

Focus on TGF- β SignallingTGF- β superfamily members and ovarian follicle development

Phil G Knight and Claire Glister

*School of Biological Sciences, The University of Reading, Whiteknights, Reading RG6 6AJ, UK**Correspondence should be addressed to P G Knight; Email: p.g.knight@reading.ac.uk***Abstract**

In recent years, exciting progress has been made towards unravelling the complex intraovarian control mechanisms that, in concert with systemic signals, coordinate the recruitment, selection and growth of follicles from the primordial stage through to ovulation and corpus luteum formation. A plethora of growth factors, many belonging to the transforming growth factor- β (TGF- β) superfamily, are expressed by ovarian somatic cells and oocytes in a developmental, stage-related manner and function as intraovarian regulators of folliculogenesis. Two such factors, bone morphogenetic proteins, BMP-4 and BMP-7, are expressed by ovarian stromal cells and/or theca cells and have recently been implicated as positive regulators of the primordial-to-primary follicle transition. In contrast, evidence indicates a negative role for anti-Mullerian hormone (AMH, also known as Mullerian-inhibiting substance) of pre-granulosa/granulosa cell origin in this key event and subsequent progression to the antral stage. Two other TGF- β superfamily members, growth and differentiation factor-9 (GDF-9) and BMP-15 (also known as GDF-9B) are expressed in an oocyte-specific manner from a very early stage and play key roles in promoting follicle growth beyond the primary stage; mice with null mutations in the *gdf-9* gene or ewes with inactivating mutations in *gdf-9* or *bmp-15* genes are infertile with follicle development arrested at the primary stage. Studies on later stages of follicle development indicate positive roles for granulosa cell-derived activin, BMP-2, -5 and -6, theca cell-derived BMP-2, -4 and -7 and oocyte-derived BMP-6 in promoting granulosa cell proliferation, follicle survival and prevention of premature luteinization and/or atresia. Concomitantly, activin, TGF- β and several BMPs may exert paracrine actions on theca cells to attenuate LH-dependent androgen production in small to medium-size antral follicles. Dominant follicle selection in monovular species may depend on differential FSH sensitivity amongst a growing cohort of small antral follicles. Changes in intrafollicular activins, GDF-9, AMH and several BMPs may contribute to this selection process by modulating both FSH- and IGF-dependent signalling pathways in granulosa cells. Activin may also play a positive role in oocyte maturation and acquisition of developmental competence. In addition to its endocrine role to suppress FSH secretion, increased output of inhibin by the selected dominant follicle(s) may upregulate LH-induced androgen secretion that is required to sustain a high level of oestradiol secretion during the pre-ovulatory phase. Advances in our understanding of intraovarian regulatory mechanisms should facilitate the development of new approaches for monitoring and manipulating ovarian function and improving fertility in domesticated livestock, endangered species and man.

Reproduction (2006) **132** 191–206**Introduction**

The two key functions of the ovary are the generation of fertilizable, developmentally competent oocytes and the secretion of steroid hormones necessary to prepare the reproductive tract for fertilization and the establishment of pregnancy. Follicles are the functional units of the ovary and each follicle consists of an oocyte surrounded by one or more layers of somatic cells. For their steroidogenic and ovulatory potential to be realized, follicles must progress through an extended and highly coordinated series of developmental stages. The fetal

ovary (neonatal ovary in some species) is endowed with several million primordial follicles, consisting of an oocyte surrounded by a single layer of flattened pre-granulosa cells. The majority of these follicles degenerate in pre- or post-natal life, while still in a quiescent state and never embark on the complex developmental pathway that may, or may not, culminate in ovulation. Of those 'resting' primordial follicles that survive and are recruited into the growing follicle population, very few (<0.1%) are destined to ovulate; the vast majority will degenerate at some point along this lengthy developmental continuum (duration ~2 weeks

in rodents to >3 months in human and sheep). Progression through successive stages of follicle development requires bi-directional communication between oocyte and granulosa cells, and granulosa and theca cells (Eppig 2001); many of the extracellular-signalling molecules implicated in this dialogue belong to the transforming growth factor- β (TGF- β) superfamily. The later stages of follicle development, including the process of follicle selection, are also dependent on appropriately timed endocrine signals, notably, pituitary gonadotrophins and metabolic hormones, which act on receptors on the two somatic cell types and interact with a myriad of locally produced factors operating in an autocrine/paracrine manner to coordinate and control cell function. Once again, members of the TGF- β superfamily feature prominently amongst these locally produced factors (see Fig. 1).

In this review, we focus on the accumulating evidence implicating TGF- β superfamily members as key regulators of follicle development in mammals. Although the fundamental process of folliculogenesis is highly similar across species, it is worth reminding the reader that most of the evidence reported thus far has been generated using rodent models and there is a relative paucity of data on other species including man. Many detailed aspects of ovarian biology differ considerably between species and such added complexity will almost certainly prove to be the case with regard to the intraovarian roles of individual TGF- β superfamily members; indeed, several species differences have already emerged. This research field has been tremendously active in recent years and the attention of the reader is drawn to a number of other recent reviews focusing on the role of various TGF- β superfamily members (Findlay *et al.* 2002, Durlinger *et al.* 2002b, Shimasaki *et al.* 2004, Knight &

Glister 2003, Shimasaki *et al.* 2004, Juengel & McNatty 2005, Visser & Themmen 2005) as well as other growth factors (Kezele *et al.* 2002, Fortune 2003, Skinner 2005) implicated as intraovarian regulators.

Overview of the TGF- β superfamily

Ligands

The TGF- β superfamily is a structurally conserved but functionally diverse group of proteins with at least 35 members in vertebrates; these proteins are widely distributed throughout the body and function as extracellular ligands involved in numerous physiological processes during both pre- and postnatal life (Massague & Wotton 2000). A common feature shared by the members of this family is that the mature bioactive forms are homo- or hetero-dimers corresponding to the cleaved carboxy-terminal regions of larger pre-proteins; in most cases these dimers are covalently linked by an interchain disulphide bond between conserved Cys residues. On the basis of additional structural characteristics, members of the superfamily have been further classified into several subfamilies. These include the prototypic TGF- β subfamily (comprising TGF- β 1, TGF- β 2, TGF- β 3), an extensive bone morphogenetic protein (BMP) subfamily (with some 20 members), the growth and differentiation factor (GDF) subfamily (at least nine members), the activin/inhibin subfamily (including activins A, AB, B, inhibins A, B), the glial cell-derived neurotrophic factor (GDNF) subfamily (including GDNF, artemin and neuturin), as well as several additional members such as anti-Mullerian hormone (AMH; also known as Mullerian-inhibiting substance, MIS) and nodal.

