Protein Patterns on a Corpus Luteum during Pregnancy in Korean Native Cows

Hak-Jae Chung†, Dong-Min You, Hyo-Ju Kim, Hye-Young Choi, Myeong-Suk Lee, Jin-Bum Kim, Suck-Dong Lee, Jung-Yong Park and Myeung-Sik Lee

Molecular Animal Reproduction Lab, Hanwoo Experiment Station, National Institute of Animal Science, RDA, Pyongchang 232-950, Korea

ABSTRACT

Luteal cells produce progesterone that supports pregnancy. Steroidogenesis requires coordination of the anabolic and catabolic pathways of lipid metabolism. In the present study, the corpus luteum (CL) in early pregnancy established from luteal phase and pregnant phase was analyzed. The first study determined progesterone changes in the bovine CL at day 19 (early maternal recognition period) and day 90 in mid-pregnancy and compared them to the CL from day 12 of the estrous cycle. CL 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 CL from luteal phase to those from pregnant phase counterparts, significant changes in expression level were found in 23 proteins. Of these proteins 17 were not expressed in pregnant phase CL but expressed in luteal phase counterpart, whereas, the expression of the other 6 proteins was limited only in pregnant phase CL. Among these proteins, vimentin is considered to be involved in regulation of post-implantation development. In particular, vimentin may be used as marker for CL development during pregnancy because the expression level changed considerably in pregnant phase CL tissue compared with its luteal phase counterpart. Data from 2-DE suggest that protein expression was disorientated in mid pregnancy from luteal phase, but these changes was regulated with progression of pregnancy. These findings demonstrate CL development during mid-pregnancy from luteal phase and suggest that alternations of specific CL protein expression may be involved in maintenance of pregnancy.

(Key words : Hanwoo, Luteal and pregnancy phase, 2-DE, Protein expression patterns)

INTRODUCTION

The function of CL is a temporary endocrine gland formed in the ovary following ovulation and contributes to estrus/menstrual cycle regularity and the successful of pregnancy (Niswender and Nett, 1994). The life cycle of the CL is a strictly regulated process. In cattles, the CL develops, functions, and begins to regress within 21 days after ovulation during the estrus cycle but retains a functional lifespan of more than 200 days during pregnancy (Niswender *et al.*, 2000). Actually, in most placental mammals the continuation of luteal function associated with pregnancy extends well beyond the period of a single estrus or menstrual cycle and is required for the establishment of pregnancy for a significant portion of gestation. The patterns of activity and mRNA abundance of the steroidogenic enzymes, cytochrome P450 side-chain cleavage (Rodgers *et al.*, 1986, 1987), 3β-hydroxysteroid dehydrogenase (Couët *et al.*, 1990), and $P450_{17\alpha}$ hydroxylase (Rodgers *et al.*, 1987) have been described for the CL during the bovine luteal phase. In general, the mRNA coding for steroidogenic enzymes increase during luteal development and are strongly expressed during the mid-luteal phase (Rodgers *et al.*, 1987; Couët *et al.*, 1990). Furthermore, these information weaken during luteal regression *in vivo* in the ewe (Hawkins *et al.*, 1993), cow (Tian *et al.*, 1994) and in luteinized porcine granulose cells in culture treated with prostaglandin $F_{2\alpha}$ (Li *et al.*, 1993).

Proteins in the CL of some mammals, such as ewe (Bott *et al.*, 2010; Chowdhury *et al.*, 2010), human (Hirsch *et al.*, 1977; Channing *et al.*, 1980; Bersinger *et al.*, 2009; Dickinson *et al.*, 2009), sheep (Campbell *et al.*, 2009) have been the subjects of many previous studies that sought to understand how the biochemistry of the ovary and uterine adapts to the presence, and supports the maintenance of pregnancy. The factors responsible for the

* This work was supported in part by a Grant-in-Aid (PJ006456) from the Rural Development Administration.

† Corresponding author : Phone: +82-33-330-0617, E-mail: hakjaena@korea.kr

progressive changing in pregnant CL from luteal cows are still unknown. In the present study, we used the excellent resolution afforded by two-dimensional polyacrylamide gel electrophoresis (2-DE) and matrix associated laser desorption/inoozation time-to-flight mass spectrometry (MALDI-TOF-MS) to separating complex polypeptide mixtures to identify proteins in luteal phase and pregnant phase CL that are associated with during pregnancy.

