise from abnormal and poorly developed placenta. In the present study the placental proteome in late pregnancy established from *in vitro* fertilization (IVF) and nuclear transfer (NT) was analysed. Proteome alternation was tested using two-dimensional polyacrylamide gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Comparing placenta from NT embryos to those from IVF counterparts, significant changes in expression level were found in 18 proteins. Of these proteins 12 were not expressed in NT placenta but expressed in IVF counterpart, whereas the expression of the other 6 proteins was limited only in NT placenta. Among these proteins, cytokeratin 8 and vimentin are considered to be involved in regulation of post-implantation development. In particular, cytokeratin 8 and vimentin may be used as makers for placental development during pregnancy because their expression levels changed considerably in NT placental tissue compared with its IVF counterpart. Data from 2-DE suggest that protein expression was disorientated in late pregnancy from NT, but this distortion was eliminated with progression of pregnancy. These findings demonstrate abnormal placental development during late pregnancy from NT and suggest that alterations of specific placental protein expression may be involved in abnormal function of placenta.**

**(Key words: Cloned cow, 2-DE, Placentome, Vimentin, Cytokeratin 8, Aldose reductase, PRP-1)**

## INTRODUCTION

The successful production of viable offspring by cloning with somatic cell nuclear transfer (NT) has been achieved in ungulates, including, goat (Baguisi et al., 1999; Zou et al., 2001; Keefer et al., 2002), sheep (Wilmut et al., 1997), pig (Polejaeva et al., 2000; Boquest et al., 2002), cattle (Kato et al., 1998; Wells et al., 1999), rabbit (Chesne et al., 2002), cat (Shin et al. 2002) and mice (Wakayama et al., 1998; Wakayama and Yanagimachi, 1999). The potential applications of cloning in medicine, agriculture, and the propagation of rare animals clearly have great commercial and conservational benefit (Wall et al., 1997; Wilmut, 1998). Howerver, NT from somatic cells still has limited efficiency in terms of live calves born due to high fetal loss after transfer and the somatic cell NT technique is the high rate of abortion as well as fetal abnormal(Well et al., 1998; Hill JR et al., 2000; De Sousa et al, 2001).

The long-lasting effects of cloning are associated with excessive accumulation of allantoic fluid and increased fetal or birth weight (Well D, 1999; Hill, 1999; Lee, 2004). This syndromeis similar to large offspring syndrome (LOS), which has been reported previously for *in vitro*-derived blastocysts in sheep and cattle. LOS has been initiated in bovine or ovine embryos after exposure to unusual environments both *in vivo* and *in vitro* (Hasler et al., 1995; Kruip et al, 1997; Wagtendonk et al., 1998; Young et al., 1998). This phenomenon has been demonstrated that bovine blastocysts produced *in vitro* display abnormal expression of a number of genes that are of importance for embryonic development (Hill et al., 1999; Niemann and

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Wrenzycki, 2000; Heyman et al., 2002) as well as high incidence of chromosomal aberrations (Viuff et al., 1999; 2000; 2001).

Pregnancy failure occurs throughout the entire gestation, from embryo transfer to parturition (Wells et al., 1998; Boquest et al., 2002). The pregnancy failure associated with NT in cattle and sheep is believed to be either failure of the placenta to form or abnormal placenta development and function. Recently, reported that the abnormal placenta development has been demonstrated in cloned mice (Wakayama and Yanagimachi, 1999; Eggan et al., 2001) and cows (Hill et al., 1999; Lazzari et al., 2002; Hashizume et al., 2002; Heyman et al., 2002; Lee et al., 2004). It has been postulated that the documented abnormalities could either be related to abnormal imprinting or methylation of genes in donor somatic cell and / or early embryonic manipulation during *in vitro* culture (Kang et al., 2001; Moore, 2001; Ohgane et al., 2001).

In the bovine, fetal trophoblast attaches to a specific area of the endometrium celled the caruncle, and the contact causes the fetal chorion in the said area to form a cotyledon. The union of both maternal and fetal tissue is called a placentome. A unique feature of this type of placentation is themigration of fetal chorionic binucleate cells (BNC) throughout pregnancy through the chorionic tight junction to fuse with the uterine epithelial cells (Wooding, 1982; 1992).

