RESEARCH ARTICLE

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Expression Patterns of Bone Morphogenetic Proteins (BMP) in PGF2a Induced Luteolysis in Ovine Corpus Luteum *

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The aim of the present study was to assess the mRNA expression of the BMP system in the induced luteolysis of ovine corpus luteum (CL). CL samples were obtained from the induced luteolysis groups. In this study, we used the induced luteolysis model, the ewes were treated with PGF2α on the 12th day of the cycle, and luteal tissues were collected at 0 hours (without PGF2α treatment, n= 4), 4 h (PG4, n=4), and 16 h (PG16, n=4) after injection. mRNA expression levels of BMP components were quantified using RT-qPCR. SDHA was used as a reference control for the normalization of qPCR data. Expression of BMP4 mRNA was found to be greater in PG16 than in PG4 ($P<0.01$). Expression of BMP6 was shown to be greater in PG16 than in C12 ($P<0.01$) but did not differ between C12 and PG4 ($P>0.05$). Expression of BMP6 and BMPR1A mRNA was found to be higher in PG16 than in PG4 ($P<0.05$). While expression of BMPR1B mRNA was greater in PG16 than in PG4 and C12 ($P<0.0001$), it was shown to be lower in PG4 than in C12 ($P<0.05$). Although BMPR2 mRNA was found to be lower in PG16 and PG4 than in C12, this was not statistically significant ($P>0.05$). We may suggest that expression patterns of some BMP genes regulate luteolysis of ovine CL.

Key Words: *Corpus luteum, gene expression, luteolysis, BMP*

Koyun Korpus Luteumun'da PGF2a ile İndüklenmiş luteoliziste Kemik Morfogenetik Proteinlerinin (BMP) Ekspresyonu

Bu çalışmanın amacı, koyun korpus luteum'unun (KL) indüklenmiş luteolizisinde BMP sisteminin mRNA ekspresyonunu değerlendirmekti. İndüklenmiş luteolizis gruplarından KL örnekleri alındı ve indüklenmiş luteolizis modeli için, koyunlara döngünün 12. gününde PGF2a enjekte edildi ve luteal dokular 0. saatte (PGF2a enjeksiyonu yok, n= 4), 4. saatte (PG4, n=4) ve 16. saate enjeksiyondan sonra toplandı (PG16, n=4). BMP bileşenlerinin mRNA ekspresyon seviyeleri, RT-qPCR kullanılarak değerlendirilmiştir. SDHA, qPCR verilerinin normalleştirilmesi için referans gen kontrol olarak kullanıldı. BMP4 mRNA ekspresyonu PG16'da PG4'ten daha fazla olduğu bulundu ($P<0.01$). BMP6 ekspresyonunun PG16'da C12'den daha fazla olduğu gösterildi ($P<0.01$) ancak C12 ve PG4 arasında farklılık bulunmadı ($P>0.05$). BMP6 ve BMPR1A mRNA ekspresyonu PG16'da PG4'e göre daha fazla olduğu bulundu ($P<0.05$). BMPR1B mRNA ekspresyonu PG16'da PG4 ve C12'den daha büyük iken ($P<0.0001$), PG4'te C12'den daha düşük olduğu gösterilmiştir ($P<0.05$). BMPR2 mRNA'sının PG16'da PG4 ve C12'ye göre daha düşük bulunmasına rağmen, bu istatistiksel olarak anlamlı değildi ($P>0.05$). Bu çalışma, bazı BMP genlerinin ekspresyonunun koyun KL'sinde luteolizisini düzenleyebileceğini önermektedir.

Anahtar Kelimeler: *Korpus luteum, gen ifadesi, luteolizis, BMP*

Introduction

Corpus Luteum (CL), temporary endocrine structure, can be resulted from functional remodeling of follicular structure following ovulation. The key function of CL is to generate and secrete progesterone (P4) for early embryonic development and maintenance of pregnancy (1, 2). When pregnancy is not established, luteolysis initiates immediately to allow another upcoming estrous cycle to occur (3, 4). In ruminants, luteolysis is defined as functional whereby decrease in P4 and structural regression of CL (5), and mediated by the pulsative release of prostaglandin F2α (PGF2α) in elevating concentrations from the non-pregnant uterus (6). On the contrary, P4 released from CL is needed to sustain pregnancy (7-9).

