Characterization of EST Gene in the Bovine Corpus Luteum during the Estrous Cycle

Eunyoung Lee, Sang Hwan Kim, Byung-Gak Kim and †Jong Taek Yoon

Dept. of Animal Life Science, Hankyong National University, Ansung 456-749, Korea

ABSTRACT : The objective of this study was to investigate the expression of bovine luteum expressed sequence tags (ESTs), vascular endothelial growth factor (VEGF), and tumor necrosis factor receptor 1 (TNFR1) and the presence of functional ESTs in the bovine corpus luteum (CL) during different stages of the estrus cycle. Reverse transcription-polymerase chain reaction (RT-PCR) analysis showed a difference in the expression of ESTs during the CL stage. Concentration of ESTs in the CL tissue increased significantly from the mid-luteal stage and decreased thereafter. RT-PCR analysis showed higher levels of the EST genes in the CL of the mid-luteal stage than in other stages, and the same level of expression of VEGF. Immunohistochemistry analysis of the tissue from CL formation to regression showed low cytosol and aggregation of the nucleus. And activity caspase 3 (apoptosis detector) was most strongly detected in the CL1 stage of bovine. During the estrous cycle, the cytosol was magnified and differentiation of the nucleus was clearly manifested. The ESTs affected the CL, and the relationship between VEGF and TNFR1 played a pivotal role for CL development and activation, dependent on the stage of CL. These results suggest local production of ESTs, the presence of functional ESTs in the bovine CL, and that ESTs play a role in regulating the function of cell death in bovine CL.

Key words : Corpus luteum, Bovine, VEGF, TNF-alpha, Apoptosis

INTRODUCTION

Hemodynamic changes are involved in the cyclic remodeling of ovarian tissue that occurs during final follicular growth, ovulation, and new corpus luteum (CL) development (Acosta et al., 2002; Brannstrom et al., 1998; Collins et al., 1991; Moor et al., 1975). Extensive vascular formation (angiogenesis) and cell differentiation (luteinization) occur in the follicular wall during the period between the luteinizing hormone (LH) surge and early CL development. At ovulation, follicles collapse and release the ovum and antral fluid. The intensity of angiogenic processes reaches a peak 2−3 days later, in early CL (Reynolds et al., 2000).

Color Doppler ultrasonography is a useful, non-invasive technique for evaluation of ovarian vascular function, allowing a visual observation of blood flow in a delimited area in the wall of preovulatory follicles or within the CL (Acosta et al., 2002; Miyazaki et al., 1998).

The regression of a cyclic CL is a very complex process, which involves major changes in tissue composition. At 24−48 h after induced luteolysis in the bovine CL, there is pronounced oligonucleosome formation with histological signs of luteal cell degeneration (Cavender & Murdoch, 1988; Juengel et al., 1995). The CL is a heterogeneous gland,

which is composed of fibroblasts and immune cells, in addition to steroidogenic luteal cells and endothelial cells. The immune cells and their cytokine products are involved in the regulation of luteal function (Pate, 1994; Penny et al., 1999).

The transition from pre-ovulatory follicle to CL is a dynamic process involving a series of biochemical and morphological changes following the LH surge, which includes angiogenesis, the formation of new blood vessels from existing vessels (Reynolds & Redmer, 1999). Indeed, the rates of luteal growth and angiogenesis are such that they are only rivaled by the fastest growing tumors, and in the mature CL, the majority of luteal cells are adjacent to one or more capillaries (Reynolds & Redmer, 1999). While luteal and follicular angiogenesis are likely to be broadly similar, there are several important differences. Firstly, the breakdown of the basement membrane following ovulation enables endothelial cells and pericytes to invade and vascularize the luteinizing granulosa cells. Secondly, the timescale of luteal development is much shorter and, thus, angiogenesis is more intense. Indeed, up to 85% of the proliferating cells in the developing CL are thought to be related to vascular remodeling, since there is extensive intermingling of all cell types (Stocco et al., 2007). The extensive vascularization of the CL enables it to receive one of the highest blood flows per unit tissue mass. Luteal blood flow remains at pre-ovulation levels in the collapsed follicle, but thereafter gradually increases in parallel with increases in luteal volume and coincides with increases in progesterone (Acosta et al., 2002). Angiogenic factors produced by the CL of cows, pigs, and sheep are primarily heparin binding and can be neutralized with antibodies against fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and tumor necrosis factor (TNF). Angiogenesis involves a complex series of cellular processes and molecular changes. In adults, it is largely limited to pathological situations such as tumor growth and wound healing. However, the ovary undergoes continual cyclical changes and, thus, requires continual angiogenesis (Fraser & Lunn, 2001; Reynolds & Redmer, 1999). VEGF is a potent mitogen for endothelial cells and a stimulator of vascular permeability. These biological activities are important in the cascade of events leading to angiogenesis, which is essential for the developing follicle and CL. In addition, VEGF is the most important factor in the regulation of normal and abnormal angiogenesis.

