

Postnatal expression of bone morphogenetic proteins and their receptors in the mouse testis

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Short title: Bone morphogenetic proteins and their receptors in mice testis

Summary

TGF- β superfamily members including bone morphogenetic proteins (BMPs) and their receptors (BMPR-1A, -1B and -2) have been shown to be important for reproductive function in both males and females, while information on the role of BMPs in males is limited. Functional studies on select BMPs and BMP receptors have demonstrated vital roles for these proteins in somatic and germ cell proliferation, steroidogenesis and overall fertility.

In order to gain insight into the importance of these genes during postnatal reproductive development in males, our study was undertaken to specify the distribution of BMP and BMPR mRNA in male reproductive and steroidogenic tissues and quantify these genes in the testis using the mouse as our model. We screened testis at two, four, six and eight weeks of age for the expression of ten BMPs and three BMP receptors using RT-qPCR. All three BMP receptor mRNAs - *Bmpr1a*, *Bmpr1b* and *Bmpr2*, and ten BMP mRNAs - *Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b* and *Bmp15* were expressed in mouse testis at all stages screened. Testicular expression of genes varied within age groups and at specific developmental stages. Our study establishes an extensive BMP system in mouse reproductive and steroidogenic tissues.

Key words: male reproduction, growth factors, bone morphogenetic proteins, testis

Introduction

Bone morphogenetic proteins (BMPs) belong to the decapentaplegic-Vg-related (DVR) family, which forms the largest subgroup of growth factors in the Transforming growth factor- β (TGF- β) superfamily (McDonald and Hendrickson 1993). BMP signalling occurs via heterodimerization of type I (BMPR-1A, BMPR-1B) and type II (BMPR-2) serine/threonine receptors (Koenig *et al.* 1994; Ebisawa *et al.* 1999), which activate SMAD1, SMAD5 and/or SMAD8 signal transducers (Hoodless *et al.* 1996; Liu *et al.* 1996; Nishimura *et al.* 1998; Aoki *et al.* 2001; Kersten *et al.* 2005). Reproductive functions of BMPs and their putative receptors in males include the modulation of testosterone synthesis (Teixeira *et al.* 1999), germ cell maturation (Zhao *et al.* 1998), sperm quality (Hu *et al.* 2004), integrity of reproductive tissues (Hu *et al.*, 2004; Zhao *et al.* 1998) and epithelial secretory function (Settle *et al.* 2001).

In mice, knockouts for *Bmpr1a* (*Alk-3*) and *Bmpr2* are embryonically fatal (Mishina *et al.* 1995; Beppu *et al.* 2000), while *Bmpr1b* (*Alk-6*) deficiency leads to infertility in males and females (Yi *et al.* 2001). In male mice *Bmpr1b* deficiency resulted in compromised fertility attributed to defective development of the seminal vesicles (Yi *et al.* 2001). Seminal vesicles have been shown to express *Bmpr1a* and *Bmpr1b* mRNA in immature mice (Settle *et al.* 2001), however, whether these mutants had altered testicular function has not been reported. On the other hand partial dysfunction of *Bmpr1b* as seen in the Booroola merino strain of sheep results in significantly fewer primordial follicles (Ruoss *et al.* 2009) yet higher ovulation rate in females and no apparent effects in males (Piper and Bindon 1982; Wilson *et al.* 2001).

Expression of mutant *Bmp4* (Hu *et al.* 2004), *Bmp7* (Zhao *et al.* 2001), *Bmp8a* (Zhao *et al.* 1998) and *Bmp8b* (Zhao *et al.* 1996) resulted in either compromised fertility or infertility in male mice, while *Bmp15* null male mice were reported to have normal fertility (Yan *et al.* 2001). *Bmp2*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b* and *Bmpr1a* have been shown to be expressed in embryonic mouse testis (Dewulf *et al.* 1995; Ross *et al.* 2007). Mouse mRNA expression of *Bmp2* and *Bmp4* was detected in immature testis (Itman and Loveland 2008), *Bmp7*, *Bmp8a* and *Bmp8b* in immature and adult testis (Zhao *et al.* 1998; Zhao *et al.* 2001; Itman and Loveland 2008), *Bmp5* and *Bmp6* in adult testis (Lyons *et al.* 1989; Marker *et al.* 1997), while in adult mouse testis *Bmp15* mRNA and *Bmp3b* (*Gdf10*) were reported to be absent (Dube *et al.* 1998; Katoh and Katoh 2006).

