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Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality

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Oocyte quality is a key limiting factor in female fertility, yet we have a poor understanding of what constitutes oocyte quality or the mechanisms governing it. The ovarian follicular microenvironment and maternal signals, mediated primarily through granulosa cells (GCs) and cumulus cells (CCs), are responsible for nurturing oocyte growth, development and the gradual acquisition of oocyte developmental competence. However, oocyte–GC/CC communication is bidirectional with the oocyte secreting potent growth factors that act locally to direct the differentiation and function of CCs. Two important oocyte-secreted factors (OSFs) are growth-differentiation factor 9 and bone morphogenetic protein 15, which activate signaling pathways in CCs to regulate key genes and cellular processes required for CC differentiation and for CCs to maintain their distinctive phenotype. Hence, oocytes appear to tightly control their neighboring somatic cells, directing them to perform functions required for appropriate development of the oocyte. This oocyte–CC regulatory loop and the capacity of oocytes to regulate their own microenvironment by OSFs may constitute important components of oocyte quality. In support of this notion, it has recently been demonstrated that supplementing oocyte *in vitro* maturation (IVM) media with exogenous OSFs improves oocyte developmental potential, as evidenced by enhanced pre- and post-implantation embryo development. This new perspective on oocyte–CC interactions is improving our knowledge of the processes regulating oocyte quality, which is likely to have a number of applications, including improving the efficiency of clinical IVM and thereby providing new options for the treatment of infertility.

Key words: oocyte quality; oocyte-secreted factors; oocyte–cumulus cell communication; growth-differentiation factor 9; bone morphogenetic protein 15

Introduction

There is a growing awareness in the field of reproductive medicine that oocyte quality is a key limiting factor in female fertility. This is particularly evident from the steadily rising age to first conception for mothers, as it is well known that increasing maternal age has a negative impact on the ability of an oocyte to support early embryo development. Oocyte quality is reflected in an oocyte's intrinsic developmental potential. This refers to the biochemical and molecular state that allows a mature oocyte to be fertilized and develop to an embryo, which upon transfer will enable healthy development to term. In accordance with this, poor oocyte quality results in either polyspermy and/or arrested embryonic development or spontaneous abortion. Increasingly, it is also believed that developmental programming of embryos and fetuses by environmental factors (Fleming *et al*., 2004) is mediated by oocyte developmental potential (Maloney and Rees, 2005; Thompson, 2006). One of the great challenges that remain in the fields of reproductive biology and reproductive medicine is understanding the nature of the molecular and cellular processes that control oocyte quality.

Improving the outcomes of clinical oocyte in vitro maturation

A key practical reason to improve our understanding of the determinants of oocyte quality is to enhance the clinical implementation of oocyte *in vitro* maturation (IVM). IVM is a reproductive technology that enables oocytes to be matured *in vitro* from ovaries that have received either no or low levels of gonadotrophin stimulation (Edwards, 1965; Smitz *et al*., 2004). A small proportion of these mature oocytes have full developmental potential to term (Schroeder and Eppig, 1984). There is potentially a great demand for IVM in clinical practice, because of the reduced use of stimulatory hormones, and hence cheaper treatment with fewer risks of adverse side effects, and the additional patient groups that would gain access to artificial reproductive technologies (ART). Furthermore, IVM is an important ART as it has the potential to capture the vast supply

of oocytes within an ovary. In domestic animals, IVM success rates are relatively high and therefore are more widely accepted, especially as an important platform technology for artificial breeding, embryonic stem cell technologies, cloning and transgenic animal production. Success rates, however, are much lower in humans and so far this has restricted its widespread clinical implementation. The success of the technology in humans is slowly improving with time and further improvements in IVM efficiency has the potential to revolutionize ART technologies, whereby IVM together with natural-cycle IVF could become the first-line of treatment of human infertility (Chian *et al*., 2004; Edwards, 2007). However, with the first IVM pregnancy some 16 years ago (Cha *et al*., 1991), progress has been disappointingly slow. The key factor contributing to the lower pregnancy rates from IVM, compared with traditional IVF, is poor oocyte quality from IVM, which post-IVF manifests in low embryo developmental potential. Any new knowledge gained of factors regulating oocyte quality can be applied to improve the efficiency of clinical IVM and thereby provide new options for the treatment of infertility.

The follicular microenvironment determines oocyte developmental potential

Mammalian oocytes grow and develop in an intimate and mutually dependent relationship with adjacent somatic cells. The bulk of oocyte growth occurs in pre-antral follicles where the oocyte is closely associated with relatively undifferentiated granulosa cells (GCs). Upon follicular antrum formation, which approximately corresponds to the end of the oocyte growth phase, the GCs differentiate into two anatomically and functionally distinct lineages: the mural GCs (MGCs) that line the wall of the follicle and that have principally a steroidogenic role and the cumulus cells (CCs), which form an intimate association with the oocyte. CCs possess highly specialized trans-zonal cytoplasmic projections which penetrate through the zona pellucida and form gap junctions at their tips with the oocyte, forming an elaborate structure called the cumulus–oocyte complex (COC) (Albertini *et al*., 2001).

During the course of antral follicular development, the oocyte gradually and sequentially acquires meiotic and developmental competence (Eppig, 1992; Lonergan *et al*., 1994; Schramm and Bavister, 1995; Gilchrist *et al*., 1997). It is during this phase of oogenesis that the oocyte acquires the molecular and cytoplasmic machinery it requires to fully support embryo development (Brevini-Gandolfi and Gandolfi, 2001; Sirard *et al*., 2006), and as such this process has been termed 'oocyte capacitation' (Hyttel *et al*., 1997). In particular, it is well known that the CCs nurture the oocyte through the final phases of its development. However, we still have only a limited understanding of the nature and diversity of compounds that transfer between the CCs and the oocyte via gap junctions during antral development, and whether or not dynamic changes in gap-junctional communication or the extent of molecular transfer impacts on the acquisition of developmental competence (Albertini *et al*., 2001; Thomas *et al*., 2004). Furthermore, a new perspective is emerging, which will be the focus of this review, that the differentiation and critical functions of CCs is controlled by the oocyte itself and that this relationship in turn affects oocyte development. Hence, unraveling the intricate oocyte–somatic cell relationship is likely to generate new insights into the fundamental molecular communication events that determine oocyte quality.

Oocyte-secreted factors

It has been a long-held perception that the mammalian oocyte is passive in terms of its relationship with follicular somatic cells. However, in recent years a new paradigm has emerged in oocyte biology. It has recently become evident that the oocyte in fact is a central regulator of follicular cell function and thereby plays a critical role in the regulation of oogenesis, ovulation rate and fecundity (reviews; Eppig, 2001; Gilchrist *et al*., 2004a; McNatty *et al*., 2004; Gilchrist and Thompson, 2007). The oocyte achieves this by secreting soluble growth factors, oocyte-secreted factors (OSFs), which act on neighboring follicular cells to regulate a broad range of GC and CC functions. The pioneering studies by Nalbandov *et al*. showed premature luteinization of rabbit antral follicles *in vivo* after aspiration of the COC (el-Fouly *et al*., 1970). GCs cultured in close proximity to oocytes appeared to be less luteinized than those cultured without oocytes (Nekola and Nalbandov, 1971). From these studies, these authors were the first to propose the concept that the oocyte secretes factor(s) that act to prevent follicular luteinization. This concept was essentially ignored for the ensuing two decades, until four studies emerged in the same year, in 1990, all of which demonstrated the concept that oocytes have the capacity to regulate GC or CC function *in vitro* (Buccione *et al*., 1990; Salustri *et al*., 1990a,b; Vanderhyden *et al*., 1990). Many subsequent studies utilizing these 'OSF bioassays' (see 'Bioassays of native oocyte-secreted factors') went on to firmly establish the concept that there is a critical bidirectional communication axis between the mammalian oocyte and its somatic cells.

More recently, there has been considerable attention on specific oocyte-secreted molecules that form the basis of this communication axis. Two landmark studies demonstrated that absence of two oocyte-specific growth factors, growth-differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15), causes sterility (Dong *et al*., 1996; Galloway *et al*., 2000). There is currently a great deal of interest in GDF9 and BMP15 biology as these are newly discovered members of the transforming growth factor β (TGFβ) superfamily, and apart from being required for early folliculogenesis, these molecules are central regulators of GC/CC differentiation, are potential contraceptive targets and may be associated with the pathogenesis of ovarian dysfunction (Gilchrist *et al*., 2004a; Shimasaki *et al*., 2004; Juengel and McNatty, 2005; McNatty *et al*., 2007). However, as GDF9 and BMP15 are newly discovered molecules, much of their cellular biology remains poorly understood. Moreover, even less is known about the interaction of these molecules with each other, with other lesser known OSFs and with traditional hormonal regulators of folliculogenesis.

The objectives of this review are to examine in detail the follicular context of oocyte paracrine signaling to, and regulation of, follicular somatic cells, from the perspective that an intricate oocyte–somatic cell interaction is required for appropriate programming of the oocyte to support early development. In this sense, this review will focus in particular on the regulation of CC differentiation by OSFs in cooperation with endocrine/local

hormones, and how this impacts on oocyte developmental potential.

