12-1-2009

Oocyte peptides as paracrine tools for ovarian stimulation and oocyte maturation.

David G Mottershead

Andrew J Watson

Journal: MOLECULAR HUMAN REPRODUCTION

Article id: gap085

Article Title: Oocyte peptides as paracrine tools for ovarian stimulation and oocyte maturation

First Author: David G. Mottershead

Corr. Author: Andrew J. Watson

AUTHOR QUERIES - TO BE ANSWERED BY THE CORRESPONDING AUTHOR

The following queries have arisen during the typesetting of your manuscript. Please answer these queries by marking the required corrections at the appropriate point in the text.

Q1	Please check that the author surnames have been correctly identified by a pink background. If this is incorrect, please identify the full surname of the relevant authors.	
Q2	Remember that any funding used while completing this work should be highlighted in a separate Funding section. Please ensure that you use the full official name of the funding body.	
Q3	Please provide the volume number for reference "Figueira et al., 2009".	
Q4	Please provide volume number and page range for the following references: Ref. Li *et al*. (2009), Sugiura *et al*. (2009) and Vloeberghs *et al*. (2009).	

Molecular Human Reproduction, Vol.00, No.0 pp. 1–6, 2009
doi:10.1093/molehr/gap085

MHR Basic science of reproductive medicine

COMMENTARY

Oocyte peptides as paracrine tools for ovarian stimulation and oocyte maturation

David G. Mottershead[1] and Andrew J. Watson[2,3,4]

[1]Robinson Institute, School of Paediatrics and Reproductive Health, Medical School, The University of Adelaide, Adelaide 5005, Australia [2]Department of Obstetrics and Gynaecology, Children's Health Research Institute-Lawson Health Research Institute, The University of Western Ontario, London, ON, Canada [3]Department of Physiology and Pharmacology, Children's Health Research Institute-Lawson Health Research Institute, The University of Western Ontario, London, ON, Canada

[4]Correspondence address. E-mail: awatson@uwo.ca

ABSTRACT: Recent studies report the production and isolation of a stable bioactive recombinant human bone morphogenetic protein 15 (rhBMP15) that is appropriately processed in HEK-293 cells and activates the SMAD 1/5/8 pathway in mouse granulosa cell cultures. Further, the purified rhBMP15 induces the expression of genes associated with cumulus expansion. Thanks to recent research, we have a greater understanding of the importance of the dialogue that occurs between the oocyte and the granulosa cell layer with regard to regulating folliculogenesis and the acquisition of oocyte developmental competence and maturation. BMP15 is one of the critical components of these intra-follicular communication pathways. The production of recombinant human BMP15 is important for understanding the biochemistry of this specific pathway and for also fully understanding its functional contributions to mediating oocyte development. The production of a stable recombinant human BMP15 is also important for use in experiments aimed at optimizing ovarian stimulation protocols and *in vitro* oocyte maturation methods. This is required to improve oocyte and embryonic developmental competence and increase our ability to effectively use *in vitro* methods for animal production and the treatment of human infertility.

Key words: growth factors / protein biochemistry / folliculogenesis / assisted reproductive technology / embryo transfer

Introduction

Controlled ovarian stimulation with gonadotrophins is an important method of stimulating follicular development, and for collecting more than the normal numbers of maturing oocytes from a wide variety of mammalian species (Cantineau *et al*., 2007; Horcajadas *et al*., 2007; Loutradis *et al*., 2008; Delvigne, 2009; Klemmt *et al*., 2009; Lussiana *et al*., 2009; Verberg *et al*., 2009). This method has been applied to literally dozens of species although perhaps most routinely to the mouse (for research purposes), the cow, sheep and pig (for animal production purposes) and the human (for addressing infertility; Cantineau *et al*., 2007; Fong *et al*., 2007; Bell *et al*., 2008; De Roover *et al*., 2008). In all cases, however, the outcome from all ovarian stimulation protocols is unpredictable. This occurs due to a number of factors including ovarian cycle synchronization or suppression, ovarian reserve, subject age and variation in pharmacokinetics between subjects and genetic factors to name a few (Cantineau *et al*., 2007; Aboulghar, 2009; Delvigne, 2009; Fauzdar *et al*., 2009; Figueira *et al*., 2009; Klemmt *et al*., 2009; Raziel *et al*., 2009; Sismanoglu *et al*., 2009).

An additional and quite likely a predominant factor that is perhaps not fully appreciated by all concerned is that the dialogue between the oocyte and the associated follicle may be suboptimal at the time of retrieval after gonadotrophin stimulation (Eppig *et al*., 1997; Eppig, 2001; Matzuk *et al*., 2002). In fact, the number and quality of the follicles and their oocytes that develop may not be determined by the hypothalamus and the pituitary, but instead be regulated by intra-ovarian factors (McNatty *et al*., 2004). Therefore, to standardize ovarian stimulation protocols and to achieve reproducible and reliable outcomes, it will be necessary to ensure the levels of intra-ovarian factors are optimal and if not, supplement those levels by administering recombinant peptides.