TNF, a nonglycosylated protein with a molecular mass of 17 kDa, first described as a tumoricidal factor produced by activated macrophages, has a lower concentration in the bovine endometrium during the early luteal stage than at other luteal stages (Miyamoto et al., 2000). Extensive research during the last decade suggests that TNF plays one or more physiological roles in the CL in a variety of species. It has been demonstrated that TNF inhibits gonadotropin-supported progesterone production by murine, porcine, and bovine luteal cells and stimulates prostaglandin synthesis by bovine luteal cells. These findings imply that the actions of TNF on CL function are concerned with luteal regression. Indeed, the interaction of TNF and inflammatory cells is postulated to promote the regression of the CL (Bagavandoss et al., 1988). Hence, if TNF has one or more roles in luteolysis, functional TNF receptors (TNFRs) should be present in the CL, at least during luteal regression.

The identification of genes expressed within the cells of a given tissue is a basic step in the determination of gene function and the analysis of tissue physiology. One efficient approach to the characterization of gene transcripts is the expressed sequence tag (EST). ESTs are generated by large-scale sequencing of randomly selected clones from cDNA libraries, constructed from mRNA. While the functions of many genes can be deduced from their homology to known genes in databases, the functions of most emerging genes and regulatory sequences are unknown. Sequence information from ESTs can be used in many other applications, such as the generation of physical maps of chromosomes, the development of genetic markers by microsatellite tagging, identifi-

cation of single nucleotide polymorphisms, and development of microarrays for functional genomics (Murakawa et al., 1994; Serapion et al., 2004). The Basic Local Alignment Search Tool (BLAST) is a well-established software program in bioinformatics research used to compare a sequence against libraries of known sequences to determine sequence similarity. A snapshot of genes expressed in a given tissue and at a given developmental stage. Therefore, ESTs provide valuable information for functional genomics and bioinformatics research. Although major database (DB) websites, including NCBI, provide BLAST services and EST data, a local DB search system is needed for better performance and security.

Here, we present animal EST DBs and a local BLAST search system. The EST DBs in NCBI Genbank were divided by animal species using the perl script which we developed. We also built extended DB search systems for new data (Local animal BLAST search system), which was constructed on a high capacity PC cluster system for better performance.

MATERIALS AND METHODS

1. Animals and CL collection

Corpora lutea were removed transvaginally from regularly cycling dairy cows with physiological saline (PBS), cut open along their longitudinal axes, and processed according to the protocols described below. The CL were cut into 4 pieces, 3 of which were immediately snap frozen in liquid nitrogen, transported to the laboratory, and stored at −80°C until RNA extraction was performed. In the first experiment, CL's were removed at 5 stages of the estrous cycle; corpora hemorrhagic CH2 (1−2 cm), CH3 (>2 cm), CL (1−2 cm), CL3 (>2 cm).

2. Total RNA extraction

Frozen tissue collected for the first experiment was homogenized in TRIzol (Brinkman instruments, Westbury, NY) reagent using a polytron tissue homogenizer. In the second experiment, RNA from cultured cells was isolated by adding TRIzol reagent directly to culture flasks. Following tissue homogenization or cell lysis, total cellular RNA was isolated according to procedures outlined by the manufacturer. The final RNA precipitate was resuspended in double-distilled water containing 0.1% diethyl pyrocarbonate (DEPC), and the RNA concentration was determined using an ultraviolet spectrophotometer at 260- and 280-nm absorbance. The A260:A280 ratio was >1.8 for all RNA samples. The RNA integrity was assessed by examining the 28S and 18S ribosomal RNA bands on ethidium bromide stained agarose formaldehyde gels. The samples were stored at −80°C until use.