Experimental models for studying OSFs

A number of differing experimental models have been employed to study OSFs, and each model has its inherent strengths and limitations (Table I). Studies examining animals that are genetically deficient in GDF9 and/or BMP15 have provided us with critical insights into the central role of these OSF molecules. Female mice and sheep deficient in GDF9 (Dong *et al*., 1996; Hanrahan *et al*., 2004) or sheep deficient in BMP15 (Galloway *et al*., 2000) are sterile due to a complete block in folliculogenesis at the primary stage of folliculogenesis, demonstrating the absolute requirement for oocyte expression of these molecules. However, because the primary lesion in ovarian function in these animals is the block in folliculogenesis, these experimental models afford us limited insight into the roles of GDF9/BMP15 during the crucial later stages of oocyte development when oocyte capacitation occurs. Hence, the development of conditional knock-outs should prove a powerful approach. On the other hand, *in vivo* immunization experiments against GDF9/BMP15 is a powerful approach yielding important new information on the role of these oocyte factors *in vivo* in regulating folliculogenesis and ovulation rate (Juengel *et al*., 2002, 2004; McNatty *et al*., 2007).

An alternative approach is *in vitro* experiments treating ovarian cells with recombinant OSFs, such as GDF9, BMP15, etc (Table I). Although this approach has yielded significant new knowledge, so far it has also been fraught with deficiencies in experimental tools. As GDF9 and BMP15 are newly discovered, still today, the field is suffering from a widespread lack of commercial grade, purified recombinant growth factors, reliable antibodies and immunoassays. Variation between laboratories in 'in-house' preparations of GDF9 and BMP15 and the lack of suitable experimental controls and standards is generating inconsistencies between laboratories. Furthermore, the recombinant GDF9 and BMP15 preparations currently in use are thought to be mature proteins separated from the proregions and these may not represent the native forms of GDF9 and BMP15 secreted by oocytes (see 'Paracrine signaling by native OSFs'). Furthermore, unlike the TGFβ superfamily in general, *in vitro* artifacts may be generated when non-homologous recombinant GDF9 and BMP15 preparations are used, e.g. sheep GC progesterone production is inhibited by recombinant ovine GDF9, but enhanced by murine GDF9 (McNatty *et al*., 2005b). An alternative or supplementary *in vitro* experimental approach to using the putative OSFs in recombinant form is to use an OSF bioassay, as outlined below.

Bioassays of native OSFs

The basic principal of an OSF bioassay is primary cultures of ovarian GCs are co-cultured with denuded oocytes (DOs), and then the responses of those GCs are compared with those cultured without DOs (Fig. 1). Presence of the DOs in co-culture dramatically alters the function of MGCs and CCs *in vitro* (Table II), and because the two cell types are generally not in physical contact with each other, this demonstrates that the effect is mediated by soluble factors (OSFs) secreted into the medium by the DOs. There are a number of further proofs of the soluble, paracrine nature of OSFs. First, culture medium can be conditioned by DOs (5–24 h), and subsequently that oocyte-conditioned medium (OCM) added to GCs to elicit a biological response (Fig. 1A) (Buccione *et al*., 1990; Salustri *et al*., 1990b). Secondly, in co-culture, DOs operate in a concentration-dependent manner: responses of

Table I. Experimental models to study candidate OSFs.

Experimental model	Model species or system	Selected references
Genetic models	Mouse	Dong *et al*. (1996), Elvin *et al*. (1999b), Yan *et al*. (2001), Yi *et al*. (2001) and Su *et al*. (2004)
	Sheep	Davis *et al*. (1992), Galloway *et al*. (2000), Mulsant *et al*. (2001), Souza *et al*. (2001), Wilson *et al*. (2001) and Hanrahan *et al*. (2004)
	Human	Di Pasquale *et al*. (2004), Montgomery *et al*. (2004), Dixit *et al*. (2006), Laissue *et al*. (2006) and Palmer *et al*. (2006)
Immunization models	Sheep	Juengel *et al*. (2002), Juengel *et al*. (2004), and McNatty *et al*. (2007)
Bioassays of native OSFs[a]	A: GC+OCM	Buccione *et al*. (1990), Salustri *et al*. (1990b), Vanderhyden *et al*. (1990, 1992), Canipari *et al*. (1995) and Coskun *et al*. (1995)
	B: GC+DO	Salustri *et al*. (1990a), Eppig *et al*. (1997), Lanuza *et al*. (1999), Li *et al*. (2000), Gilchrist *et al*. (2001, 2003, 2004b, 2006), Hickey *et al*. (2005) and Diaz *et al*. (2006, 2007b)
	C: OOX+DO	Buccione *et al*. (1990), Salustri *et al*. (1990b), Vanderhyden *et al*. (1993), Coskun *et al*. (1995), Vanderhyden and Macdonald (1998), Li *et al*. (2000), Dragovic *et al*. (2005), Hussein *et al*. (2005), Sugiura *et al*. (2005), Diaz *et al*. (2006, 2007b) and Dragovic *et al*. (2007)
	D: COC+DO	Hussein *et al*. (2006)
Bioassays using candidate recombinant OSFs	TGFβ	Salustri *et al*. (1990a), Gilchrist *et al*. (2003, 2006) Vanderhyden *et al*. (2003) and Dragovic *et al*. (2005)
	GDF9	Elvin *et al*. (1999a), Hayashi *et al*. (1999), Vitt *et al*. (2000a,b), Hreinsson *et al*. (2002), Yamamoto *et al*. (2002), Kaivo-Oja *et al*. (2003), Gilchrist *et al*. (2004b, 2006), Dragovic *et al*. (2005), Hickey *et al*. (2005), Hussein *et al*. (2006), Dragovic *et al*. (2007) and Yeo *et al*. (2007)
	BMP15	Otsuka *et al*. (2000, 2001c), Otsuka and Shimasaki (2002a), Moore *et al*. (2003), Hussein *et al*. (2005, 2006), McNatty *et al*. (2005a,b), Yoshino *et al*. (2006) and Sugiura *et al*. (2007)
	BMP6	Otsuka *et al*. (2001b), Glister *et al*. (2004), Hussein *et al*. (2005) and Gilchrist *et al*. (2006)

[a]See Fig. 1 for illustration of models A–D and see Table II for full listing of references.
DO, denuded oocyte; OCM, oocyte conditioned medium; GC, granulosa cell; OOX, oocytectomized complexes; CC, cumulus cells.

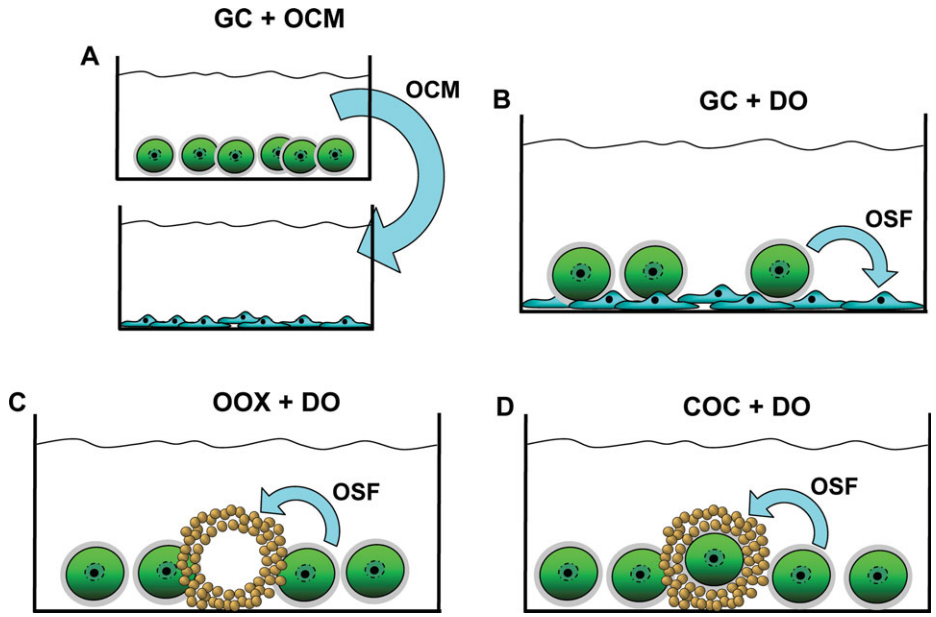

Figure 1: Bioassays of native OSFs
Illustrated are the differing experimental models that have been developed for studying native OSFs. In all models, the source of OSFs is DOs, generated by mechanically removing CCs from COCs, which are then cultured at high density (~1 DO/μl). Target somatic cells can be exposed to OSFs, either by treating them with medium conditioned by DOs (**A**) or by co-culturing with DOs (**B** and **C**). Because most OSFs appear to be quite labile, using OCM is a less efficient method. The DOs used to produce the OSFs can be in varying states; e.g. growing, fully grown, meiotically arrested, meiotically maturing oocytes, etc. Somatic cells typically examined include pre-antral GCs, MGCs or CCs cultured as isolated or clumped attached cells (**A** or **B**), or non-attached CCs either as OOXs (C) or intact COCs (**D**). GC, granulosa cell; COC, cumulus–oocyte complex; OOX, oocytectomized complex; DO, denuded oocyte; OSF, oocyte-secreted factor; OCM, oocyte-conditioned medium; CC, cumulus cell; MGC, mural granulosa cell

GCs to OSFs can be increased by increasing the concentration of DOs (Fig. 2) (Lanuza *et al*., 1998; Hussein *et al*., 2005; Gilchrist *et al*., 2006). As the typical operating range of concentration of DOs is from 0.5 to 4 DOs/μl, these experiments are normally performed in micro-drops and still require many hundreds of oocytes per experiment. Figure 1 illustrates the main types of OSF bioassays in use, where the target GCs are in three different states, either as: a mono-layer or clumps usually of pre-antral or MGCs (Fig. 1B), oocytectomized complexes (OOX) of CCs where the oocyte has been microsurgically removed (Fig. 1C), or as intact COCs (Fig. 1D) (see figure caption for description).