Findings about the expression of BMP receptor mRNAs in the mouse testis or cells derived thereof have been inconclusive, perhaps due to assay sensitivity. Pellegrini *et al.* (2003), Dewulf *et al.* (1995) and ten Dijke *et al.* (1994) reported not finding *Bmpr1b* mRNA in mouse testis using Northern blotting and *in situ* hybridization, while Gouedard *et al.* (2000) reported *Bmpr1b* expression in mouse testis and testicular cell lines MA-10 cells and SMAT-1 cells derived from Leydig cell tumors and immature Sertoli cells respectively using polymerase chain reaction (PCR). In Sertoli cells of immature mice Puglisi *et al.* (2004) detected *Bmpr1a* using the Ribonucleas Protection Assay and *Bmpr2* expression by Northern blot, while on the contrary Pellegrini *et al.* (2003) did not readily detect the same mRNAs in Sertoli cells. In spermatogonia of immature mice Pellegrini *et al.* (2003) identified BMPR-IA protein and *Bmpr1a* and

Bmpr2 transcripts using Northern blot, and while Puglisi *et al.* (2004) also found *Bmpr1a* expressed in spermatogonia, they did not detect *Bmpr2*.

Pellegrini *et al.* (2003) demonstrated that BMP-4 increased proliferation of spermatogonia while its transcripts were expressed by Sertoli cells but not germ cells, indicating BMP-4 had a paracrine function in germ cell signalling. *Bmp4* expression decreased progressively from postnatal day 4 to 17 (Pellegrini *et al.* 2003). In mice before 3 weeks of age *Bmp8a* and *Bmp8b* mRNAs have been detected in spermatogonia and spermatocytes and at 3 weeks *Bmp8a* and *Bmp8b* mRNAs were localized in stage 6-8 round spermatids, demonstrating a development shift (Zhao and Hogan 1996; Zhao *et al.* 1996). *Bmp8b* homozygous mutants had greater germ cell degeneration than *Bmp8a* mutants, additionally homozygous *Bmp8b^{tm1blh}* mutant mice exhibited progressive depletion of germ cells due to increased germ cell apoptosis and were rendered infertile (Zhao *et al.* 1996; Zhao *et al.* 1998), indicating a function in germ cell survival and maintenance.

As significant roles for BMPs and their receptors are emerging in male reproductive function, the aim of this study was to investigate the distribution of these genes in adult reproductive and steroidogenic tissues using RT-PCR and quantify the relative gene expression of BMP receptors - *Bmpr1a*, *Bmpr1b* and *Bmpr2*, and BMPs - *Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b* and *Bmp15* in the mouse testis at two, four, six and eight weeks of age using reverse transcription quantitative PCR (RT-qPCR) analysis of mRNA. By examining the expression profiles of BMP/BMP receptor mRNAs at four specific time points we provide greater clarity to what functions individual genes may serve at specific developmental stages, as well as

providing comparisons between different genes to enhance understanding of relative abundance and how that may affect normal physiology of the testis.

Methods

Animals

The University of New England Animal Ethics Committee authorized the use of animals needed to conduct this research, which was in accordance with the National Health and Medical Research Council: Australian code of practice for the care and use of animals for scientific purposes 7th Edition 2004. Male Swiss Quackenbush mice (Physiology Animal House, University of New England, NSW, Australia) were housed in sanitary conditions in a light controlled room (12:12) at a constant temperature of 21 °C and had access to a constant supply of standard rodent chow and water.

At the age of 2, 4, 6 and 8 weeks mice were sacrificed by asphyxiation with CO₂ and approximately 100 mg testis placed in RNALater (Ambion, Austin TX) and incubated at 4 °C overnight then stored at -80 °C until RNA extraction. Epididymis, vas deferens, seminal vesicles, coagulating gland, prostate, adrenal gland and visceral adipose tissue of mature mice were also collected for RNA extraction and processed as for testis. Seminal vesicle and testis weights of mice sacrificed weekly from 2 to 8 weeks of age were measured to have a biological indicator of reproductive development. At 2 weeks mice were considered immature, at 4 weeks early pubertal, at 6 weeks late pubertal and at 8 weeks mature. N = 5.