The study by Li *et al*. (2009) brings us closer to that goal as they describe the production and isolation of a stable bioactive recombinant human bone morphogenetic protein 15 (rhBMP15). The rhBMP15 peptide is appropriately processed in HEK-293 cells and the purified protein activates the SMAD 1/5/8 pathway in primary cultures of mouse granulosa cells. Further, the purified rhBMP15 induces the expression of genes associated with cumulus expansion, namely *Ptx3*, *Has2*, *Tnfaip6* and *Ptgs2*. Thus, in total, this new purified rhBMP15 protein mimics a great number of known BMP15 functions and represents an important new agent for regulating folliculogenesis and oocyte developmental competence.

Intra-ovarian factors

Although the hypothalamic–pituitary–gonadal axis plays an indisputable and vital role in regulating folliculogenesis, the concerns regarding the variability in response following the administration of gonadotrophin-based stimulation protocols extend well beyond the simple variation in follicle or oocyte numbers (Cantineau *et al*., 2007; Horcajadas *et al*., 2007; De Roover *et al*., 2008; Loutradis *et al*., 2008; Aboulghar, 2009; Fauzdar *et al*., 2009; Figueira *et al*., 2009; Verberg *et al*., 2009; Vloeberghs *et al*., 2009). There is an increasing concern that oocytes generated from ovarian stimulation protocols do not achieve a normal developmental competence likely due to variations in oocyte mRNA and protein pools, and possibly due to abnormal variations in epigenetic patterning (Fauque *et al*., 2007; Sato *et al*., 2007; Meng *et al*., 2008). Since these components of oocyte developmental competence are likely influenced or regulated by intra-ovarian factors, it may well be necessary to employ recombinant intra-ovarian factors in combination with gonadotrophins to achieve optimal oocyte developmental competence *in vivo* and *in vitro*.

Growth differentiation factor 9 and BMP15 as key oocyte-derived factors

Research in recent years has focused on defining the molecular dialogue that occurs between the oocyte and the granulosa cells as the oocyte grows (Gilchrist *et al*., 2008). Oocyte paracrine growth factors acting on neighboring granulosa cells are important regulators of follicular growth (Eppig, 2001). Attention has primarily focused on the members of the transforming growth factor-β (TGF-β) family, including members such as growth differentiation factor 9 (GDF9) and BMP15. The deletion or mutation of *Gdf9* or *Bmp15* can greatly affect fertility (Dong *et al*., 1996; Galloway *et al*., 2000). BMP15 null mice display cumulus cell dysfunction, whereas GDF9 null mice display failed folliculogenesis (Dong *et al*., 1996; Yan *et al*., 2001). Thus, these factors are not only required for early folliculogenesis, but they are also potent mediators of granulosa cell differentiation (Gilchrist *et al*., 2004a; Juengel and McNatty, 2005; McNatty *et al*., 2007). There are important differences between species, e.g. mice are able to function without BMP15, however this is an essential factor for both human and sheep fertility (Montgomery *et al*., 2001; Yan *et al*., 2001; Teixeira Filho *et al*., 2002; McNatty *et al*., 2003; Di Pasquale *et al*., 2004; Hanrahan *et al*., 2004; Moore *et al*., 2004; Dixit *et al*., 2006). Interestingly, *Gdf9* or *Bmp15* heterozygosity results in increased fertility in sheep (Montgomery *et al*., 2001; Hanrahan *et al*., 2004), but once again no obvious effect is observed on mouse fertility (Dong *et al*., 1996; Yan *et al*., 2001). In humans, abnormal expression of GDF9 has been linked to polycystic ovarian syndrome (Teixeira Filho *et al*., 2002), and mutations in *Gdf9* and *Bmp15* are associated with premature ovarian failure (Di Pasquale *et al*., 2004; Dixit *et al*., 2006) and dizygotic twinning (Montgomery *et al*., 2004; Palmer *et al*., 2006).

As with all members of the TGF-β family, GDF9 and BMP15 are produced in a pro-form which is proteolytically processed during synthesis and secretion (Massagué, 1990). This means that the final product consists of an amino-terminal pro-region and a smaller biologically active carboxy-terminal mature region. A particular characteristic of GDF9 and BMP15 is that they lack the fourth conserved cysteine residue which is normally present in TGF-β family members to enable the formation of an inter-monomer disulphide bridge (McPherron and Lee, 1993; Dube *et al*., 1998; Laitinen *et al*., 1998). Thus, GDF9 and BMP15 form non-covalently associated homodimers and intriguingly they may be able to form heterodimers in culture (Liao *et al*., 2003; McNatty *et al*., 2003, 2004), which raises the possibility of greater molecular interactions *in vivo*. These factors signal via TGF-β receptors and activate SMAD cascades (Kaivo-oja *et al*., 2006). Within the ovary, the granulosa cells express most components of the TGF-β family signaling system, including type-II receptors and ALK type-I receptors, co-receptors such as β-glycan, binding proteins such as follistatin and the SMAD and co-SMAD intracellular messengers (Juengel and McNatty, 2005; Kaivo-oja *et al*., 2006). Clearly, GDF9 and BMP15 are among the most important intra-ovarian factors as their roles in mediating oocyte–granulosa cell interactions during folliculogenesis would indicate. It is thus critical that we develop molecular tools that will enable a full understanding of their biochemistry and function.