Proteins in the placenta of some mammals, such as sheep (Gootwine, 2004), human (Van et al., 2001; Hass and Sohn, 2003) and mice (Abigail, 2003; An et al., 2004) have been the subjects of many previous studies that sought to understand how the biochemistry of the fetal and maternal adapts to the presence, and supports the maintenance of pregnancy. The factors responsible for the progressive exchanging in placental proteins among pregnant nuclear transferred to cows are still unknown.

Therefore, this study analyzed several proteins in placenta tissues of late pregnancy and the incidence of large placentomes between fetal-maternal derived from nuclear transferred embryos produced *in vitro*. In the present study, we used the excellent resolution afforded by two-dimensional polyacrylamide gel electrophoresis (2-DE) and matrix associated laser desorption/ionization time--to-flight mass spectrometry (MALDI-TOF-MS) to separating complex polypeptide mixtures to identify proteins in IVF and NT fetal-placentome that are associated with during around parturition.

## MATERIALS AND METHODS

### Nuclear Transfer (NT) Embryo Production and *In Vitro* Fertilization (IVF)

The NT embryos were generated essentially using the same ovarian follicular cell line from cows in this study. The cells used for NT derived from a small ear skin biopsy in 2-year-old cows. Ear skin fibroblast cells were cultured on 50-ml tissue culture flask in DMEM supplemented with 10% FBS. After 5 days of culture, the cells were 0.05% trypsinized (0.05% trypsin, Sigma. St. Louis, MO) and washed three times with $Ca^{2+}$, $Mg^{2+}$ -free PBS (Gibco BRL, USA) by centrifugation at 300×g for 5 min. The pellet was re-suspended and cultured in 50-ml tissue culture flasks in DMEM containing 10% FBS. Fibroblast cells were cultured over several passages (2 to 12) and used as donor cells for nuclear transfer. After injection of the donor cells into the perivitelline space, fusion between karyoplast and cytoplast was accomplished by the use of electric pulse. The karyoplast and cytoplast complexes were placed in the Zimmerman Cell fusion medium for equilibration and then placed between the electrofusion needles (Fujihira, Japan). Cell fusion was induced with a single DC pulse of 25V/mm for 10μ sec. After the fusion treatment, the Karyoplast-cytoplast complexes were washed in H-TCM 199 supplemented with 20% FBS, and the fusion was evaluated by microscopic examination after 30 min. The fused embryos were activated in CR2 containing 1.5 mg/μl BSA, 5% FBS and 10μM calcium ionophore for 5 min, and followed by 2mM 6-dimethylaminopurine (DMAP) for 3 hr. After activation, fused embryos performed *in vitro* embryo production in CR2 medium with fatty acid-free bovine albumin (3mg/ml) and NT embryos judged to be of sufficiently good quality were selected for transfer into recipients on Day 7 after fusion.

To compare with cloned embryos, the embryos fertilized *in vitro* was examined by fertilizing some oocytes matured with frozenthawed semen according to the method described by Otoi et al. (1993) with minor modifications. Briefly, after maturation, the cumulusoocyte complexes were fertilized *in vitro* with frozen semen and BO medium (Brackett and Oliphant, 1975). The semen was used from the same bull.

Recipient cows were raised in the Suwon Branch of National Livestock Research Institute. After 7 to 8 days culture of embryos produced by NT and IVF, hatching blastocysts were transported from the institute to the Branch in TCM 199 supplemented with 20% FBS. The hatching blastocysts were loaded into 0.25ml straw using embryo transfer freezing medium (Gibco BRL, USA) supplemented with 20% FBS and then non-surgically transferred to recipient cows using cassou gun on day 7 after natural estrus. Experimental protocols were approved by the National Livestock Research Institute, Rural Development Administration Animal Care and Use Committee.

### Pregnancy Monitoring and Morphometric Measurement

After embryo transferred with IVF and NT embryos, all recipient cows examined for the presence of plasma progesterone at 21 days postovulation by pregnancy diagnosis kit (Greencross Life Science Co., Yong-In, Korea).

All recipients were performed for the presence or absence of a viable fetus at Day 50±2days by transrectal ultrasonography (Sonoace SA-600, Medison Co., Korea) using a linear 3.5~5.0 MHz probe. All pregnant recipients were then repeatedly checked on days 50, 100, 150 and 250 of pregnancy. Overdue at parturition in NT recipients were delivered by cesarean section (day280±2; day of estrus) and for used to control samples. Both newborn calves weight and total placentomes weight were recorded.