Bone morphogenetic proteins (BMPs) are among the subfamily members of the transforming growth factor and are regarded as multifunctional growth factors. BMPs have functional roles in embryonic development, including cartilage, neural development, and postnatal bone formation (10). Besides their function in organs, BMPs function in different biological events in the ovary such as folliculogenesis, ovulation, and steroidogenesis (11, 12). BMPs compose the extensive part of the transforming growth factor (TGFβ) superfamily members. Some of the member of BMPs (BMP-2, - 3, -4, -6, -7) which forms heterodimer initiate BMP signaling through receptors of BMP; type I (BMP-IA and BMPR-IB) and type II (BMPR-II). Once ligand interaction implemented, signal transduction is accomplished via phosphorylation of type I receptor by type II receptor.

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Recently, during spontaneous regression, some of BMPs and their own receptors have been shown to be expressed in the human corpus luteum and are adversely modulated by the human chorionic gonadotropin (13). Contrary to the widespread role in folliculogenesis, limited number of studies (13-15) have focused on the modulation and function of BMPs throughout luteinization and luteolysis. Accordingly, we aimed to assess the expression patterns of BMP genes in the induced luteolysis of ovine CL.

Material and Methods

Animal Design of the Study: The experimental design of animal procedures was carried out in the breeding season of 2016. Animal diets for 3- to 5-year-olds ewes were adjusted to satisfy the National Research Council (NRC) Nutrient Requirements of Small Ruminants (16), including grass, dry clover, and concentrated feed. All other supplementals (vitamin and mineral with salt mix, and marble powder) were given ad libitum during the study (16). Bahri Dağdaş Research Center Ethical Committee (Number: 29/01/2016–49–7) approved the study procedures. In this study, we used induced luteolysis design. The ewes were divided into three groups following injection of PGF2α and PGF2α exposure was at 4 (PG4, n= 4) and 16 (PG16, n= 4) hours on day 12 of the cycle. We used cFOS and steroidogenic pathway mRNA expression to validate early challenge of CL to PGF2α treatment. The CL samples obtained on day 12 were allocated as zero h (C12, without PGF2a treatment, n= 4). We kept CL tissues at –80°C.

RNA Isolation and cDNA Synthesis: We extracted RNA as defined earlier (17). Shortly, 20 mg of CL tissues in 800 mL Trizol (PureZOL™ RNA Isolation Reagent-Bio-Rad) were smashed and 260 µL chloroform was used to obtain separation phase by centrifugation at 11,000 G for 12 min. We precipitated RNA using 500 µL isopropanol (Sigma-Aldrich) and removed supernatant and subsequently washed the pellet with 70% ethanol. Immediately, we eluted RNA in 40 µL of RNAse-DNAse free water. We confirmed RNA concentration and quality using NanoDrop (Thermo Scientific™ NanoDrop™ 2000) checking the absorbance values. We used one microgram RNA for conversion to cDNA through a kit (I-Script, BioRAD).

Gene Expression: We evaluated BMPs mRNA expression levels with the aid of qPCR with specific primers of BMP genes (Table 1). We prepared qPCR mix as follows: 10 µl qPCR master mix (Luna® Universal Protocol, #M3003, NEB), 2 µL cDNA, 2 µL primer (10 pMol), and 6 µL water to final volume of 20 µL (18). PCR conditions were arranged as previously defined (19). We ran melting analysis as follows: 95°C for 2 min, the fluorescence signal was captured at 1°C increment starting from 55°C up to 95°C (Bio-Rad CFX96; Bio-Rad, USA). To normalize the gene expression data, SDHA was employed as a housekeeping gene for gene expression analysis (20). Positive and negative control runs were included to eliminate genomic contamination. We performed each gene duplicate.

Statistical Analysis: qPCR (Ct) data were used to calculate relative expression (18). The method of $2^{-\Delta\Delta Ct}$ was employed to calculate relative expression (21). qPCR normalized data were analyzed by t-test and ANOVA with Tukey's post hoc test. $P<0.05$ value was set as significance.