3. Semi-quantitative RT-PCR

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) was carried out with the housekeeping gene, lyceraldehydes 3-phosphate dehydrogenase (GAPDH), as an internal standard. GAPDH is constitutively expressed and has been used effectively in studies on the regulation of gene expression in ovarian cells (Chomczynski & Sacchi, 1987; Spanel-Borowski & van der Bosch, 1990; Tsai, Wiltbank, & Bodensteiner, 1996). The RT-PCR amplification was calibrated to determine the optimal number of cycles to allow detection of transcripts while keeping the amplification of these genes in the log phase (primer dropping method). GAPDH oligonucleotide primer pair (sense 5′-CAATGAACCCCTTCATTGACC-3′; antisense 5′-AGCA CCTCCCTGAATACTGG-3′) and the EST gene oligo-nucleotide primer pair (sense 5′-CTCAGCCCAACATGA GGATA-3′; antisense 5′-TTTCCCAGGACTGCTCCTTT-3′) were synthesized according to bovine sequences. The TNFR1 primer pairs (sense 5′-CACCACCACCATCTGC TT-3′; antisense 5′-TCTGAACTGGGGTGCAGA-3′) and the VEGF primer pairs (sense 5′-CACCACCACCATCT GCTT-3′; antisense 5′-TCTGAACTGGGGTGCAGA-3′) were synthesized according to bovine cDNA for TNFR1 and VEGF. The expected PCR product lengths were 452 base pairs (bp) for GAPDH, 117 bp for the ESTs, 257 bp for TNFR1, and 117 bp for VEGF. With the exception of

CL, 35 cycles of PCR were conducted for GAPDH, ESTs, TNFR1, and VEGF.

4. Southern blot

The PCR amplified products of selected individual lines of three independent from wild type, mutant, and transformed strains of Haemophilus influenzae and digested to completion with PvuII. After agarose gel electrophoresis, Southern blot analysis with CL cDNA was performed as described elsewhere (Chomczynski & Sacchi, 1987).

5. Immunohistochemistry of apoptosis during bovine CL at various stages

Immunohistochemistry of apoptosis was performed on 5 μm tissue sections mounted on silanized slides. Briefly, paraffin sections were dewaxed with a xylene substitute (Polyclear solvent; Polysciences, PA, USA) and rehydrated in a graded series of ethanol. Antigen retrieval was performed by heating at 95°C in 10 mM sodium citrate (pH 6.0). Endogenous peroxidases were quenched with 0.3% hydrogen peroxide in methanol for 5 min at room temperature. After 3 times washed in 1× PBS buffer, the sections were blocked with 1% goat and 3% horse serum for 1 hr at room temperature. Sections were exposed to anti-rabbit caspase 3 polyclonal antibodies (diluted 1:100; Cat No. ab4051; Abcam) in blocking solution overnight at 4°C. Sections were washed and incubated with anti-rabbit secondary antibodies (diluted 1:300) for 1 hr at room temperature, and then rinsed and incubated with ABC detection (Vector) for 10 min. Diaminobenzidine (DAB) was used as a substrate for HRP. Sections were counterstained with PAS reagent and Harris hematoxylin containing 4% acetic acid. Tissues were dehydrated, cleared, and coverslipped using Permount (Fisher, PA, USA).

RESULTS

BLAST analysis of EST genes in day 5 CL (Fig. 1, 2)