These OSF bioassays have the disadvantage that, generally [with the exception of COC+DO (Fig. 1D)], the intricate physical association between oocytes and CCs through trans-zonal projections and gap junctions is lost (see 'Oocyte–CC physical interaction'). However, OSF bioassays have the distinct advantage over treating GCs with putative recombinant growth factors, such as GDF9 or BMP15, as in the OSF bioassay, the GCs/CCs are exposed simultaneously to the multitude of molecules secreted by the oocyte and the oocyte-secreted growth factors may be in forms more closely resembling those actually secreted by oocytes *in vivo*. Hence, for the purposes of this review, such OSFs will be referred to as 'native OSFs' to distinguish these from recombinant OSFs (Table I).

Oocyte regulation of GC and CC function

Bioassays of native OSFs have proved an extremely valuable and powerful experimental approach and have provided significant new insights into oocyte–somatic cell communication. The following section examines in detail the fundamental aspects of GC and CC function regulated by OSFs, which are summarized in Table II.

Regulation of GC kit ligand

Kit ligand is produced by pre-antral GCs and promotes oocyte growth through the Kit receptor located on the oolemma (Packer *et al*., 1994). Co-culturing growing oocytes with pre-antral GCs stimulates GC expression of *Kitl*, whereas fully grown oocytes suppress expression (Joyce *et al*., 2000). Although *Kitl* expression by rodent GCs *in vitro* has been shown to be inhibited by GDF9 and stimulated by BMP15 (Joyce *et al*., 2000; Otsuka and Shimasaki, 2002a), the complexity of regulation of oocyte growth is compounded by differential regulation and activities of two Kit ligand isoforms and by the fact that FSH regulates *Bmp15* expression via Kit signaling (Thomas *et al*., 2005). These studies demonstrate the multi-faceted complexity of oocyte–somatic cell regulatory loops (Shimasaki *et al*., 2004; Thomas and Vanderhyden, 2006).

Stimulation of GC/CC proliferation

Oocytes are potent stimulators of MGC and CC DNA synthesis and cellular proliferation. This has been determined using a number of different experimental approaches *in vitro*, including up-regulation of *Ccnd2*, the transcript encoding cyclin D2, stimulation of [^{3}H] thymidine uptake as a measure of DNA synthesis, increases in total DNA content from GC cultures and increased numbers of GCs (Vanderhyden *et al*., 1992; Lanuza *et al*., 1998; Li *et al*., 2000; Gilchrist *et al*., 2001, 2003, 2004b, 2006;

Table II. Catalog of the GC and/or CC genes or functions regulated by native OSFs.

Effect of native OSFs on GC, CC or oocyte function	References[a]
Signaling cascades	
Activation of GC/CC SMAD signaling	Gilchrist *et al*. (2006)
Activation of MAPK signaling	Su *et al*. (2003)
Oocyte growth	
Stimulation/suppression *KitL*	Joyce *et al*. (1999)
CC/MGC Proliferation	
Stimulation of *Ccnd2*	Gilchrist *et al*. (2006)
Stimulation of GC/CC DNA synthesis, cell number or follicle growth	Vanderhyden *et al*. (1992), Lanuza *et al*. (1998), Li *et al*. (2000), Gilchrist *et al*. (2001, 2003, 2004b, 2006), Eppig *et al*. (2002), Brankin *et al*. (2003), Glister *et al*. (2003) and Hickey *et al*. (2005)
Interaction of OSFs with IGF-I	Lanuza *et al*. (1998), Li *et al*. (2000), Brankin *et al*. (2003), Gilchrist *et al*. (2003) and Hickey *et al*. (2005)
Stimulation of CC *Ar*	Diaz *et al*. (2007b)
CC apoptosis	
Prevention of CC apoptosis	Hussein *et al*. (2005)
CC/MGC luteinization	
Regulation of MGC/CC progesterone or estradiol production	Nekola and Nalbandov (1971), Vanderhyden *et al*. (1993), Coskun *et al*. (1995), Vanderhyden and Tonary (1995), Vanderhyden and Macdonald (1998), Lanuza *et al*. (1999), Li *et al*. (2000) and Glister *et al*. (2003)
Suppression of CC *Cyp11a1*	Diaz *et al*. (2007b)
Suppression of FSH-induced *Lhcgr*	Eppig *et al*. (1997)
Regulation of MGC inhibin–follistatin–activin production	Lanuza *et al*. (1999) and Glister *et al*. (2003)
Stimulation of CC *Amh*	Salmon *et al*. (2004) and Diaz *et al*. (2007b)
Suppression of CC *Cd44*	Diaz *et al*. (2007b)
CC metabolism	
Stimulation of CC glycolysis	Sutton *et al*. (2003) and Sugiura *et al*. (2005)
Stimulation of CC AA transport	Eppig *et al*. (2005)
CC expansion	
Enabling FSH/EGF-stimulated CC expansion (CEEF–murine)	Buccione *et al*. (1990), Salustri *et al*. (1990b), Vanderhyden *et al*. (1990), Dragovic *et al*. (2005, 2007)
Production of CEEF (non-murine)	Prochazka *et al*. (1991), Singh *et al*. (1993), Vanderhyden (1993), Ralph *et al*. (1995) and Prochazka *et al*. (1998)
Enabling FSH/EGF-induction of *Has2, Ptgs2, Ptx3, Tnfaip6* and secretion of hyaluronic acid	Salustri *et al*. (1990a,b), Joyce *et al*. (2001), Dragovic *et al*. (2005, 2007) and Diaz *et al*. (2006)
Regulation of plasminogen activator	Canipari *et al*. (1995) and D'Alessandris *et al*. (2001)
Oocyte quality	
IVM additive increasing blastocyst development	Hussein *et al*. (2006) and Yeo *et al*. (2007)
IVM additive increasing fetal survival	Yeo *et al*. (2007)

[a]Studies using native OSFs.
GC, granulosa cell; CC, cumulus cell; MAPK, mitogen-activated protein kinase, OSF, oocyte-secreted factors; MGC, mural granulosa cell; AA, amino acid; CEEF, cumulus-expansion enabling factors; EGF, epidermal growth factor; IVM, *in vitro* maturation.

Brankin *et al*., 2003; Glister *et al*., 2003; Hickey *et al*., 2005). Murine (Gilchrist *et al*., 2006), porcine (Hickey *et al*., 2005) and bovine oocytes (Gilchrist *et al*., 2003) all promote DNA synthesis in a dose-dependent manner when co-cultured with their homologous MGCs, providing a simple and robust bioassay of oocyte mitogens (Fig. 2A; Gilchrist *et al*., 2006). Furthermore, the oocyte-derived mitogens interact with well-known GC mitogens in a species-dependent manner. In the absence of other growth factors or steroids, murine oocytes are potent stimulators of GC proliferation (Fig. 2A) and this activity is not enhanced by IGF-I (Gilchrist *et al*., 2001). In contrast, bovine and porcine oocytes display low growth-promoting activity in their own right, but interact substantially with IGF-I (but not FSH) to become potent CC/MGC growth-promoters (Lanuza *et al*., 1998; Li *et al*., 2000; Brankin *et al*., 2003; Gilchrist *et al*., 2003; Hickey *et al*., 2005). In the case of the pig follicle at least, this interaction is further enhanced by androgens acting directly through the androgen receptor, suggesting that there is some type of interaction between androgen and OSF signaling and that the growth-promoting effects of androgens on follicles require oocyte participation (Hickey *et al*., 2005). These *in vitro* demonstrations of oocyte-secreted mitogens are substantiated by an elegant experiment, where Eppig *et al*. (2002) re-aggregated *in vitro* mouse GC complexes with oocytes at various stages of development, which were subsequently transplanted *in vivo*, and demonstrated that the rate of folliculogenesis is dictated by the oocyte.