Recombinant GDF9 and BMP15

GDF9 was the first of these two oocyte-secreted factors to be produced as a recombinant protein (Elvin *et al*., 1999; Hayashi *et al*., 1999). However, neither of these two reports were the recombinant protein purified, being used in various bioassays as an impure preparation. This situation, and the fact that impure preparations have been used throughout the published literature (Elvin *et al*., 1999; Hayashi *et al*., 1999; Kaivo-Oja *et al*., 2003, 2005; Gilchrist *et al*., 2004b; Hickey *et al*., 2005; McNatty *et al*., 2005a, b), may well be the prime cause for the differing results that have been reported over the years concerning the bioactivity of GDF9 (Elvin *et al*., 1999; Dragovic *et al*., 2005). Indeed, only in 2008, the characterization of a purified recombinant GDF9 has been published (Mottershead *et al*., 2008). There have also been sporadic reports of the use of a purified commercially available bacterially produced GDF9 (Martins *et al*., 2008; Huang *et al*., 2009; Shi *et al*., 2009). The issue with any bacterially produced TGF-β family member is the question of what percentage of the refolded protein is correctly folded to give biologically active protein. Further, a bacterially produced protein will lack post-translational modifications, such as glycosylation and phosphorylation, which are of particular relevance for BMP15 and GDF9 (McMahon *et al*., 2008; Saito *et al*., 2008). Hence, any bacterially produced GDF9 or BMP15 should preferably be compared with a purified mammalian cell produced version of the protein, before being confident of having a correctly folded protein with full bioactivity.

The purification and initial characterization of a recombinant BMP15 were first published in 2000 by the Shimasaki laboratory (Otsuka *et al*., 2000). This material was a version of the human BMP15 protein with a carboxy-terminal FLAG tag, instrumental for the purification of the protein via immunoaffinity chromatography. However, as pointed out by Li *et al*. (2009), there has been a concern over the effect of the carboxy-terminal FLAG tag on BMP15 protein stability and bioactivity, as previously it has been found that a carboxy-terminal tag fused to TGF-β (Wakefield *et al*., 1991), or GDF9 (Mottershead *et al*., 2008) is detrimental for bioactivity. The recombinant human BMP15

produced by Di Pasquale and associates (Di Pasquale *et al*., 2004; Bodin *et al*., 2007; Rossetti *et al*., 2009) also suffers from the same issue; in this case, the carboxy-terminal tag is even longer, consisting of both the Myc and His6 tags in tandem. The new report from the Matzuk laboratory (Li *et al*., 2009) has placed the affinity purification tag (in this case, the FLAG tag) close to the amino-terminus of the mature region of hBMP15, leaving the carboxy-terminus unmodified. Further, and most importantly, in this current study, the authors have compared their purified N-FLAG tagged hBMP15 with a recently commercially available untagged hBMP15 mature region. Hence, we can be confident that the bioactivity of the epitope-tagged growth factor represents that also of the untagged native sequence growth factor. The effect of an epitope tag on the bioactivity of both human and mouse GDF9 has been clearly described previously (Mottershead *et al*., 2008).

Apart from one recent study (McIntosh *et al*., 2008), the issue of the role/effect of the pro-region of BMP15 or GDF9 on the bioactivity of the corresponding mature region is yet to be tackled by the workers in this field. Within the TGF-β family, the effect of the pro-region on the bioactivity of the corresponding mature region covers the full spectrum from potentiation (AMH/MIS; Wilson *et al*., 1993) to no effect (BMP9; Brown *et al*., 2005) to inhibition (TGF-β; McMahon *et al*., 1996). This is why it will be important to determine the situation for GDF9 and BMP15, by comparing the bioactivity of the purified (under native conditions) pro-mature complex for each growth factor with that of the corresponding purified mature region. Currently, commercially available mammalian cell produced forms of mGDF9 and hBMP15 contain just the mature regions of the respective proteins. Therefore, data based solely on experiments using these proteins should be interpreted cautiously given that we do not know how the pro-region affects the bioactivity of the mature region. To date, all the published, well-characterized purified recombinant BMP15 proteins (Saito *et al*., 2008; Li *et al*., 2009) have contained both the pro-region as well as the mature region. The situation for the published recombinant GDF9s (Elvin *et al*., 1999; Hayashi *et al*., 1999; Kaivo-Oja *et al*., 2003, 2005; Gilchrist *et al*., 2004b; Liao *et al*., 2004; Hickey *et al*., 2005; McNatty *et al*., 2005a, b) is most likely to be similar, although one study has produced and characterized the purified GDF9 mature region (Mottershead *et al*., 2008), and a side-by-side comparison with a pro-mature complex awaits future studies.