Score = 693 bits (375), Expect = 0.0
Identities = 389/396 (98%), Gaps = 1/396 (0%)
Strand=Plus/Plus

```
Query 1    TCTGGGGGCGGACTCTTGGTTTATTTAGAAAGGAGCTATATACTCCCCTTTCTGTCTCCA 60
           ||| |||||||||||| ||||||||||||||||||||||||||||||||||||||||||
3263       TCTTGGGGCGGACTCTTTGTTTATTTAGAAAGGAGCTATATACTCCCCTTTCTGTCTCCA 3322
61         TCTGGGGCATCTTCCGCCGCACGGGACATCTCTGGTGCCTCGGGGTAT-TTTGACAGCTA 119
           ||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||||
3323       TCTGGGGCATCTTCCGCCGCACGGGACATCTCTGGTGCCTCGGGGTATCTTTGACAGCTA 3382
120        ACTTTCTCAGTCCTCTCTGGGTGCACCCCAGCCTNTAGTGCAGCTCACCTCTCATTTCCC 179
           ||||||||||||||||||||| ||||||||||||| ||||||||||||||||||||||||
3383       ACTTTCTCAGTCCTCTCTGGCTGCACCCCAGCCTCTAGTGCAGCTCACCTCTCATTTCCC 3442
180        AGGACTGCTCCTTTCTCCCTGATGACTGTGCCCCATCATTCATCTCAGCAGCAAGCGTGG 239
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
3443       AGGACTGCTCCTTTCTCCCTGATGACTGTGCCCCATCATTCATCTCAGCAGCCAGCGTGG 3502
240        GCTTTCCTCCATGTCCCCCGCCCTCGTCACACATCCTCATGTTGGGCTGAGAAGCATCTG 299
           ||||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||
3503       GCTTTCCTCCATGTCCCCCGCCCTCGTCACACATCCTCATGCTGGGCTGAGAAGCATCTG 3562
300        CTGGACTTGGGTCTTAGGGTCCCCTTCACACCCGCCACAGCTGATTATAGTATGTCCACT 359
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

Fig. 1. Comparison of nucleotide sequences of ESTs (1–395 bp) using BLAST.

Score = 619 bits (335), Expect = 1e-176
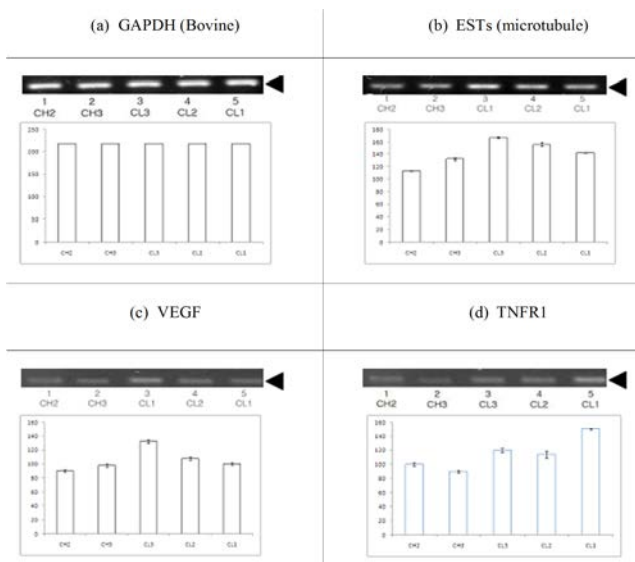Identities = 335/335 (100%), Gaps = 0/335 (0%)
Strand=Plus/Plus