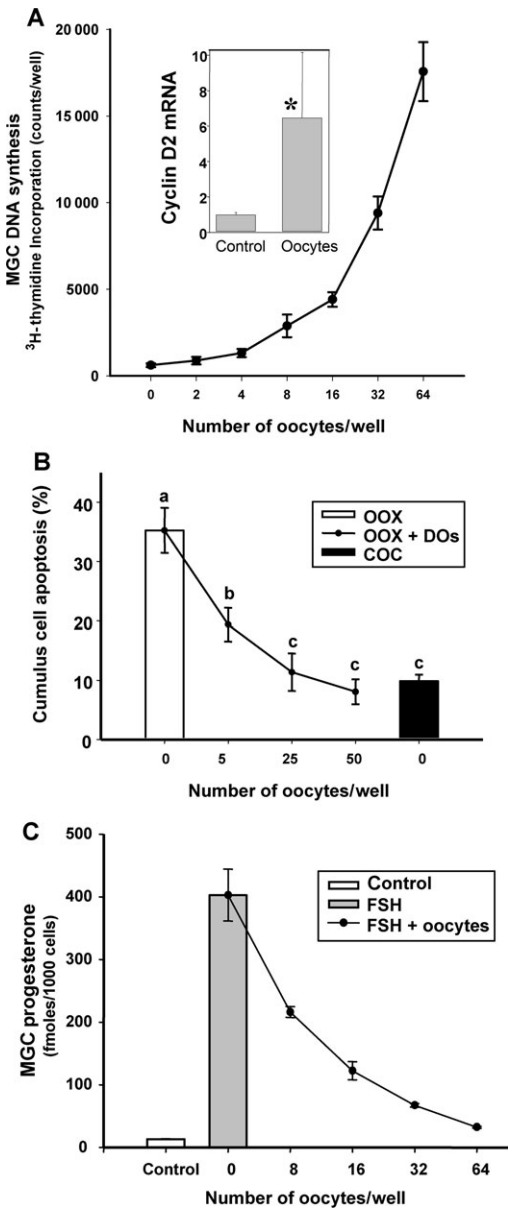

Figure 2: Oocyte regulation of GC/CC functions
(A) Growth-promoting activity of OSFs. Co-culturing mouse MGCs with increasing numbers of DOs per well leads to a dose-dependent increase in MGC [^{3}H]-thymidine incorporation, which is used as a marker of MGC DNA synthesis. Inset: DOs co-cultured with MGCs induces expression of *Ccnd2* mRNA, the transcript encoding the cell cycle protein cyclin D2. Data modified from Gilchrist *et al*. (2006). **(B)** Anti-apoptotic activity of OSFs. An intact bovine COC matured for 24 h in the absence of FSH and serum has a low proportion of apoptotic CCs (~10%). CCs cultured for 24 h in the absence of the oocyte (OOX) generated by microsurgical removal of the oocyte from the COC exhibit a substantially higher proportion of apoptosis (~35%). Exposing CCs to OSFs by co-culturing OOXs with DOs dose-dependently decreases the incidence of CC apoptosis. These results demonstrate that the low incidence of apoptosis within the COC is maintained by the oocyte secreting potent, locally acting, anti-apoptotic factors. Figure modified from Hussein *et al.* (2005). **(C)** OSFs inhibit FSH-induced luteinization. In the absence of the oocyte, FSH stimulates mouse MGC and CC progesterone production *in vitro*, which in turn is inhibited by co-culture with DOs in a dose-dependent fashion. A critical function of OSFs is to counter the luteinizing effects of FSH (Gilchrist RG and Ritter LJ, unpublished data). MGC, mural granulosa cell; CC, cumulus cell; DO, denuded oocyte; COC, cumulus–oocyte complex; OOX, oocytectomized complex

Prevention of CC apoptosis

While promoting growth, oocytes also actively prevent CC death. We have recently shown that microsurgical removal of the oocyte from a COC to generate an OOX increases CC apoptosis, and that this is reversed by exposing OOXs to OSFs (Hussein *et al*., 2005). DOs co-cultured with CCs induce a dose-dependent suppression of CC apoptosis, in unexpanded as well as FSH-stimulated expanding complexes (Hussein *et al*., 2005) (Fig. 2B). Oocytes achieve this in part by promoting the expression of anti-apoptotic Bcl-2 proteins and suppressing pro-apoptotic Bax proteins in CCs. The anti-apoptotic effect of OSFs is so potent that they are able to counter the effects of an external apoptotic insult. These oocyte effects are acutely localized, such that the corona radiata (CCs immediately surrounding the oocyte) have a lower incidence of apoptosis compared with the CCs on the outer side of the COC, which in turn have a lower incidence than the MGCs on the other side of the follicle (Hussein *et al*., 2005). This may account for the fact that the COC is the last compartment of the ovarian follicle to be affected by advanced atresia.

Inhibition of CC luteinization

In 1970, it was first proposed (el-Fouly *et al*., 1970) that the oocyte has the capacity to prevent follicular luteinization, and this hypothesis is now widely accepted. The hallmark of GC luteinization is steroidogenic production (progesterone in particular) and COCs produce very low levels of progesterone relative to their MGC counterparts from the same follicle (Li *et al*., 2000). Microsurgical removal of the oocyte from the COC leads to dramatic luteinization of the CCs in OOXs, as evidenced by dramatic FSH-induced increases in transcripts encoding the LH receptor, *Lhcgr* (Eppig *et al*., 1997), the steroidogenic enzyme P450 side chain cleavage, *Cyp11a1* (Diaz *et al*., 2007b), leading to increased CC progesterone secretion from murine, porcine and bovine OOXs (Vanderhyden *et al*., 1993; Coskun *et al*., 1995; Li *et al*., 2000). All these markers of luteinization are, however, restored to COC levels when OOXs are co-cultured with DOs, demonstrating that oocytes prevent luteinization of their CCs via the actions of soluble OSFs. The capacity of OSFs to prevent follicular luteinization is also clearly demonstrated by the dose-dependent suppression of FSH-stimulated progesterone production by MGCs (Fig. 2C). In the mouse, OSFs also stimulate CC estradiol production by a mechanism which is proposed to be independent from the suppression of progesterone production (Vanderhyden *et al*., 1993; Vanderhyden and Tonary, 1995; Vanderhyden and Macdonald, 1998). In contrast, FSH-stimulated estradiol production by bovine MGCs is suppressed by exposure to OSFs (Glister *et al*., 2003).

Oocytes also regulate a number of other CC/GC functions that can be broadly categorized as inhibiting luteinization, but their exact role in the regulation of COC function is not yet clear. The immune marker, *Cd34*, is expressed in high levels in MGCs but expression is suppressed in COCs by OSFs (Diaz *et al*., 2007b). Conversely, OSFs promote CC expression of *Amh*, the transcript encoding anti-Müllerian hormone (Salmon *et al*., 2004; Diaz *et al*., 2007b). Interestingly, oocytes also play a role in the regulation of the GC inhibin–follistatin–activin system. Co-culture of DOs with MGCs has been shown to increase inhibin B production (Lanuza *et al*., 1999) and to antagonize

FSH- and IGF-induced MGC production of inhibin A, activin A and follistatin (Glister *et al*., 2003). Interestingly, MGC production of inhibin-related peptides is also potently regulated by recombinant GDF9 and BMP15 (Otsuka *et al*., 2001c; McNatty *et al*., 2005a) and BMP15 bioactivity is antagonized by follistatin (Otsuka *et al*., 2001a; Hussein *et al*., 2005). Although the effects of native or recombinant OSFs on the production of inhibin-related peptides have not yet been examined in CCs, these findings hint at a complex local regulatory network between OSFs and the inhibin–follistatin–activin system that may have implications for the extracellular regulation of OSF bioactivity and/or the broader control of follicle selection/growth.

Regulation of CC metabolism

The cellular compartments of the COC have remarkably different metabolic activities and requirements (review; Thompson *et al*., 2007). At least in large antral follicles, the fully grown oocyte is totally dependent on oxidative phosphorylation for ATP production and has an inability to oxidize glucose (Biggers *et al*., 1967; Rieger and Loskutoff, 1994; Cetica *et al*., 2002). Whether this is a characteristic of all oocytes throughout follicle development is not known. It was initially believed that oocytes in preantral follicles probably existed in a severely hypoxic, even anoxic, microenvironment (Gosden and Byatt-Smith, 1986), but this has recently been refuted with the more likely explanation that as the follicle grows, a gradient of oxygen develops from the theca to the oocyte and that formation of the follicular antrum is associated with prevention of hypoxic conditions (Hirshfield, 1991). In contrast, CCs have a significant ability to uptake and utilize glucose, via aerobic glycolysis (Gardner *et al*., 1996; Sutton *et al*., 2003; Sutton-McDowall *et al*., 2004). The metabolism of glucose within CCs to provide carboxylic acids as substrates for oxidative phosphorylation within the oocyte has been a long-held and entirely appropriate view, as it fits well with known data (Biggers *et al*., 1967; Sutton *et al*., 2003). As a consequence, little oxygen is utilized by the CCs themselves. Indeed, Clarke *et al*. (2006) using mathematical modeling have demonstrated that in large antral follicles, the O_2 partial pressure at the surface of the oocyte is only slightly lower than in follicular fluid.

This contrast in metabolic requirements between oocytes and CCs suggests that the metabolic preference between these two cell types may be a regulated phenomenon. Suguira *et al*. (2005) observed that several glycolytic enzymes were up-regulated in mouse CCs of large antral follicles compared with corresponding MGCs, which they confirmed by *in situ* hybridization. Furthermore, they showed that oocytectomy decreased cumulus glycolytic enzyme mRNA levels and glycolytic activity, which was restored upon treatment with OSFs by co-culturing OOXs with fully grown oocytes. In contrast, activity was not restored with growing oocytes from secondary follicles (Sugiura *et al*., 2005). In another study, the same laboratory demonstrated a similar phenomenon for a sodium-coupled neutral amino acid transporter, SLC38A3 (Eppig *et al*., 2005). As with the glycolytic enzymes, mRNA levels and transporter activity were up-regulated in CCs compared with MGCs, and OSFs were responsible for this up-regulation. These studies illustrate the intimate relationship between the oocyte and the CCs, whereby the oocyte directs its somatic cells to supply it with metabolites for its own development that it is unable to generate itself. However, this phenomenon may be restricted to certain species, as Sutton *et al*. (2003) were unable to detect metabolic differences in CC metabolism between intact bovine COCs, OOXs and OOXs treated with OSFs.

Promotion of CC mucification and expansion

Initiation of cumulus expansion is dependent upon two signaling events: (i) stimulation by gonadotrophins or epidermal growth factor (EGF)-like peptides and (ii) paracrine signals secreted by the oocyte termed the cumulus-expansion enabling factors (CEEFs), which act on CCs, enabling them to respond to the gonadotrophin/EGF signal to synthesize extracellular matrix (ECM) molecules. Hyaluronan makes up the major structural backbone of the cumulus ECM and is synthesized by the enzyme hyaluronan synthase 2 (HAS2). Other important components of the cumulus matrix include the cross-linking proteins tumor necrosis factor alpha-induced protein 6 (TNFAIP6) and pentraxin 3 (PTX3), and the proteoglycan versican (Russell and Salustri, 2006). Mucification and expansion of the COC in response to the LH surge is absolutely required for ovulation and hence for fertility, as failure to synthesize components of the cumulus matrix leads to reduced fertility or sterility (reviewed; Russell and Robker, 2007).