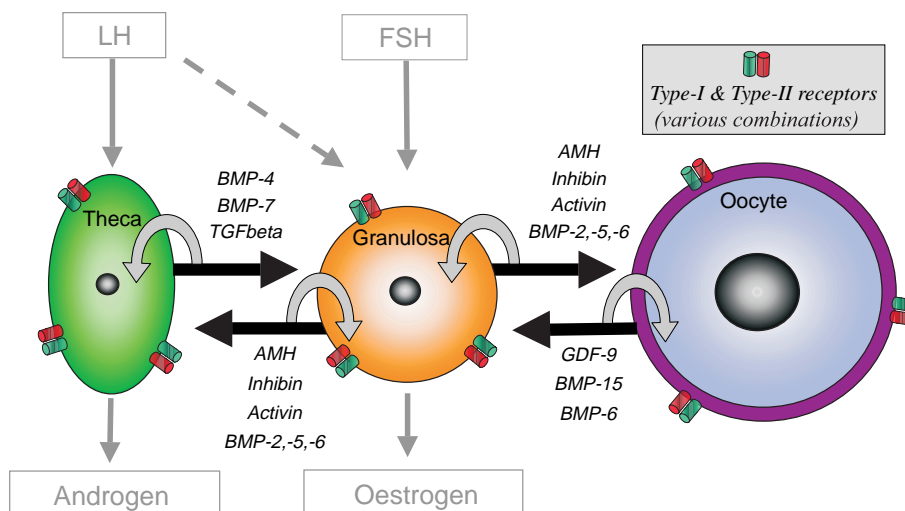


Figure 1 Members of the TGF- β superfamily feature prominently amongst the growing list of extracellular ligands implicated in the bi-directional communication between theca and granulosa cells, and granulosa cells and oocyte. Both autocrine (thick grey arrows) and paracrine (thick black arrows) signalling events are likely, depending on the expression of appropriate combinations of type-I and type-II receptors on the cell surface.

Signalling receptors

Most TGF- β superfamily members, with the exception of the GDNF subfamily and inhibin, exert their effects on target cells by binding to and forming hetero-tetrameric complexes with two types of Ser/Thr kinase receptor on the cell surface designated type-I and type-II (Massague & Wotton 2000, Miyazawa *et al.* 2002, Chang *et al.* 2002). In mammals there are seven known type-I and five type-II receptors associated with TGF- β signal transduction; different TGF- β superfamily ligands can form active signalling complexes by binding to the extracellular domain of one or more combination of type-I and type-II receptors. Receptor activation through phosphorylation of their intracellular kinase domains leads to the phosphorylation of downstream-signalling molecules called receptor-regulated Smads (R-Smads). These associate with a common partner Smad and translocate to the nucleus to modulate target gene expression through interaction with various transcription factors, co-activators and co-repressors.

Non-signalling binding proteins

Other cell-surface molecules, including β -glycan (also known as TGF- β type-III receptor), can act as non-signalling co-receptors for certain TGF- β superfamily ligands, including TGF- β and inhibin. Although β -glycan does not contain an intracellular signalling motif, it can bind TGF- β isoforms and inhibin with high affinity and greatly enhance the presentation of these ligands to type-II receptors on the cell surface, thus potentiating their action(s) (Lewis *et al.* 2000). There are other secreted (e.g. follistatin, noggin, chordin and gremlin) and membrane-bound (e.g. BMP and activin membrane-bound inhibitor BAMBI) proteins that can modulate TGF- β signalling (Gumienny & Padgett 2002). While such binding proteins are generally regarded as 'ligand traps' that diminish the interaction of ligands with their cognate signalling receptors, in some instances (particularly when the binding affinity is low), they may serve to enhance the presentation of the ligand by maintaining a locally high ligand concentration at or near the cell surface (Sugino *et al.* 1994).

A detailed description of TGF- β superfamily receptors and their associated signal transduction machinery and ancillary-binding proteins is beyond the scope of this article, but may be found elsewhere in this special issue (Lin *et al.* 2006).

Putative intraovarian roles of TGF- β superfamily members

Studies on several mammalian species, principally rodents, indicate that a number of ligands, receptors, signalling intermediaries and binding proteins associated with the TGF- β superfamily are expressed by

oocytes and ovarian somatic cells in a developmental stage-related manner (Shimasaki *et al.* 1999, Drummond *et al.* 2003, Erickson & Shimasaki 2003, Bristol & Woodruff 2004, McNatty *et al.* 2005). An increasing body of experimental evidence supports their having key roles in multiple aspects of follicle development, including primordial follicle recruitment, granulosa and theca cell proliferation/atresia, steroidogenesis, gonadotrophin receptor expression, oocyte maturation, ovulation, luteinization and corpus luteum (CL) formation. Further clues about the roles of these molecules have emerged from genetic studies involving targeted gene deletions in mice and naturally occurring mutations in sheep. This evidence is presented below in four subsections corresponding to progressive stages of follicle development.

Activation of 'resting' primordial follicles

In most mammals, the ovarian endowment of primordial follicles (ovarian reserve) is fully established before birth. Likewise, the recruitment of resting primordial follicles into the growing follicle population commences in fetal life (delayed until a few days after birth in rodents) and continues throughout much of postnatal life until the ovarian reserve is depleted and folliculogenesis ceases (i.e. human menopausal transition). The precise mechanisms that 're-awaken' these growth-arrested follicles remain to be elucidated although it has been recognized that the rate at which primordial follicles join the growing pool is positively related to the size of the ovarian reserve (Peters 1979, Gougeon 1996), an observation compatible with the notion of intraovarian signalling between growing follicles, resting follicles and ovarian stroma. Certainly, there is compelling evidence that bi-directional communication between the oocyte and surrounding granulosa cells (epithelial-derived), and granulosa cells and thecal-interstitial cells (stromal/mesenchymal-derived) is obligatory for normal follicle recruitment and development (Eppig 2001, Skinner 2005).

The pre-granulosa cells that surround the oocyte in primordial follicles express a number of peptide factors unrelated to the TGF- β superfamily, including kit ligand (KL, also known as stem cell factor, SCF) and leukemia inhibitory factor (LIF) that have been shown *in vitro* to promote the transition of primordial follicles into primary follicles, stimulate oocyte growth and the recruitment and proliferation of theca cells from the surrounding stromal tissue (Nilsson *et al.* 2002, Nilsson & Skinner 2003, 2004). The receptor for KL (c-kit) is expressed by oocyte and thecal-interstitial cells, enabling them to respond to this growth factor. Some of the mesenchymal cells surrounding primordial follicles (precursor-theca cells) have been shown to produce another peptide called keratinocyte growth factor (also called fibroblast growth factor-7, FGF-7) that may, in turn, act on the pre-granulosa cells and/or

granulosa cells to upregulate KL expression, and thus amplify its positive effects on pre-theca cell/theca cell proliferation and oocyte growth (Kezele *et al.* 2005). Another member of the fibroblast growth factor (FGF) family, FGF-2 (also called basic FGF), is expressed by oocytes from the primordial follicle stage and has been shown to upregulate KL expression in pre-granulosa cells and promote the primordial-to-primary follicle transition in cultured neonatal rat ovary (Nilsson & Skinner 2004).

Theca/stroma-derived BMPs

With regard to TGF- β superfamily members, evidence has emerged in rodents to support positive roles for BMP-4 and BMP-7 of pre-thecal and/or stromal cell origin in promoting the primordial-to-primary follicle transition and enhancing follicle survival. Lee *et al.* (2001) have reported the *in vivo* effects of injections of recombinant BMP-7 into the ovarian bursa of rats. BMP-7 treatment decreased the numbers of primordial follicles, yet increased the numbers of primary, preantral, and antral follicles. Similarly, *in vitro* exposure of neonatal rat ovaries to BMP-4 raised the proportion of developing primary follicles and lowered the number of resting primordial follicles (Nilsson & Skinner 2003). On the other hand, exposing a neutralizing antibody to BMP-4 resulted in smaller ovaries accompanied by a progressive loss of oocytes and primordial follicles, and increased cellular apoptosis (Nilsson & Skinner 2003).