```
443    GAGAGAAGTAAACTCCAGAAGGGAACCAGGAACTCCTCCCTTCCCTGGTGAGTATTTCCA 502
       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3706   GAGAGAAGTAAACTCCAGAAGGGAACCAGGAACTCCTCCCTTCCCTGGTGAGTATTTCCA 3765
503    TTATTCCGTTAAGGTTTAATATGCATTCAGATTACTTTTACTAAATAGTACACCATAAAG 562
       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3766   TTATTCCGTTAAGGTTTAATATGCATTCAGATTACTTTTACTAAATAGTACACCATAAAG 3825
563    CTTTTGTTATATATTAAATGTAAACTGAAAGGAATGTAAACAAATGTATTGTTAATTATA 622
       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3826   CTTTTGTTATATATTAAATGTAAACTGAAAGGAATGTAAACAAATGTATTGTTAATTATA 3885
623    AATATAGATAAGTAATGACATAATAGATGAAAAGGTCTTATTCAGATGTATCACATTCAT 682
       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3886   AATATAGATAAGTAATGACATAATAGATGAAAAGGTCTTATTCAGATGTATCACATTCAT 3945
683    TTTACATTACCCATCTATTGTCGCATGGTAGAATAGTTTTTTGTCTCTGAATATGTGAAT 742
       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3946   TTTACATTACCCATCTATTGTCGCATGGTAGAATAGTTTTTTGTCTCTGAATATGTGAAT 4005
743    AACTTGACTTGCGTTTATCTTTTTACATATTTAAT 777
       |||||||||||||||||||||||||||||||||||
```
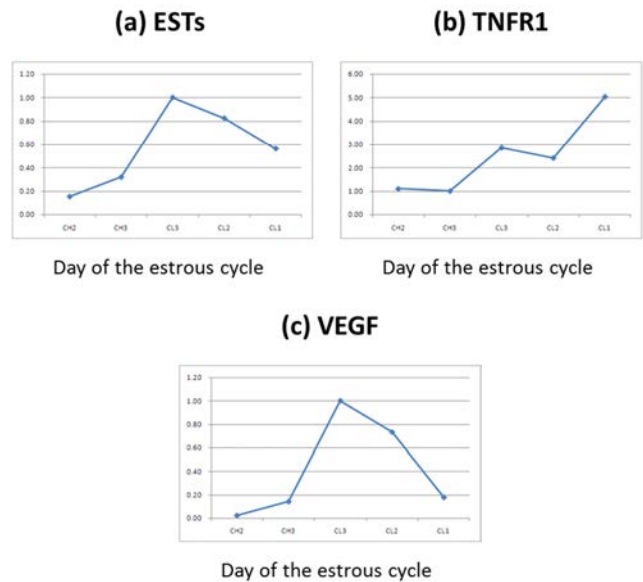
Fig. 2. Comparison of nucleotide sequences of ESTs (443–777 bp) using BLAST.

identified a bovine microtubule gene. Representative examples of RT-PCR-amplified ESTs, VEGF, and TNFR1 are shown in
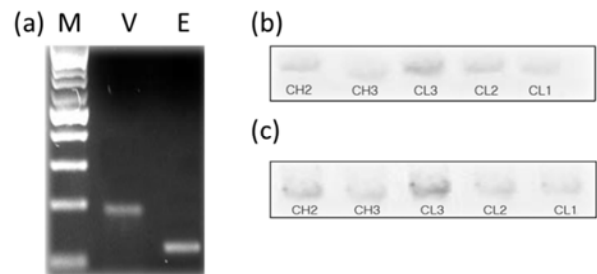
Fig. 3. There are distinct differences in receptor expression during early, middle, and late (days 13–18) luteal phases. The analysis of EST mRNAs is shown in Fig. 3. VEGF expression was high at the middle luteal stage (CL3) and significantly decreased during the late stage. The expression of TNFR1 was very low during the activation of ESTs and VEGF. However, its expression gradually increased during the degradation of CL2–CL1 (Fig. 4, 5). Bovine CL at various stages were used for IHC (Immunohistochemistry) (Fig. 6), semi-quantitative (SQ)-PCR (Fig. 3), real-time PCR (Fig. 4), and Southern Blot hybridization (Fig. 5). We also analyzed the expression pattern of the relationship between the ESTs and VEGF (a growth factor), and TNFR1 (cell death-related). ESTs and VEGF showed a similar expression pattern, suggesting that the appearance of ESTs might affect the expression pattern of VEGF. Staining of the CL tissue with IHC (Fig. 6) from CL formation to regression showed low cytosol and aggregation of the
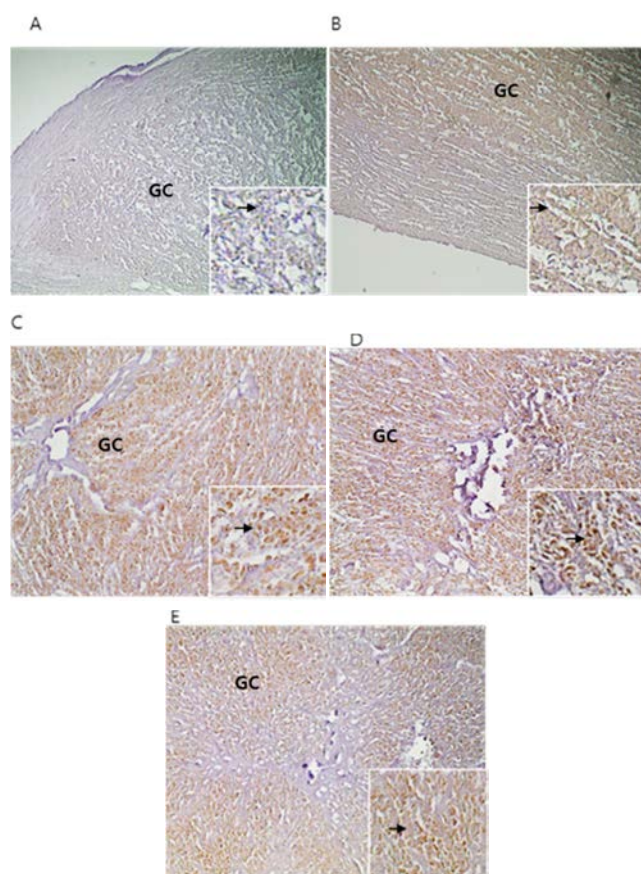

Fig. 4. Expression of cDNA by RT-PCR. Densitometrically analyzed RT-PCR results in bovine CL tissue during different luteal phases and pregnancy. (a) Expression of ESTs mRNA. (b) Expression of TNFR1 mRNA. (c) Expression of VEGF mRNA.

Fig. 3. Representative sample of specific semi-quantitative (SQ)-PCR products for (a) EST gene of GAPDH, (b) EST gene (117 bp), (c) EST gene of VEGF, and (d) EST gene of TNFR1 in bovine tissue during different luteal phases with DNA mass ladders. Lanes 1–2; early luteal phases, lanes 3–4; middle luteal phase, lane 5; last phase.

Fig. 5. Analysis of PCR Southern hybridization. (a) Electrophoresis of the probe and the fragments resolved on 1% agarose gel. Lane 1 = 100-bp molecular weight ladder, lane 2 = VEGF, lane 3 = EST. (b) 5-Day Southern blot hybridization of a probe for EST (c) Southern blot hybridization of a probe for VEGF.

nucleus. During the estrous cycle, the cytosol was magnified and differentiation of the nucleus was clearly manifested. At this time, we noted large granulosa cells death and blood vessel formation. We surmised that ESTs affect the cell, and that the relationship of VEGF and TNFR1 plays a pivotal role in cell nutrition and activation, depending on