In the mouse, the process of cumulus expansion is critically dependent upon the oocyte secreting the soluble CEEFs. Ablation of CEEFs, either by physically removing the oocyte (Buccione *et al*., 1990; Salustri *et al*., 1990b; Vanderhyden *et al*., 1990) or by using inhibitors of oocyte signaling (see 'Paracrine signaling by native OSFs'; Diaz *et al*., 2007b; Dragovic *et al*., 2007), eliminates FSH- or EGF-induced CC expansion. Cumulus expansion can be fully restored in FSH-stimulated CCs or OOX complexes by co-culturing with DOs, demonstrating the secretion of and requirement for CEEFs for cumulus expansion (Buccione *et al*., 1990; Salustri *et al*., 1990b; Vanderhyden *et al*., 1990; Dragovic *et al*., 2005). Furthermore, the CEEFs are required for FSH- or EGF-induced expression of transcripts required for each of the major ECM components; *Has2, Tnfaip6* and *Ptx3* (Dragovic *et al*., 2005, 2007; Diaz *et al*., 2006). Prostaglandin synthesis is also required for cumulus expansion (Davis *et al*., 1999) and OSFs are required to enable expression of *Ptgs2* (Joyce *et al*., 2001; Diaz *et al*., 2006; Dragovic *et al*., 2007), which encodes the rate-limiting enzyme prostaglandin-endoperoxide synthase 2, otherwise known as cyclooxygenase-2. Once the cumulus ECM is formed, OSFs may also contribute to matrix stability for a short period by preventing the actions of proteases. FSH induces MGC protease activity; however, OSFs appear to counter these actions of FSH in COCs by inhibiting FSH-induced plasminogen activator activity (Canipari *et al*., 1995). The regulation of cumulus expansion by the paracrine actions of oocytes can be imitated *in vitro* by a number of growth factors, including TGFβ1 (Salustri *et al*., 1990a; Vanderhyden *et al*., 2003), GDF9 (Elvin *et al*., 1999a; Dragovic *et al*., 2005), BMP15 (Yoshino *et al*., 2006) and activins (Dragovic *et al*., 2007), and presumably some combination of these growth factors make up the CEEFs (see 'Paracrine signaling by native OSFs').

Considerable attention has been paid to the mouse oocyte-secreted CEEF in recent years, and hence it is noteworthy that COCs from all other species examined to date (rat, cow and

pig), readily undergo FSH-stimulated expansion in the absence of the oocyte (Prochazka *et al*., 1991; Singh *et al*., 1993; Vanderhyden, 1993; Ralph *et al*., 1995; Prochazka *et al*., 1998). Hence, the absolute requirement for the CEEF for cumulus expansion to proceed may be restricted to the mouse. Even though these species do not require the CEEF, interestingly, their oocytes produce the CEEF, as these oocytes are capable of enabling FSH-induced expansion of mouse OOXs. The regulation of cumulus expansion in non-human primates and women is relatively poorly understood and it is still unknown if human cumulus expansion requires paracrine signals from the oocyte.

The molecular basis of oocyte paracrine signaling

As listed in Table II, there is now a body of evidence illustrating which GC/CC functions are regulated by OSFs. However, the molecular mechanisms underpinning the oocyte-to-CC paracrine communication axis are far less clear. Specific OSFs have recently been identified and at least some of their signaling pathways in GCs and CCs characterized. To date, the focus has been almost entirely on members of the TGFβ superfamily as constituting the key OSFs (reviews; Vanderhyden *et al*., 2003; Gilchrist *et al*., 2004a). From the TGFβ superfamily, oocytes appear in general to express TGFβ1, TGFβ2, activins, GDF9, BMP15 and BMP6, although not in all species and there may be notable species differences (Juengel and McNatty, 2005). However to date scant attention has been paid to candidate OSF molecules from outside the TGFβ superfamily. A recent study showing for the first time a critical interaction between BMP15 and oocyte-secreted fibroblast growth factor (FGF) 8B illustrates this point (Sugiura *et al*., 2007), and highlights our current rudimentary understanding of the molecular mechanisms regulating oocyte–somatic cell signaling.

Growth-differentiation factor 9 and bone morphogenetic protein 15

GDF9 and BMP15 (also known as GDF9b) are two closely related members of the TGFβ superfamily, which are expressed and translated in oocytes as preproproteins, consisting of a signal peptide, a large proregion and a mature region (Shimasaki *et al*., 2004). Members of the TGFβ superfamily invariably function as homodimers of the mature regions, and presumably this is also the case for GDF9 and BMP15. There are a number of features of these oocyte growth factors that are particularly noteworthy in terms of oocyte–somatic cell interactions.

a) First, oocyte expression of GDF9 and BMP15 are required for female fertility as homozygous carriers of mutations in either *Gdf9* (Dong *et al*., 1996; Hanrahan *et al*., 2004) or *Bmp15* (Braw-Tal *et al*., 1993; Galloway *et al*., 2000) are sterile due to a block at the primary stage of folliculogenesis (Table I). There are notable species variations in the requirement for these oocyte factors: in sheep (and perhaps in mono-ovular species in general; McNatty *et al*., 2003; Moore *et al*., 2004), both GDF9 and BMP15 are required for folliculogenesis (Galloway *et al*., 2000; Hanrahan *et al*., 2004), whereas BMP15 is not essential in the mouse as *Bmp15* null mice are fertile (Yan *et al*., 2001). Moreover, *Gdf9* or *Bmp15* heterozygosity lead to increased fertility in sheep (Montgomery *et al*., 2001; Hanrahan *et al*., 2004), but have no overt effect on murine fertility (Dong *et al*., 1996; Yan *et al*., 2001). Data are emerging, illustrating that GDF9 and BMP15 also play an important role in the regulation of human fertility, including aberrant expression of GDF9 may be associated with polycystic ovarian syndrome (Teixeira Filho *et al*., 2002), rare mutations in *Gdf9* and *Bmp15* contribute to premature ovarian failure (Di Pasquale *et al*., 2004; Dixit *et al*., 2006; Laissue *et al*., 2006), as well as mutations in *Gdf9* are associated with dizygotic twinning (Montgomery *et al*., 2004; Palmer *et al*., 2006) (Table I).
b) Secondly, GDF9 and BMP15 are widely thought of as oocyte-specific growth factors—oocytes certainly express exceptionally high levels of mRNA and protein throughout most of folliculogenesis and in many species ovarian GDF9 and BMP15 expression is restricted exclusively to oocytes (Juengel and McNatty, 2005). There are, however, a number of exceptions to this, most notable GDF9 and BMP15 are expressed in high levels in testes (Fitzpatrick *et al*., 1998; Aaltonen *et al*., 1999). Low-level expression of GDF9 and/or BMP15 mRNA and possibly protein has been reported in MGCs and CCs in a number of species (Sidis *et al*., 1998; Prochazka *et al*., 2004; Silva *et al*., 2005), as well as GDF9 and BMP15 mRNA expression in the pituitary (Fitzpatrick *et al*., 1998; Otsuka and Shimasaki, 2002b). Non-ovarian expression of GDF9 and BMP15 is highly variable between species and so far a physiological role has not been described.
c) The third distinguishing feature of GDF9 and BMP15 is that both these molecules lack the fourth cysteine residue, that is otherwise common throughout the TGFβ superfamily, that is required for intersubunit disulfide bridge formation (McPherron and Lee, 1993; Laitinen *et al*., 1998). Hence, most unusually for the superfamily, GDF9 and BMP15 form homodimers that are linked non-covalently, and using *in vitro* or modeling systems, GDF9 and BMP15 appear to be capable of forming a GDF9/BMP15 heterodimer (Liao *et al*., 2003; McNatty *et al*., 2004). Although it is unclear if a GDF9/BMP15 heterodimer forms *in vivo*, the proteins are co-located and at times probably co-secreted, and furthermore synergize substantially to regulate certain GC functions (McNatty *et al*., 2005a,b), and hence the biological actions of these two growth factors should be considered in unison.
d) Finally, from a local ovarian perspective, GDF9 and BMP15 are significant because when added to GCs or CCs *in vitro*, these growth factors can mimic nearly all the demonstrated actions of oocytes on GC/CC functions as outlined in 'Oocyte regulation of GC and CC function' (Table II), and so GDF9 and BMP15 are often equated with native OSF bioactivity, although this is undoubtedly an over-simplistic view (see 'Paracrine signaling by native OSFs').

