A comparative study on the role of bone morphogenetic protein in ovarian development and function in mice and ewes

by

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Declaration of originality

Accept where acknowledged, this thesis is entirely my own work and contains no material that has been accepted for an award of any degree or diploma at any university.

To the best of my knowledge and belief, this thesis does not contain any material previously published or submitted by another person, except where due reference is given in the text.

I certify that any help given in the preparation of this thesis, and all sources, have been acknowledged in this thesis.

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Dedication

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List of abbreviations

ACVR/ActR	Activin receptor
ALK	Activin-like kinase
AMH	Anti Mullerian hormone
AMHR2	Anti Mullerian hormone receptor 2
BAMBI	BMP and activin membrane bound inhibitor
BFGF	Basic fibroblast growth factor
BMP	Bone morphogenetic protein
BMPR	Bone morphogenetic protein receptor
β	beta
cAMP	cyclic adenosine monophosphate
cDNA	complementary DNA
CG	Chorionic gonadotropin
Co_2	Carbon dioxide
Co- Smad	Co-mediator Smad
E2	Estradiol
eCG	equine chorionic gonadotropin
ERβ	oestrogen receptors type β
FSH	Follicle stimulating hormone
FSHR	Follicle stimulating hormone receptor
GCs	Granulosa cells
GDF	Growth differentiation factor
GnRH	Gonadotropin releasing hormone
hCG	human chorionic gonadotropin
HRT	Hormone replacement therapy
IGF I	Insulin-like growth factor I
I-Smad	Inhibitory Smad
IVF	In vitro fertilization
KGF	Keratinocyte growth factor
KL	Kit ligand
LH	Luteinizing hormone
LHR	Luteinizing hormone receptor
LIF	Leukemia inhibitory factor
MIS	Mullerian inhibiting substance
mRNA	messenger ribonucleic acid
PCOS	Polycystic ovary syndrome
PGCs	Primordial germ cells
PMSG	Pregnant mare serum gonadotropin
RGM	repulsive guidance molecule
R-Smad	Receptor-regulated Smad
RT-PCR	Reverse transcription polymerase chain reaction
SCF	Stem cell factor

SEM	Standard error of the mean
TGF-β	Transforming growth factor-β
TβR	Transforming growth factor-β receptor
TSH	Thyroid-stimulating hormone
VEGF	Vascular endothelial growth factor

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Conference abstracts

AL-Ali, I.; Almahbobi, G.; McFarlane, J.R. (2012): The role of bone morphogenetic protein receptor 1B (BMPR-1B) in mouse ovarian follicular development .The annual scientific meeting of the Endocrine Society of Australia and the Society for Reproductive Biology 26th- 29th August 2012, Gold coast.

Ciller, I.M.**;** Athiappan Palanisamy, S.K.; Ciller, I.M.; AL-Ali, I.; McFarlane, J.R. (2012): BMPR-IB Modulates Steroidogenic Enzyme Genes Differentially in Pubertal and Adult Male Mice. The annual scientific meeting of the Endocrine Society of Australia and the Society for Reproductive Biology, 26th- 29th August 2012. Gold Coast.

AL-Ali, I.; Palanisamy, S.K.A.; McFarlane, J.R. (2013): The Effect of Passive Immunization against Bone Morphogenetic Receptor 1B on Ovarian Gene Expression. The annual scientific meeting of the Endocrine Society of Australia and the Society for Reproductive Biology 25th - 28th August 2013, Sydney.

AL-Samerria, S.; AL-Ali, I.; McFarlane, J.R.; Almahbobi, G. (2013): Passive immunization is a useful model to study the role of bmps in the regulation of ovarian follicle development in mice. The annual scientific meeting of the Endocrine Society of Australia and the Society for Reproductive Biology 25th - 28th August, Sydney.

Abstract

Fertility in females is totally dependent on the growth and proliferation of primordial follicles to mature into Graafian follicles. Several factors are involved in the regulation of ovarian follicle development from the follicle recruitment to ovulation. Among these regulatory factors is the bone morphogenetic protein family (BMP).

BMPs are a group of proteins that belong to the transforming growth factor-β super family (TGF-β). The stimulatory role of BMP-4 in primordial follicle recruitment has been confirmed in passively immunized mice (Tanwar *et al*., 2008) and a mutation in the gene encoding bone morphogenetic protein receptor-1B (BMPR-1B) has been linked with increased ovulation rate in Booroola sheep. The aim of this study was to further investigate the effect of immunization against BMP-4 and BMPR-1B on ovarian follicle development and atresia as well as to study the long-term effect on ovarian gene expression in mice. The features examined in this study were the number of primordial, primary and developing follicles in mice. In addition transcription levels of *Bmp2*, *Bmp4*, *Gdf9*, *Amh*, *Amhr2*, *Bmpr1a*, *Bmpr1b*, *Bmpr2*, *Acvr1a*, *Acvr1b*, *Acvr2a* and *Acvr2b* RT-qPCR measured during 4, 5, 9, 13 and 25 weeks of age.

We studied the effect of four different concentrations of equine chorionic gonadotropin (eCG) on follicle recruitment in female Swiss mice, which demonstrated that there were significant differences in regard to the number of primordial, developing and atretic follicles with different doses of eCG administered.

In vivo immunization against BMP-4 in immature mice increased the number of primordial follicles and significantly decreased *Amh* transcript levels and increased the expression of

Acvr1c compared with the control group. In addition, anti-BMPR-1B treatment increased the number of primordial follicles and significantly decreased the mRNA expression of *Bmp2*, *Bmp4*, *Bmpr1a*, *Bmpr1b* and *Acvr1a* in contrast with the control group. At 25 weeks, the mRNA expression of *Bmp2*, *Bmp4*, *Gdf9*, *Amh*, *Amhr2*, *Bmpr1*b, *Bmpr2 Acvr1a*, *Acvr1b* and *Acvr2b* were significantly reduced in untreated mice compared with the 5 week old untreated mice. Immunization against BMP-4 and BMPR-1B significantly decreased the mRNA expression levels of *Amh*, *Amhr2* and *Bmpr1b* at 5 weeks old compared with the control group. At 9 weeks anti-BMP-4 and anti-BMPR-1B immunization significantly decreased the mRNA expression of *Bmp4*, *Gdf9*, *Bmpr1a*, *Bmpr1b*, *Bmpr2*, *Acvr1a*, *Acvr1c*, *Acvr2a* and *Acvr2b* compared with the control group. There was no significant effect on the transcription levels of *Bmp2*, *Bmp4*, *Gdf9*, *Amh* and *Bmpr1b* while there was a significant increase in the mRNA expression level of *Amhr2* in anti-BMP-4 and anti-BMPR-1B treated groups at 13 weeks of age. In addition, the mRNA expression of *Bmp4*, *Bmpr1a*, *Bmpr1b* and *Bmpr2* was significantly increased ($P < 0.05$). However, the expression of *Amhr2*, *Acvr1b* and *Acvr2b* was significantly decreased in anti-BMP-4 and anti-BMPR-1B treated groups compared with the control group at 25 weeks of age.

Six months after treatment, our data shows that *in vivo* immunization against BMP-4 and BMPR-1B had no significant effect on body mass, ovaries, oviduct, uterus, spleen, kidney, liver, adrenal gland and heart, in comparison with control groups. Histological examination of tissues exhibited normal structure in immunized mice. The immunization against BMP-4 in sheep in early life did not have a significant effect on the total number of primordial follicles in both Booroola and Merino ewes.