Results

BMP2 mRNA steady-state levels were demonstrated in C12 and induced luteolysis of PG4, and PG16 in Figure 1. When we evaluated, *BMP2* mRNA seemed to be higher in PG4 than in C12 and PG16, but found to be similar among the groups, compared to each other ($P>0.05$). *BMP4* mRNA steady-state levels were demonstrated in C12 and induced luteolysis of PG4, and PG16 in Figure 2. Expression of *BMP4* mRNA was found to be greater in PG16 than in PG4 ($P<0.05$). However, the level of *BMP4* mRNA did not change between induced luteolysis groups (PG4 and PG16) and C12 ($P>0.05$). *BMP6* mRNA steady-state levels were demonstrated in C12 and induced luteolysis of PG4, and PG16 in Figure 3. Expression of *BMP6* was shown to be greater in PG16 than in C12 ($P<0.05$) but did not differ between C12 and PG4 ($P>0.05$). Expression of *BMP6* mRNA was found to be higher in PG16 than in PG4 ($P<0.05$).

BMPR1A mRNA steady-state levels were demonstrated in C12 and induced luteolysis of PG4, and PG16 in Figure 4. Expression of *BMPR1A* mRNA was found to be greater in PG16 than in PG4 ($P<0.05$). However, the level of *BMPR1A* mRNA did not change between induced luteolysis groups (PG4 and PG16) and C12 ($P>0.05$). *BMPR1B* mRNA steady-state levels were demonstrated in C12 and induced luteolysis of PG4, and PG16 in Figure 5. While expression of *BMPR1B* mRNA was greater in PG16 than in PG4 and C12 ($P<0.05$), it was shown to be lower in PG4 than in C12 ($P<0.05$). *BMPR2* mRNA steady-state levels were demonstrated in C12 and induced luteolysis of PG4, and PG16 in Figure 6. Although *BMPR2* mRNA was found to be lower in PG16 than in PG4 and C12 ($P<0.05$), this was not significant ($P>0.05$). Expression of *BMPR2* mRNA did not change between C12 and PG4 ($P>0.05$).

Table 1. BMP gene primers for qPCR

Oligonucleotide ID	Nucleotide Sequence	Product Size (bp)	Accession
BMP2 F	5'- ACCCGGCGATTCTTCTTTAAT-3'	99	XM_004014353.3
BMP2 R	5'- CAAAGCTTCGGGCATATGTTTC-3'		
BMP4 F	5'- GGAGAAGCAGCCCAACTATG-3'	104	XM_015096688.1
BMP4 R	5'- CTTGAGGTAACGATCGGCTAAT-3'		
BMP6 F	5'- GAGTTCCGAATCTACAAGGACTG-3'	95	XM_015102858.1
BMP6 R	5'- CTGTGCTGATGCTCCTGTAA-3'		
BMPR1A F	5'- CCTCTATGACTTCCTGAAGTGTG-3'	115	NM_001280714.1
BMPR1A R	5'- CCCTTGAGTGCCGTAGATTT-3'		
BMPR1B F	5'- CATTGCTGCAGATATCAAAGGG-3'	100	NM_001009431.1
BMPR1B R	5'- GGTGGACTTCAGGTAATCATAGAG-3'		
BMPR2 F	5'- GTACCAGTTCTAGCTTGCCTTAC-3'	116	NM_001306123.1
BMPR2 R	5'- GGGTGGGACATCAGGAATTAAA-3'		

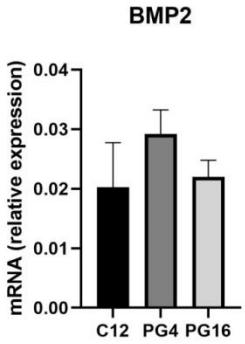

Figure 1. Expression of BMP2 mRNA in C12, PG4, and PG16. Data was presented as mean±standard error of means (SEM)

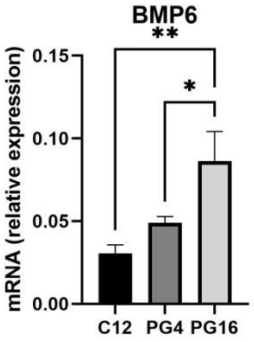

Figure 3. Expression of BMP6 mRNA in C12, PG4, and PG16. *: P<0.05 and **: P<0.01. Data was presented as mean±standard error of means (SEM)

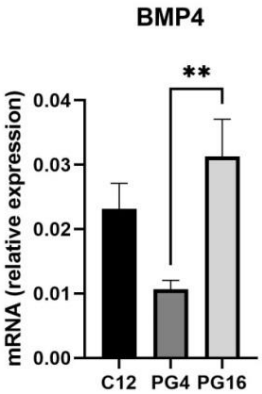

Figure 2. Expression of BMP4 mRNA in C12, PG4, and PG16. **: P<0.01. Data was presented as mean±standard error of means (SEM)

