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Expression of bone morphogenetic protein 2, 4, and related components of the BMP signaling pathway in the mouse uterus during the estrous cycle*

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Abstract: The objective was to investigate the expression of bone morphogenetic protein (BMP) family members in the mouse uterus during the estrous cycle by real-time polymerase chain reaction (PCR) and immunohistochemistry. Uterine samples from Swiss ICR mice were collected and dissected free of surrounding tissue. One uterine horn was snap frozen in liquid nitrogen immediately after collection and stored at −80 °C for RNA extraction, and the other was fixed in 40 mg/ml paraformaldehyde at room temperature for immunolocalization of BMP2 protein. Real-time PCR analysis showed that the expression level of *Bmp2* was significantly higher at proestrus than at estrus and metestrus (P<0.05). The relative abundance of *Bmp4* exhibited significant fluctuations, but there were no statistically significant differences between the expression levels of *Bmp2* and *Bmp4* (P>0.05). The expression levels of *Bmpr1a* and *Bmpr2* remained unchanged during estrous cycles. However, the level of *Bmpr1b* mRNA decreased significantly at estrus (P<0.05), increasing subsequently at metestrus. Furthermore, the level of *Bmpr1b* mRNA was significantly lower than those of *Bmpr1a* and *Bmpr2* mRNA at the corresponding stages (P<0.05). All three receptor-regulated Smads (*R-Smads*) detected were differentially expressed in the mouse uterus and the expression levels of *Smad1* and *Smad5* were significantly higher than that of *Smad8* (P<0.05). In addition, the expression level of *Smad4* did not change substantially throughout the estrous cycle. Immunohistochemical experiments revealed that BMP2 protein was differentially expressed and localized mainly in the uterine luminal and glandular epithelial cells throughout the estrous cycle. In conclusion, our results provide information about the variation in the mRNA levels of *Bmp2* and *Bmp4* and related components of the BMP signaling pathway. The data provide quantitative and useful information about the roles of endometrial BMP proposed and demonstrated by others, such as the degradation and remodeling of the endometrium.

Key words: Bone morphogenetic protein (BMP), BMP receptor, SMAD, Uterus, Estrous cycle
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1 Introduction

The uterus is a major female hormone-responsive organ necessary for many pivotal reproductive events, such as embryo implantation, decidualization, placentation, and labor (Dey, 2004; Fernandez-Valdivia *et al.*, 2007; Franco *et al.*, 2008; Abdallah *et al.*, 2012; Kong *et al.*, 2012). Endometrial tissues undergo remarkable periodic growth, degeneration, and leucocytosis of the epithelial cells during the estrous cycle. These histological events are controlled by ovarian steroids. In addition, many of these processes are considered to be regulated by cytokines and growth factors, several of which are members of the

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transforming growth factor-β (TGF-β) superfamily (Liu *et al.*, 2004; Omwandho *et al.*, 2010; Bukowska *et al.*, 2011; Tanwar and McFarlane, 2011; Argañaraz *et al.*, 2013).

The bone morphogenetic protein (BMP) family is the largest subgroup of ligands in the TGF-β superfamily, which is closely associated with biological processes and cellular events involved in uterine cell proliferation, differentiation, apoptosis, and tissue remodeling (Shimasaki *et al.*, 2004; Shimizu *et al.*, 2004; Jones *et al.*, 2006). More than 20 BMP isoforms have been identified, and they can be divided into four distinct subgroups based on the properties of their binding to type I receptors, including BMP2/4, BMP5/6/7/8, growth/differentiation factor (GDF)-5/6/7, and BMP9/10. Homo- or hetero-dimers of the BMP family ligands bind to and phosphorylate two types of serine/threonine kinase receptors to form complexes of transmembrane type I and type II receptors, which subsequently transmit signals from the cell surface to the cytoplasm (Aoki *et al.*, 2001; Hu *et al.*, 2003; Cai *et al.*, 2012).