### Tissue Collection

IVF and NT placentome tissues were collected from calves at birth by cesarean section (day 280±2; day of estrus). NT placentomes obtained by selected abnormal fetus at parturition ([IVF] $n$=3, [NT] $n$=4) and from the vicinity of the umbilical cord (Fig. 1).

The placentome tissues were completelywashed two times in ice-cold phosphate-buffered saline (pH=7.2) and dissected into small fragments. Both fetal cotyledons, maternal caruncles and tissues from intact placentomes were snap-frozen in Liquid nitrogen and stored at 70°C until experimental analysis.

### Tissue Preparation and 2-D Polyacrylamide Gel Electrophoresis

Placentome tissues were homogenated directly by motor-driven homogenizer (PowerGen125, Fisher Scientific) in sample buffer composed with 7M urea, 2M Thiourea containing 4% (w/v) 3-[(3-cholamidopropy) dimethyammonio]-1-propanesulfonate (CHAPS), 1% (w/v) dithiothreitol (DTT) and 2% (v/v) pharmalyte and 1mM benzamidine. Proteins were extracted for 1 hr at room temperature with vortexing. After centrifugation at 15,000 × g for 1 hr at 15℃, insoluble material was discarded and soluble fraction was used for two-dimensional gel electrophoresis. Protein loading was normalized by Bradford assay (Bradford et al., 1976).

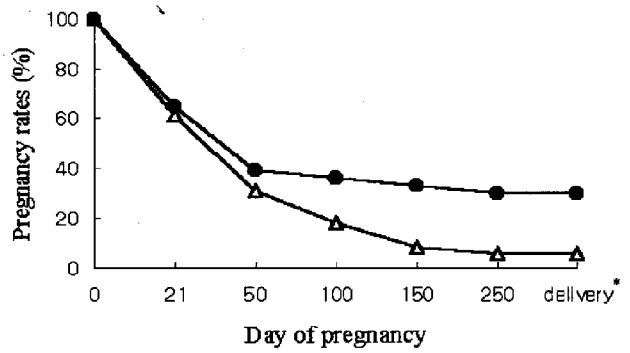


**Fig. 1. Rates of pregnant after embryo transfer with IVF (●) or NT (△) embryos.** Pregnancy was determined either by pregnancy diagnosis kit or ultrasound scanning. Within the same row, percentages with different superscripts are significantly different ($P$<0.05). Day280±2; day of estrus.
* Day280±2; day of estrus.

IPG dry strips were equilibrated for 12~16 hrs with 7M urea, 2M thiourea containing 2% 3-[(3-cholamidopropy) dimethyammonio]-1- propanesulfonate (CHAPS), 1% dithiothreitol(DTT), 1% pharmalyte and respectively loaded with 200ug of sample. Isoelectric focusing (IEF) was performed at 20 ℃ using a Multiphor II electrophoresis unit and EPS 3500 XL power supply (Amersham Biosciences) following manufacturer's instruction. For IEF, the voltage was linearly increased from 150 to 3,500V during 3 hrs for sample entry followed by constant 3,500V, with focusing complete after 96kVh. Prior to the second dimension, strips were incubated for 10 minutes in equilibration buffer (50mM Tris-HCl, pH 6.8 containing 6M urea, 2% SDS and 30% glycerol), first with 1% DTT and second with 2.5% iodoacetamide. Equilibrated strips were inserted onto SDS-PAGE gels (20~24cm, 10~16%). SDS-PAGE was performed using Hoefer DALT 2-D system (Amersham Biosciences) following manufacturer's instruction. 2-D gels were run at 20 ℃ for 1,700Vh. And then 2-D gels were silver stained as described by Oakley et al (1980) but fixing and sensitization step with glutaraldehyde was omitted.

Quantitative analysis of digitized images was carried out using the PDQuest software (version 7.1, Bio-Rad, USA) according to the protocols provided by the manufacturer. Quantity of each spot was normalized by total valid spot intensity. Protein spots were selected for the significant expression variation deviated over two fold in its expression level compared with IVF placentome tissues.