MATERIALS AND METHODS

Animals

These experiments were carried out at Hanwoo Experiment Station and at Reproduction Science department of National Institute of Animal Science. The National Institute of Animal Science Agricultural Animal Care and Use Committee approved animal procedures used in the experiments. The present study used 25 pubertal Hanwoo cows at 2~3 years of age. We raised Hanwoo cows under semi-intensive range condition and fed (07:00 h and 16:00 h) with a mixture of locally available gress, corn silage, and concentrates (TDN 54.4% and CP 12.8% on DM basis).

Experimental Design

In this study, cows were used based on palpation per rectum of genitalia and ovaries to assess normality and the number of follicular waves detected by transrectal ultrasonography (Aloka 500V, Aloka, Wallingford, CT, USA) equipped with a 5.0-MHa linear-array transducer probe. Therefore, we checked on the condition of the ovary and follicles every two days using ultrasonography during the previous estrous cycle and confirmed the number of follicular waves. The animals were divided into the following three groups: ovariectomy during the estrous cycle (not artificial inseminated) on day 12 and 16, with five cows on each day, respectively, and ovariectomy of five cows after artificial insemination on day 17. Whereas, a control group containing seven cows at day 50 of pregnancy was included in the study. To determine the exact day of ovulation, all cows received GnRH (0.01 mg Buserelin, 2.5 ml of Receptal™, Intervet, Unterschleißheim, Germany), $PGF_2\alpha$ (0.5 mg Cloprostenol, 2.0 ml Estrumate™, Essex Tierznei, Munich, Germany) 7 days later and then GnRH 48 hrs after $PGF_2\alpha$. The inseminated cows in the day 60 and day 90 pregnant groups were artificial inseminated 12 and 24 hrs after GnRH application. Blood samples were collected into 10 ml tubes containing 0.3M EDTA (BD Vacutainer™, Belliver Industrial Estste, Plymouth, UK) at 08:00~10:00 hrs daily by puncture of the coccygeal vessels throughout the estrous cycle until the cows were ovariectomized. Blood samples were immediately centrifuged for 20 min at 2,500×g, and the plasma was then stored at −20℃ until assayed.

Preparation of Ovaries

Ovaries were obtained from pubertal Hanwoo cows at the abattoir of National Institute of Animal Science in Suwon. CL from non-pregnant animals was classified into three stages by a careful examination of the reproductive tract as described and validated by Ireland *et al.* (1980). Briefly, CL-1 phase includes the interval between ovulation and time when the epithelium grows over the rupture point. During CL-2 phase the CL is enclosed in epithelium, blood vessels are visible around the periphery and the apex is red. In CL-3 phase the ovaries contain at least one large follicle and the CL has decreased in size and has no blood vessels visible on the surface. Ireland *et al.* (1980) estimated that the days of the cycle corresponding to these different stages were as follow: CL-1 phase, days 1-5; CL-2 phase, days 6~13; CL-3 phase, days 14~20. CL were also collected from early pregnant phase (at day 60 of pregnancy) to mid pregnant phase (at day 90 of pregnancy) cows in which the duration of pregnancy was estimated from the crown-rump length of the fetus. In addition preovulatory follicles and whole ovaries from cyclic cows containing follicles but not luteal tissue were obtained.

Treatment of Ovaries

Whole ovaries were frozen on solid CO_2 immediately after collection (10~20 min from slaughter) and stored at −20℃. After partial thawing, the CL or preovulatory follicles were dissected from the rest of the ovarian stroma and weighed. The earlier classification was checked by bisecting the CL. A small piece (10~20 mg wet weight) was removed for progesterone analysis. The remainder (still mainly frozen) was sliced into cold extraction medium consisting of 15% (v/v) trifluoroacetic acid, 5% (v/v) formic acid, 1% (w/v) sodium chloride in 1M HCl and homogenized. Extraction was performed on individual CL as described by Osnes *et al.* (1993).

Measurement of Progesterone

Aliquots of each freeze-dried ovarian tissue extract were measured in specific ELISA for progesterone. The progesterone content of the ovarian tissue was estimated by a scaled-down version of the technique described by Axelson *et al.* (1975). The extraction medium (1.5ml chloroform: methanol, 1:1 v/v) was added to the frozen tissue sample (10~20 mg) which was then homogenized for two 15-sec bursts. The homogenized was placed in an ultrasonic bath for 15 min, mixed then centrifuged at 10,000×g for 3 min to remove the se-

diment. The supernatant was dried down under a stream of air the extract was re-dissolved in 1ml progesterone assay buffer. Progesterone concentration was measured by a specific Immunoassay kit (DELFIA®, Boston, MA, USA) according to the procedure described by Miyamoto *et al.* (1992).

2-DE Polyacrylamide Gel Electrophoresis

The corpus luteum tissues were homogenized directly by motor-driven homogenizer (PowerGen125, Fisher Scientific) in sample buffer composed with 7 M 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 1 mM 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).