The Smad-dependent pathway involves canonical BMP signaling and mediates signals from the cytoplasm to the nucleus (Wen *et al.*, 2011). SMAD proteins, the major intracellular signal transducers for the BMP family receptors, can be classified into three types: receptor-regulated SMADs (R-SMADs: SMAD1, SMAD5, and SMAD8), inhibitory SMADs (I-SMADs: SMAD6 and SMAD7), and a common-mediator SMAD (SMAD4) (Massague *et al.*, 2005; Miyazono *et al.*, 2010; Cai *et al.*, 2012; Pangas, 2012). Once a ligand-receptor complex is formed, type-I receptors phosphorylate down-stream R-SMAD proteins. Phosphorylated R-SMADs form heteromeric complexes with SMAD4 (Co-SMAD) and translocate to the nucleus to regulate BMP-stimulated alterations in target gene expression through interaction with other factors, including transcription factors and transcriptional coactivators or corepressors (Xia *et al.*, 2005; Bruce and Sapkota, 2012).

Several molecular and functional studies have suggested the presence of BMP family members in the mammalian uterus (Paria *et al.*, 2001; Erickson *et al.*, 2004; Lee *et al.*, 2007). *In situ* hybridization results revealed that *Bmp7* was highly expressed in the endometrium of the non-pregnant rat uterus (Erickson *et al.*, 2004). A low level of *Bmp5* mRNA has been detected in the stroma close to the myometrium and in the myometrial connective tissue (Paria *et al.*, 2001). *Bmpr* mRNA appeared to show dynamic expression (Erickson *et al.*, 2004), whereas *Smad4* mRNA was constitutively expressed in the glandular and luminal epithelium throughout the estrous cycle (Liu *et al.*, 2004). The differential distribution and expression of BMP family members in the uterus indicate their differing roles in the regulation of uterine function and development. There is an increasing evidence that BMP2 affects proliferation, differentiation, and apoptosis of endometrial cells (Lee *et al.*, 2007; Franco *et al.*, 2011) and that BMP4 may play an important role in the maintenance of the uterine endometrium (Tanwar and McFarlane, 2011). Although a series of studies have focused on BMP signaling, there is a lack of quantitative descriptions of BMP family members in the mouse uterus. To gain more experimental clues clarifying the molecular mechanisms of BMP signaling during the estrous cycle, we analyzed the temporal and spatial expression patterns of BMP family members by real-time polymerase chain reaction (PCR) and immunohistochemistry.

2 Materials and methods

2.1 Experimental animals and sample preparation

A total of 30 female Swiss ICR mice of similar age and weight were purchased from the Laboratory Animal Center of Nanjing Medical University, China. They were raised in an animal room with controlled temperature (20–25 °C) and lighting (12-h light/dark cycle), and were provided with commercial feed and tap water *ad libitum*. The various stages of the estrous cycle were monitored by vaginal smears. The body weights of the mice were recorded immediately before they were sacrificed. Uterine samples (at least seven mice per time point) were collected and dissected free of surrounding tissue. One uterine horn was snap frozen in liquid nitrogen immediately after collection and stored at −80 °C for RNA extraction, and the other was fixed in 40 mg/ml paraformaldehyde at room temperature for immunolocalization of BMP2 protein. All experiments were carried out in accordance with the principles for the Care and Use of Laboratory Animals approved by the Institutional

Animal Care and Use Committee of Nanjing Agricultural University, China.

2.2 RNA isolation and reverse transcription

Total RNA was extracted from all samples using a commercial RNA isolation kit (TaKaRa code: D9108A, Dalian, China) according to the manufacturer's instructions. Total RNA was dissolved in 60 μl RNase-free deionized water (dH_2O) and stored at −80 °C. Subsequently, 1 μg of total RNA of high integrity (not degraded) and high purity (no contaminants) was reverse-transcribed into complementary DNA (cDNA) using reverse transcription reagent kits (TaKaRa code: RR047A, Dalian, China). Each PCR reaction contained 2.0 μl 5× genomic DNA (gDNA) eraser buffer, 1.0 μl gDNA eraser, 1 μg total RNA, 1.0 μl PrimeScript™ RT enzyme mix 1, 1.0 μl RT primer mix, 1.0 μl RT primer mix, 4.0 μl 5× PrimeScript™ buffer 2, and sufficient RNase-free dH_2O in a total volume of 20 μl. Reverse transcription cycle conditions were: 42 °C for 2 min and 37 °C for 15 min. The reaction was terminated by incubation at 85 °C for 5 s and cooling at 4 °C. The cDNA was stored at −80 °C or used for real-time PCR immediately.