### MALDI-TOF Analysis and Database Search

Protein analysis were performed using a Ettan MALDI-TOF (Amersham Biosciences). Peptides were evaporated with a N2 laser at 337nm, and using a delayed extraction approach. They were accelerated with 20Kv injection pulse for time of flight analysis. Each spectrum was the cumulative average of 300 laser shots. The search program ProFound, developed by the Rockefeller University (http://129.85.19.192/profound_bin/WebPro Found .exe), was used for protein identification by peptide mass fingerprinting. Spectra were calibrated with trypsin autodigestion ion peak m/z(842.510, 2211.1046) as internal standards.

### Statistical Analysis

Placentome weights of IVF and NT to the late gestation were using a Student's T-test. Analysis of calves weight measurements was by a one way-ANOVA test on log transformed data followed by paired by Tukey-kramer comparison.

## Results

### Pregnancy Monitoring after Embryo Transferred with IVF and NT Embryos

Pregnant monitoring assessed by ultrasonography or pregnant diagnosis kit (only day 21). The pregnancy results for the two groups (IVF and NT) of recipients are presented in Table 1. Fig. 2 showed that transferred of IVF embryos and NT embryo resulted in the similar range of initiated pregnancy rates at day 21 (IVF; 65%, NT; 61%). However, evolution of pregnancy rates was quite different between IVF and NT. Confirmed pregnancy rates by day 50 were significantly lower in NT group (31%) compared with the IVF group (51.7%). Whereas, at days 50~250, pregnancy rates were significantly declined in NT (31%→ 5.7%) and maintained a pregnancy rates in IVF (52.7%→ 48.9%). Proportions of calves born differed significantly between IVF group (48.9%) and NT group (5.7%).

### Proteomic Analysis of IVF and NT Placentome Tissues

The proteins obtained from placentome tissues of IVF and NT at parturition wasapplied to 2-DE. The protein spots visualized with silver stain. After staining, the number of spots was estimated by using PDQuest software; approximately 2,000~2,500 spots were determined. The 2-DE experiment was repeated at least 10 times on both IVF ($n$ =3) and NT ($n$=4) placentome tissue samples. Quantity of each spot was normalized by total valid spot intensity.

**Table 1. Monitoring of pregnancy after embryo transferred with NT and IVF embryos**

| Groups | No. of transferred embryo | No. of recipients | Day of pregnancy | | | | | Live offspring |
|---|---|---|---|---|---|---|---|---|
| | | | *21 | *50 | *150 | *250 | ** Delivery | |
| | | | Pregnancy failure | | | | | |
| NT | 280 | 140 | 54 | 42 | 32 | 4 | 8 | 3 |
| IVF | 220 | 110 | 40 | 13 | 3 | 1 | 53 | 53 |

* The day of pregnancy diagnosis was examined.
** Day 280±2; Day of estrus, delivered by cesarean section; [NT] $n$=4, [IVF] $n$=3.

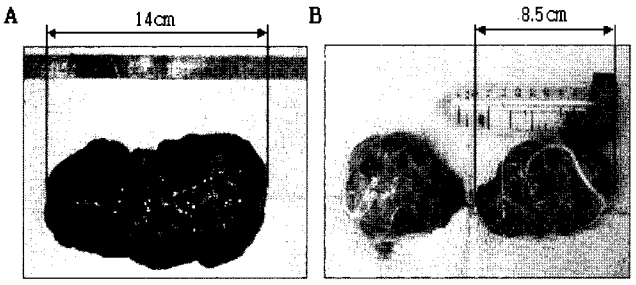


**Fig. 2. Comparison of shape from NT (A) and IVF (B) placentomes.** Each placentomes were collected from calves at birth by cesarean section (day 280±2). The placentomes were obtained from the vicinity of the umbilical cord. The placentomes weight; (A: 320 g, B: 210g).

Additionally, protein spots were selected for the significant expression variation deviated over three fold in NT placental proteins profile expression level compared with control placental proteins. Only the protein spots that fulfilled $P<0.05$ were selected. In this way, we found 34 spots that exhibit statistically significant variations. (Fig. 3) After the statistical analysis, MALDI-TOF mass spectrometric analysis was performed for all of the selected protein spots. This analysis was performed at least three times for each protein spot, and 18 proteins were clearly identified (Table 2).

