Research in Veterinary Science 118 (2018) 371–388

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Transcriptional and translational abundance of Bone morphogenetic protein (BMP) 2, 4, 6, 7 and their receptors BMPR1A, 1B and BMPR2 in buffalo ovarian follicle and the role of BMP4 and BMP7 on estrogen production and survival of cultured granulosa cells

G. Rajesh[a,d], S.R. Mishra[a], A. Paul[a], M. Punetha[a], G.M. Vidyalakshmi[a], K. Narayanan[b], S. Bag[a], S.K. Bhure[c], V. Singh Chouhan[a], V.P. Maurya[a], G. Singh[a], Mihir Sarkar[a,*]

[a] Physiology & Climatology Division, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243122, India
[b] Animal Reproduction Division, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243122, India
[c] Animal Biochemistry division, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243122, India
[d] Department of Veterinary Physiology and Biochemistry, Veterinary College and Research Institute, TANUVAS, Tirunelveli, India

ARTICLE INFO

Keywords:
Buffalo
Follicle
BMP
Estradiol
Granulosa cell

ABSTRACT

BMPs and their receptors modulate the granulosa cell (GC) function in the follicle of domestic animals. Since little is known on BMPs in the buffalo, the present study was aimed to investigate the expression of BMP2, 4, 6, 7 and their receptors BMPR1A, BMPR1B, BMPR2 in the GC and theca cells (TC) of ovarian follicles and the role of BMP4 and BMP7 on buffalo GC. Follicles were classified into four groups based on size and E_2 level in the follicular fluid as follows: (i) Group1(4–6 mm; < 0.5 ng/mL) (ii) Group 2 (7–9 mm; 0.5–5 ng/mL) (iii) Group 3 (10-13 mm; 5–40 ng/mL) and (iv) Group 4 (dominant follicle) (> 13 mm; > 180 ng/mL). The results revealed that except BMP6, BMP2, 4 7 and receptors BMPR1A, BMPR1B and BMPR2 showed a minimum of 1.5–2 fold increase in mRNA expression in the GC of dominant follicle as compared to other follicle classes. In the dominant follicle, a two-fold increase in BMP4 and BMP7 expression was observed in the TC. At 100 ng/mL, the BMP4 and BMP7 either alone or in combination maximally down-regulated CASPASE3 and stimulated the transcripts of PCNA, FSHR and CYP19A1 that was supported by E_2 secretion in the granulosa cell culture suggesting their role in cell survival and E2 production. In conclusion, GC and TC of dominant follicles express BMP 2, 4, 6, 7 and their receptors BMPR1A, BMPR1B and BMPR2. BMP4 and BMP7 stimulate E_2 production and promote GC survival.

1. Introduction

Water buffalo is one of the most important dairy animals in the Asian countries. Indian buffaloes contribute to 57.8% of total population and India produces about 68% of the world's buffalo milk production (FAOSTAT, n.d.). The high milk fat content, lean meat and better conversion of roughage are the strengths of buffalo production. There is no religious taboo in the consumption of buffalo meat. Despite the merits, certain inherent reproductive problems like delayed puberty, silent heat, poor conception rate and long postpartum anoestrus period limit the lifetime productivity of the buffalo (Madan & Prakash, 2007; Perera, 2008).

Bone morphogenetic proteins (BMPs) are pleiotrophic growth factors that belong to the transforming growth factor-beta (TGF-β) super family. To date, over 20 BMPs are identified and shown to be involved in the regulation of cell proliferation, survival, differentiation and apoptosis, chondrogenesis, osteogenesis and embryogenesis. The BMPs have attracted much attention in the field of ovarian physiology. The expression of a range of BMPs within the different cell types of the antral follicle has been demonstrated in a variety of species including rodents, humans and ruminants (Elvin et al., 2000; Erickson & Shimasaki, 2003; Glister et al., 2004; Shimasaki et al., 2004). The granulosa cell-derived BMP2, BMP6 and the theca cell-derived BMP4 and BMP7 were found to promote granulosa cell (GC) proliferation, follicle survival and prevention of premature luteinization in the cow (Knight & Glister, 2006). The BMP receptor mRNAs are present in the ovary, with the strongest expression in GC and oocyte, which is consistent with the BMP actions observed on the GC *in vitro* (Shimasaki