2.3 Real-time PCR

The mRNA expression patterns of BMP2, BMP4, and components of the BMP signaling pathway were assessed using a commercial real-time PCR kit (TaKaRa code: RR820A, Dalian, China) on an ABI StepOne™ real-time PCR machine (Applied Biosystems, Life Technologies Corporation, Grand Island, NY, USA). The primer pairs used for amplification of the target genes are listed in Table 1. The PCR amplification was performed in a total volume of 20 μl containing 10 μl SYBR Premix Ex Taq II, 6.0 μl RNase-free dH_2O, 2 μl cDNA, 0.4 μl ROX reference dye, and 0.8 μl each of forward and reverse primer pairs. Each PCR cycle included a denaturation step at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and extension at 60 °C for 34 s, and a dissociation step consisting of 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. The real-time PCR was performed with three technical replicates per animal sample. Finally, the relative expression of each gene was standardized to glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*).

Table 1 Primer sequences, GenBank accession Nos., and product sizes of genes selected for real-time PCR

Target gene	Accession No.	Primer sequence (5'→3')	Product size (bp)
Gapdh	NM_008084.2	F: CATGTTCCAGTATGACTCCACTC	136
		R: GGCCTCACCCCATTTGATGT	
Bmp2	NM_007553.2	F: TGACTGGATCGTGGCACCTC	112
		R: CAGAGTCTGCACTATGGCATGGTTA	
Bmp4	NM_007554.2	F: TTTGTTCAAGATTGGCTCCCAAG	101
		R: AAACGACCATCAGCATTCGGTTA	
Bmpr1a	NM_009758.4	F: ATGGCCATTGCTTTGCCATTA	126
		R: TCCTGCGTAGCTGGGCTTTC	
Bmpr1b	NM_007560.3	F: CTACGTTGTAAATGCCACCACCA	118
		R: AGGTGACAACAGGCATTCCAGA	
Bmpr2	NM_007561.3	F: CAAATATCCTGGATGGCAGCAGTA	141
		R: TGGAGATGACCCAGGTGGAC	
Smad1	NM_008539.3	F: ACCTGTGGCTTCCGTCTC	97
		R: ATCGTGGCTCCTTCGTC	
Smad4	NM_008540.2	F: CAGCACTACCACCTGGACTGGA	145
		R: CTGGAATGCAAGCTCATTGTGAA	
Smad5	NM_001164041.1	F: AAGTAGATTCTGCCTGGGATT	197
		R: AGACGGTGGTGGGATGG	
Smad8	NM_019483.5	F: ATCCCTGGCAATCTGTA	169
		R: CCCTGGCTGTCCTGTAA	

2.4 Immunolocalization of BMP2 protein

Immunohistochemistry was carried out using the streptavidin-biotin peroxidase complex (SABC) method to examine the immunolocalization of BMP2 protein in the mouse uterus during the estrous cycle. Paraffin-embedded uteri were cut into 6-μm-thick sections and mounted on slides. Sections were de-paraffinised and hydrated by a consecutive series of xylene and ethanol, and treated with 9 mg/ml hydrogen peroxide to inactivate endogenous peroxidase activity. Sections were then incubated with 50 mg/ml bovine serum albumin (BSA) over 1 h to block non-specific staining. Subsequently, sections were incubated with a rabbit polyclonal antibody to BMP2 (Abcam Inc., Cambridge, MA, USA) at a dilution of 1:400 overnight at 4 °C, and then incubated with a secondary antibody, goat anti-rabbit IgG, diluted in phosphate buffer solution (PBS) over 2 h in a wet box at room temperature. Finally, immunoreactive signals were visualized by addition of diaminobenzidine substrate (DAB; Sigma Chemical Co., USA). Negative control sections were incubated with normal rabbit serum omitting the primary antibody. To distinguish the staining intensity of BMP2 protein among the different uterine structural components and cell types, sections were examined using a method similar to that described previously (Shi *et al.*, 2004; Ding *et al.*, 2012; Li *et al.*, 2014): −, no staining; +, weak staining; ++, moderate staining; +++, strong staining. Relative levels of immunostaining were evaluated at least three times for each sampling stage.