RNA extraction, RT PCR and qPCR

RNA was extracted using TRI Reagent (Sigma-Aldrich Co, St Louis MO) according to manufactures instructions. RNA integrity was checked on a 1 % RNA

agarose gel (CSBC 2011) and quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE).

Reverse transcription was performed by annealing 2 µg of total RNA with 20 ng Oligo(dT)₁₅Primer (Fisher Biotec, Subiaco WA) at 70 °C for 5 min then placed on ice. This was followed by extension carried out at 40 °C for 60 min using 10 mM dNTP Mix (Fisher Biotec), 1 x RT Buffer, 400 U M-MLV Reverse Transcriptase (Promega, Alexandria NSW) and 1 U rRNasin(R)RNase Inhibitor (Promega).

Gene specific primers (Table 1.) (GeneWorks Pty Ltd, Hindmarsh SA) were designed using NCBI Primer-BLAST (Ye *et al.* 2009). A routine PCR using 80 ng cDNA was carried out to confirm the specificity of primers and their expected product lengths were checked using 2 % agarose gel electrophoresis to confirm the presence and amplification of the gene of interest. The PCR products were gel eluted and sequenced at Ramachiotti Centre for Genomics, UNSW, Sydney for confirmation.

Quantitative PCR (qPCR) reactions were set up in duplicate using 16 ng cDNA, in Fast EvaGreen qPCR Mix (Biotium, Hayward CA) using a CAS-1200 automated PCR Setup robot (Corbett Robotics, Eight Mile Plains, QLD) and qPCR performed using a Rotor-Gene R6 6000 Real-time Analyzer (Corbett Life Science, Concorde NSW).

Data Analysis

Data analysis of qPCR Ct values was performed by the $2^{-\Delta\Delta Ct}$ method using β -actin as the reference gene. Statistical analysis was performed using a general linear model procedure in SAS statistical software (SAS Institute Inc. Cary, NC, USA). The data were evaluated using one-way ANOVA followed by the Student-Newman Keuls *post hoc* test.

Values were considered to be significantly different at $P < 0.05$ and presented as mean \pm standard error (SE).

Results

Testis and seminal vesicle weight

As a measure of reproductive development we measured testis and seminal vesicle (SV) weight in 2 to 8 week old mice as shown in figure 1 panel A and B. Testis and SV weight increased significantly ($P < 0.05$) on a weekly basis from 2 weeks to 7 weeks at which time point weight had plateaued and was no longer significantly different at 8 weeks.

Comparative analysis of BMP receptor gene expression

The relative changes in the BMP receptor gene expression at the 4 time points examined are shown in figure 2. *Bmpr2* and *Bmpr1a* were the most abundant genes at all ages tested, while *Bmpr1b* was expressed in considerably lower amounts. Overall the pattern of change with age was similar in all three receptors with a marked decline in expression at 4 weeks followed by a slight rise at 8 weeks. At 2 weeks *Bmpr1a* expression was relatively high at $60.3 \pm 12\%$, while at 4 weeks *Bmpr1a* expression had been reduced 23 fold ($P = 0.0003$) and continued to decline although not significantly to six weeks. By 8 weeks *Bmpr1a* significantly increased by 2 fold ($P = 0.016$) over the expression at 6 weeks (Figure 2). At 2 weeks of age *Bmpr1b* was expressed at significantly lower amounts ($1.4 \pm 0.4\%$) than either *Bmpr1a* ($60.3 \pm 12\%$) or *Bmpr2* ($295 \pm 75\%$). The drop in expression level at 4 and 6 weeks from 2 weeks of age was also much higher (182 and 255 fold) than the decline seen with *Bmpr1a* but similar to that observed with *Bmpr2*. The expression of *Bmpr2* was the highest of the 3 receptors at 2 weeks ($296 \pm 75\%$), and followed a similar expression pattern with age as did *Bmpr1b*,

with a large drop at 4 weeks and remaining low at 6 weeks followed by a slight but significant rise ($P < 0.0001$) at 8 weeks.