Oocyte-derived TGF- β ligands

Three other TGF- β superfamily members GDF-9, BMP-15 (also known as GDF-9B) and BMP-6 have been found to be selectively expressed by oocytes from early-stage follicles – primary follicles in rodents and primordial follicles in cows and sheep (McGrath *et al.* 1995, Jaatinen *et al.* 1999, Bodensteiner *et al.* 1999, Elvin *et al.* 2000, McNatty *et al.* 2001). The various type-I and type-II receptors through which each of these ligands can signal are expressed by pre-granulosa cells/granulosa cells of the corresponding early follicle stages, making these cells potential targets for paracrine signalling. Mice, with a null mutation in the *gdf-9* gene, are infertile and show arrested follicle development at the primary stage (Dong *et al.* 1996, Carabatsos *et al.* 1998), indicating that oocyte-derived GDF-9 is essential for further follicle progression. This conclusion is reinforced by the finding that GDF-9 treatment *in vivo* (Vitt *et al.* 2000b) or *in vitro* (Nilsson & Skinner 2002) enhances the progression of early to late-stage primary follicles in the rat. However, there is some dispute whether GDF-9 affects the primordial-to-primary follicle transition; the *in vivo* study by Vitt *et al.* (2000b) supported this by showing a GDF-9-induced reduction in primordial follicle number, whereas the *in vitro* study by Nilsson & Skinner (2002) found no evidence of a GDF-9-induced increase in the primordial-to-primary follicle transition.

The arrested follicles observed in GDF-9 knockout mice had abnormal granulosa cells with increased expression of KL and they failed to acquire a theca layer, indicating that GDF-9 exerts a paracrine action on the surrounding somatic cells (Dong *et al.* 1996, Carabatsos *et al.* 1998, Elvin *et al.* 1999). Oocyte growth and zona pellucida formation proceed, but other aspects of oocyte differentiation are perturbed and these mice remain infertile. In contrast to GDF-9 knockout, null mutations in the *bmp-15* (*gdf-9b*) or *bmp-6* gene have minimal effects on follicle development and fertility (Solloway *et al.* 1998, Yan *et al.* 2001). However, species differences in the role of BMP-15 are evident, since naturally occurring point mutations of either the *bmp-15* or *gdf-9* gene in sheep affect fertility profoundly (Galloway *et al.* 2002, McNatty *et al.* 2005). These mutations are thought to result in reduced production of mature protein or impaired binding to cell-surface receptors. Ewes heterozygous for either of these mutations show an increase in ovulation rate, while homozygotes are infertile. In addition, the ovarian phenotype of homozygotes is similar to that seen in ewes actively immunized against BMP-15 and GDF-9, with follicles failing to develop beyond the primary stage (Juengel *et al.* 2002, McNatty *et al.* 2005). This ovarian phenotype also has many similarities with that in GDF-9 null mice (Carabatsos *et al.* 1998). It has recently been established that GDF-9 signalling involves interaction with TGF- β RI (activin-like kinase 5, ALK5) and bone morphogenetic protein receptor II (BMPRII) on the target cell surface, while BMP-15 signalling involves BMPRII (ALK6) and BMPRII. Expression of each of these receptor types has been confirmed in granulosa cells from the primordial/primary stage onwards consistent with responsiveness to these ligands (see Juengel & McNatty 2005). Thus, the evidence points to oocyte-derived GDF-9 (rodents) or both GDF-9 and BMP-15 (sheep) as having critically important effects on follicular somatic (pre-granulosa and/or granulosa) cells of primordial and/or primary follicles that are essential for further follicle progression. The mechanism(s) through which GDF-9 and/or BMP-15 promote follicle progression are obscure. The observation that GDF-9 can upregulate KL expression by bovine granulosa cells (Nilsson & Skinner 2002) is notable, since KL can enhance theca cell recruitment from the surrounding stromal cells (Parrot & Skinner 2000) and has been implicated in early oocyte growth (Manova *et al.* 1993). However, as mentioned earlier, KL expression by granulosa cells is upregulated in GDF-9 null mice and may account for the accelerated growth and early demise of oocytes in these mutants (Elvin *et al.* 1999).

AMH

Firm evidence indicates an inhibitory role for another TGF- β superfamily member, AMH, in the initiation of primordial follicle growth (Durlinger *et al.* 2002b). AMH (also known as Mullerian-inhibiting substance, MIS) was identified in a different context many years ago as the hormone produced

by Sertoli cells of the fetal testis that promotes regression of the Mullerian ducts during differentiation of the male reproductive tract. More recently, it was discovered that AMH is also expressed by granulosa cells of the female gonad. *in vitro* exposure of neonatal mouse ovaries to AMH was found to halve the number of growing follicles (Durlinger *et al.* 2002a). Conversely, mice with targeted deletion of the *amh* gene show an increased rate of recruitment of primordial follicles resulting in a premature depletion of their ovarian reserve (Durlinger *et al.* 1999). The observation that AMH expression is absent in primordial follicles, but detected in granulosa cells of follicles from the primary stage through to the small antral stage, supports the concept that growing follicles exert an inhibitory feedback influence on resting primordial follicles. The pattern of expression of AMH type II receptors in the ovary indicates that this inhibitory action of AMH is most likely exerted at the level of pre-granulosa/granulosa cells rather than the oocyte, although this requires confirmation. As discussed later, AMH has also been shown to attenuate follicle-stimulating hormone (FSH)-dependent follicle function at more advanced stages of development.

A schematic representation of some of the potential signalling interactions involved in the primordial-to-primary follicle transition is shown in Fig. 2.

Progression of primary follicles to the early-antral stage

The development of primary follicles to the late preantral/early antral stage involves oocyte enlargement,

zona pellucida formation, extensive granulosa cell proliferation to form a multilayered tissue, formation of a basal lamina, condensation of stromal cells around the basal lamina to form an enclosing theca layer and the development of fluid-filled spaces that gradually coalesce to form a single antral cavity. Inevitably, there are species differences in the timing of this progression; for example, rodent follicles acquire a well-defined theca layer at a much earlier stage than ruminant or primate follicles. In addition, as discussed later, rodent follicles appear to become gonadotrophin-dependent in the late preantral stage, whereas in ruminants and primates this dependency does not develop until the mid-antral stage. Although there is good evidence that gonadotrophins influence early preantral follicle progression (e.g. Dufour *et al.* 1979, Cortvrindt *et al.* 1997), their role is considered permissive rather than essential. Instead, evidence supports the proposition that local factors, including several members of the TGF- β superfamily, regulate the primary-to-secondary follicle transition and subsequent follicle growth to the late preantral/early antral stage.