2.5 Statistical analysis

All data were collected using Origin Pro 8.0 software (Origin Lab Corporation, USA). Data are presented as mean±standard error of the mean (SEM). For statistical evaluation of data, one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test was applied to detect differences among stages for each gene. Paired-sample *t*-tests were performed for two-gene comparisons. Means identified with the same letter are not significantly different ($P>0.05$).

3 Results

3.1 Body weights of mice during the estrous cycle

As shown in Table 2, the body weight of each mouse was nearly 30 g, and there were no significant differences among different stages of the estrous cycle.

Table 2 Body weights of mice during the estrous cycle

Stage of estrous cycle	Number of animals	Body weight* (g)
P	7	28.47±0.59
E	7	30.46±0.62
M	8	28.84±0.93
D	8	29.72±0.81

* Data are expressed as mean±SEM. P: proestrus; E: estrus; M: metestrus; D: diestrus

3.2 mRNA expression patterns of *Bmp2*, *Bmp4*, and components of the signaling pathway during the estrous cycle

To investigate the mRNA levels of BMP family members, real-time PCRs were performed in the mouse uterus during the estrous cycle. Our results showed that different members of the BMP family were differentially expressed in all studied stages of the estrous cycle.

The expression level of *Bmp2* was significantly higher at proestrus (P) than at estrus (E) and metestrus (M) ($P<0.05$; Fig. 1). The relative abundance of *Bmp4* showed significant fluctuations, strongly decreasing at estrus and diestrus (D) ($P<0.05$), and strongly increasing at metestrus ($P<0.05$). Paired-sample *t*-tests revealed that there were no statistically significant differences between the expression levels of *Bmp2* and *Bmp4*.

The expression levels of *Bmpr1a* and *Bmpr2* remained unchanged during an entire estrous cycle. However, the level of *Bmpr1b* mRNA decreased significantly at estrus ($P<0.05$), increasing subsequently at metestrus ($P<0.05$). Furthermore, the level of *Bmpr1b* mRNA was significantly lower compared with that of *Bmpr1a* and *Bmpr2* mRNA at the corresponding stages ($P<0.05$).

The expression level of *Smad1* did not change significantly at proestrus or estrus, whereas it increased markedly at metestrus ($P<0.05$). The relative quantity of *Smad4* did not change substantially throughout the estrous cycle. The expression level of *Smad5* was significantly higher at metestrus than at other stages ($P<0.05$). The relative quantity of *Smad8* was lower at estrus than at diestrus ($P<0.05$). In