GDF9 and BMP15 signaling

GDF9 and BMP15 have recently been shown to signal through known TGFβ superfamily receptors to activate the SMAD intracellular cascade (see reviews; Shimasaki *et al*., 2004; Juengel and McNatty, 2005; Kaivo-oja *et al*., 2006). TGFβ superfamily growth factors, in the form of homodimers or heterodimers, bind

to either a type-I receptor referred to as an activin receptor-like kinase (ALK) or a type-II receptor, and subsequent receptor heteromerization leads to ALK phosphorylation, followed by phosphorylation of intracellular receptor-regulated signal transducers called SMADs (reviews; Massague, 2000; Shimasaki *et al*., 2004). Ligand-induced gene transcription is mediated by a heterodimeric complex of receptor-regulated SMADs and receptor-independent co-SMADs, such as SMAD4. Intracellular signaling by TGFβ superfamily growth factors can be broadly divided into two distinct groups: those utilizing the TGFβ/activin signaling pathway leading to activation of the SMAD2 and SMAD3 proteins and those using the BMP pathway leading to activation of SMAD1, SMAD5 and/or SMAD8 molecules (Massague, 2000). Ovarian GCs possess a large compliment of the TGFβ superfamily signaling system, including; most of the type-II receptors and ALK type-I receptors, co-receptors such as betaglycan, binding proteins such as follistatin and the SMAD and co-SMAD intracellular messengers (Juengel and McNatty, 2005).

BMP15 and BMP6 use the classic BMP pathway to signal in GCs: binding the BMP type-II receptor (BMPR-II) and ALK6, and activating the SMAD1/5/8 intracellular pathway (Moore *et al*., 2003; Shimasaki *et al*., 2004) (Fig. 3). In contrast, GDF9 utilizes an unusual hybrid combination of the two TGFβ superfamily signaling systems, namely GDF9 binds BMPR-II (Vitt *et al*., 2002) but utilizes the TGFβ type-I receptor, ALK5 (Mazerbourg *et al*., 2004; Kaivo-Oja *et al*., 2005), leading to activation of SMAD2 and SMAD3 signal transducers (Kaivo-Oja *et al*., 2003, 2005; Roh *et al*., 2003; Mazerbourg *et al*., 2004). Hence, even though GDF9 binds a BMP type-II receptor, it induces a TGFβ-like intracellular response in terms of SMAD activation. In addition to SMAD signaling, GDF9 and BMP15 may also activate alternate pathways, particularly when acting synergistically or with non-superfamily members such as the FGFs (Sugiura *et al*., 2007).

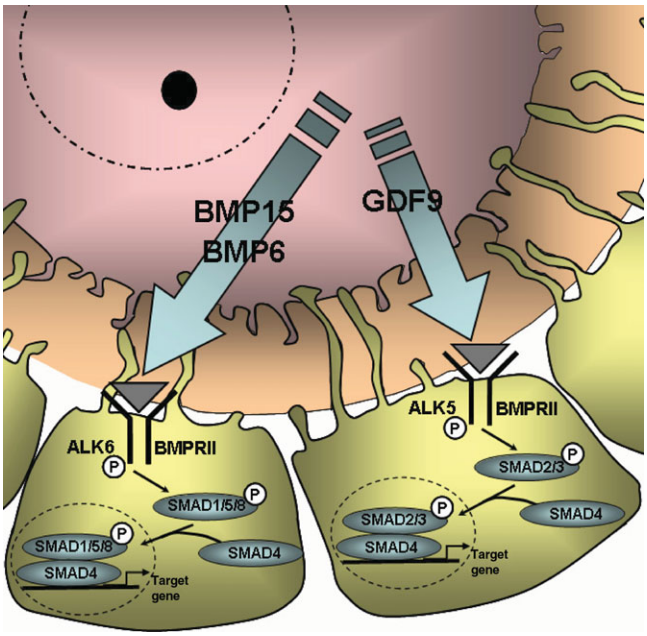

Figure 3: Molecular basis of oocyte–CC paracrine signaling
Details of the molecules mediating oocyte paracrine signaling to CCs are still emerging; however, TGFβ superfamily signaling is central to this communication axis. Key OSFs include GDF9, BMP15 and possibly BMP6. GCs and CCs express a large compliment of TGFβ superfamily receptors, co-receptors and intracellular signal transducer molecules (SMAD). BMPRII is the critical type-II receptor utilized by all three OSF ligands, although BMP6 also binds ActRIIA. GDF9 binding BMPRII/ALK5 leads to activation of ALK5 which in turn phosphorylates SMAD two-three. SMAD two-three associates with the common SMAD4 and then this complex translocates to the nucleus to interact with specific DNA motifs and transcriptional regulators, leading to expression of target genes. BMP15 and BMP6 also bind BMPRII; however, recruitment and activation of ALK6 leads to signaling through the BMP pathway mediated by activated SMAD1/5/8. OSFs also appear to activate the MAPK pathway in CCs, although the signaling cascade is currently unclear. GDF9, growth-differentiation factor 9; BMP15, bone morphogenetic protein 15; BMPRII, bone morphogenetic protein receptor type-II; ALK, activin receptor-like kinase; TGFβ, transforming growth factor β; MAPK, mitogen-activated protein kinase; OSF, oocyte-secreted factor; CC, cumulus cell

Paracrine signaling by native OSFs

The molecular mechanisms by which oocytes produce soluble molecules that direct the function of their neighboring GCs or CCs is still emerging. As the concept of oocyte regulation of GC/CC function is based on the original bioassays of OSFs (Salustri *et al*., 1990a,b; Vanderhyden *et al*., 1990), much attention has focused on candidate growth factors that can mimic the effects of oocytes on GCs *in vitro*. The main focus has been on members of the TGFβ superfamily, and in the 1990s, prior to the availability of GDF9 and BMP15, attention focused on TGFβ. Recombinant TGFβ1 and TGFβ2 are able to completely substitute for oocytes in modulating many oocyte-regulated GC/CC functions. For example, like native OSFs, recombinant TGFβ1/β2 enables FSH-induced CC hyaluronic acid production, mucification and expansion, TGFβ1 regulates cumulus cell steroidogenesis and promotes granulosa cell proliferation (Salustri *et al*., 1990a; Gilchrist *et al*., 2003, 2006; Vanderhyden *et al*., 2003; Dragovic *et al*., 2005). In all these studies, however, TGFβ antagonists (either pan-specific TGFβ neutralizing antibodies or soluble forms of TGFβ receptors) had no effect on the capacities of oocytes to regulate these GC/CC functions. These studies illustrate some important principles that the OSF bioassay is an effective approach to dissect the mechanisms of oocyte paracrine signaling and that OSF activity can be mimicked by members of the TGFβ superfamily which may not account for the actual native oocyte factors. Our laboratory has focused on exploiting this approach (using mouse, cattle and pig models) to investigate specifically the roles of native GDF9 and BMP15 (i.e. actually secreted by the oocyte, as opposed to in recombinant form) in the specification of GC/CC functions.

To exploit the approach of neutralizing native oocyte-secreted GDF9 and/or BMP15 in the *in vitro* OSF bioassay, specific GDF9 and BMP15 antagonists were required, and these emerged with the characterization of the GDF9 and BMP15 receptors and intracellular signaling pathways. Follistatin was identified as a BMP15 binding protein (Otsuka *et al*., 2001a) and a GDF9 monoclonal antibody was characterized as an effective GDF9 neutralizing antibody (Gilchrist *et al*., 2004b). Furthermore, a specific inhibitor of the kinase activities of ALKs 4/5/7 (Inman *et al*., 2002; Laping *et al*., 2002) completely antagonize GDF9 bioactivity, without affecting activity of the BMPs which signal through ALK6 (Gilchrist *et al*., 2006). Using these GDF9 and BMP15 antagonists, we attempted to investigate the roles of these specific molecules in oocyte regulation of key GC/CC functions, namely proliferation,

cumulus expansion and apoptosis. We have determined that activation of the SMAD two-three pathway in GCs/CCs by oocytes is central to oocyte regulation of GC function (Gilchrist *et al.*, 2006; Dragovic *et al.*, 2007) (Fig. 3). Mouse OSFs are capable of phosphorylating GC SMAD two-three molecules, but curiously did not appear to activate the SMAD 1/5/8 pathway utilized by BMP15 and BMP6 (Gilchrist *et al.*, 2006). This latter result may be explained by the recent discovery that mouse oocytes may not actually secrete bioactive processed BMP15 until just prior to ovulation (Yoshino *et al.*, 2006). It remains to be determined whether non-murine oocytes activate the SMAD 1/5/8 pathway in GCs, although this would seem likely.

Using neutralizing antibodies directed against putative native OSFs, we determined that the potent growth-promoting effects of oocytes on CCs and GCs appear to be mediated by multiple TGFβ superfamily members, including ~50% accounted for by GDF9 (Gilchrist *et al.*, 2004b), with essentially no mitogenic activity from oocyte-secreted TGFβ1/β2 or BMP6 (Gilchrist *et al.*, 2003, 2006). The potent oocyte-secreted mitogens can be completely ablated *in vitro* by either a soluble form of the BMP type-II receptor or the ALK 4/5/7 kinase inhibitor (Gilchrist *et al.*, 2006). Likewise, OSF-activation of the SMAD two-three signaling pathway is required for oocyte-enabled FSH- or EGF-stimulated CC expansion (Dragovic *et al.*, 2007). The identities of the oocyte factors enabling expansion remain controversial, but it appears to involve some combination of oocyte-secreted GDF9 and BMP15 (but not BMP6) (Dragovic *et al.*, 2005; Gui and Joyce, 2005; Yoshino *et al.*, 2006). Conversely, oocyte-secreted GDF9 provides little of the anti-apoptotic effects of bovine oocytes on CCs, whereas BMP15 and BMP6 appear to play important roles (Hussein *et al.*, 2005). Other key GC/CC functions regulated by OSFs, such as the regulation of steroidogenesis and metabolism, have not yet been characterized to specific OSFs. Although these studies have identified key oocyte paracrine signaling pathways belonging to the TGFβ superfamily, in particular, the receptors BMPR-II and ALK 4/5 leading to activation of the SMAD two-three cascade (Fig. 3), it seems quite likely that other oocyte-secreted molecules from outside the TGFβ superfamily are also likely to participate, and further research is required in this area to elucidate the full molecular nature of the oocyte–somatic cell communication axis.