IPG dry strips were equilibrated for 12~16 hrs with 7M urea, 2M thiourea containing 2% 3-[(3-cholamidopropy) dimethyammonio]-1-propanesulfonate (CHAPS), 1% (w/v) dithiothreitol (DTT), 1% pharmalyte and respectively loaded with 200 μg of sample. Isoelectric focusing (IEF) was performed at 20℃ suing a Multiphor II electrophoresis unit and EPS 3500 XL power supply (Amersham Biosciences, USA) following manufacturer's instruction. For IEF, the voltage was linearly increased from 150 to 3,500 V during 3 hrs for sample entry followed by constant 3,500 V with focusing complete after 96 kVh. Prior to the second dimension, strips were incubated for 10 min in equilibration buffer (50 mM Tris-HCl, pH 6.8 containing 6 M urea, 2% SDS and 30% glycerol), first with 1% DTT and second with 2.5 iodoacetamide. Equilibrated strips were inserted onto SDS-PAGE gels (20~24 cm, 10~16%). SDS-PAGE was performed suing Hoefer DALT 2-DE system (Amersham Biosciences, USA) following manufacturer's instruction. 2-DE gels were run at 20℃ for 1,700 Vh. And then 2-DE 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. Qantity od each spot were selected for the significant expression variation over two fold in its expression level compared with non-pregnant CL tissues.

MALDI-TOF Analysis Database Search

Protein analysis was performed by using Ettan MALDI-TOF (Amersham Biosciences, USA). Peptides were evaporated with a N_2 laser at 337 nm, and using a delayed extraction approach. They were accelerated with 20 kv 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/WebProfound.exe), was used for protein identification by peptide mass fingerprinting. Spectra were calibrated with trypsin auto-digestion Ion peak m/z (842.510, 2211. 1046) as internal standards.

Statistical Analysis

All data are presented as means±SEM. Plasma and CL progesterone concentrations were compared using Student's *t*-test. All analysis of data was by a one way-ANOVA test on log transformed data followed by paired by Tukey-Kramer comparison.

RESULTS

Progesterone Level

The plasma progesterone concentrations increased equally in both the cyclic and pregnant cows up to day 18. However, in comparison with the pregnant cows, a significant decline in the plasma progesterone concentrations of the cyclic cows occurred on day 19.

The visual classification of the CL was supported by the data on their progesterone content (Fig. 1A). The progesterone levels of the CL removed at each stage increased between stage of CL1 and CL2 ($p<0.05$), and then dropped precipitously by CL3 stage ($p<0.001$). On the other hand, progesterone levels in pregnant cows were similar to those in mid-cycle (Fig. 1B).

Proteomic Analysis of Cyclic and Pregnant Corpus Luteum Tissues

The proteins obtained from CL tissue of luteal phase (CL-2) and at day 90 of pregnant was applied to 2-DE. The protein spots visualized with silver stain. After staining, the number of spots was estimated by using PDQuest software; approximately 600~900 spots were determined. The 2-DE experiment was repeated at least 7 times on both luteal (n=5) and pregnant (n=4) CL tissue samples. Quantity of each spot was normalized by total valid spot intensity. In Hanwoo, luteal phase (CL-2) and pregnant luteal tissue samples to the investigation of the differential protein expression surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Micromass, UK) technique was used (Fig. 2).

Additionally, protein spots were selected for the significant expression variation deviated over two fold in pregnant CL proteins profile expression level compared with luteal phase CL proteins. Only the protein spots that fulfilled $p<0.05$ were selected. In this way, we fo-