Comparative analysis of BMP gene expression in mouse testis during postnatal development

The relative changes in BMP gene expression at the 4 time points examined are shown in figure 3 panel A and B. With the exception of *Bmp5* all genes reduced their expression from 2 to 4 weeks. *Bmp15* was the least expressed gene at 2 weeks. Genes that significantly increased their expression from 4 to 8 weeks were *Bmp3*, *Bmp5*, *Bmp7*, *Bmp8a* and *Bmp8b*. Genes that did not change significantly from 4 to 8 weeks were *Bmp2*, *Bmp4*, *Bmp6* and *Bmp15*, while *Bmp3b* was the only gene to significantly decrease its expression by 8 weeks. At 4 weeks *Bmp3b* was reduced by 12 fold ($P \leq 0.04$), *Bmp4* by 22 fold ($P \leq 0.0007$), *Bmp7* by 5 fold ($P = 0.0008$), *Bmp8a* by 5 fold ($P \leq 0.02$) and *Bmp8b* by 9 fold ($P = 0.0463$), while *Bmp2* was reduced 8 fold to less than 0.1 % ($P \leq 0.0001$), and *Bmp3* was reduced 65 fold to less than 0.01 % were $P = 0.009$. *Bmp3* expression declined the most radically of all the BMP mRNAs becoming the least expressed gene. By 6 weeks *Bmp5*, *Bmp8a* and *Bmp8b* significantly increased their expression were $P \leq 0.0001$, $P = 0.003$ and $P \leq 0.004$ respectively, and *Bmp3b* and *Bmp7* expression reduced significantly were $P \leq 0.03$ and $P = 0.02$ respectively from 4 weeks. Expression of *Bmp2*, *Bmp3*, *Bmp4*, *Bmp6* and *Bmp15* did not alter and *Bmp3* remained the least expressed gene. At 8 weeks expression of *Bmp7* and *Bmp8a* increased by 4 fold ($P \leq 0.005$) and 1 fold ($P = 0.04$) respectively. Expression of *Bmp2*, *Bmp3*, *Bmp3b*,

Bmp4, *Bmp5*, *Bmp6*, *Bmp8b* and *Bmp15* did not change, however *Bmp15* was the least expressed gene at 8 weeks, being expressed at only 0.004 % of the housekeeping gene.

Bmp receptor and bmp screening in male reproductive and steroidogenic tissues

Having detected mRNA expression of *Bmpr1a*, *Bmpr1b*, *Bmpr2*, *Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b* and *Bmp15* in mouse testis, we examined the expression of these genes in the epididymis, vas deferens, seminal vesicles, coagulating gland, prostate, adrenal gland and visceral adipose tissue of adult mice (Figure 4). All genes were detected in all tissues screened with the exception of *Bmp3* and *Bmp5* in vas deferens of which expression was too low to detect.

Discussion

We have demonstrated that there is widespread expression of BMPs and BMP receptors in male reproductive and steroidogenic tissues, which included finding testicular expression of *Bmpr1b*, *Bmpr2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6* and *Bmp15* at all developmental stages. It is likely that *Bmpr1b* mRNA had been reported absent in mouse testis by numerous researchers (ten Dijke *et al.* 1994; Dewulf *et al.* 1995; Pellegrini *et al.* 2003) with one exception (Gouedard *et al.* 2000), because its expression is low both relative to the housekeeping gene and to *Bmpr1a* and *Bmpr2* as determined in this study using highly sensitive RT-qPCR. This is supported in part by Belville *et al.* (2005) who found that *Bmpr1b* was expressed at a significantly lower level than *Bmpr1a* in SMAT-1 cells. We found *Bmpr1a* and *Bmpr2* mRNA in relatively high levels at all developmental stages, however BMP receptor expression was significantly higher in immature animals than in adult animals. Similarly, Puglisi *et al.* (2004) reported that *Bmpr1a* and *Bmpr2* expression in the testis decreased significantly with age, however they were unable to detect *Bmpr2* mRNA by 30 days of age (~ 4 weeks) or older. Given that *Bmpr2* was one of the most abundant genes tested in our study the reason for this discrepancy is unclear.