### Proteins Differentially Expressed in NT Placentome Tissue

From to the group of 36 spots, 18 spots were identified by MALDI-TOF MS. The identified proteins are listed in Table 3 and their position on the 2-D gel map are marked by

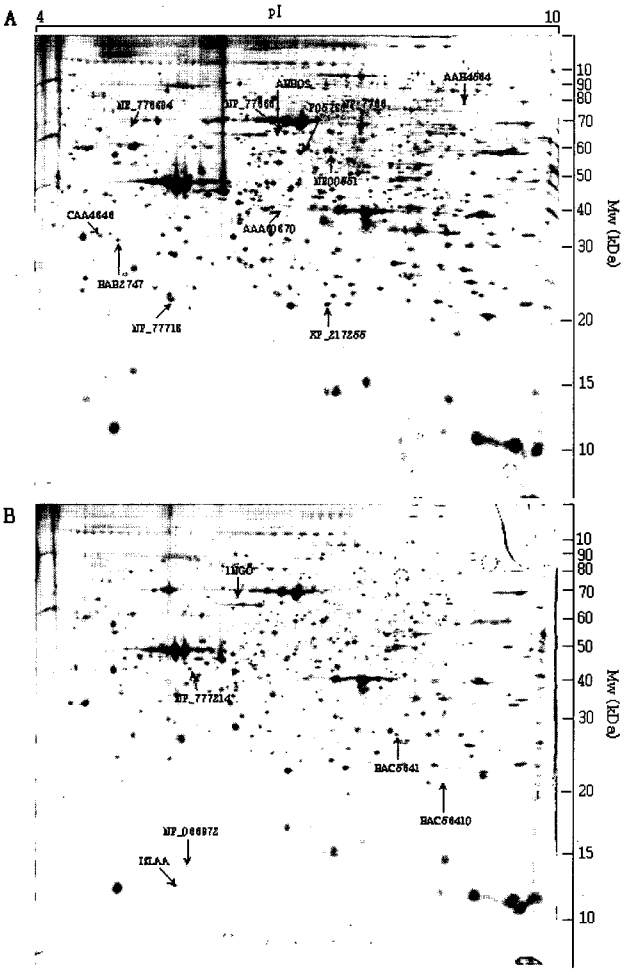


**Fig. 3. 2-DE map of placental proteins obtained from IVF (A) and NT (B) placentome at parturition.** 2-DE was performed on an immobilized pH 4~10 strip, followed by the second-dimensional separation on SDS-PAGE gel (10~16%). As the spots marked with an arrow were each differently expressed proteins, labeled by the SWISS-PROT accession number. As seen in Tables 2 for relevant information about the protein spots.

**Table 2. The 18 proteins for which expression differed between IVF and NT placentome tissue**

| AN[a] | Protein name[b] | MM (kDa) | pI[c] | IVF | NT |
|---|---|---|---|---|---|
| | **IVF** | | | | |
| NP_777188 | Preoviredoxin 2 | 22.21 | 5.4 | ++ | - |
| P05766 | Cyrokeratin 8 | 42.4 | 5.1 | +++ | - |
| NP_776394 | Vimentin | 53.71 | 5.2 | +++ | - |
| AAA30370 | Aldose reductase | 34.35 | 5.7 | ++ | - |
| ABBOS | Serum albumin precursor | 71.25 | 5.8 | + | - |
| NP_776365 | myxovirus resistance 1 | 75.19 | 5.6 | + | - |
| XP_217255 | Similar to RIKEN cDNA 1810013B01 | 22.71 | 5.6 | +++ | - |
| CAA43469 | Laminin-binding protein | 31.9 | 4.8 | ++ | - |
| AAH45641 | Glutamine-fructose-6-phosphate transaminase 1 | 77.54 | 6.4 | ++ | - |
| NP_776600 | Seryl-tRNA synthetase | 59.10 | 5.9 | + | - |
| NP_005511 | Heterogeneous nuclear ribonucleoprotein H1 | 49.50 | 5.9 | + | - |
| BAB27476 | Unnamed protein product | 25.08 | 5.0 | +++ | - |
| | **NT** | | | | |
| ISLAA | Galectin-1 (S-Lectin) Hexagonal Crystal | 14.94 | 5.3 | - | ++ |
| AAH30634 | Heat shock 70kD protein 9B (mortalin-2 | 74.12 | 6.0 | - | + |
| NP_066972 | Coactosin-like 1 | 16.04 | 5.5 | - | +++ |
| BAC56410 | Similar to prolactin related protein 1 | 16.17 | 6.3 | - | +++ |
| NP_777214 | Serine (or cysteine) proteinase inhibitor | 42.89 | 5.4 | - | ++ |
| BAC56419 | Similar to phosphoglycerate mutase | 15.56 | 7.9 | - | ++ |

[a] AN, SWISS-PROT accession number.
[b] Proteins identified with a significance of P<0.05 ( PDQuest software ver. 7.0).
[c] Isoelectric point(+++; high level, ++; middle level, +; low level, -; not detected or very small quantities).