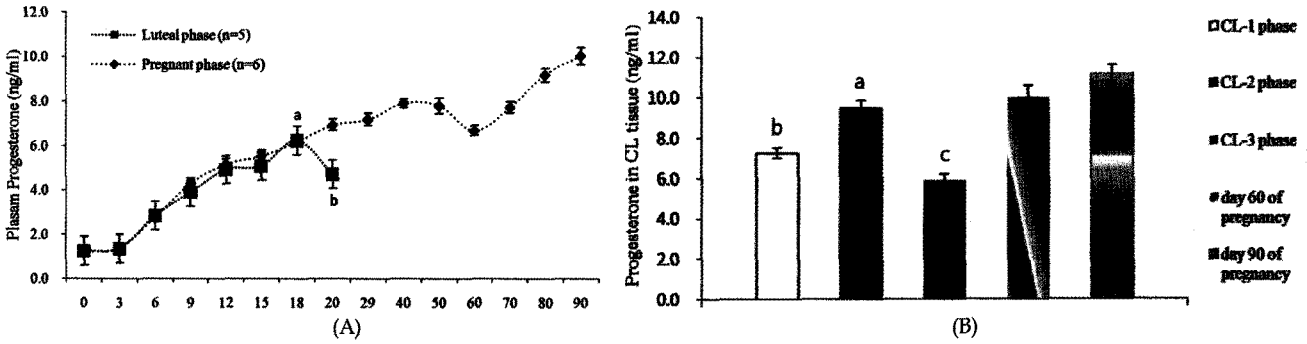

Fig. 1. Changes of plasma progesterone concentrations are indicated with white bars during the estrous cycle and black bars during pregnancy. (A) In the cyclic cows, 5 samples are indicated for cyclic days 1~13, and 6 samples are indicated for cyclic days 14~20. (B) Luteal phase progesterone concentrations during the estrous cycle on CL-1(day 1~5, n=5), CL-2(6~13, n=5), CL-3(14~20, n=5) and on day 60(n=5) and on day 90(n=5) of pregnancy. All values are shown as means±SEM.

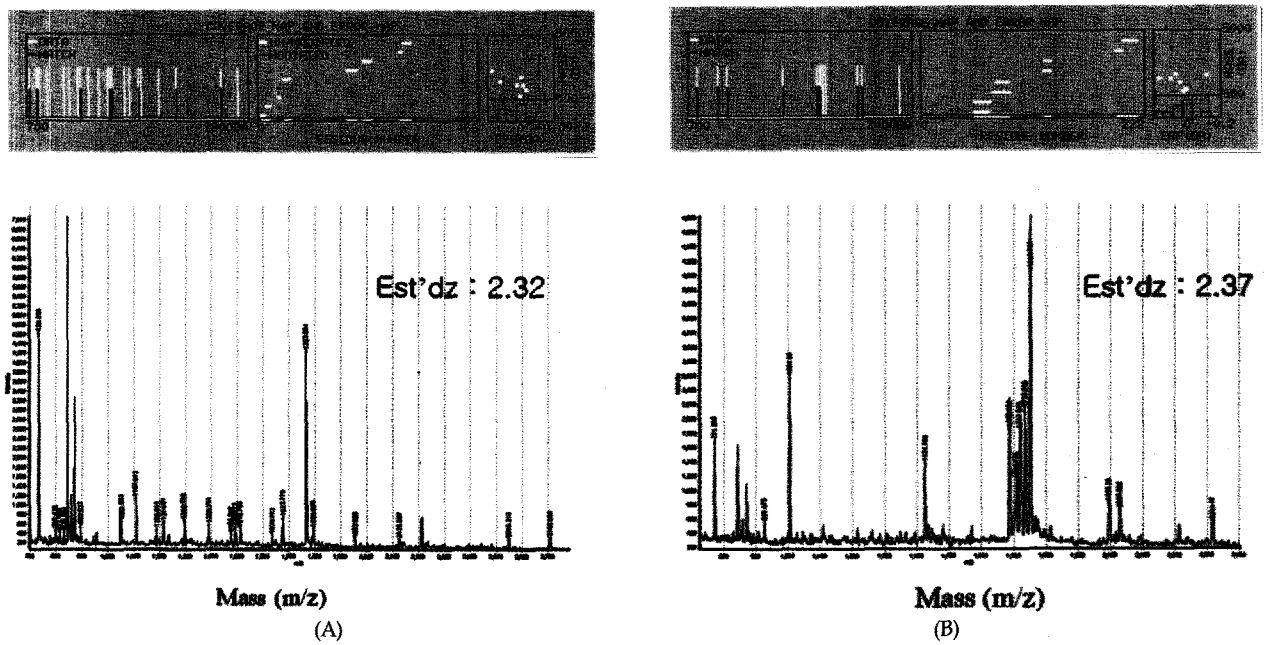

Fig. 2. Peptide fingerprint obtained by mass spectrometry of protein spots that showed in luteal phase (CL-3) and at day 90 of pregnancy. (A) The peak shown on the peptide fingerprint correspond to fumarate hydratease, (B) the peaks shown on the peptide fingerprint correspond to manganous superoxide dismutase. Protein information retrieved from NCBI web site.

und 32 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 23 proteins were clearly identified (Table 1).

Proteins Differentially Expressed in Pregnant Luteal Tissue

From the group of 32 spots, 23 spots were identified by MALDI-TOF MS. The identified proteins are listed in Table 1 and their position on the 2-DE gel map was marked by SWISS-PROT accession number (Fig. 3). Proteins of all listed in Table 1 provided with their molecular mass, experimental corresponding percentile in an estimated random match population (Est'dz score). A comparison of the density of the spots identified on the reference map between the luteal phase and pregnant CL tissues showed that 6 protein spots were significantly increased (Fig. 3A and Table 1), 17 protein spots were significantly decreased (Fig. 3B and Table 1) in pregnant CL tissue.