In the current study by Li *et al*. (2009), the purified rhBMP15 protein induces the expression of a number of genes in mural granulosa cells, a subset of which are associated with cumulus expansion, namely *Ptx3*, *Has2*, *Tnfaip6* and *Ptgs2*. This raises the issue of the involvement of GDF9 and BMP15 in the process of cumulus expansion, a controversial area where differing results have been obtained by groups using their own versions of unpurified (Elvin *et al*., 1999) or partially purified (Dragovic *et al*., 2005; Dragovic *et al*., 2007) recombinant GDF9, as well as one study using a purified BMP15 (Yoshino *et al*., 2006). Although any extensive discussion as to the nature of the cumulus cell expansion enabling factor (CEEF) is beyond the scope of this review, it is hoped that now with the availability of purified recombinant BMP15 and GDF9 (Li *et al*., 2009; Sugiura *et al*., 2009) that some of the issues surrounding the identity of the CEEF (Pangas and Matzuk, 2005) will be addressed. A further issue that should be investigated now that these proteins are available is the presence or absence of various post-translational modifications on the recombinant BMP15 and GDF9 proteins, both of which recently have been reported to be phosphorylated (McMahon *et al*., 2008). This modification is crucial to investigate further, as it is the first time that any member of the TGF-β family has been reported to be phosphorylated, and further, it was stated that this particular modification was necessary for biological activity.

BMP15 and GDF9 as reagents for *in vitro* oocyte maturation

In vitro oocyte maturation has been employed for producing preimplantation embryos from predominantly agricultural species (cow, sheep, goat and pig) for over 25 years. It is now increasingly being employed as a method to treat human infertility. The main, well-documented concern with this approach is that embryos produced from oocytes matured *in vitro* display a reduced developmental capacity than embryos produced from *in vivo* matured oocytes (Banwell and Thompson, 2008; Duranthon *et al*., 2008; Rinaudo and Lamb, 2008; Watkins *et al*., 2008; Watkins and Fleming, 2009; Wells *et al*., 2009). At the molecular level, variations in gene expression patterns and abnormalities in epigenetic programming are increasingly being documented in embryos and fetuses derived from oocytes matured *in vitro* (Banwell and Thompson, 2008; Duranthon *et al*., 2008; Rinaudo and Lamb, 2008; Watkins *et al*., 2008; Watkins and Fleming, 2009; Wells *et al*., 2009). Our understanding of the reasons for these differences in embryos produced from *in vitro* and *in vivo* matured oocytes is rudimentary at best but likely lies in important variations in the environment in which the oocyte develops. The most obvious difference is that *in vitro* matured oocytes are removed from the follicular environment and are therefore likely missing or are underexposed to the critical intra-ovarian factors that control the acquisition of oocyte developmental competence (Banwell and Thompson, 2008; Duranthon *et al*., 2008; Rinaudo and Lamb, 2008; Watkins *et al*., 2008; Watkins and Fleming, 2009; Wells *et al*., 2009).

Recent studies have indicated that the addition of BMP15 (Hussein *et al*., 2006) or GDF9 (Hussein *et al*., 2006; Yeo *et al*., 2008) increases developmental competence when added within an *in vitro* maturation setting. In particular, Yeo *et al*. (2008) demonstrated that addition of GDF9 to oocyte *in vitro* culture medium has a dramatic effect on the number of day 15 mouse fetuses, indicating that if the oocyte is in good shape at the start of the procedure, the chances of obtaining a viable and healthy pregnancy increase as well. This beneficial effect on the fetal development rate by supplementing *in vitro* oocyte culture medium with ovarian paracrine factors is quite likely to apply to BMP15 supplementation as well, given the effect of BMP15 on the blastocyst rate and quality (Hussein *et al*., 2006). However, a limitation of both of these studies (Hussein *et al*., 2006; Yeo *et al*., 2008) is that they involved the use of only partially purified recombinant BMP15 and GDF9, and it is very likely that some of the beneficial effects of GDF9 and BMP15 have been masked by inhibitory components from the 293H cell conditioned media.

Thus, the production of a purified recombinant human BMP15 will allow for studies aimed at optimizing *in vitro* oocyte maturation protocols. The expected benefits include an increase in oocyte

developmental competence, improved embryo quality and pregnancy rates following embryo transfer, and likely even increased fetal viability.

Conclusions

In the past 10 years, research has revealed great insight into the dialogue that occurs between the oocyte and the granulosa cell layer, raising our understanding of the importance of paracrine and autocrine intra-ovarian factors in regulating folliculogenesis and acquisition of oocyte-developmental competence and maturation. BMP15 is one of the critical components of these intra-follicular communication pathways. The production of recombinant human BMP15 will afford an important opportunity to investigate the biochemistry of this specific pathway and will provide an opportunity to fully understand its functional contributions to mediating oocyte development. In addition, the production of a stable recombinant human BMP15 provides an important agent for experiments aimed at optimizing ovarian stimulation and *in vitro* oocyte maturation protocols. Such optimization should provide for improved embryo developmental competence and increase our ability to use *in vitro* methods for increasing animal production and the treatment of human infertility. Finally, the production of recombinant BMP15 will allow for experiments aimed at rescuing follicular defects in BMP15 null mice as a prelude to demonstrating the therapeutic value that recombinant BMP15 could have on the health of human patients.