Local TGF- β superfamily members implicated as positive regulators of preantral follicle growth, include GDF-9 and BMP-15 of oocyte origin, activins of granulosa origin, BMP-4 and BMP-7 of thecal origin and TGF- β from theca and granulosa cells. In contrast, firm evidence indicates a negative role for AMH in preantral follicle development. The expression of mRNA and/or protein for each of the above-mentioned ligands

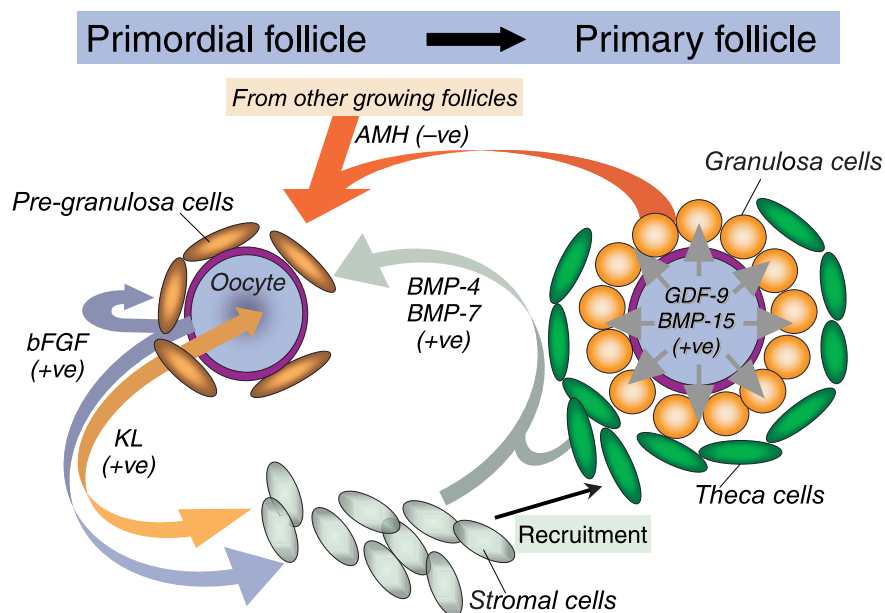


Figure 2 Potential signalling interactions (+ve, stimulatory; -ve, inhibitory) involved in the primordial-to-primary follicle transition; kit ligand (KL) and basic fibroblast growth factor (bFGF) secreted by pre-granulosa cells and oocyte respectively, have mutual stimulatory effects on oocytes and granulosa cells; they also promote recruitment of theca cells from the surrounding stromal/interstitial cell population. Stromal/interstitial cells and theca cells secrete BMP-4 and BMP-7, which promote follicle activation and survival. GDF-9 and/or BMP-15 secreted by the oocyte of the activated follicle promote granulosa cell proliferation, KL expression and theca formation. Granulosa cells of growing follicles secrete AMH that appears to act as a 'brake' on primordial follicle recruitment.

and indeed, for many of their receptors and intracellular signal transduction components, has been documented in theca, granulosa or oocyte of growing preantral follicles of several species (McNatty *et al.* 1999, Erickson & Shimasaki 2003, Drummond *et al.* 2003, Bristol & Woodruff 2004). Such anatomical evidence consolidates the findings of functional studies *in vitro* and *in vivo*, and also accords with ovarian histological observations in animals with targeted deletions or inactivating mutations of some of the relevant genes (reviews: Matzuk 2000, McNatty *et al.* 2001, 2005).

GDF-9 and BMP-15

In vitro exposure of rodent (Hayashi *et al.* 1999, Nilsson & Skinner 2002, 2003, Wang & Roy 2004) and human (Hreinnsson *et al.* 2002) ovarian tissue to GDF-9 has been shown to promote primary follicle progression. Conversely, follicle development beyond the primary stage occurs neither in GDF-9 null mice (Dong *et al.* 1996) nor in ewes homozygous for naturally occurring inactivating mutations in the *gdf-9* gene (Hanrahan *et al.* 2004) or in ewes actively immunized against GDF-9 (Juengel *et al.* 2002) indicating an obligatory role for this oocyte-derived growth factor. Another oocyte-derived factor BMP-15 has been shown to stimulate proliferation of undifferentiated granulosa cells in an FSH-independent manner (Otsuka *et al.* 2000). However, mice with null mutations in the *bmp-15* gene are only mildly subfertile with a weak ovarian phenotype (Yan *et al.* 2001). In contrast, ewes homozygous for inactivating *bmp-15* mutations are completely infertile with follicle development arrested at the primordial stage (Juengel *et al.* 2002, Hanrahan *et al.* 2004). Thus, species variation is evident in the role of oocyte-derived BMP-15 in early preantral follicle development.

BMP-4 and BMP-7

BMP-4 and BMP-7 are expressed in rat thecal cells from the primary/secondary stage onwards (Erickson & Shimasaki 2003), implying a functional role. The *in vivo* finding in rats (Lee *et al.* 2004) that intrabursal administration of BMP-7 reduced primordial follicle number while increasing the number of primary, preantral and antral follicles supports a positive paracrine action of theca-derived BMP-7 on granulosa cells of growing preantral follicles. Similarly, BMP-4 has been shown to increase the number of developing preantral follicles in cultured neonatal rat ovaries (Nilsson & Skinner 2003). However, it cannot be ruled out that these effects of BMP-7 and BMP-4 are entirely due to promotion of the primordial-to-primary transition; null mutations in the *bmp-7* gene in mice are peri-natally lethal, precluding comparison of postnatal ovarian follicle development. A similar constraint applies to BMP-4 null mice that die during embryogenesis. As discussed later, there is ample evidence that BMP-4 and BMP-7 exert autocrine/paracrine actions in antral follicles.

Activins

Expression of activin β A and β B subunits, type-I and type-II activin receptors and follistatin (activin-binding protein) has been detected in follicles from an early stage (primary–secondary according to species), indicative of local autocrine/paracrine roles in early follicle progression (Rabinovici 1991, McNatty 2000, Pangas *et al.* 2002, Drummond *et al.* 2002). Activin β A and β B subunits and follistatin are primarily expressed by granulosa cells, while activin receptors (both type-I and type-II) are expressed by theca cells, granulosa cells and oocyte. There is very little information on potential differential functions and bioactivities of the three different activin isoforms (A, AB and B); most studies to date have been confined to measurements of activin A production and/or assessing the effects of activin A treatment. Rodent preantral follicles secrete activin A *in vitro* (Smitz & Cortvrindt 1998) and exogenous activin A enhanced preantral follicle growth and granulosa cell proliferation in a follistatin-reversible manner (Li *et al.* 1995, Smitz *et al.* 1998, Liu *et al.* 1999, Zhao *et al.* 2001). However, activin A had no effect on early follicle progression when added to cultured pieces of bovine ovarian cortex (Fortune *et al.* 2000). Inhibin α -subunit null mice over-produce activin protein which is thought to explain the uncontrolled proliferation of granulosa cells and ovarian tumor development seen in these animals (Matzuk *et al.* 1992). In contrast, follicle development is arrested at the early antral stage in null mutant mice deficient in activin type-IIB receptor, further supporting a role for activin in promoting granulosa cell proliferation/differentiation (Matzuk *et al.* 1996).

TGF- β

Expression of TGF- β mRNA/protein in preantral follicles has been documented in several species including rodents, human, sheep and cattle (Teerds & Dorrington 1992, Schmid *et al.* 1994, Roy & Kole 1998, Nilsson *et al.* 2003, Juengel *et al.* 2004). Considerable species variation is evident in the spatio-temporal expression pattern of individual isoforms (TGF- β 1, TGF- β 2 and TGF- β 3) and of type-I and type-II TGF- β receptors amongst theca cells, granulosa cells and oocyte making it difficult to draw generalizations. Likewise, the results of functional *in vitro* studies involving ovarian explants are often contradictory; several reports indicate an inhibitory effect of TGF- β 1 on primary follicle survival and/or progression to the late preantral/early antral stage. However, other studies indicate positive effects or a lack of effect (Fortune 2003, Juengel & McNatty 2005).