DISCUSSION

The validity of this study dependent on the accuracy

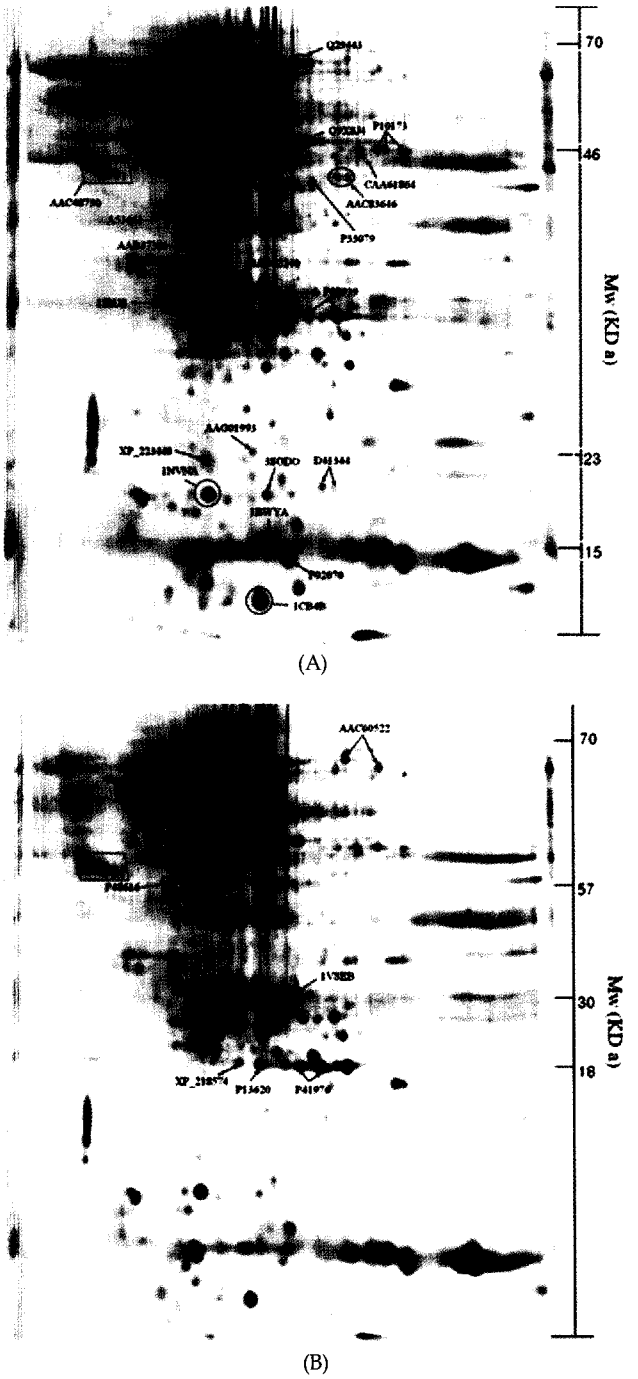

Fig. 3. 2-DE map of luteal proteins obtained from luteal phase (A) and corpus luteum at day 90 of pregnancy (B). Blue square boxes are control marker.

with which the CL were dated and this subject therefore merits a careful consideration. The comparison has been made between the blood and progesterone contents of CL in this study with previously reported values (Veenhuizen *et al.*, 1972; Axelson *et al.*, 1975; Ireland *et al.*, 1980). In the present study, during CL-1 phase the CL still has a soft consistency and may contain a pool of blood. When dissecting the ovary fresh it is more difficult to be certain that all the tissue has been obtained, particularly if the CL is removed at laparotomy rather than at slaughter. As shown in Fig. 1, the plasma progesterone concentrations on day 17 of the pregnant cows significant deviated from those of luteal phase cows on day 17. This means that the significant deviation occurred at the time of expected onset of luteolysis. This result correlates with findings of Chagas and Lopes (2005), who indicated that the plasma progesterone concentrations of pregnant heifers (by artificial insemination or embryo transfer) significantly deviate from those of non-pregnant and non-bred heifers on day 17. On the other hand, no change in the tissue progesterone concentrations could be observed between the CL of the cyclic and pregnant cows, which is in agreement with a previous reported (Erb *et al.*, 1968). Although luteal progesterone appears to be produced to maintain early pregnancy during the mid-luteal phase in cyclic cows, there are some independent mechanisms between production of progesterone within the CL and secretion of progesterone into the circulation. Nevertheless, the mechanism regulating progesterone release into the circulation is still unknown.