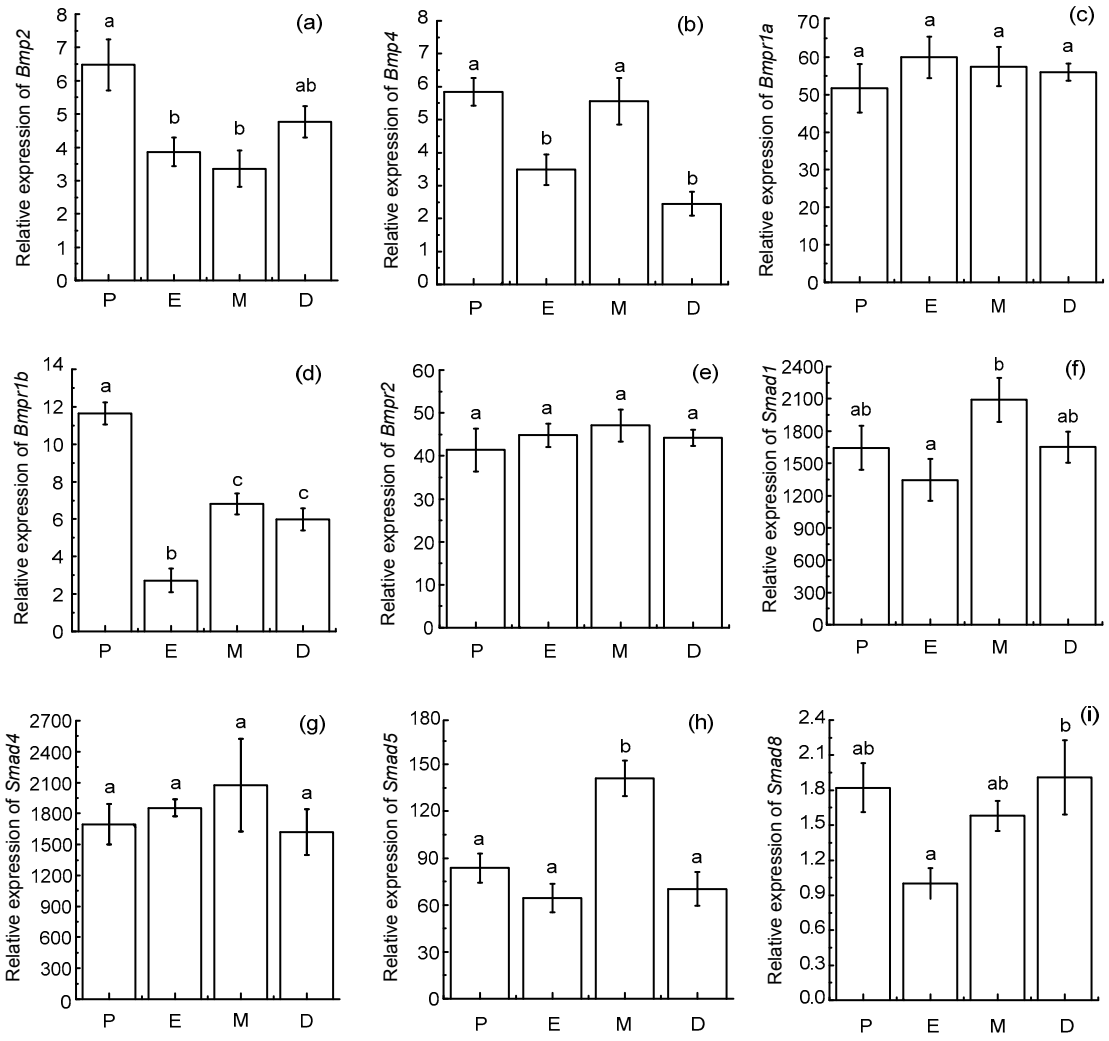

Fig. 1 Changes in mRNA levels for *Bmp2*, *Bmp4*, and components of the signaling pathway throughout the estrous cycle

Uterine samples were collected at various stages of the estrous cycle (P: proestrus; E: estrus; M: metestrus; D: diestrus). Total RNA was extracted from all samples, and 1 μg total RNA was analyzed by real-time PCR. The relative expression of each gene was standardized to glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*). Then, all data were normalized on the basis of level of *Smad8* mRNA at estrus. All results were presented as mean±SEM (at least seven mice each stage). Means identified with the same letter are not significantly different (P>0.05)

addition, the expression levels of *Smad1* and *Smad5* were significantly higher than that of *Smad8* (P<0.05).

3.3 Immunolocalization of BMP2 protein during the estrous cycle

To provide more evidence illustrating the important role of BMP signaling during the estrous cycle, the BMP2 protein in the mice uterus was examined using immunohistochemistry. Our results showed that BMP2 protein was detected throughout the estrous cycle (Fig. 2). Specifically, it was moderately immunolocalized to the uterine luminal and glandular epithelial cells at proestrus (Figs. 2a and 2e; Table 3), estrus (Figs. 2b and 2f; Table 3), and diestrus (Figs. 2d and 2h; Table 3), whereas it was weak at metestrus (Figs. 2c and 2g; Table 3). Furthermore, individual stromal cells and myometrium were weakly stained BMP2 protein positive (Fig. 2; Table 3). No specific staining was detected in the negative control sections (Figs. 2i and 2j).