Q2 Funding

References

Aboulghar M. Symposium: update on prediction and management of OHSS prevention of OHSS. *Reprod Biomed Online* 2009;**19**:33–42.

Banwell KM, Thompson JG. In vitro maturation of Mammalian oocytes: outcomes and consequences. *Semin Reprod Med* 2008;**26**:162–174.

Bell CE, Calder MD, Watson AJ. Genomic RNA profiling and the programme controlling preimplantation mammalian development. *Mol Hum Reprod* 2008;**14**:691–701.

Bodin L, Di Pasquale E, Fabre S, Bontoux M, Monget P, Persani L, Mulsant P. A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. *Endocrinology* 2007;**148**:393–400.

Brown MA, Zhao Q, Baker KA, Naik C, Chen C, Pukac L, Singh M, Tsareva T, Parice Y, Mahoney A *et al.* Crystal structure of BMP-9 and functional interactions with pro-region and receptors. *J Biol Chem* 2005;**280**:25111–25118.

Cantineau AE, Cohlen BJ, Heineman MJ. Ovarian stimulation protocols (anti-oestrogens, gonadotrophins with and without GnRH agonists/antagonists) for intrauterine insemination (IUI) in women with subfertility. *Cochrane Database Syst Rev* 2007: CD005356.

Delvigne A. Symposium: update on prediction and management of OHSS epidemiology of OHSS. *Reprod Biomed Online* 2009;**19**:8–13.

De Roover R, Feugang JM, Bols PE, Genicot G, Hanzen C. Effects of ovum pick-up frequency and FSH stimulation: a retrospective study on seven years of beef cattle in vitro embryo production. *Reprod Domest Anim* 2008;**43**:239–245.

Di Pasquale E, Beck-Peccoz P, Persani L. Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (BMP15) gene. *Am J Hum Genet* 2004;**75**:106–111.

Dixit H, Rao LK, Padmalatha VV, Kanakavalli M, Deenadayal M, Gupta N, Chakrabarty B, Singh L. Missense mutations in the BMP15 gene are associated with ovarian failure. *Hum Genet* 2006;**119**:408–415.

Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 1996;**383**:531–535.

Dragovic RA, Ritter LJ, Schulz SJ, Amato F, Armstrong DT, Gilchrist RB. Role of oocyte-secreted growth differentiation factor 9 in the regulation of mouse cumulus expansion. *Endocrinology* 2005;**146**:2798–2806.

Dragovic RA, Ritter LJ, Schulz SJ, Amato F, Thompson JG, Armstrong DT, Gilchrist RB. Oocyte-secreted factor activation of SMAD 2/3 signaling enables initiation of mouse cumulus cell expansion. *Biol Reprod* 2007;**76**:848–857.

Dube JL, Wang P, Elvin J, Lyons KM, Celeste AJ, Matzuk MM. The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. *Mol Endocrinol* 1998;**12**:1809–1817.

Duranthon V, Watson AJ, Lonergan P. Preimplantation embryo programming: transcription, epigenetics, and culture environment. *Reproduction* 2008;**135**:141–150.

Elvin JA, Clark AT, Wang P, Wolfman NM, Matzuk MM. Paracrine actions of growth differentiation factor-9 in the mammalian ovary. *Mol Endocrinol* 1999;**13**:1035–1048.

Eppig JJ. Oocyte control of ovarian follicular development and function in mammals. *Reproduction* 2001;**122**:829–838.

Eppig JJ, Chesnel F, Hirao Y, O'Brien MJ, Pendola FL, Watanabe S, Wigglesworth K. Oocyte control of granulosa cell development: how and why. *Hum Reprod* 1997;**12**(Suppl 11):127–132.

Fauque P, Jouannet P, Lesaffre C, Ripoche MA, Dandolo L, Vaiman D, Jammes H. Assisted reproductive technology affects developmental kinetics, H19 imprinting control region methylation and H19 gene expression in individual mouse embryos. *BMC Dev Biol* 2007;**7**:116.

Fauzdar A, Halder A, and Kumar A. Effect of gonadotropins on chromosome aneuploidy, chromosome mosaicism & sex ratio in mouse preimplantation embryos. *Indian J Med Res* 2009;**129**:669–675.

Figueira RD, Braga DP, Nichi M, Madaschi C, Semiao-Francisco L, Iaconelli A Jr, Borges E Jr. Poor ovarian response in patients younger than 35 years: is it also a qualitative decline in ovarian function? *Hum Fertil (Camb)* 2009;1–6. **Q3**

Fong B, Watson PH, Watson AJ. Mouse preimplantation embryo responses to culture medium osmolarity include increased expression of CCM2 and p38 MAPK activation. *BMC Dev Biol* 2007;**7**:2.