In the present study, we examined the analysis of progesterone levels of luteal and pregnant phase, and report the protein patterns of luteal-pregnant phase. The physiological activity of most CL existed for maintenance of pregnancy. In addition to proteomics is becoming a widely used approach to study cellular mechanisms. The study of proteins and post-translational 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 luteal phase and pregnant CL tissues (see Fig. 3, panels A and B). This report identified 23 proteins of which expression levels were significantly different between luteal phase and pregnant phase CL tissues. These identified results were comprised of post-translational protein.

Ulp 1 protease family (small ubiquitin-like modifier, SUMO), have been identified in eukaryotic organisms (Melchior, 2000; Schwartz and Hochstrasser, 2003; Dohmen, 2004). SUMOylation influences stability, interaction, cellular localization, and activity of proteins and thereby regulates processes such as DNA repair (Hoege *et al.*, 2002; Pfander *et al.*, 2005), nuclear transport (Stade *et al.*, 2002), transcription regulation (Ross *et al.*, 2002; Sapetschnig *et al.*, 2002), chromosome segregation (Nacerddine *et al.*, 2005), and ion channel activity (Rajan *et al.*, 2005). Interestingly, vimentin related intermediated filament proteins in luteal phase CL tissue were not detected on 2-DE analysis (Fig. 3). Vimentin expression is required for maintenance of pregnant CL in cows (Fenyves *et al.*, 1993). Vimentin coexpressed in tropho-

Table 1. The 23 proteins for which expression differed between luteal phase and corpus luteum at day 90 of pregnancy

Spot No.	AN*	Protein name	Est'dz**
		Expression of luteal phase	
2324	BAD32240	mKIAA0518 protein [*Mus musculus*]	1.84
2331	1IRUS	Chain S, crystal structure of the mammalian 20s proteasome At 2.75 A Resolution	1.67
2524	A53661	Protein-tyrosine-phosphatase (EC 3.1.3.48), receptor type O precursor - rabbit	0.87
3000	AAG01993	Similar to homo sapiens mRNA for KIAA0120 gene with GenBank Accession Number D21261.1	1.3
3109	XP_223443	Similar to bM573K1.5 (novel Ulp1 protease family member) [*Rattus norvegicus*]	1.23
3407	AAB37381	IgG1 heavy chain constant region [*Bos taurus*]	1.87
5113	3SODO	Chain O, Cu, Zn superoxide dismutase (E.C.1.15.1.1) mutant with Cys 6 replaced by Ala (C6a)	1.71
5215	1BWYA	Chain A, Nmr study of bovine heart fatty acid binding protein	2.11
6004	P02070	Hemoglobin beta chain	1.19
6523	P33097	Aspartate aminotransferase, cytoplasmic (Transaminase A) (Glutamate oxaloacetate transaminase-1)	2.23
6606	Q9XSJ4	Alpha enolase (2-phospho-D-glycerate hydro-lyase) (NNE) (Enolase 1) (Phosphopyruvate hydratase)	2.39
6814	Q29443	Serotransferrin precursor (Transferrin) (Siderophilin) (Beta-1-metal binding globulin)	2.37
7135_R	D41344	Lutropin-choriogonadotropin receptor precursor (splice form D)	1.02
7513	AAC83646	Dystrophin [*Canis familiaris*]	1.17
7814	CAA61864	put. 26S protease subunit [*Sus scrofa*]	2.34
8633	P10173	Fumarate hydratase, mitochondrial (Fumarase)	2.32
6226	P00939	Triosephosphate isomerase (TIM)	1.45
		Expression of pregnany phase	
2509	P48616	Vimentin	2.37
4223	XP_218574	Similar to BC013491 protein [*Rattus norvegicus*]	1.03
5212	P13620	ATP synthase D chain, mitochondrial	2
6311	1V9EB	Chain B, Crystal structure analysis of bovine carbonic anhydrase Ii	2.3
6224	P41976	Superoxide dismutase [Mn], mitochondrial precursor	2.38
7813	AAC60522	Manganous superoxide dismutase; MnSOD [*Bos taurus*]	2.37

* AN: Accession Number (NCBI search)
** Est'dz: Z score (corresponding percentile in an estimated random match population 2.326=99%, 1.65=95%, 1.282=90%).

blast cells in human (Vicovac and Aplin, 1996) amd mouse (Souza and Katz, 2001). Therefore, aberrant expression of vimentin was likely indicative of future CL in pregnant cows. The vimentin appear to be useful diagnostic marker of functional CL during pregnancy.