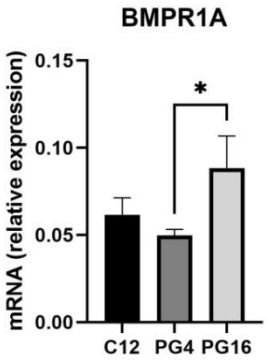

Figure 4. Expression of BMPR1A mRNA in C12, PG4, and PG16. *: P<0.05. Data was presented as mean±standard error of means (SEM)

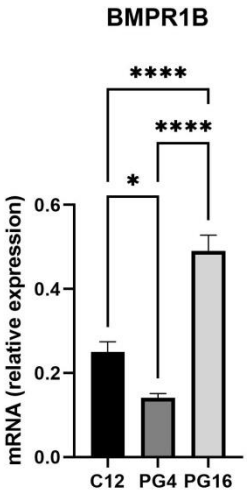

Figure 5. Expression of BMPR1B mRNA in C12, PG4, and PG16. *: P<0.05 and ****: P<0.0001. Data was presented as mean±standard error of means (SEM)

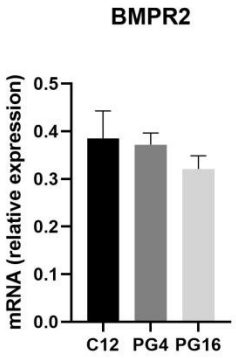

Figure 6. Expression of BMPR2 mRNA in C12, PG4, and PG16. Data was presented as mean±standard error of means (SEM)

Discussion

Our study apparently demonstrated that the mRNA expression of some BMPs genes were detected using qPCR in induced luteolysis (PG4 and PG16). The CL tissues obtained facilitated us to evaluate *BMP2, BMP4, BMP6, BMPR1A, BMPR1B,* and *BMPR2* mRNA expressions in ovine CL.

Throughout the oestrous cycle, luteal regression in ruminants and other species is induced by the release of PGF2α, which reaches the CL from the uterus. Luteolysis is prevented when the embryo is present and emerges in cyclic animals (21, 22). Endogenous or exogenous administration of PGF2α facilitates a signalling of events causing to irreversible death of CL. In the entire process, CL undergoes significant changes in terms of its steroidogenic capacity, vascularization, extracellular matrix regeneration and cell viability (23, 24).

In recent years, many functional genomic studies have elucidated the underlying mechanism of PGF2α over luteolysis (25, 26). Studies reveal that factors produced by uterine or exogenous PGF2α mediate a variety of processes from decreased steroid production to apoptotic cell death. Factors such as bone morphogenic proteins (BMP), tumor necrosis factor-alpha (TNFα), and activin A may have inhibitory effects on StAR expression (27-30).

It has been reported that some BMPs cause luteolysis by suppressing *StAR* expression and P4 generation in women and granulosa cells (13, 31). In our study, this may be consistent with the regulation of BMP genes that we showed *BMP6* mRNA were upregulated in PG16 compared to C12. Also, we previously reported that *StAR* mRNA was shown to be sharply decreased in PG16, thus explaining BMPs' inhibitory effects on *StAR* as identified previously in humans CL (13). *BMP2* and *BMP4* mRNA expression was demonstrated to be increased in induced luteolysis of cattle CL after 12 hours after PGF2α treatment, but *BMP6* was after 2 hours (32). *BMP2* abundance was reported to link/be linked with diminished P4 in cattle CL (33). However, we have not observed any changes in the expression of *BMP2* and *BMP4* mRNA levels in our study. BMPRs assessed in the current study were expressed in the ovine CL during luteolysis. We have not detected significant regulation for *BMPR2* as in induced luteolysis of bovine CL (32). However, *BMPR1A* mRNA were not regulated in induced luteolysis against C12, whereas *BMPR1B* mRNA was upregulated in PG16. In line with our study, in rats, the greater levels of BMPR1B was reported in CL regression which implies its involvement in luteolysis (14).

In conclusion, considering mRNA expression of *BMP2, BMP4, BMP6, BMPR1A, BMPR1B,* and *BMPR2*, BMPs appeared to have functional role in corpus luteum. We may suggest that expression patterns of some BMP genes regulate luteolysis of ovine CL.

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