Bmp2, *Bmp4* and *Bmp7* mRNAs were present at all ages screened but had their highest expression in immature testis. Itman and Loveland (2008) reported *Bmp2*, *Bmp4* and *Bmp7* mRNA in 5 day old mouse testis, and demonstrated that BMP-2 and BMP-4 treatment stimulated signalling of SMAD 1, 5 and 8 in Sertoli cell and spermatogonial cultures. Furthermore, BMP-2 had a role in the proliferation of spermatogonia in concert with FSH but not alone, and BMP-7 had a role in the proliferation of Sertoli cells in the

presence of FSH (Puglisi *et al.* 2004), while BMP-4 increased proliferation of spermatogonia independent of gonadotrophins (Pellegrini *et al.* 2003). BMP-4 has been shown to be important for sperm quality with heterozygous mutation of *Bmp4* resulting in diminished sperm counts and motility (Hu *et al.* 2004). This demonstrates a great diversity of actions by these closely related BMPs and demonstrates how they can have vastly different functions under different conditions including the presence or absence of gonadotrophin stimulation.

Gene expression of *Bmp7* was highest in immature testis, lowest during early puberty and then significantly increased during late puberty and more so in adult testis. This suggests upon translation BMP-7 may have a role in the initiation of germ cell proliferation and maintenance of late stage spermatogenesis. Our findings are in agreement with Zhao *et al.* (2001) who found abundant expression of *Bmp7* in spermatogonia of immature mice, while in adult mice *Bmp7* mRNA was found mainly in spermatids and suggested to have a supporting role for maintenance of spermatogenesis. Similar to Zhao *et al.* (1998) we found *Bmp8a* was significantly less expressed than *Bmp8b* in pubertal testis but not in adult testis. This supports the suggestions that BMP-8a is important in late stage spermatogenesis with mRNA being identified in round spermatids (Zhao *et al.* 1998), while BMP-8b was shown to be necessary for both initiation and maintenance of spermatogenesis being expressed in spermatogonia and spermatids of pubertal mice and at high levels in round spermatids of adult mice (Zhao and Hogan 1996).

In humans *BMP3B* (*GDF10*) mRNA has been detected in the testis (Hino *et al.* 1996) and by using *in-silico* expression analysis Katoh and Katoh (2006) identified

BMP3B in human testis but reported the gene absent in mouse testis. We found low-level expression of *Bmp3* and significantly higher expression of *Bmp3b* in all age groups. *Bmp3b* expression was highest in immature mice, reduced significantly during early puberty and late puberty and remained unchanged in adult testis. Based on its expression patterns we hypothesis *Bmp3b* may have a role in germ cell proliferation while factors released from spermatids and/or spermatozoa present at 6 weeks of age (Seok *et al.* 2004) may down regulate its expression.

Bmp5 mRNA has been detected in adult mouse spermatogonia (Marker *et al.* 1997) and of interest *Bmp5* was the only gene we tested that had a lower expression at 2 weeks than all other age groups and increased significantly between 4 and 6 weeks which suggests a possible role in late stage spermatogenesis. *Bmp6* has been reported to be expressed in mature mouse testis (Lyons *et al.* 1989), and in our study *Bmp6* expression stayed relatively high compared with other BMP genes indicating a likely role in testicular functioning. We also detected *Bmp15* mRNA in mouse testis, which was previously not detected using Northern blotting (Dube *et al.* 1998). Compared to other BMP genes, *Bmp15* was expressed at a low level throughout development.

In humans *BMPR2* and *BMP15* mRNAs have been detected in the testis (Rosenzweig *et al.* 1995; Aaltonen *et al.* 1999), and *BMPR1B* expression was shown to be elevated in testicular cancer (Fustino *et al.* 2011). As the expression of BMPs is widespread in the testis, over-expression of receptors is likely to result in heightened sensitivity to low expressed ligands resulting in altered cell responses. Altered expression of BMPs is also characteristic of prostate cancers (Harris *et al.* 1994; Barnes *et al.* 1995; Buijs *et al.* 2007). Given that altered BMPR-1B signalling by putative BMPs is

implicated in reproductive cancers including testicular cancer (Miyazaki *et al.* 2004; Bokobza *et al.* 2009; Fustino *et al.* 2011; Neumann *et al.* 2011), which is one of the most common cancers affecting young Caucasian men in developed countries (Rosen *et al.* 2011), a mouse model profiling the postnatal expression of BMP and BMP receptor genes in the testis may be useful for understanding normal physiology and provide a comparison for altered physiology in cancerous tissues.