Galloway SM, McNatty KP, Cambridge LM, Laitinen MP, Juengel JL, Jokiranta TS, McLaren RJ, Luiro K, Dodds KG, Montgomery GW *et al.* Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nat Genet* 2000;**25**:279–283.

Gilchrist RB, Ritter LJ, Armstrong DT. Oocyte-somatic cell interactions during follicle development in mammals. *Anim Reprod Sci* 2004a;**82–83**:431–446.

Gilchrist RB, Ritter LJ, Cranfield M, Jeffery LA, Amato F, Scott SJ, Myllymaa S, Kaivo-Oja N, Lankinen H, Mottershead DG *et al.* Immunoneutralization of growth differentiation factor 9 reveals it partially accounts for mouse oocyte mitogenic activity. *Biol Reprod* 2004b;**71**:732–739.

Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. *Hum Reprod Update* 2008;**14**:159–177.

Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R, Galloway SM. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biol Reprod* 2004;**70**:900–909.

Hayashi M, McGee EA, Min G, Klein C, Rose UM, van Duin M, Hsueh AJ. Recombinant growth differentiation factor-9 (GDF-9) enhances growth and differentiation of cultured early ovarian follicles. *Endocrinology* 1999; **140**:1236–1244.

Hickey TE, Marrocco DL, Amato F, Ritter LJ, Norman RJ, Gilchrist RB, Armstrong DT. Androgens augment the mitogenic effects of oocyte-secreted factors and growth differentiation factor 9 on porcine granulosa cells. *Biol Reprod* 2005;**73**:825–832.

Horcajadas JA, Diaz-Gimeno P, Pellicer A, Simon C. Uterine receptivity and the ramifications of ovarian stimulation on endometrial function. *Semin Reprod Med* 2007;**25**:454–460.

Huang Q, Cheung AP, Zhang Y, Huang HF, Auersperg N, Leung PC. Effects of growth differentiation factor 9 on cell cycle regulators and ERK42/44 in human granulosa cell proliferation. *Am J Physiol Endocrinol Metab* 2009;**296**:E1344–E1353.

Hussein TS, Thompson JG, Gilchrist RB. Oocyte-secreted factors enhance oocyte developmental competence. *Dev Biol* 2006;**296**: 514–521.

Juengel JL, McNatty KP. The role of proteins of the transforming growth factor-beta superfamily in the intraovarian regulation of follicular development. *Hum Reprod Update* 2005;**11**:144–161.

Kaivo-Oja N, Bondestam J, Kamarainen M, Koskimies J, Vitt U, Cranfield M, Vuojolainen K, Kallio JP, Olkkonen VM, Hayashi M *et al.* Growth differentiation factor-9 induces Smad2 activation and inhibin B production in cultured human granulosa-luteal cells. *J Clin Endocrinol Metab* 2003;**88**:755–762.

Kaivo-Oja N, Mottershead DG, Mazerbourg S, Myllymaa S, Duprat S, Gilchrist RB, Groome NP, Hsueh AJ, Ritvos O. Adenoviral gene transfer allows Smad-responsive gene promoter analyses and delineation of type I receptor usage of transforming growth factor-beta family ligands in cultured human granulosa luteal cells. *J Clin Endocrinol Metab* 2005;**90**:271–278.

Kaivo-oja N, Jeffery LA, Ritvos O, Mottershead DG. Smad signalling in the ovary. *Reprod Biol Endocrinol* 2006;**4**:21.

Klemmt PA, Liu F, Carver JG, Jones C, Brosi D, Adamson J, Mardon HJ, McVeigh E. Effects of gonadotrophin releasing hormone analogues on human endometrial stromal cells and embryo invasion in vitro. *Hum Reprod* 2009;**24**:2187–2192.

Laitinen M, Vuojolainen K, Jaatinen R, Ketola I, Aaltonen J, Lehtonen E, Heikinheimo M, Ritvos O. A novel growth differentiation factor-9 (GDF-9) related factor is co-expressed with GDF-9 in mouse oocytes during folliculogenesis. *Mech Dev* 1998;**78**:135–140.

Li Q, Rajanahally S, Edson MA, Matzuk MM. Stable expression and characterization of N-terminal tagged recombinant human bone morphogenetic protein 15. *Mol Hum Reprod* 2009. **Q4**

Liao WX, Moore RK, Otsuka F, Shimasaki S. Effect of intracellular interactions on the processing and secretion of bone morphogenetic protein-15 (BMP-15) and growth and differentiation factor-9. Implication of the aberrant ovarian phenotype of BMP-15 mutant sheep. *J Biol Chem* 2003;**278**:3713–3719.

Liao WX, Moore RK, Shimasaki S. Functional and molecular characterization of naturally occurring mutations in the oocyte-secreted factors bone morphogenetic protein-15 and growth and differentiation factor-9. *J Biol Chem* 2004;**279**:17391–17396.