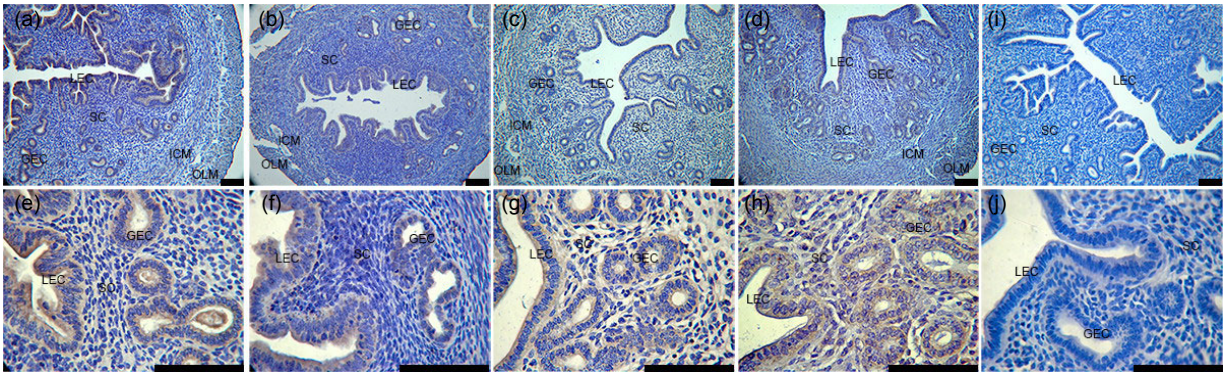

Fig. 2 BMP2 protein in the mouse during the estrous cycle: low magnification (a–d) and high magnification (e–h)
Uterine sections were immunostained with a rabbit polyclonal antibody to BMP2 and counterstained with haematoxylin. A positive reaction was observed as brown staining and the counterstaining background appeared blue. BMP2 protein was moderately immunolocalized to the uterine luminal and glandular epithelial cells at proestrus (a, e), estrus (b, f), and diestrus (d, h), whereas it was weak at metestrus (c, g). Furthermore, individual stromal cells and myometrium were weakly stained BMP2 protein positive. Negative control sections showed no specific staining (i, j). LEC: luminal epithelial cells; GEC: glandular epithelial cells; SC: stromal cells; ICM: inner circular smooth muscle layer; OLM: outer longitudinal smooth muscle layer. Scale bar: 10 μm (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

Table 3 Relative abundances of BMP2 protein in the mouse uterus during the estrous cycle

Uterine structural component and cell type	Staining intensity			
	Proestrus	Estrus	Metestrus	Diestrus
Glandular epithelial cell	++	++	+	++
Luminal epithelial cell	++	++	+	++
Myometrial cell	+	+	+	+
Stromal cell	+	+	+	+

+: weak staining; ++: moderate staining; +++: strong staining

4 Discussion

Our study provided novel information regarding the expression of *Bmp2* and *Bmp4* members of the BMP family and components of the signaling pathway at the mRNA level in the mouse uterus during the estrous cycle. To minimize individual differences, female Swiss ICR mice of similar age and body weight were used. Our findings strongly suggest that the *Bmp2* and *Bmp4* signaling pathway might play key roles in the regulation of cyclic changes in the mouse uterus.

A wealth of evidence has indicated that the mRNAs encoding a number of BMP family members are abundantly and dynamically expressed in the murine uterus, and are associated with cell proliferation, differentiation, and apoptosis (Paria *et al.*, 2001; Erickson *et al.*, 2004; Liu *et al.*, 2004; Xia *et al.*, 2005; Li *et al.*, 2007; Large and DeMayo, 2012; Wetendorf and DeMayo, 2012; Nagashima *et al.*, 2013). In our study, we examined the mRNA levels of *Bmp2* and *Bmp4* during the estrous cycle. Our results showing that the expression level of *Bmp2* was significantly higher at proestrus than at estrus and metestrus were inconsistent with Erickson *et al.* (2004). This inconsistency might be caused by differences between animals and experimental techniques used for study. *Bmp2* ablation affects proliferation, differentiation, and apoptosis in the decidualizing uterus (Lee *et al.*, 2007; Franco *et al.*, 2011). Results of *in vitro*