* Corresponding author.
E-mail address: msarkar24@gmail.com (M. Sarkar).

https://doi.org/10.1016/j.rvsc.2018.04.002
Received 11 September 2017; Received in revised form 13 March 2018; Accepted 13 April 2018
0034-5288/ © 2018 Elsevier Ltd. All rights reserved.

Table 1
Target gene, primer sequences and amplicon length used in the qRT-PCR study.

Gene	Sequence of nucleotide (5′-3′)	Efficiency (%)	Amplicon length (bp)	EMBL accession No. or reference
BMP2	Forward: AAGGCCCTTGCTTGTCACTTT Reverse: TGCTTGCCGCTTTTCTCTTC	102.3	73	NM_001099141.1
BMP4	Forward: TTTATGAGGTTATGAAGCCCCCGGC Reverse: AGTTTCCCACCGCGTCACATTGTG	104.2	104	NM_001045877.1
BMP6	Forward: GGCCCCGTTAACTCGACTGTGACAA Reverse: TTGAGGACGCCGAACAAAACAGGA	101.2	108	XM_600972.2
BMP7	Forward: GGCAGGACTGGATCATCG Reverse: GAGCACAGAGATGGCATTGA	99.7	191	NM_001206015.1
BMPR1A	Forward: TCAGCGAACTATTGCCAAACAG Reverse: CCCATCCACACTTCTCCGTATC	103.6	75	NM_001076800.1
BMPR1B	Forward: TGGATGTCTAGGACTAGAAGGCTC Reverse: CAAAATCTCTGTTTTTCAGCGGA	98.9	149	NM_001 1 05328.1
BMPR2	Forward: AACACCACTCAGTCCACCTC Reverse: GTCAGCATCCTATATCCAAAGCA	100.7	120	NM_001304285.1
FSHR	Forward: AATTTGTCACACTCGTGGAGG Reverse: GTTCCCAGTGATGGCCAG	98.6	222	L22319
CYP19A1	Forward: AAGCCTTAGAGGATGATGTC Reverse: GGTCTCGTCTGGATGCAAGG	97.4	326	Mishra et al. (2017) (Mishra et al., 2017)
PCNA	Forward: ACCTGCAGAGCATGGACTCGTC Reverse: CATGCTGGTGAGGTTCACGCCCA	98.6	160	Mishra et al. (2016a) (Mishra et al., 2016a)
CASPASE3	Forward: CAGGGTGCCCAGGACTTTAG Reverse: AGAAAGCTCACGGGAACCAG	97.3	165	Mishra et al. (2016b) (Mishra et al., 2016b)
RPS15A	Forward: AGGGCTGGGAAAATTGTTGTGAA Reverse: TGAGGGGATGGGAGCAGGTTAT	104.8	125	Mishra et al. (2016c) (Mishra et al., 2016c)
Beta actin	Forward: AGTTCGCCATGGATGATGA Reverse: TGCCGGAGCCGTTGT	104.4	54	Singh et al. (2015) (Singh et al., 2015)

Abbreviations: **BMP**,Bone morphogenetic protein; **FSHR**, Follicle stimulating hormone receptor; **CYP19A1**, Cytochrome P450 family 19 subfamily A member 1; **PCNA**, Proliferating cell nuclear antigen; **CASPASE3**, Cysteine aspartic acid protease3; EMBL, European molecular biology laboratory.

et al., 1999; Wilson et al., 2001).