Loutradis D, Vomvolaki E, Drakakis P. Poor responder protocols for in-vitro fertilization: options and results. *Curr Opin Obstet Gynecol* 2008;**20**:374–378.

Lussiana C, Guani B, Restagno G, Rovei V, Menato G, Revelli A, Massobrio M. Ovarian hyper-stimulation syndrome after spontaneous conception. *Gynecol Endocrinol* 2009;1–5.

Martins FS, Celestino JJ, Saraiva MV, Matos MH, Bruno JB, Rocha-Junior CM, Lima-Verde IB, Lucci CM, Bao SN, Figueiredo JR. Growth and differentiation factor-9 stimulates activation of goat primordial follicles in vitro and their progression to secondary follicles. *Reprod Fertil Dev* 2008;**20**:916–924.

Massagué J. The transforming growth factor-beta family. *Annu Rev Cell Biol* 1990;**6**:597–641.

Matzuk MM, Burns KH, Viveiros MM, Eppig JJ. Intercellular communication in the mammalian ovary: oocytes carry the conversation. *Science* 2002; **296**:2178–2180.

McIntosh CJ, Lun S, Lawrence S, Western AH, McNatty KP, Juengel JL. The proregion of mouse BMP15 regulates the cooperative interactions of BMP15 and GDF9. *Biol Reprod* 2008;**79**:889–896.

McMahon GA, Dignam JD, Gentry LE. Structural characterization of the latent complex between transforming growth factor beta 1 and beta 1-latency-associated peptide. *Biochem J* 1996;**313**:343–351.

McMahon HE, Sharma S, Shimasaki S. Phosphorylation of bone morphogenetic protein-15 and growth and differentiation factor-9 plays a critical role in determining agonistic or antagonistic functions. *Endocrinology* 2008;**149**:812–817.

McNatty KP, Juengel JL, Wilson T, Galloway SM, Davis GH, Hudson NL, Moeller CL, Cranfield M, Reader KL, Laitinen MP *et al.* Oocyte-derived growth factors and ovulation rate in sheep. *Reprod Suppl* 2003; **61**:339–351.

McNatty KP, Moore LG, Hudson NL, Quirke LD, Lawrence SB, Reader K, Hanrahan JP, Smith P, Groome NP, Laitinen M *et al.* The oocyte and its role in regulating ovulation rate: a new paradigm in reproductive biology. *Reproduction* 2004;**128**:379–386.

McNatty KP, Juengel JL, Reader KL, Lun S, Myllymaa S, Lawrence SB, Western A, Meerasahib MF, Mottershead DG, Groome NP *et al.* Bone morphogenetic protein 15 and growth differentiation factor 9 co-operate to regulate granulosa cell function. *Reproduction* 2005a; **129**:473–480.

McNatty KP, Juengel JL, Reader KL, Lun S, Myllymaa S, Lawrence SB, Western A, Meerasahib MF, Mottershead DG, Groome NP *et al.* Bone morphogenetic protein 15 and growth differentiation factor 9 co-operate to regulate granulosa cell function in ruminants. *Reproduction* 2005b;**129**:481–487.

McNatty KP, Hudson NL, Whiting L, Reader KL, Lun S, Western A, Heath DA, Smith P, Moore LG, Juengel JL. The effects of immunizing sheep with different BMP15 or GDF9 peptide sequences on ovarian follicular activity and ovulation rate. *Biol Reprod* 2007;**76**:552–560.

McPherron AC, Lee SJ. GDF-3 and GDF-9: two new members of the transforming growth factor-beta superfamily containing a novel pattern of cysteines. *J Biol Chem* 1993;**268**:3444–3449.

Meng LH, Xiao SQ, Huang XF, Zhou Y, Xu BS. A study on bisulfite sequencing method for methylation status of imprinted genes in single human oocytes. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2008; **25**:289–292.

Montgomery GW, Galloway SM, Davis GH, McNatty KP. Genes controlling ovulation rate in sheep. *Reproduction* 2001;**121**:843–852.

Montgomery GW, Zhao ZZ, Marsh AJ, Mayne R, Treloar SA, James M, Martin NG, Boomsma DI, Duffy DL. A deletion mutation in GDF9 in sisters with spontaneous DZ twins. *Twin Res* 2004;**7**:548–555.

Moore RK, Erickson GF, Shimasaki S. Are BMP-15 and GDF-9 primary determinants of ovulation quota in mammals? *Trends Endocrinol Metab* 2004;**15**:356–361.

Mottershead DG, Pulkki MM, Muggalla P, Pasternack A, Tolonen M, Myllymaa S, Korchynskyi O, Nishi Y, Yanase T, Lun S *et al.*

Characterization of recombinant human growth differentiation factor-9 signaling in ovarian granulosa cells. *Mol Cell Endocrinol* 2008;**283**:58–67.

Otsuka F, Yao Z, Lee T, Yamamoto S, Erickson GF, Shimasaki S. Bone morphogenetic protein-15. Identification of target cells and biological functions. *J Biol Chem* 2000;**275**:39523–39528.