experiments had suggested that BMP2 promoted differentiation of endometrial stromal cells (Li *et al.*, 2007). Thus, the expression of *Bmp2* in the uterus implies that BMP2 might have regulatory functions in the degradation and remodeling of the endometrium during the estrous cycle. However, the specific mechanism underlying this effect needs confirmation. In comparison to *Bmp2*, the relative abundance of *Bmp4* showed significant fluctuations, strongly decreasing at estrus and diestrus, and strongly increasing at metestrus. We speculate that the significant change in *Bmp4* expression might be tightly correlated with the cyclic changes in the endometrium. This suggestion is nearly identical to that proposed by Tanwar and McFarlane (2011). Increasing evidence suggests that there is a subtle relationship between steroids and BMP signaling. Expression of uterine *Bmp7* was abolished in response to estradiol treatment in mice (Shimasaki *et al.*, 2004; Tanwar and McFarlane, 2011). Similarly, exogenous treatment with progesterone receptor antagonist RU486 has been shown to markedly down-regulate the level of *Bmp2* mRNA in the decidual uterus (Li *et al.*, 2007). Further studies are needed to confirm a functional link between steroids and BMP signaling in the uterus. Paired-sample *t*-tests revealed that there were no statistically significant differences between the expression levels of *Bmp2* and *Bmp4*, which suggests the possibility that both play important roles in the control of uterine functions. Collectively, these findings suggest that both *Bmp2* and *Bmp4* might contribute to the degradation and remodeling of the endometrium during the estrous cycle.

BMP receptors are essential mediators of BMP signal transduction from the cell surface to the cytoplasm. BMPR1A and BMPR1B are receptors specific for BMP2 and BMP4, whereas BMPR2 appears to bind exclusively to BMP ligands among all BMP receptors identified in mammals (Shimasaki *et al.*, 2004; Miyazono *et al.*, 2010). Although much is known about BMP receptors, little is known about their roles. In the current study, the mRNA levels of the three *Bmprs* were confirmed by real-time PCR. *Bmpr1a* and *Bmpr2* showed extremely stable expression during an entire estrous cycle. Our findings suggest that both *Bmpr1a* and *Bmpr2* are constitutively expressed in the uterus. In contrast, we found that the level of *Bmpr1b* mRNA appeared to change significantly throughout the estrous cycle. The stage-specific changes in *Bmpr1b* mRNA expression might be closely associated with the growth and development of the endometrium (Kim *et al.*, 2003; Erickson *et al.*, 2004). In support of this deduction, mice deficient in *Bmpr1b* exhibited a failure in endometrial gland formation and severe destruction of the uterine structure (Yi *et al.*, 2001). Furthermore, our results showed that the level of *Bmpr1b* mRNA was significantly lower than that of *Bmpr1a* and *Bmpr2* mRNA. *In situ* hybridization results showed that *Bmpr1a* and *Bmpr2* mRNA appeared to be widely presented in the endometrium and myometrium, whereas *Bmpr1b* message was restricted to the luminal and glandular epithelial cells (Erickson *et al.*, 2004). Thus, epithelial restricted expression of *Bmpr1b* could be one of the reasons for the low expression level of *Bmpr1b* compared to *Bmpr1a* and *Bmpr2*. Our findings, together with the studies cited above, suggest that *Bmpr1b* is essential for maintenance of normal uterine functions. Our results strongly suggest that three murine BMP receptors differentially contribute to the control of growth and development of the endometrium during the estrous cycle.

Smad1, *Smad5*, and *Smad8* are *R-Smads* mediating the intracellular signaling of BMP. Earlier studies have reported their critical functions in different models (Chen *et al.*, 1997; Chang *et al.*, 1999; Ying and Zhao, 2000; Huang *et al.*, 2009). However, there is a lack of understanding of the role of each *R-Smad* in the mouse uterus during the estrous cycle. Our results showed that all the three *R-Smads* were differentially expressed in the mouse uterus, demonstrating their differing roles during the estrous cycle. Further analysis revealed that the expression levels of *Smad1* and *Smad5* were significantly higher than that of *Smad8*. Thus, we conjecture that *Smad1* and *Smad5* are the predominant *R-Smads* involved in the regulation of uterine function during the estrous cycle. Our results revealed also that *Smad4* was highly expressed throughout the estrous cycle. Our results are largely similar to those of previous works (Lin *et al.*, 2004; Liu *et al.*, 2004). The marked expression of *Smad4*

might be closely correlated with its molecular roles. *Smad4*, a common partner for all *R-Smads*, works through combination with *R-Smads* to control gene transcription (Wan *et al.*, 2002; Shimasaki *et al.*, 2004). Combining the relative level of *Smad4* in the current study with previous results in rodents, we speculate that the persistent and high-level expression of *Smad4* in the uterus is an essential factor for mediation of BMP signal during the estrous cycle.