This study establishes an extensive BMP system in mouse testis throughout postnatal development at the mRNA level. Further examination of BMP and BMP receptor mRNAs in Leydig cells, Sertoli cells and germ cells will need to be carried out to elucidate what their individual roles are likely to be. Additionally, studies of protein expression will be needed to confirm the translation and abundance of the BMP and BMP receptor proteins not already detected. Given the predominance of BMP and BMP receptor genes reported in our study, and based on available research findings, it is likely that many of these genes have vital roles in germ and somatic cell proliferation, cellular homeostasis and steroid production, aspects we are currently investigating.

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Table 1. The forward and reverse primer sequences used for qPCR

Gene	NCBI reference #	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon length (bp)
<i>β-actin</i>	NM_007393.3	CGTCGACAACGGCTCCGGCATG	TGGGCCTCGTCACCCACATAG	150
<i>Bmpr1a</i>	NM_009758.4	AGGTCAAAGCTGTTCGGAGA	CTGTACACGGCCCTTTGAAT	178
<i>Bmpr1b</i>	NM_007560.3	TCAATGTCGTGACACTCCCATTCCT	TGCTGTACCGAGGTCGGGCT	245
<i>Bmpr2</i>	NM_007561.3	CACCCCCTGACACAACACCACTC	GACCCCGTCCAATCAGCTCCAG	243
<i>Bmp2</i>	NM_007553.2	ACCCCCAGCAAGGACGTCGT	AAGAAGCGCCGGGCCGTTTT	197
<i>Bmp3</i>	NM_173404.3	AGCAGTGGGTCGAACCTCGGA	ACCCCCACCGCTCGCACTAT	199
<i>Bmp3b</i>	NM_145741.2	GGCAACACCGTCCGAAGCTTCC	AGGAGGCGGCAGGATGCGTT	199
<i>Bmp4</i>	NM_007554.2	GACTACTGGACACCAGACTAGTCC	CTTCCCGGTCTCAGGTATCA	180
<i>Bmp5</i>	NM_007555.3	ATCAGGACCCCTCCAGGATGCC	TGATCCAGTCCTGCCATCCCAGATC	120
<i>Bmp6</i>	NM_007556.2	GCAGAGTCGCAACCGGTCCA	GGTGCAATGATCCAGTCCTGCC	153
<i>Bmp7</i>	NM_007557.2	CAAGCAGCGCAGCCAGAATCG	CAATGATCCAGTCTGCCAGCCAA	161
<i>Bmp8a</i>	NM_007558.2	TTGGCTGGCTGGACTGGGTCA	GCTGTCATAGTACAGCACAGAGGTG	209
<i>Bmp8b</i>	NM_007559.4	GGCTGGCTGGACTCTGTCATTGC	AGCTCAGTAGGCACACAGCACAC	176
<i>Bmp15</i>	NM_009757.4	GCCGTCGGCCAACACAGTAAG	AGAAGGTAAGTGCTTGGTCCGGCA	202