The cellular basis of oocyte paracrine signaling

A key challenge facing reproductive biologists currently is the integration of this new knowledge about OSFs into coherent physiological mechanisms of how oocytes govern folliculogenesis, CC function, oocyte and embryo development, and fecundity. Although key OSFs have been identified, in particular GDF9 and BMP15, understanding their modes of action is substantially complicated by multiple interactions between maternal and oocyte signaling molecules, as well as the constantly changing state of physical interactions between the oocyte and its companion somatic cells throughout folliculogenesis.

Oocyte–CC physical interactions

An important deficiency in our current knowledge of oocyte–CC communication and the determinants of oocyte quality is the interaction between paracrine and gap-junctional signaling within the COC. Throughout most of the course of oogenesis, oocytes are physically and metabolically coupled to somatic cells through gap junctions. Gap junctions facilitate the transfer of small molecular weight molecules between GCs/CCs and the oocyte and also between CCs (Herlands and Schultz, 1984). Molecules that pass via gap junctions include ions, metabolites and amino acids that are necessary for oocyte growth, as well as small regulatory molecules such as cAMP that control oocyte nuclear maturation, and gap-junctional signaling is a key means of disseminating local and endocrine signals to the oocyte via CCs (Albertini *et al.*, 2001). This mode of somatic cell–oocyte communication is essential for development as genetic deletion of the oocyte-specific gap junctional subunit, connexin-37, leads to female sterility (Simon *et al.*, 1997).

An intriguing feature of oocyte–CC gap-junctional communication is that the oocyte and CCs are physically separated a considerable distance by the zona pellucida surrounding the oocyte (Fig. 4). To overcome this distance and to allow gap-junctional communication to occur, CCs have developed highly specialized trans-zonal cytoplasmic projections, which penetrate through the

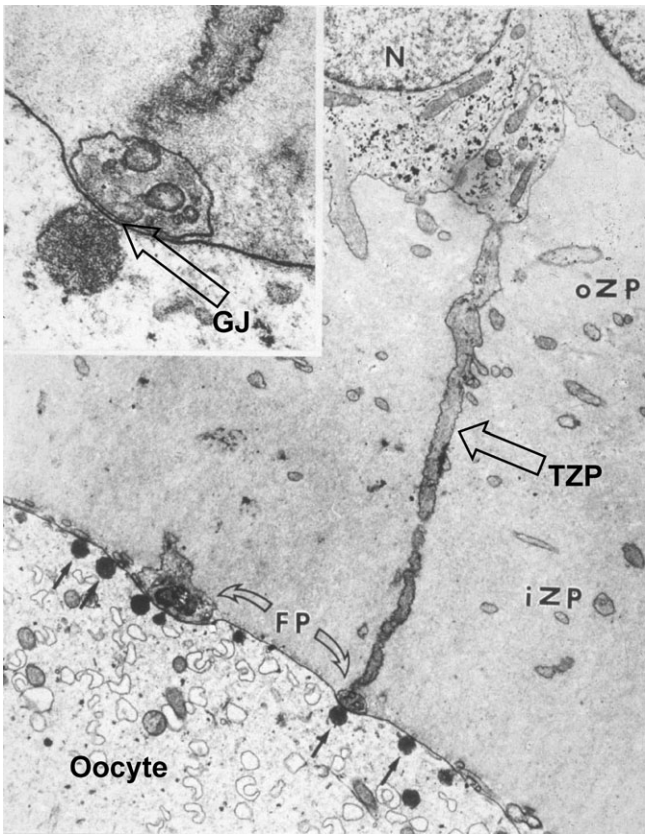

Figure 4: CC–oocyte coupling through trans-zonal projections and gap junctions

Transmission electron micrograph of a marmoset monkey COC at the oocyte–CC interface. Illustrated is a CC trans-zonal cytoplasmic projection (TZP) that penetrates the zona pellucida (ZP) and forms a foot-process (FP) abutting the oolema. Inset: high magnification of the foot process shows the presence of organelles in the end of the TZP as well as the intimate physical association of the two membranes and the site of CC–oocyte gap junctions (GJ). A cortical granule is shown directly below the foot process (solid arrow). Reproduced from Gilchrist (1996)

zona pellucida and abut the oocyte membrane, forming gap junctions at the ends of these projections (Fig. 4; Gilchrist, 1996). Details of the role and regulation of trans-zonal projections function are scant; however, it is known that trans-zonal projections contain cytoplasmic organelles and that the structure of trans-zonal projections changes throughout folliculogenesis and during oocyte maturation (Albertini *et al*., 2001). It is noteworthy that in three of the four OSF bioasaays (Fig. 1A–C) and also using the approach of adding recombinant OSFs such as GDF9 to isolated CCs, CC trans-zonal projections and GC/CC–oocyte gap-junctional communication are experimentally destroyed. Hence, a considerable limitation of the common *in vitro* approaches to study OSFs is that the intricate physical interplay between CCs and the oocyte is lost. The roles and relative significances of trans-zonal projections and gap junctions in the oocyte–paracrine communication axis are entirely unclear at this stage, and it would be fascinating to know if trans-zonal projections possess the key OSF receptors, BMPR-II, ALK4/5 and ALK6, and thereby if OSFs act in an acutely localized manner within the zona pellucida via trans-zonal projections to regulate CC functions.

OSF interactions with follicular signals

A further aspect of COC biology that is poorly understood is the interaction between OSFs and maternal follicular signals. Folliculogenesis is driven and governed by a stage-specific, highly coordinated interaction between endocrine hormones and local follicular-derived growth factors and steroids. As it is now clear that GC/CC functions and follicular growth are also regulated by oocyte paracrine signaling, this represents an additional layer of complexity on top of this traditional perspective on processes regulating folliculogenesis and ovulation rate (McNatty *et al*., 2004). Currently, we have a limited understanding of how OSFs interact with well-characterized key maternal regulators of folliculogenesis, such as FSH, LH, IGF-I, estradiol, androgens and inhibin-activin.

Three examples provide an insight into the mutual cooperation required between oocyte and maternal signaling to regulate GC/CC function. First, mouse cumulus expansion requires these simultaneous signaling events: OSF activation of CC SMAD two-three signaling (Dragovic *et al*., 2007) as well as EGF/FSH-induced activation of mitogen-activated protein kinase (MAPK) (Su *et al*., 2003; Diaz *et al*., 2006), and neither signal alone is sufficient to stimulate cumulus expansion. Secondly, the oocyte-derived mitogens in some species appear to synergize with IGF-I and androgens to promote GC/CC growth (Lanuza *et al*., 1998; Li *et al*., 2000; Brankin *et al*., 2003; Gilchrist *et al*., 2003; Hickey *et al*., 2005). Thirdly, an important feature of differentiation of the CC lineage is the capacity of OSFs to antagonize the luteinizing effects of FSH (Diaz *et al*., 2007b). These recent studies illustrate the important concept that OSFs operate in a fully integrated manner with maternal signals to regulate folliculogenesis.

Ontogeny of OSF bioactivity

In attempting to place the oocyte–GC/CC communication axis in a physiological context governing fertility, it is important to gain an understanding of the dynamic nature of this relationship throughout oogenesis and folliculogenesis. Crucial to this understanding is the knowledge that the capacity of the oocyte to regulate GC/CC functions (Table II) changes dramatically throughout

Table III. Contrast between profiles of OSF bioactivity and OSF expression: the oocyte's capacity to regulate specific GC or CC functions changes throughout oocyte development and contrasts the oocyte expression profile of OSFs.

OSF regulation of GC or CC function[a]	Stage of oocyte development			References
	Growing Oocyte[b]	Fully Grown Oocyte[c]	MII Oocyte	
Stimulation of KL	+++	−		Joyce *et al*., (1999)
Suppression of KL	−	+++		Joyce *et al*., (1999)
Stimulation of proliferation	−	+++	+	Gilchrist *et al*., (2001)
Stimulation of glycolysis	−	+++		Sugiura *et al*., (2005)
Stimulation of AA uptake	−	+++		Eppig *et al*., (2005)
Suppression of LHR	+	+++	+	Eppig *et al*., (1997)
Steroid regulation	+ +	+++	+++	Vanderhyden and Macdonald, (1998)
Enable CC expansion	−	+++	+	Vanderhyden *et al*., (1990); Nagyova *et al*., (2000)
Enable ECM expression	−	+++		Joyce *et al*., (2001); Diaz *et al*., (2006)
Suppression of uPA	−	+++	++	Canipari *et al*., (1995)
tPA activity		+	+++	D'Alessandris *et al*., (2001)
Expression of OSF[d]				
GDF9	• • •	• • •	•	Prochazka *et al*., (2004); Juengel and McNatty, (2005); Li *et al*., (2006)
BMP15	• • •	• • •	• •	Shimasaki *et al*., (2004); Juengel and McNatty, (2005); Li *et al*., (2006)
BMP6	• • •	• • •		Shimasaki *et al*., (2004); Juengel and McNatty, (2005)

'+++', maximum GC response to oocyte; '−', minimum GC response to oocyte; empty cell, not determined.
• • •, high mRNA and/or protein expression; •, low mRNA and/or protein expression.
[a]Regulation of specific GC or CC function by native OSFs.
[b]Meiotically incompetent GV oocyte from secondary preantral follicle.
[c]Meiotically competent immature oocyte from antral follicle.
[d]Generalized mRNA and/or protein expression profile throughout oocyte development.

the course of folliculogenesis. To generalize, the oocyte's capacity to regulate GC/CC activities is low or more frequently absent during its growing phase in secondary pre-antral follicles, is then highest throughout the antral phase of folliculogenesis and then declines soon after the LH surge and with the re-initiation of meiosis (Table III). This developmental coordination of OSF bioactivity can be exemplified by the growth-promoting capacity of oocytes, whereby (i) growing oocytes from pre-antral follicles have a low capacity to promote GC proliferation, despite pre-antral GCs being highly responsive to oocyte factors, (ii) fully grown meiotically immature oocytes in antral follicles potently stimulate MGC/CC growth and (iii) this activity declines over the course of oocyte maturation such that this activity is all but lost in zygotes (Gilchrist *et al*., 2001).