AMH

Granulosa cells continue to express AMH until the early (mouse), mid-antral (human) or pre-ovulatory (sheep) stage (Durlinger *et al.* 2002a,b, Visser & Themmen

2005), implying a continued functional involvement in follicle development. Recombinant AMH has been shown to inhibit FSH-dependent growth of late preantral mouse follicles (Durlinger *et al.* 2001) and, as mentioned earlier, AMH-null mice show a marked increase in recruitment of primordial follicles into the growing pool (Durlinger *et al.* 1999). These observations support a negative effect of endogenous AMH on preantral follicle development beyond the primordial-to-primary transition.

Antral follicle growth and the follicle selection mechanism

Follicle progression through the antral stage of development is associated with continued proliferation of granulosa and theca cells, increased thecal vascularisation, further oocyte enlargement and a relatively rapid increase in diameter and volume. The increasing size and histotypic complexity of the follicle will impose limits on the diffusion-dependent transfer of secreted signalling molecules between cells in different intrafollicular compartments. As illustrated in Fig. 3, different follicular cell types are presumably exposed to very different 'cocktails' of signalling molecules depending on their relative position.

As mentioned earlier, FSH can influence the development of early-mid-stage preantral follicles. Growth beyond the late-preantral/small-antral stage (depending

on species), however, becomes critically dependent on FSH support. Evidence, mostly from studies in rodents, indicates an autocrine/paracrine role for granulosa-derived activin and BMP-6, and a paracrine role of oocyte-derived GDF-9, BMP-15 and BMP-6 in promoting granulosa cell proliferation and modulating FSH-dependent follicle function. Differential exposure to these factors may be one of the ways in which certain follicles are sensitised to FSH and thus selected to become the dominant follicle(s) that continue growth to the pre-ovulatory stage. For instance, in cultures of undifferentiated rat granulosa cells, activin promotes FSH receptor expression (Hasegawa *et al.* 1988, Xiao *et al.* 1992), whereas mice overexpressing follistatin (Guo *et al.* 1998) or those with null mutations in ActRIIB (Nishimori & Matzuk 1996), exhibit arrested follicle development. This implies a role for activin in the cyclic recruitment of follicles.

In contrast, AMH reduces the FSH responsiveness of preantral and small antral follicles and may thus have a negative role in the cyclic recruitment of follicles and dominant follicle selection process (Durlinger *et al.* 2002a,b, Visser & Themmen 2005). Circulating AMH concentrations in women are well correlated with the number of antral follicles detectable by transvaginal ultrasonography (de Vet *et al.* 2002, van Rooij *et al.* 2002) and, by inference, with the size of the ovarian reserve. As would be predicted from this, serum AMH concentrations in normal cycling women decrease with

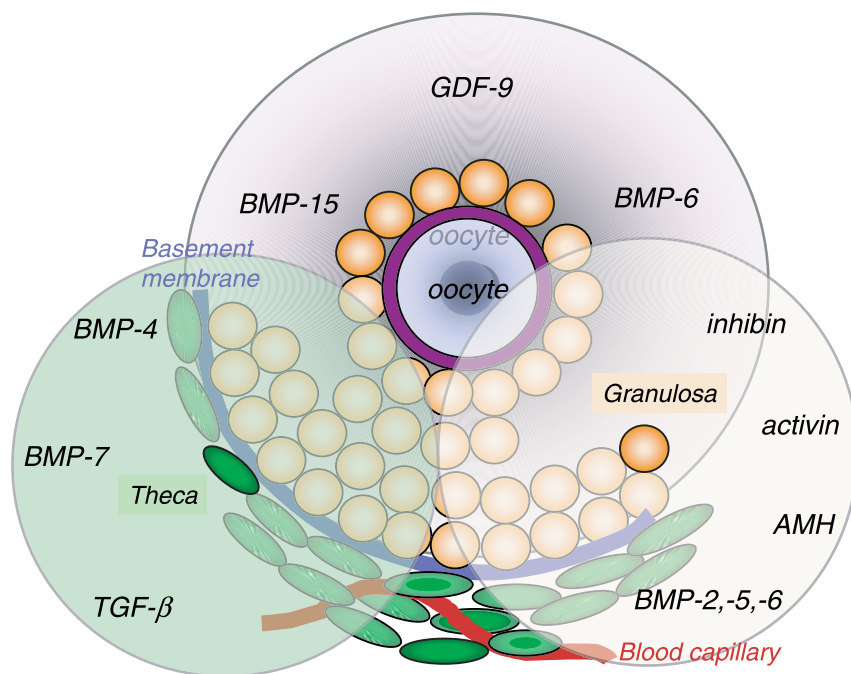


Figure 3 The secretory activity of different follicular cell types (theca cells, mural granulosa cells, cumulus granulosa cells, oocyte) will generate intrafollicular concentration gradients of intraovarian signalling molecules, including the TGF- β superfamily members depicted here; it is thus likely that individual cells are exposed – and respond – to very different ligand 'cocktails' depending on their relative position within the growing follicle and whether they express receptors of appropriate specificity. Likewise, proximity to thecal capillaries will influence access to systemic signals such as gonadotrophins and insulin.

age and are undetectable in post-menopausal women (Visser & Themmen 2005). Thus, the measurement of serum AMH may have great utility as a clinical marker of ovarian reserve.

Inhibin–activin system

Whilst granulosa cells have the capacity to synthesise inhibins and activins from an early stage of follicle development (McNatty *et al.* 2000, Montgomery *et al.* 2001), there is evidence that smaller follicles preferentially produce more activin relative to inhibin, whilst larger selected follicles secrete proportionally more inhibin (Schwall *et al.* 1990, Yamoto *et al.* 1992, Glister *et al.* 2006). However, a sharp increase in activin A:inhibin A and activin A:follistatin ratios occurs in bovine antral follicles as they reach the size at which the FSH-dependent follicle selection mechanism operates (Glister *et al.* 2006). So far, there is no corresponding information available on the intrafollicular concentrations of other ligands (BMPs, TGF- β isoforms) although, as will be discussed later, these proteins have also been clearly implicated in the autocrine/paracrine regulation of granulosa and theca cell function in antral follicles. Similarly, there is a paucity of information on the spatial and temporal pattern of expression of BMP-binding proteins in developing follicles, despite their probable role in the modulation of BMP action.

Other studies have shown that activin A (Hsueh *et al.* 1987, Hillier & Miro 1993, Wrathall & Knight 1995), TGF- β (Cortvrindt *et al.* 1997) and BMP-4, BMP-6 and BMP-7 (Glister *et al.* 2005) can attenuate LH-dependent androgen production by theca cells of small to medium-size antral follicles; the response to activin A can be reversed by follistatin. Granulosa-derived inhibin A, that is produced in increasing quantities by selected antral follicles approaching pre-ovulatory status, can also override the inhibitory action leading to enhanced LH-induced androgen production (Hsueh *et al.* 1987, Hillier & Miro 1993, Wrathall & Knight 1995, Campbell & Baird 2001). In this way, granulosa cells are able to maintain sufficient supply of thecal androgen required for their greatly increased oestrogen synthesis during the pre-ovulatory phase.

Activins may also have an important role in oocyte development within growing antral follicles. Cumulus granulosa cells surrounding the oocyte express inhibin/activin subunits (α , β A, β B) and follistatin (Roberts *et al.* 1993, Sidis *et al.* 1998, Izadyar *et al.* 1998, Silva *et al.* 2003), and the oocyte expresses both type-I and type-II activin receptors (Cameron *et al.* 1994, Izadyar *et al.* 1998, Sidis *et al.* 1998). In both rodents and primates, activin A accelerates oocyte maturation (Sadatsuki *et al.* 1993, Alak *et al.* 1996, 1998) and in the bovine, oocyte developmental competence is improved (Silva & Knight 1998). Conversely, inhibin A, or its free α -subunit has a negative effect on both oocyte

maturation (O *et al.* 1989) and developmental competence (Silva *et al.* 1999).