SWISS-PROT accession number (Fig. 3). Proteins of all listed in Table 2 provided with their molecular mass, experimental isoelectric point (pI) accession number. In some cases, proteins did not run in the gel according to their molecular mass or pI, and these proteins may have undergone modifications that change their position in the 2-D gel. A comparison of the density of the spots identified on the reference map between the IVF and NT placentome tissues showed that 6 protein spots were significantly increased (Fig. 3B and Table 2), 12 protein spots were significantly decreased (Fig. 3A and Table 2) in NT placentome tissue. These identified results were comprised of intermediate filament proteins (cytokeratin 8, vimentin and coactosin -like protein 1), antioxidant protein (peroxiredoxin 2), metabolic related proteins (glutamine-fructose-6-phosphate transaminase 1, similar to phosphoglycerate mutase and aldose reductase), virus resistance protein (myxovirus resistance 1), proteins associated with stress response (heat shock 70kD protein), extra cellular matrix related protein (laminin binding protein), pregnancy related protein (prolactin related protein), angiogenesis related protein (serin proteinase inhibitor), mitochondrial protein synthesis systems (seryl-tRNA synthetase), along with serum albumin precursor, heterogeneous nuclear ribonucleoprotein

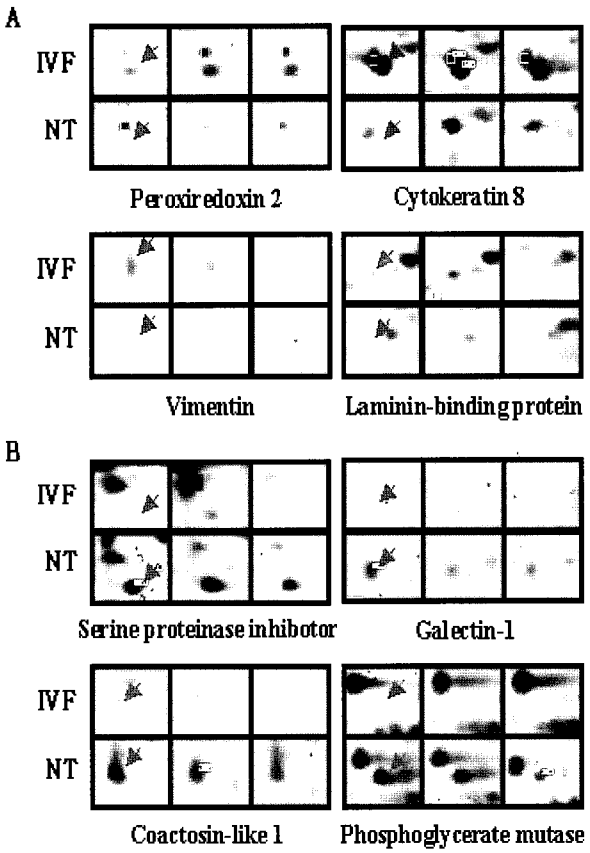


**Fig. 4. Patterns of change in the proteins expression from IVF and NT placentome tissues.** 2-D spots image of typical proteins showing significant changes in the protein expression; significantly increased (A) and significantly decreased (B) in NT placentome tissues. The differential expression of the proteins marked the spots with an arrow.

H1, galectin-1 and two proteins were unnamed (Table 2). Particularly, high level (++ or +++) proteins in 2-D gels showed that IVF placentome tissues detected cytokeratin8, vimentin, aldose reductase, proxiredoxin 2 and NT placentome tissues detected coactosin-like 1, prolactin related protein, serine proteinase inhibitor (Table 2). Fig. 4 visualized by examples of protein spots on 2-D gel in each IVF and NT placentome tissues (IVF; $n$=3 and NT; $n$=4).