We investigated the immunolocalization of BMP2 protein using immunohistochemistry. Our observation that BMP2 protein was localized mainly in the uterine luminal and glandular epithelial cells throughout the estrous cycle appears to contrast with the results of Erickson *et al.* (2004). This discrepancy might be a result of differences between animals and experimental techniques. Our findings demonstrated that both uterine luminal and glandular epithelium were primary targets for BMP signaling. Differences in the staining intensity of BMP2 protein were observed during different stages of the estrous cycle. Thus, we surmised that BMP2 protein contributed to the degradation and remodeling of the endometrium in a stage-specific manner.

5 Conclusions

In conclusion, we detected the temporal expression patterns of *Bmp2* and *Bmp4* members of the BMP family and components of the signaling pathway in the mouse uterus during the estrous cycle. Also, we examined the histological location of BMP2 protein using immunohistochemistry. Our results provide information about the variation in the mRNA levels of *Bmp2* and *Bmp4* and components of the BMP signaling pathway. Our findings also provide quantitative and useful information about the roles of endometrial BMP proposed and demonstrated by others, such as the degradation and remodeling of the endometrium.

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Compliance with ethics guidelines

Yan LI, Quan-wei WEI, Jian-gang FENG, Mu-lin XU, Rui-hua HUANG, and Fang-xiong SHI declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要：

本文题目：BMP2，BMP4及 BMP 信号通路相关成员在发情周期小鼠子宫中的表达

Expression of bone morphogenetic protein 2, 4, and related components of the BMP signaling pathway in the mouse uterus during the estrous cycle

研究目的：研究骨形态发生蛋白（BMP）2，4及 BMP 信号通路相关成员在发情周期小鼠子宫中的表达模式。

创新要点：运用实时荧光定量聚合酶链式反应（PCR）系统地研究了 *Bmp2*和 *Bmp4*及其 BMP 信号通路相关成员在小鼠子宫中 mRNA 水平表达模式，同时运用免疫组化方法研究了 BMP2蛋白在小鼠子宫中的定位模式。

研究方法：收集发情周期各个时期小鼠子宫，一侧子宫角贮存于液氮或−80 °C 冰箱用于实时荧光定量 PCR，另一侧子宫角用40 mg/ml 多聚甲醛固定用于 BMP2蛋白免疫组化定位。

重要结论：实时荧光定量 PCR 结果表明，*Bmp2*的表达水平在发情前期显著高于发情期和发情后期（$P<0.05$），*Bmp4*的表达水平呈现显著波动，但 *Bmp2*与 *Bmp4*差异不显著（$P>0.05$）。*Bmpr1a* 和 *Bmpr2*的表达水平在整个发情周期无显著变化（$P>0.05$）。然而，*Bmpr1b* 的 mRNA 水平在发情期显著下降（$P<0.05$），在发情后期上升。此外，*Bmpr1b* 的 mRNA 水平显著低于相应时期 *Bmpr1a* 和 *Bmpr2*的 mRNA 水平（$P<0.05$）。三种 *R-Smads* 差异地表达于小鼠子宫，并且 *Smad1*和 *Smad5*的表达水平显著高于 *Smad8*（$P<0.05$）。此外，*Smad4*的表达水平在整个发情周期无显著变化。免疫组化结果显示，BMP2蛋白在整个发情周期差异表达，并主要定位于子宫腔上皮细胞和腺上皮细胞。我们的结果提供了 BMP2和 BMP4及其 BMP 信号通路相关成员 mRNA 水平表达变化信息，这些数据为论证 BMP 在子宫内膜中的作用如子宫内膜的退化与重塑提供量化和有用的信息。

关键词组：骨形态发生蛋白；骨形态发生蛋白受体；SMAD 蛋白；子宫；发情周期