There is some, albeit inconsistent evidence supporting an autocrine action of inhibin on granulosa cells. Infusion of inhibin A into ewes resulted in a decrease in oestradiol secretion, although with a parallel decrease in plasma FSH levels (Campbell & Scaramuzi 1996). In accordance with this *in vivo* study, bovine granulosa cells treated with inhibin A showed a decrease in oestradiol secretion, an effect reversed when inhibin antibodies were added to the cells (Jimenez-Krassel *et al.* 2003). However, this conflicts with a report on the effects of inhibin on ovine granulosa cells showing that inhibin A enhances FSH-induced oestradiol secretion, while inhibin antiserum has the reverse effect (Campbell & Baird 2001). Hutchinson *et al.* (1987) reported that bovine inhibin A had no effect on FSH-induced steroid production by rat granulosa cells.

Taken together, these observations indicate that a changing intrafollicular balance between mutually opposing granulosa cell-derived inhibins and activins contributes to granulosa cell proliferation/differentiation, theca cell androgen synthesis and oocyte support and development. The effect of activin is subject to a further level of control by locally produced follistatin.

TGF- β

Ovarian cells have been shown to produce three isoforms of the TGF β subfamily, namely TGF- β 1, TGF- β 2 and TGF- β 3. Whilst the precise cellular distribution of these isoforms is variable depending on the species studied, stage of the follicle and most likely the detection method used (Juengel & McNatty 2005), generally, expression is first detected in preantral follicles and continues throughout the subsequent stages of follicular development. In rodents and humans, TGF- β is produced by both theca and granulosa cells, whereas in sheep, cows and pigs it is mainly produced by theca cells. The type-I and type-II TGF- β receptors appear to be ubiquitously expressed in most cell types. Reports detailing the effect of TGF- β on ovarian cells *in vitro* are also conflicting and appear to be highly dependent on the species studied, the stage of follicle differentiation, the presence of other growth factors as co-treatments and the precise cell-culture conditions. However, the three TGF- β isoforms appear to induce similar effects, albeit with different potencies depending on the cell type studied (Juengel & McNatty 2005). Similar to activin A, TGF- β can stimulate FSH receptor expression (Dunkel *et al.* 1994), amplify FSH-induced aromatase activity, inhibin production, progesterone production and LH receptor induction (Hutchinson *et al.* 1987, Zhang *et al.* 1988, Kim & Schomberg 1989, Dorrington *et al.* 1993, Drummond *et al.* 2000). TGF- β has also been shown to suppress thecal P450c17 expression and androgen production, in a similar

manner to activin A (Demeter-Arlotto *et al.* 1993, Fournet *et al.* 1996, C Glister & PG Knight, unpublished observations).

BMPs and GDF-9

In addition to their well-documented roles in early follicular development discussed earlier, there is increasing evidence to support critical role(s) of BMPs and GDF-9 in growing antral follicles. The expression of a range of BMPs and GDF-9 within different compartments 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, Juengel & McNatty 2005). Briefly, BMP-6, BMP-15 and GDF-9 are expressed by oocytes, BMP-2, BMP-5 and BMP-6 by granulosa cells and BMP-2, BMP-3b, BMP-4 and BMP-7 by theca cells.

It has been hypothesised that within antral follicles, the oocyte continues to influence the behaviour of the granulosa cells that surround it via the production of specific oocyte-secreted factors, hence regulating its own microenvironment (Eppig 2001, Gilchrist *et al.* 2004). BMP-15 and GDF-9, exclusively produced by oocytes, along with BMP-6, are prime candidates for this role. The expression of BMP receptors in oocytes (Souza *et al.* 2002, Erickson & Shimasaki 2003, Glister *et al.* 2004) further suggests a role for BMPs in modulating oocyte development and maturation. However, a recent study by Fatehi *et al.* (2005) found that bovine oocytes treated with exogenous BMP-2 and BMP-4 showed no response in terms of oocyte nuclear maturation, cumulus cell expansion or blastocyst yield. The ability of BMPs secreted by the oocyte itself (i.e. BMP-6, -15 and GDF-9) to influence oocyte development cannot be ruled out at this stage.

Immunoneutralization studies in sheep suggest that BMP-15 is required for follicle development to the ovulatory stage (Juengel *et al.* 2002). Both BMP-6 and BMP-15 have been shown to attenuate FSH action on rat granulosa cells; BMP-15 may act by suppressing FSH receptor expression (Otsuka *et al.* 2000), while BMP-6 may act by downregulating adenylate cyclase activity (Otsuka *et al.* 2001a). Intriguingly, a dramatic loss in BMP-6 mRNA levels has been noted at the time of dominant follicle selection (Erickson & Shimasaki 2003). These authors proposed that as BMP-6 can suppress FSH action, its absence at this time would be necessary for continued follicle development via the action of FSH. They also suggested that FSH itself may be responsible for the observed downregulation in BMP-6 expression.

Similar to BMP-15, GDF-9 may exert its effect via regulation of gonadotrophin action. Vitt *et al.* (2000a) demonstrated that GDF-9 could suppress FSH-stimulated progesterone and oestradiol production and attenuate FSH-induced LH receptor formation. Indeed, BMP-6, BMP-15 and GDF-9 all inhibit gonadotrophin-

stimulated progesterone secretion (Otsuka *et al.* 2001a,b), but only GDF-9 suppresses P450arom activity (Vitt *et al.* 2000a, Yamamoto *et al.* 2002). Recently, McNatty *et al.* (2005) studied the effect of these factors on ruminant (sheep and cow) granulosa cells. Overall, GDF-9 and BMP-15 alone and in combination suppressed FSH-stimulated progesterone production and stimulated cell proliferation, as previously seen in the rat and human. A marked synergy between the actions of GDF-9 and BMP-15 was also evident. As both these oocyte-secreted factors are co-expressed at most stages of follicular development, they should perhaps be regarded as one functional signalling unit as opposed to separate entities.

With regard to BMPs of presumptive granulosa origin, BMP-2 was shown to promote oestradiol and inhibin A secretion from cultured ovine granulosa cells (Souza *et al.* 2002). BMP-6 (also expressed by oocytes) inhibited FSH-induced progesterone production by rat granulosa cells but had no effect on P450arom expression or oestradiol secretion (Otsuka *et al.* 2001b). With bovine granulosa cells, BMP-6 enhanced basal and IGF-stimulated oestradiol, inhibin A, activin A and follistatin secretion and cell number, but suppressed progesterone secretion (Glister *et al.* 2004); the same group found no effect of BMP-6 on FSH-induced hormone secretion or cell number (C Glister & PG Knight, unpublished observations) although BMP-2 in porcine (Brankin *et al.* 2005a) and BMP-5 in rat (Pierre *et al.* 2005) were reported to reduce FSH-stimulated progesterone production. Collectively, these experiments highlight the ability of multiple BMPs to interact in a complex manner with both IGF- and FSH-dependent signalling pathways. It appears that BMP-2, BMP-5 and BMP-6 act on granulosa cells to promote follicle survival by maintaining cell proliferation and preventing premature luteinization and/or atresia, as with oocyte-derived GDF-9 and BMP-15.