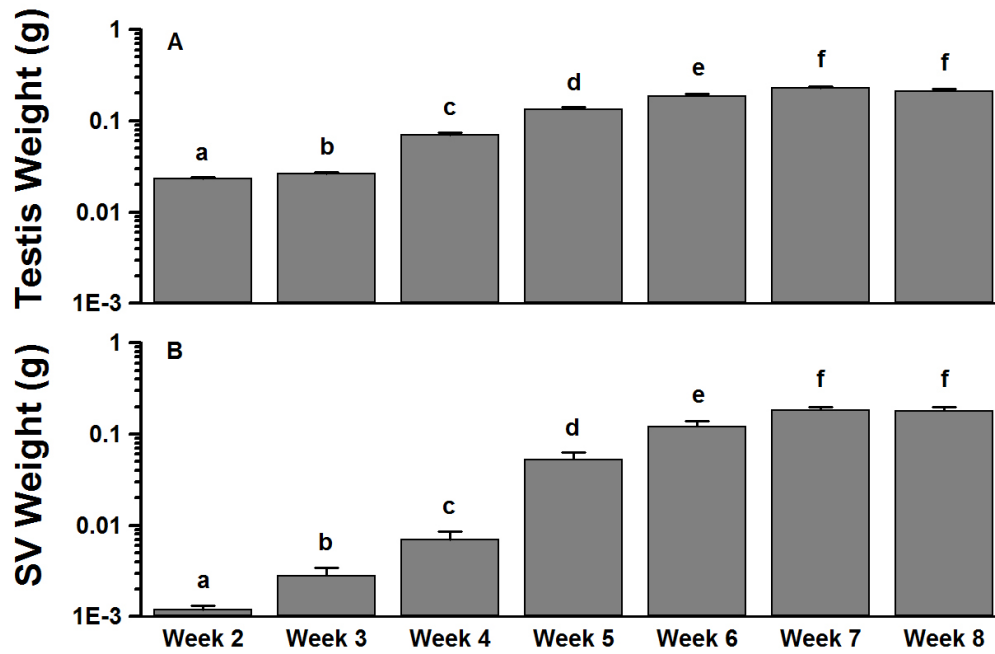


Figure 1. (Panel A) Testis and (Panel B) seminal vesicle weights (g) from 2, 3, 4, 5, 6, 7 and 8 week old mice. Results expressed as Mean \pm SE (n = 5). Different superscripts denote different levels of significance ($P < 0.05$).

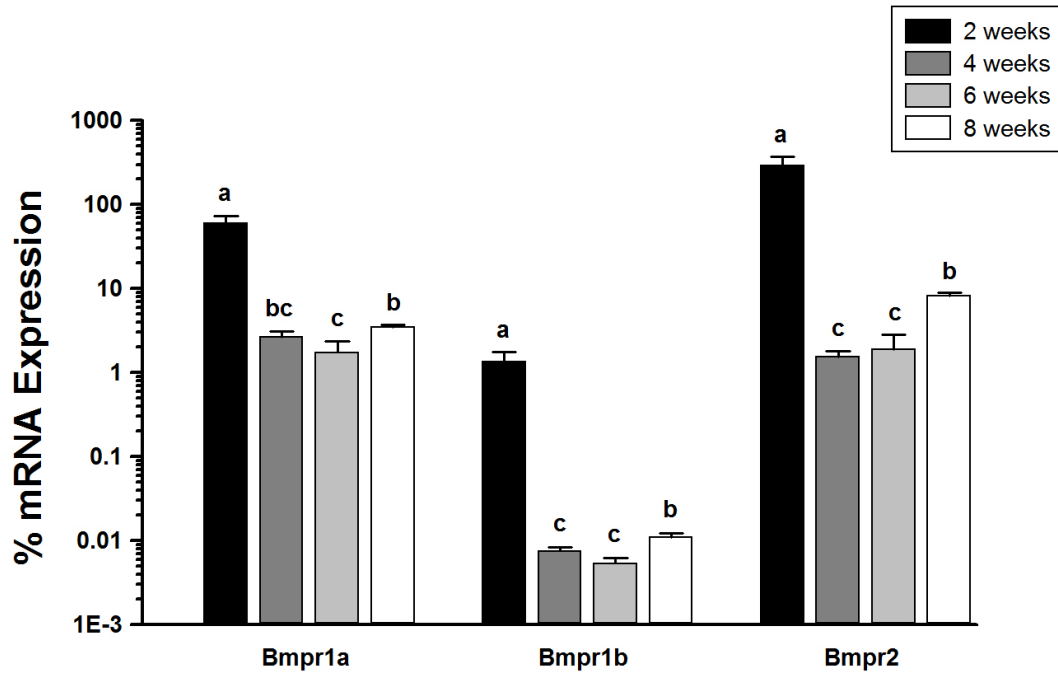


Figure 2. *Bmpr1a*, *Bmpr1b* and *Bmpr2* expression in 2, 4, 6 and 8 week old mouse testis. Results were calculated as a percentage of the housekeeping gene β -actin and presented as Mean \pm SE (n = 5 in duplicate). Different superscripts denote different levels of significance (P < 0.05) of individual genes throughout postnatal development.