The BMPs mediate the effects through activating the membrane bound serine/threonine kinase receptors (Xiao et al., 2007). The signal transduction of BMP is mainly mediated *via* the classical BMPs–Receptor–Smads signal pathway (Nohe et al., 2004). Binding of BMP ligands to the BMPR2 initiates the phoshorylation cascade. First, BMPR1A/B is phosphorylated, that subsequently phosphorylates receptor-activated Smad proteins (R-Smads), which associate with common mediator-Smad (co-Smad) and enter the nucleus, where they regulate gene expression. The Smad proteins regulate promoter activity by interacting with transcriptional co-activators or co-repressors to positively or negatively control gene expression (Xiao et al., 2007; Miyazono et al., 2005). A mitogen activated protein kinase mediated (MAPK-mediated) Smad independent pathway has also been reported to be involved in the BMP signaling (Cook & Evans, 2014).

The BMP4 and BMP7 regulate the process of follicle development through GCs in the cow (Shimizu et al., 2012a). The BMPs function as luteinization inhibitors by suppressing luteinizing hormone (LH) receptor expression in GC (Shimasaki et al., 2004). Additionally, the BMP system was shown to play a crucial role in folliculogenesis in human (Shi et al., 2009; Shi et al., 2010; Shi et al., 2011). Mounting evidence suggests that actively growing follicles that are destined for ovulation are the major targets of BMPs in the cow (Glister et al., 2004; Shimasaki et al., 1999). Given their role in ovarian follicular function, we hypothesized that BMPs and their receptor are expressed in the follicle and regulate the functions of GCs in the buffalo. Therefore, the present study was done with the following objectives: A) To evaluate the transcriptional, translational profile of BMP2, 4, 6, 7 and their receptors such as BMPR1A,1B and BMPR2 in the GC and TC of ovarian follicle during different stages of development in buffalo ovary; B) To study the effects of BMP4 and/or BMP7 on estradiol (E_2) secretion, FSHR and CYP19A1 mRNA expression in the primary culture of GC and C) To study the effects of BMP4 and/or BMP7 on proliferating cell nuclear antigen (PCNA) and pro-apoptotic CASPASE3 mRNA expression in the primary culture of GC.

2. Materials and methods

All experimental protocols met the regulations of the Institutional Animal Care and Use Committee (IACUC).

2.1. Collection of follicles and preparation

Twenty genitalia of normal, healthy, cyclic buffalo cows were collected at a local slaughter house within 10 to 20 min of exsanguination and were transported on ice to the laboratory. A total of forty ovaries were isolated from them. From each ovary, only healthy follicles were isolated followed by the isolation of GCs and theca cells (TC) of different groups as described earlier (Sarkar et al., 2010). The GC and TC isolated from each follicle were transferred into separate tubes and labeled. The GC in the flushing solution was centrifuged at 3000*g* for 10 min at 4 °C. The TC and GC pellet were separately snap frozen in liquid nitrogen and stored at -80 °C until RNA and protein isolation. The FF was stored at -20 °C until determination of progesterone (P_4) and E_2. Because healthy follicles have relatively constant P_4 concentrations in the FF, only follicles with P_4 below 100 ng/mL in the FF were used for the evaluation, to exclude atretic follicles (Sarkar et al., 2010; Chouhan et al., 2013).

2.2. Follicle classification

The follicles were classified according to the E_2 content (ng/mL) in the FF as follows; (i) Group1 (FL1) < 0.5; (ii) Group 2 (FL2) 0.5–5; (iii) Group 3 (FL3) 5–40; and (iv) Group 4 (FL4) > 180 FF. The corresponding size of follicles were in the range of (i) 4–6 mm (FL1 or small); (ii) 7–9 mm (FL2 or medium); (iii) 10–13 mm (FL3 or large); (iv) >

Download English Version:

https://daneshyari.com/en/article/8503964

Download Persian Version:

https://daneshyari.com/article/8503964

Daneshyari.com