In a similar manner, theca-cell-derived BMP-4 and BMP-7 also have the potential to act as paracrine regulators of granulosa cell function although species differences are evident. In the rat, BMP-4 and BMP-7 attenuated FSH-stimulated progesterone and enhanced FSH-stimulated oestradiol secretion, without affecting cell proliferation (Shimasaki *et al.* 1999, Lee *et al.* 2001). In bovine granulosa cells, BMP-4 and BMP-7 enhanced basal and IGF-stimulated (but not FSH-stimulated) oestradiol, inhibin A, activin A and follistatin secretion and cell number, while progesterone secretion was suppressed (Glister *et al.* 2004). In ovine granulosa cells, BMP-4 reduced FSH-induced progesterone secretion, concomitant with a decrease in StAR and P450scc (Pierre *et al.* 2004); BMP-4 was also shown to inhibit the transcriptional activity of SF-1.

More recently, a potential autocrine role for theca cell-derived BMP-2, BMP-4 and BMP-7 and a paracrine role for granulosa cell-derived BMP-2 and BMP-6 on

theca cell function, has been explored. BMP-4, BMP-6 and BMP-7 potently suppressed basal and LH-induced androgen secretion and enhanced cell proliferation in bovine theca cells (Glister *et al.* 2005). This was associated with a concomitant suppression in both P450c17 mRNA and protein levels, in agreement with a previous study on a human ovarian theca-like tumour cell culture model showing a suppressive effect of BMP-4 on forskolin-induced androgen production and P450c17 expression (Dooley *et al.* 2000). Similarly, BMP-2 was found to suppress androgen secretion by porcine theca cells (Brankin *et al.* 2005b). Thus, BMPs, like activins, are implicated as negative regulators of thecal androgen secretion. As such, it has been suggested that a functional deficit in BMP and/or activin signalling could contribute to the raised ovarian androgen output associated with polycystic ovarian disease in the human (Glister *et al.* 2005).

BMP-binding proteins

In addition to analysing the temporal and spatial expression patterns of different BMPs, consideration should be given to the co-localization of BMPs with their binding proteins (e.g. follistatin, noggin, chordin, gremlin, BAMBI), an increasing number of which has been identified and implicated in the modulation of BMP action. Briefly, follistatin can bind to BMP-4, BMP-6 and BMP-7 and inhibit their effects on bovine granulosa cells (Glister *et al.* 2004) although evidently not on theca cells (Glister *et al.* 2005). Follistatin can prevent the inhibitory actions of BMP-15 on FSH receptor expression (Otsuka *et al.* 2001a,b). PRDC blocks the signalling of BMP-2 and BMP-4 in rat granulosa cells (Sudo *et al.* 2004); noggin potently neutralised BMP-2 and BMP-4 action on ovine granulosa cells (Pierre *et al.* 2005); gremlin suppressed BMP-4 signalling in mouse granulosa cells (Pangas *et al.* 2004). Given their wide tissue distribution and potent activities, these binding proteins are likely to represent another important level in the control of the intrafollicular BMP/GDF system.

Ovulation, luteinization and CL formation

For the small proportion of follicles that reach ovulatory status, folliculogenesis culminates with the release of the cumulus–oocyte complex and subsequent formation of CL by a process termed luteinization. The newly formed CL synthesises and secretes large amounts of progesterone (and oestrogen in primates) essential for support of a potential pregnancy. If fertilization and subsequent implantation of the conceptus do not occur, steroidogenesis ceases and the CL regresses. Whilst the precise mechanisms governing the dynamic morphological and functional changes a CL undergoes have yet to be defined, emerging evidence indicates a role for several members of the TGF- β superfamily. It should be

emphasized that there is no clear-cut evidence indicating a direct involvement of TGF- β superfamily members in the ovulatory process itself. Rather, ovulation failure in response to perturbation of TGF- β superfamily ligands and/or receptors can be viewed as a consequence of the failure of follicles to develop normally to the pre-ovulatory stage. Similarly, increases in ovulation rate, such as those observed in inhibin-immunized animals, reflect an increased number of follicles reaching the pre-ovulatory stage.

After ovulation and during CL formation, inhibin/activin subunit expression is downregulated in most species, with the exception of primates where the expression of α - and β A-subunits, although not the β B-subunit, is maintained (Yamoto *et al.* 1991, Roberts *et al.* 1993). A role for inhibin A and/or its free α -subunit in primate CL formation and progesterone production has been proposed. During the human cycle, the CL becomes a significant source of inhibin A, levels of which peak at the mid-luteal phase (Muttukrishna *et al.* 1994). Furthermore, in the marmoset, addition of antibodies to the inhibin α -subunit suppressed human chorionic gonadotrophin (hCG)-induced progesterone secretion (Webley *et al.* 1994). Conversely, activin A may delay granulosa cell luteinization and/or atresia and decrease basal and hCG-induced progesterone production in both cultured human and monkey granulosa-lutein cells (Rabinovici *et al.* 1990, Brannian *et al.* 1992, Di Simone *et al.* 1994), further reinforcing a positive role for inhibin in promoting luteal formation. The effects of activin A could be reversed by its binding protein follistatin (Cataldo *et al.* 1994), the expression of which was upregulated by hCG in granulosa-lutein cells (Tuuri *et al.* 1994), indicating another potential component of the gonadotrophin-dependent luteal support system.

In addition to its role in antral follicle development, TGF- β may also contribute to CL formation. TGF- β 1 and TGF- β 2 are both expressed in mouse luteal cells (Ghiglieri *et al.* 1995) and rat luteal macrophages (Matsuyama & Takahashi 1995). In rodents, a possible role for TGF- β s is through mediation of the luteotrophic actions of prolactin and subsequent inhibition of apoptosis of luteal cells. Prolactin enhances progesterone production via the suppression of 20 α -hydroxysteroid dehydrogenase (20 α -HSD) expression. In cultured rat luteal cells, both prolactin and TGF- β suppressed 20 α -HSD activity (Matsuyama *et al.* 1990). Furthermore, the suppressive effect of prolactin was attenuated by a TGF- β antibody (Matsuyama *et al.* 1990), indicating that the luteotrophic action of prolactin is at least in part mediated by TGF- β . It has also been demonstrated that prolactin can stimulate TGF- β 2 expression in rat luteal macrophages (Matsuyama *et al.* 1990). Human granulosa-lutein cells treated with TGF- β 1 showed a significant reduction in apoptosis (Matsubara *et al.* 2000). Therefore, it appears that TGF- β isoforms in concert with prolactin have the potential to