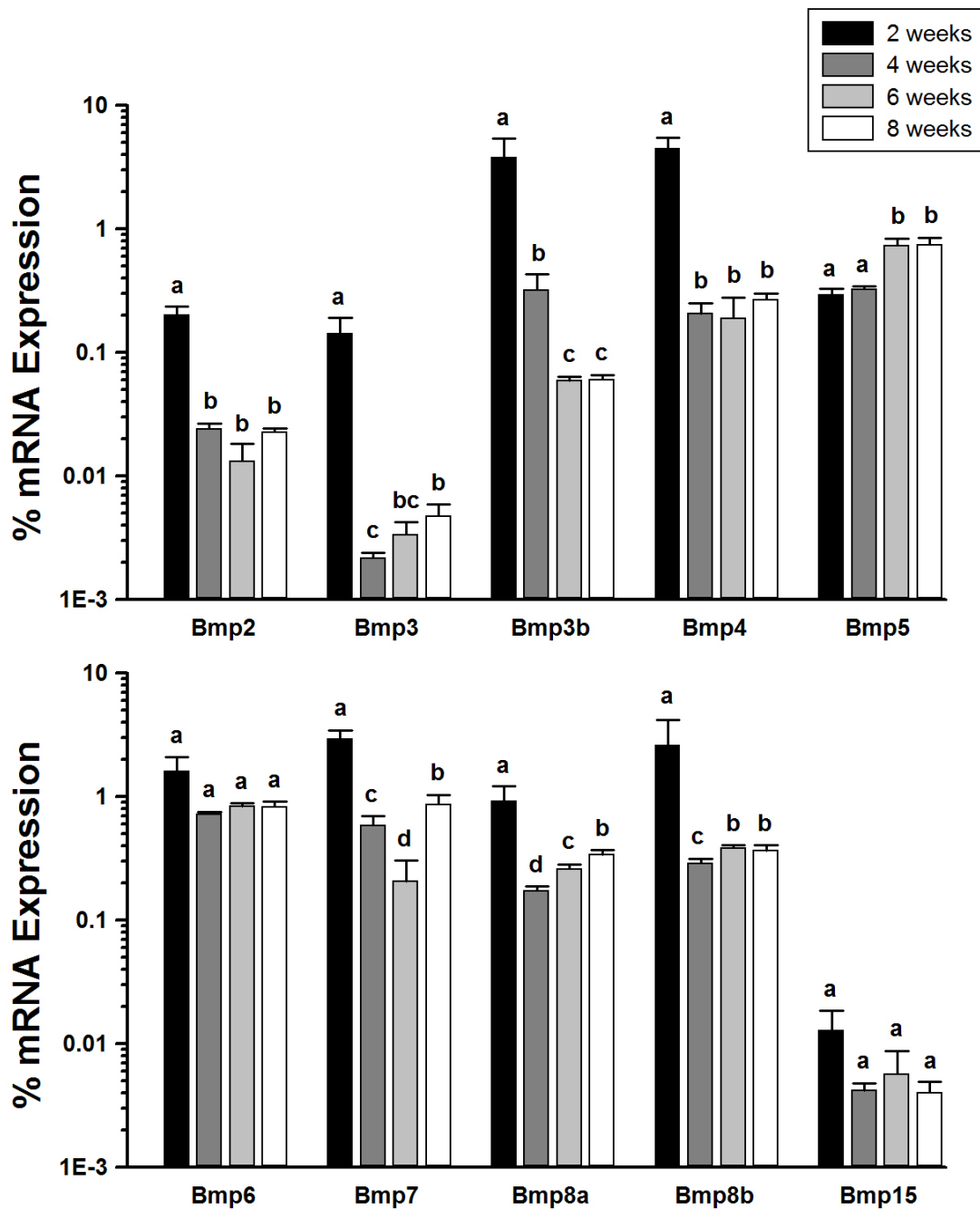


Figure 3. (Panel A) *Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4* and *Bmp5*, (Panel B) *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b* and *Bmp15* mRNA levels in 2, 4, 6 and 8 week old mouse testis. Results were calculated as a percentage of the housekeeping gene β -actin and presented as Mean \pm SE (n = 5 in duplicate). Different superscripts denote different levels of significance (P < 0.05) of individual genes throughout postnatal development.

Figure 4. BMP receptor (*Bmpr1a*, *Bmpr1b*, *Bmpr2*) and BMP (*Bmp2*, *Bmp3*, *Bmp3b*, *Bmp4*, *Bmp5*, *Bmp6*, *Bmp7*, *Bmp8a*, *Bmp8b*, *Bmp15*) mRNA expression in adult male reproductive and steroidogenic tissues. Lane: 1 = Negative control, 2 = Epididymis, 3 = Vas deferens, 4 = Seminal vesicle, 5 = Prostate, 6 = Coagulating gland, 7 = Adrenal gland, 8 = Adipose tissue.