Palmer JS, Zhao ZZ, Hoekstra C, Hayward NK, Webb PM, Whiteman DC, Martin NG, Boomsma DI, Duffy DL, Montgomery GW. Novel variants in growth differentiation factor 9 in mothers of dizygotic twins. *J Clin Endocrinol Metab* 2006;**91**:4713–4716.

Pangas SA, Matzuk MM. The art and artifact of GDF9 activity: cumulus expansion and the cumulus expansion-enabling factor. *Biol Reprod* 2005;**73**:582–585.

Raziel A, Schachter M, Friedler S, Ron-El R. Outcome of IVF pregnancies following severe OHSS. *Reprod Biomed Online* 2009;**19**:61–65.

Rinaudo PF, Lamb J. Fetal origins of perinatal morbidity and/or adult disease. *Semin Reprod Med* 2008;**26**:436–445.

Rossetti R, Di Pasquale E, Marozzi A, Bione S, Toniolo D, Grammatico P, Nelson LM, Beck-Peccoz P, Persani L. BMP15 mutations associated with primary ovarian insufficiency cause a defective production of bioactive protein. *Hum Mutat* 2009;**30**:804–810.

Saito S, Yano K, Sharma S, McMahon HE, Shimasaki S. Characterization of the post-translational modification of recombinant human BMP-15 mature protein. *Protein Sci* 2008;**17**:362–370.

Sato A, Otsu E, Negishi H, Utsunomiya T, Arima T. Aberrant DNA methylation of imprinted loci in superovulated oocytes. *Hum Reprod* 2007;**22**:26–35.

Shi FT, Cheung AP, Leung PC. Growth differentiation factor 9 enhances activin a-induced inhibin B production in human granulosa cells. *Endocrinology* 2009;**150**:3540–3546.

Sismanoglu A, Tekin HI, Erden HF, Ciray NH, Ulug U, Bahceci M. Ovulation triggering with GnRH agonist vs. hCG in the same egg donor population undergoing donor oocyte cycles with GnRH antagonist: a prospective randomized cross-over trial. *J Assist Reprod Genet* 2009;**26**:251–256.

Sugiura K, Su YQ, Li Q, Wigglesworth K, Matzuk MM, Eppig JJ. Fibroblast growth factors and epidermal growth factor cooperate with oocyte-derived members of the TGFbeta superfamily to regulate Spry2 mRNA levels in mouse cumulus cells. *Biol Reprod* 2009.

Teixeira Filho FL, Baracat EC, Lee TH, Suh CS, Matsui M, Chang RJ, Shimasaki S, Erickson GF. Aberrant expression of growth differentiation factor-9 in oocytes of women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2002;**87**:1337–1344.

Verberg MF, Macklon NS, Nargund G, Frydman R, Devroey P, Broekmans FJ, Fauser BC. Mild ovarian stimulation for IVF. *Hum Reprod Update* 2009;**15**:13–29.

Vloeberghs V, Peeraer K, Pexsters A, D'Hooghe T. Ovarian hyperstimulation syndrome and complications of ART. *Best Pract Res Clin Obstet Gynaecol* 2009.

Wakefield LM, Kondaiah P, Hollands RS, Winokur TS, Sporn MB. Addition of a C-terminal extension sequence to transforming growth factor-beta 1 interferes with biosynthetic processing and abolishes biological activity. *Growth Factors* 1991;**5**:243–253.

Watkins AJ, Fleming TP. Blastocyst environment and its influence on offspring cardiovascular health: the heart of the matter. *J Anat* 2009; **215**:52–59.

Watkins AJ, Papenbrock T, Fleming TP. The preimplantation embryo: handle with care. *Semin Reprod Med* 2008;**26**:175–185.

Wells PG, McCallum GP, Chen CS, Henderson JT, Lee CJ, Perstin J, Preston TJ, Wiley MJ, Wong AW. Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer. *Toxicol Sci* 2009;**108**:4–18.

Wilson CA, di Clemente N, Ehrenfels C, Pepinsky RB, Josso N, Vigier B, Cate RL. Mullerian inhibiting substance requires its N-terminal domain for maintenance of biological activity, a novel finding within the transforming growth factor-beta superfamily. *Mol Endocrinol* 1993; **7**:247–257.

Yan C, Wang P, DeMayo J, DeMayo FJ, Elvin JA, Carino C, Prasad SV, Skinner SS, Dunbar BS, Dube JL *et al.* Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol Endocrinol* 2001;**15**:854–866.

Yeo CX, Gilchrist RB, Thompson JG, Lane M. Exogenous growth differentiation factor 9 in oocyte maturation media enhances subsequent embryo development and fetal viability in mice. *Hum Reprod* 2008;**23**:67–73.

Yoshino O, McMahon HE, Sharma S, Shimasaki S. A unique preovulatory expression pattern plays a key role in the physiological functions of BMP-15 in the mouse. *Proc Natl Acad Sci USA* 2006;**103**:10678–10683.

Submitted on August 26, 2009; resubmitted on September 30, 2009; accepted on September 30, 2009