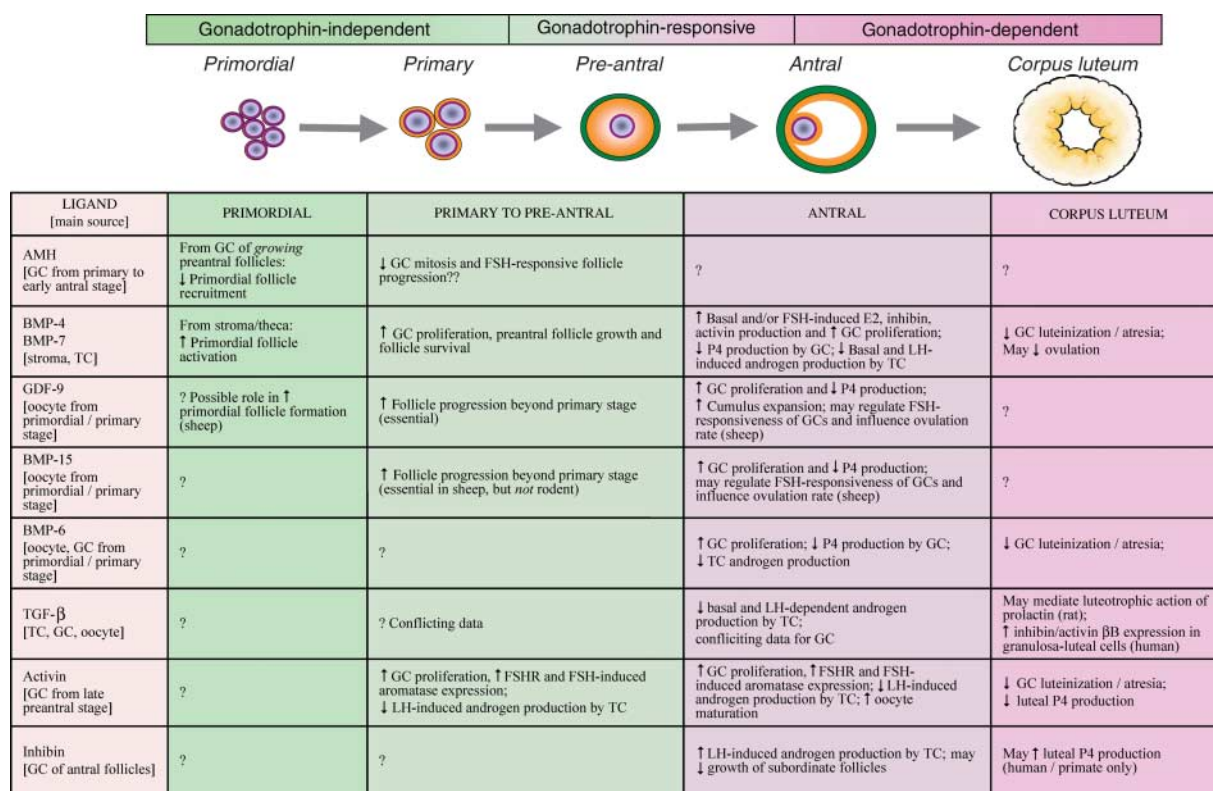


Figure 4 Summary of putative intrafollicular roles of TGF- β superfamily members at different stages of follicle development.

support the CL by enhancing progesterone production and suppressing apoptosis. TGF- β s may also regulate the inhibin–activin system within the CL. In human granulosa-lutein cells, TGF- β 1 and TGF- β 2 induce expression of the inhibin/activin β B-subunit, without affecting β A- or α -subunit expression (Eramaa & Ritvos 1996). Interestingly, the effect of TGF- β s was absent in the presence of hCG, suggesting that in human granulosa-lutein cells, gonadotrophins may have the ability to prevent β B-subunit expression. This would potentially explain the finding that β B-subunit expression, unlike α - and β A-subunit, is downregulated after ovulation in primates (Yamamoto *et al.* 1991, Roberts *et al.* 1993).

The process of luteinization is widely regarded to be under the control of oocyte-derived luteinization inhibitors; it is envisaged that these act to prevent luteinization and suppress progesterone synthesis until such a time that the oocyte is released at ovulation. Oocyte-derived BMP-6, BMP-15 and GDF-9, have the ability to act as inhibitors of luteinizing activity in cultured granulosa cells e.g. inhibit progesterone production, enhance oestradiol secretion, enhance granulosa cell proliferation (Shimasaki *et al.* 1999, Otsuka *et al.* 2001a,b, Glistler *et al.* 2004). It is highly probable that loss of these factors upon ovulation would have a significant effect on the remaining follicle cells and promote luteinization.

A recent study of the expression patterns of BMP ligands and receptors during folliculogenesis in the rat (Erickson & Shimasaki 2003) showed that follicular expression of BMP-2, BMP-3b, BMP-4, BMP-6, BMP-7 are profoundly reduced upon ovulation. Intriguingly, both BMP-2 and BMP-7 mRNAs are rapidly downregulated on ovulation and show little or no expression until luteolysis, whereupon both ligand and receptor are re-expressed. A link between BMP-7 and CL function is further reinforced by the observation that CL lifespan is extended in *BMP-7*-null mice (Yi *et al.* 2001). It has also been shown that in cultures of human granulosa-lutein cells, BMP-2 stimulates both the expression of the inhibin/activin β B-subunit and secretion of dimeric inhibin B (Jaatinen *et al.* 2002). These data suggest a possible mechanism whereby the selective expression of certain components of the BMP-system within the CL may act to regulate both luteinization and luteolysis.

Conclusions

Considerable progress has been made towards elucidating the complex intraovarian control mechanisms that, in concert with systemic signals, coordinate the recruitment and progression of primordial follicles through to ovulation and CL formation. As discussed above, and summarized in Fig. 4, many of the locally

produced signalling molecules implicated in this process belong to the extensive TGF- β superfamily. The accumulating evidence that different TGF- β superfamily ligands, receptors and binding proteins are selectively expressed in different ovarian compartments in a developmental stage-related manner has provided a rational basis on which to design functional/mechanistic studies. So far, these have mostly used *in vitro* approaches (e.g. cultured whole ovaries, ovarian cortical slices, isolated granulosa cells, theca cells, oocytes) to explore the actions of one or more ligands (or antagonists) on a range of functional endpoints (e.g. follicle progression, cell proliferation, atresia and steroid production). The application of RNA interference methodology to transiently silence expression of selected genes in ovary explants or cultured cells should prove to be of great value in dissecting these complex signalling pathways.

Despite the utility of *in vitro* techniques, there are obvious limitations to the 'reductionist' approach and a need to verify many of these *in vitro* findings using more physiological 'whole animal' models. In some cases, *in vivo* corroboration of *in vitro* findings has been forthcoming; for instance, the effects in rodents of intrabursal injection of exogenous ligands, the effects in mice of targeted deletion or overexpression of genes for particular ligands or receptors, the consequences of naturally occurring genetic mutations in sheep and the effects of active or passive immunization on ovarian function in sheep. It is anticipated that the generation of mice with conditional 'knockouts' or 'knockins' of selected genes (i.e. TGF- β ligands, receptors and binding proteins) will prove to be highly useful for assessing the importance of individual superfamily members in follicle development.

It is increasingly evident that considerable cross-talk exists between different intraovarian signalling systems; there also appears to be an element of built-in redundancy in that several locally produced ligands can elicit the same (or similar) biological response. Likewise, there is considerable promiscuity amongst the receptors through which TGF- β superfamily ligands signal and amongst the extracellular-binding proteins that serve to modulate the access of ligands to these receptors. Perhaps, this redundancy is not surprising given that ovarian folliculogenesis is essential for propagation of the species. The use of gene microarrays, serial analysis of gene expression (SAGE), proteome analyses and other emerging high-throughput technologies for monitoring simultaneously changes in expression of many genes/gene products, should facilitate rapid progress towards understanding how activation (or inactivation) of one intraovarian signalling system can modulate many other systems that contribute to the coordinated biological response.

Without doubt, the next decade will witness further leaps in our understanding of the roles of TGF- β superfamily members in ovarian function. It is

anticipated that this knowledge will lead to the development of new approaches for manipulating ovarian function and improving fertility in domesticated livestock, endangered species and man.

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