Fig. 6. Immunolocalization and expression analysis of apoptosis-Casp-3 proteins in the follicles during bovine CL various stages. A) CH2 (early phase CL), B) CH3 (early phase CL), C) CL3 (middle phase CL), D) CL2 (last phase CL), E) CL1 (last phase CL).

the stage of CL in the bovine ovary.

DISCUSSION

To the best of our knowledge, this study is the first to report on the expression of ESTs, TNFR1, and VEGF in the luteal tissue of any species. LH is one of the most important hormones secreted by the pituitary gland for normal luteal development and its function has been demonstrated in many species. For continuity, much of the following discussion focuses on the role of the pituitary in regulating luteal function in ewes. If the pituitary is removed

on day 3 of the ovine estrous cycle, the CL does not continue to increase in weight, and serum concentrations of progesterone remain at or below levels observed on day 5 (Farin et al., 1990; Juengel et al., 1995). This loss of luteal weight, when compared with that in intact control ewes, is associated with a decrease in the number of small luteal cells (SLCs) and fibroblasts and a decrease in the size of both small and large steroidogenic luteal cells (Farin et al., 1990).

The gonadotropic regulation of the expression of mRNA for VEGF in the primate CL was studied *in vivo* by treating monkeys with a potent gonadotropin-releasing hormone (GnRH) antagonist during the mid-luteal phase of the menstrual cycle. A 3-day treatment regimen brought about a significant reduction in the levels of VEGF mRNA. In rats treated with human chorionic gonadotropin (hCG), increases in VEGF transcripts were detectable both in granulosa cells and thecal stromal tissue and the early CL. Cell differentiation itself plays an important role in the regulation of stimuli for VEGF expression. VEGF enables quiescent and resting endothelial cells to degrade underlying extracellular matrix, to invade and directionally migrate toward the angiogenic stimulus, and to proliferate and organize into new, 3-dimensional capillaries.

The body of evidence implicating immune cells as regulators of luteal function is expanding. Macrophages and T lymphocytes are found in the CL of a number of species, as are the mRNAs and proteins of several T cell-derived cytokines (Penny et al., 1999). T cell cytokines such as interlukin-1β (IL-1β), the TNF family, and interferon-γ (IFN-γ) inhibit LH-stimulated steroidogenesis and induce prostaglandin (PGF2α) production in cultures of mixed luteal parenchymal cells. The cDNA sequence of the bovine TNF-α receptor has been reported only for the type 1 receptor. Therefore, the expression of mRNA for the type-1 receptor in the bovine CL was examined by use of RT-PCR in the present study. The mRNA of TNFR1 was

expressed in the bovine CL at all stages during the estrous cycle. The concentration of TNF in the bovine endometrium at the early luteal stage was lower than concentrations at other luteal stages (Miyamoto et al., 2000). Local secretion of TNF-α in the bovine CL was higher at the late stage than during the mid-stage. TNF-α concentration in the CL were dramatically increased from levels of TNF-α mRNA expression and maintained at the same level in the CL throughout the estrous cycle. In conclusion, the overall results of the present study indicate the production and presence of local ESTs as well as functional VEGF in the bovine CL during the estrous cycle, and suggest that ESTs play physiological roles in regulating bovine CL function at the time of luteal regression as well as throughout all luteal phases. Further functional analysis of the EST genes will be required to predict their role in the CL.