Manganous sueroxide dismutase (MnSOD), a reactive oxygen scavenging metalloenzyme, catalyzes the conversion of superoxide radicals to hydrogen peroxide and oxygen inside mitochondria (McCord and Fridovich, 1969). The endotoxin-induced increase in MnSOD could contribute to the reported protective effect of endotoxin against oxygen toxicity in bovine endothelial cells (Shi-

ki *et al.*, 1987). Chronic exposure to endocrine stress (pregnant) is also known to cause a variety of patho-phtsiological changes in endocrine system, resulting in changed steroidogenesis.

In conclusion, pregnant cows were noted have functionally developed CL resulting from atypical development of the luteal tissues. This was associated with atypical gene expression. Accordingly, this study carried out an extensive proteomic analysis of luteal tissues from luteal phase and pregnant phase and identified proteins showing significant differences in the expression level. These proteins were classified according to the viral factor as being involved in luteal and pregnant CL tissues. These results suggest strongly that the expression pattern of proteome in pregnant CL tissue is associated atypical protein expression.

REFERENCES

1. Axelson M, Schumacher G, Sjövall J, Gustafsson B, Lindell JO (1975): Identification and quantitative determination of steroids in bovine corpus luteum during oestrous cycle and pregnancy. Acta Endocr Copenh 80:149-164.
2. Bersinger NA, Bikhäuser MH, Yared M, Wunder DM (2009): Serum glycodelin pattern during the menstrual cycle in healthy young women. Acta Obstet Gynecol Scand 88:1215-1221.
3. Bott RC, Ashley RL, Henkes LE, Antoniazzi AQ, Bruemmer JE, Niswender GD, Bazer FW, Spencer TE, Smirnova NP, Anthony RV, Hansen TR (2010): Uterine vein infusion of interferon tau (ITNF) extends luteal life span in ewe. Biol Reprod 82:725- 735.
4. Bradford MM (1976): A repad and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254.
5. Campbell BK, Kendall NR, Baird DT (2009): Effect of direct infusion of bone morphogenetic protein 6 (BMP6) on ovarian function in sheep. Biol Reprod 81: 1016-1023.
6. Chagas e SJ, Lopes de CJ (2005): Luteotrophic influence of early bovine embryos and the relationship between plasma progesterone concentrations and embryo survival. Theriogenology 64:49-60.
7. Channing CP, Schaerf FW, Anderson LD, Tsafriri A (1980): Ovarian follicular and luteal physiology. Int Rev Physiol 22:117-201.
8. Chowdhury MW, Scaramuzzi RJ, Wheeler-Jones CP, Khalid M (2010): The expression of angiogenic growth factors and their receptors in ovarian follicles throughout the estrous cycle in the ewe. Theriogenology 73:856-872.
9. Couët J, Martel C, Dupont E, Luu-The V, Sirard MA, Zhao H-E, Pelletier G, Labrie F (1990): Changes in 3β-hydroxysteroid dehydroxygenase/$\Delta^5-\Delta^4$ isomerase mRNA, activity and protein levels during the estrous cycle in the bovine ovary. Endocrinology 127:2141-2148.
10. Dickinson RE, Stewart AJ, Myers M, Millar RP, Duncan WC (2009): Differential expression and functional characterization of luteinzing hormone receptor splice variants in human luteal cells: implications for luteolysis. Endocrinology 150:2873-2881.
11. Dohmen RJ (2004): SUMO protein modification. Biochem Biophys Acta 1695:113-131.
12. Erb RE, Estergreen VL, Gomes WR, Plotka ED, Frost OL (1968): Progestin levels in corpora lutea and progesterone in ovarian venous jugular vein blood plasma of the pregnant bovine. J Dairy Sci 51:401-410.
13. Fenyves AM, Behrens J, Spanel-Borowski K (1993): Cultured microvascular endothelial cells (MVEC) differ in cytoskeleton expression of cadherins and fibronectin matrix: A study under influence of interferon-γ. J Cell Sci 106:879-890.
14. Hirsch PJ, Fergusson IL, King RJ (1977): Protein composition of human endometrium and its secretion at different stages of the menstrual cycle. Ann N Acad Sci 11:286-248.
15. Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S (2002): RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 419:135-141.
16. Ireland JJ, Muephee RL, Coulson PB (1980): Accuracy of predicting stages of bovine estrous cycle by gross appearance of the corpus luteum. J Dairy Sci 63:155-160.
17. KcCord JM, Fridovich I (1969): Superoxide dismutase: an enzymic function for erythrocuprein (Hemocuprein). J Biol Chem 244:6049-6055.
18. Li X-M, Juorio AV, Murphy BD (1993): Prostaglandins alter the aboundance of messenger ribonucleic acid for steroidogenic enzymes in cultured porcine granulose cells. Biol Reprod 48:1360-1366.
19. Melchior F (2000): SUMO-nonclassical ubiquitin. Annu Rev Cell Biol 16:591-626.
20. Nacerddine K, Lehembre F, Bhaumik M, Artus J, Cohen-Tannoudji M, Babinet C, Pandolfi PP, Dejean A (2005): The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. Dev Cell 9:769-779.
21. Nelson PS, Han D, Rochon Y, Corthals GL, Lin B, Monson A, Nguyen V, Franza BR, Olymate SR, Aebersold R, Hood L (2000): Comprehensive analyses of prostate gene expression: convergence of expressed sequence tag database, transcript profiling and subsequent development. Electrophoresis 21:2235-2242.
22. Niswender GD, Nett TM (1994): Corpus luteum and its control in infraprimate species. In: Knobil E, Ne-