## DISCUSSION

In the present study, we demonstrates the analysis of pregnancy somatic cloning and report the feto-placentome of late pregnancy. The development of most abnormal placenta occurred later and at a higher rate than after somatic embryonic cloning. Thus, the considerable variation in abnormalities may be caused by the different cell types and lines used, the different methods of producing and culturing NT embryos, and species differences in the mode of placentation and gestational physiology. Abnormal placental development and overgrowth are hallmark pathologies associated with cloning by NT. The significantly lower number of caruncles and, higher weight of placentome in NT group (Hill et al., 2001). NTcows were noted to have poorly developed caruncles resulting from atypical development of the placentome in terns of shape and size. These reported that was associated with atypical expression of pregnancy or placental associated factors.

In this examined, describe a new logic-based discovery and validation approach that integrates newly developed, global analytical techniques to map the proteins expressed in the placental tissue.

In addition to cDNA microarrays, proteomics is becoming a widely used approach to study cellular mechanisms. The study of proteins and posttranslational modifications adds to what can be learned from genomic studies (Nelson et al., 2000). In the present work, 2-DE and MALDI-TOF MS were used as a proteomic tool, protein expression profiles were compared between NT and IVF placentome tissues (see Fig. 3, panels A and B). This report identified 23 proteins of which expression levels were significantly different between NT and IVF placentome tissues. These identified results were comprised of intermediate filament proteins, antioxidant protein, metabolic related proteins, stress-induced proteins, pregnancy related protein, angiogenesis related protein and other proteins.

Fructose is the predominant hexose in fetal fluids and fetal blood of ungulates during bovine pregnancy (Huggett et al., 1961); in the late pregnant bovine, conversion of glucose to fructose occurs on passage of glucose through the placenta. Aldose reductase, which catalyzes the conversion of glucose to sorbitol, has been purified from placenta of the late pregnancy (Hastein and Velle, 1968 1969). In the work shown in 2-D gel that aldose reductase was not detected in NT placentome tissue. In other words, abnormal expression of metabolic enzymes in NT placenta may be have influenced on placenta development.

Interestingly, vimentin and cytokeratin 8 related intermediate filament (IF) proteins in NT placentome tissue were not detected on 2-D gel and immunoblot analysis (Fig. 3). Vimentin and cytokeratin 8 expressions are required for post-hatching development of bovine embryos (Maddox-Hyttel et al.2003). These proteins coexpressed in trophoblast cells in human (Vicovac and Aplin, 1996) and mouse (Souza and Katz, 2001). Cytokeratin intermediate filaments are markers of trophoblast tissue (Daya and Sabet, 1991). Therefore, aberrant expression of these proteins waslikely indicative of future placental abnormalities in cloned cattle. These two proteins (vimentin and cytokeratin 8) appear to be useful diagnostic markers of placental development during pregnancy.

A unique feature of bovine placentation is the migration of trophoblast-derived BNCs at implantation, placentation and fetomaternal communication in ungulates that is analogous to the role played by human extravillous trophoblast and rodent giant cells (Cross et al., 1994 and Wooding, 1982;1992). BNCs are directly involved in modification of the uterine epithelium beginning at implantation and continuing until term. The BNC plays a pivotal role in feto-maternal communication in the bovine. The said cells produce an array of proteinaceous compounds including bovine placental lactogens (bPL), prolactin-related protein-1 (bPRP-1), and pregnancy- associated glycoproteins (bPAG) (Duello et al., 1986; Milosavlhevic et al., 1989;Green et al., 2000). In contrast, PRP-1 signals were comparable between cows, including NT cows carrying dead fetuses. Expression of bPRP-1 in the placentomal and interplacentomal tissues of the NT group varied considerably more, compared with the AI group at day 100 of gestation (Patel et al., 2004; Gootwine, 2004). In present study, this report found that bPRP-1 was significantly expressed in NT placentome tissue at late gestation. We results suggest that an aberration in the proliferation and differentiation of the trophoblast cells, especially the BNCs that may result from defective pregnancy-related factor transcriptional commands. The disparate patterns of the BNC-specific transcripts appear to by indirect indicators of atypical differentiation and development of the extraembryonic membranes in cloned cattle, in particular the migration of BNCs.

In conclusion, NT cows were noted to have poorly developed caruncles resulting from atypical development of the placentome. This was associated with atypical gene expression. Accordingly, this study carried out an extensive proteomic analysis of placentome tissues from IVF and NT and identified proteins showing significant differences in the expression level. These proteins were classified according to the viral factor as being involved in IVF and NT placentome tissues. These results suggest strongly that the

expression pattern of proteome in NT placentome tissue is associated atypical gene expression.

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