REFERENCES

Acosta TJ, Yoshizawa N, Ohtani M, Miyamoto A (2002) Local changes in blood flow within the early and midcycle corpus luteum after prostaglandin F (2 alpha) injection in the cow. Biol Reprod 66:651-658.

Bagavandoss P, Kunkel SL, Wiggins RC, Keyes PL (1988) Tumor necrosis factor-a (TNF-a) production and localization of macrophages and T lymphocytes in the rabbit corpus luteum. Endocrinology 122:1185-1187.

Brannstrom M, Zackrisson U, Hagstrom HG, Josefsson B, Hellberg P, Granberg S, Bourne T (1998) Preovulatory changes of blood flow in different regions of the human follicle. Fertil Steril 69:435-442.

Cavender JL, Murdoch WJ (1988) Morphological studies of the microcirculatory system of periovulatory ovine follicles. Biol Reprod 39:989-997.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159.

Collins W, Jurkovic D, Bourne T, Kurjak A, Campbell S (1991) Ovarian morphology, endocrine function and intra-follicular blood flow during the peri-ovulatory period. Hum Reprod 6:319-324.

Farin CE, Nett TM, Niswender GD (1990) Effects of luteinizing hormone on luteal cell populations in hypophysectomized ewes. J Reprod Fertil 88:61-70.

Fraser HM, Lunn SF (2001) Regulation and manipulation of angiogenesis in the primate corpus luteum. Reproduction 121:355-362.

Juengel JL, Nett TM, Tandeski TR, Eckery DC, Sawyer HR, Niswender GD (1995) Effects of luteinizing hormone and growth hormone on luteal development in hypophysectomized ewes. Endocrine 3:323-326.

Miyamoto A, Matsuyama T, Ishiguro S, Nishio A (2000) Captopril increases the affinity of bradykinin receptor binding sites in bovine coronary arterial endothelial cells. Jpn J Pharmacol 84:82-85.

Miyazaki T, Tanaka M, Miyakoshi K, Minegishi K, Kasai K, Yoshimura Y (1998) Power and colour Doppler ultrasonography for the evaluation of the vasculature of the human corpus luteum. Hum Reprod 13:2836-2841.

Moor RM, Hay MF, Seamark RF (1975) The sheep ovary: regulation of steroidogenic, haemodynamic and structural changes in the largest follicle and adjacent tissue before ovulation. J Reprod Fertil 45:595-604.

Murakawa K, Matsubara K, Fukushima A, Yoshii J, Okubo K (1994) Chromosomal assignments of 3'-directed partial cDNA sequences representing novel genes expressed in granulocytoid cells. Genomics 23:379-389.

Pate JL (1994) Cellular components involved in luteolysis. J Anim Sci 72:1884-1890.

Penny LA, Armstrong D, Bramley TA, Webb R, Collins RA, Watson ED (1999) Immune cells and cytokine production in the bovine corpus luteum throughout the oestrous cycle and after induced luteolysis. J Reprod Fertil 115:87-96.

Reynolds LP, Grazul-Bilska AT, Redmer DA (2000) Angiogenesis in the corpus luteum. Endocrine 12:1-9.

Reynolds LP, Redmer DA (1999) Growth and development of the corpus luteum. J Reprod Fertil Suppl 54: 181-191.

Serapion J, Kucuktas H, Feng J, Liu Z (2004) Bioinformatic mining of type I microsatellites from expressed sequence tags of channel catfish (*Ictalurus punctatus*). Mar Biotechnol (NY) 6:364-377.

Spanel-Borowski K, van der Bosch J (1990) Different phenotypes of cultured microvessel endothelial cells obtained from bovine corpus luteum. Study by light microscopy and by scanning electron microscopy (SEM). Cell Tissue Res 261:35-47.

Stocco C, Telleria C, Gibori G (2007) The molecular control of corpus luteum formation, function, and regression. Endocr Rev 28:117-149.

Tsai SJ, Wiltbank MC, Bodensteiner KJ (1996) Distinct mechanisms regulate induction of messenger ribonucleic acid for prostaglandin (PG) G/H synthase-2, PGE (EP3) receptor, and PGF_2 alpha receptor in bovine preovulatory follicles. Endocrinology 137:3348-3355.