ill JD (eds.), The Physiology of Reproduction. 2nd ed, New York: Raven Press pp 781-816.
23. Niswender GD, Juengel JL, Silva PJ, Rollyson MK, McIntush EW (2000): Mechanisms controlling the function and lifespan of the corpus luteum. Physiol Rev 80:1-29.
24. Osnes T, Sandstad O, Skar V, Osnes M, Kierulf P (1993): Total protein in common dust bile measured by acetonitrile precipitation and a micro bicinchoninic acid (BCA) method. Scand J Clin Lab Invest 53:757-763.
25. Pfander B, Moldovan GL, Sacher M, Hoege C, Jentsch S (2005): SUMO modified PCNA recruits Srs2 to prevent recombination during S phase. Nature 436:428-433.
26. Rajan S, Plant LD, Rabin ML, Butler MH, Goldstein SA (2005): Sumoylation silences the plasma membrane leak K^+ channel K2P 1. Cell 121:37-47.
27. Rodgers RJ, Waterman MR, Simpson ER (1986): Cytochrome P-450sec, P-$450_{17\alpha}$, adrenodoxin, and reduced in bovine follicles and corpora lutea. Changes in specific contents during the ovarian cycle. Endocrinology 118:1366-1374.
28. Rodgers RJ, Waterman MR, Simpson ER (1987): Levels of messenger RNA encoding cholesterol side-chain cleavage cytochrome P-450, 17 α-hydroxylase cytochrome P450, adrenodoxin and low density lipoprotein receptor in bovine follicles and corpora lutea throughout the ovarian cycle. Mol Endocrinol 1:274-279.
29. Ross S, Best JL, Zon LI, Gill G (2002): SUMO-1 modification represses Sp3 transcriptional activation and modulates its subnuclear localization. Mol Cell 10: 831-842.
30. Sapetschnig A, Rischitor G, Braun H, Doll A, Schergaut M, Melchior F, Suske G (2002): Transcription factor Sp3 is silenced through SUMO modification by PIAS 1. EMBO J 21:5206-5215.
31. Schwartz DC, Hochstasser M (2003): A superfamily of protein tags: SUMO and related modifiers. Trends Biochem Sci 28:321-328.
32. Shiki Y, Meyrick BO, Brigham KL, Burr IM (1987): Endotoxin increase superoxide dismutase in cultured bovine pulmonary endothelial cells. Am J Physiol 252:C436-440.
33. Souza PC, de Katz SG (2001): Coexpression of cytokeratin and vimentin in mice trophoblastic giant cells. Tissue & Cell 33:40-45.
34. Stade K, Vogel F, Schwienhorst I, Meusser B, Volkwein C, Nentwig B, Dohmen RJ, Sommer T (2002): A lack of SUMO conjugation affects cNLS-dependent nuclear protein import in yeast. J Biol Chem 277:49554-49561.
35. Tian XC, Berndtson AK, Fortune JE (1994): Changes in levels of messenger ribonucleic acid for cytochrome P450 side-chain cleavage and 3 β-hydroxysteroid dehydrogenase during prostaglandin F_2 α-induced luteolysis in cattle. Biol Reprod 50:349-356.
36. Veenhuizen EL, Wagner JF, Tonkinson LV (1972) Corpus luteum response to 6-chloro Δ6-17 acetoxyprogesterone and hCG in the cow. Biol Reprod 6: 270-276.
37. Vicovac L, Aplin JD (1996): Epithelial-mesenchymal transition during trophoblast differentiation. Acta Anat 156:202-216.

(Received: 15 September 2010 / Accepted: 20 September 2010)