This dynamic pattern of OSF bioactivity also holds for many other OSF-regulated GC functions (Table III). For example, growing mouse oocytes from pre-antral follicles are unable to regulate the following GC functions: enable FSH-induced CC expansion (Vanderhyden *et al*., 1990), including enabling expression of the ECM transcripts *Has2, Ptgs2, Ptx3 and Tnfaip6* (Joyce *et al*., 2001; Diaz *et al*., 2006), suppression of synthesis of urokinase plasminogen activator (Canipari *et al*., 1995), suppression of *Lhcgr* expression (Eppig *et al*., 1997) and stimulation of CC glycolysis (Sugiura *et al*., 2005) and amino acid uptake (Eppig *et al*., 2005). The capacity of oocytes to regulate all these GC functions is first acquired by mouse oocytes at around the time of antrum formation, as oocytes reach the end of their growth phase and acquire meiotic competence (reviewed; Gilchrist *et al*., 2004a). There are, however, two notable exceptions to this general pattern. First, oocytes appear to have the capacity to regulate GC/CC steroidogenesis throughout most of oogenesis (Vanderhyden and Macdonald, 1998). Secondly, GC expression of *Kitl* is stimulated in pre-antral follicles by growing oocytes, but then inhibited in antral follicles by fully grown oocytes (Joyce *et al*., 1999). Hence, it appears that during the course of oogenesis, an oocyte first directs its neighboring GCs to promote its own growth, and then once fully grown, the oocyte then actively prevents GCs from stimulating its further growth. Consistent with this is oocyte overgrowth in *Gdf9*-deficient mice (Carabatsos *et al*., 1998). This complex local paracrine regulatory loop between oocyte and GCs illustrates the remarkable degree of bidirectional control that exists in the oocyte–somatic cell communication axis.

In this context, it is noteworthy that the developmental coordination of OSF bioactivity throughout folliculogenesis does not match the expression profile of the key putative OSFs, GDF9 and BMP15, as these transcripts and proteins are expressed at high levels in the oocyte essentially throughout folliculogenesis (Table III). GDF9 is expressed from primordial follicles onwards in sheep, cattle, possum and hamster, and from primary follicles onwards in rodents and humans, whereas BMP15 is first expressed in primary follicles in all these species except the possum (review; Juengel and McNatty, 2005). In terms of ontogeny of expression, GDF9 and BMP15 mRNA expression generally coincides with translation to protein in the oocyte, where very high levels of the unprocessed pro-mature forms are found (Gilchrist *et al*., 2004b; Guéripel *et al*., 2006; Li *et al*., 2006).

The first data on the regulation of expression and actual secretion of biologically active mature GDF9 and BMP15 proteins from oocytes are just emerging. A number of recent studies have shown that proteolytic removal of proregions to form mature GDF9 or BMP15 may be temporally and/or hormonally regulated. Most notably, expression of the mature form of BMP15 in mouse oocytes prior to the LH surge appears to be very low or even absent, followed by an increase in the quantity of mature BMP15, but not mature GDF9, just prior to ovulation (Guéripel *et al*., 2006; Yoshino *et al*., 2006). This BMP15 expression profile may be peculiar to the mouse and it can be anticipated that ruminant and possibly primate oocytes should express and secrete BMP15 prior to the LH surge (Juengel and McNatty, 2005). Proregion processing of TGFβ superfamily growth factors normally takes place intracellularly, prior to secretion, yet curiously the unprocessed pro-mature form of GDF9 and BMP15 are the predominant forms detected in sheep follicular fluid (McNatty *et al*., 2006) and in mouse OCM (Gilchrist *et al*., 2004b). The biological significance of these intriguing findings is currently unclear. Either the unprocessed forms of GDF9 and BMP15 are biologically active, which would be most unusual for the superfamily (Shimasaki *et al*., 2004), or regulation at the post-translational and/or extracellular levels constitute a critical level of biological control of these growth factors. Examination of these hypotheses might explain the apparent discrepancy between consistent expression of GDF9/BMP15 proteins throughout folliculogenesis versus the precise developmental coordination of native OSF bioactivity (Table III).

Significance of oocyte paracrine signaling: OSFs determine the CC phenotype and regulate the COC microenvironment

What is the purpose of oocyte–paracrine signaling? An attractive and increasingly verified concept is that the oocyte secretes potent paracrine growth factors that regulate the differentiation of CCs so as to carefully control its own microenvironment. CCs and MGCs originate from common progenitor cells, yet in terms of gene expression and function, they are very different. This disparate differentiation of somatic cells within the follicle must be carefully managed as the two cell types have distinct functions: the specialized CCs are required to support the appropriate development of the oocyte and to facilitate ovulation and fertilization, whereas the MGC's principal role is steroid production and differentiation toward luteal cells. It is now recognized that the oocyte actively directs the lineage decision of its neighboring GCs toward CCs, through the paracrine actions of OSFs. Table II illustrates the large number of GC genes and functions that are under OSF control—the cumulative effect of which is the differentiation of the characteristic CC phenotype. Under the influence of FSH, the default pathway of GC differentiation is toward the more luteinized MGC phenotype (Eppig *et al*., 1997; Li *et al*., 2000). Elimination of oocyte paracrine signaling, either by physical removal of the oocyte from the COC by oocytectomy (Eppig *et al*., 1997; Li *et al*., 2000) or by ablation of oocyte-activated SMAD signaling in CCs (Gilchrist *et al*., 2006; Diaz *et al*., 2007b; Dragovic *et al*., 2007), causes CCs to lose their distinctive phenotype and to display characteristics more typical of MGCs (e.g. low proliferation index, increased LH receptor expression and steroidogenic capacity). However, CC characteristics can be fully restored in OOX complexes by treating OOXs with OSFs, importantly demonstrating that the oocyte actively abrogates FSH-induced

GC differentiation toward luteinization (Eppig *et al*., 1997; Li *et al*., 2000; Diaz *et al*., 2007b). These studies have now entirely validated the original observation that the oocyte acts to prevent follicular luteinization (el-Fouly *et al*., 1970).

Given that the primary function of OSF paracrine signaling in tertiary follicles is to promote and maintain the COC phenotype, it is perhaps not surprising that OSF bioactivity is most potent during the antral phase of folliculogenesis (see 'Ontogeny of OSF bioactivity'; Table III). In secondary (pre-antral) follicles, it appears growing oocytes do not have the capacity to direct differentiation of GCs; this capacity is first acquired by oocytes at the end of their growth phase which, in mice, is coincident with antrum formation. Hence, OSFs are crucial in the pre-antral to antral transition period to drive the differentiation of pre-antral GCs surrounding the oocyte into CCs (Diaz *et al*., 2006,2007a). As the antral follicle then continues to grow, the GCs lining the wall of the follicle differentiate into steroidogeneic MGCs under the influence of FSH, although these effects of FSH are countered by OSFs only in those cells in close proximity to the oocyte (Hussein *et al*., 2005; Diaz *et al*., 2007b). Hence, it would seem that OSFs act in an extremely localized manner, establishing a morphogenic gradient of OSFs within the COC. We have recently tested this hypothesis by examining the gradient of anti-apoptotic activity of oocytes within the layers of a COC (Hussein *et al*., 2005). Figure 5 illustrates that the incidence of CC apoptosis is lowest in the inner most layers of CCs and is higher on the outer layer of the COC, and moreover it is well known that the COC has a lower incidence of apoptosis than MGCs, especially in atretic follicles. Removal of OSFs by oocytectomy led to an increase in apoptosis in all layers of the COC. However, when OOXs were co-cultured with DOs and thereby exposed to native OSFs from outside the complex, the gradient of apoptosis was reversed, with the outer CC layer having the lowest, and the inner layer the higher, incidence of apoptosis (Hussein *et al*., 2005).

These findings support the hypothesis that OSFs establish and maintain a morphogenic gradient across the follicle acting in an acutely localized manner within the antral follicle, in which CCs appear to be the primary recipients of OSFs. MGCs are clearly far less influenced by OSFs as otherwise they would be re-differentiated to function as CCs (Eppig *et al*., 1997; Li *et al*., 2000; Diaz *et al*., 2007b). OSFs either do not reach MGCs because of the gradient of OSFs, or reach MGCs but in an inactive form or MGCs have some mechanism to counter their actions. Further studies are required to investigate this hypothesis. However, it is now clear that the principal function or purpose of oocyte paracrine signaling in antral follicles is to drive differentiation of CCs and to maintain their distinctive functions, thereby actively regulating a highly specialized microenvironment immediately surrounding the oocyte that is distinct from the rest of the ovarian follicle.

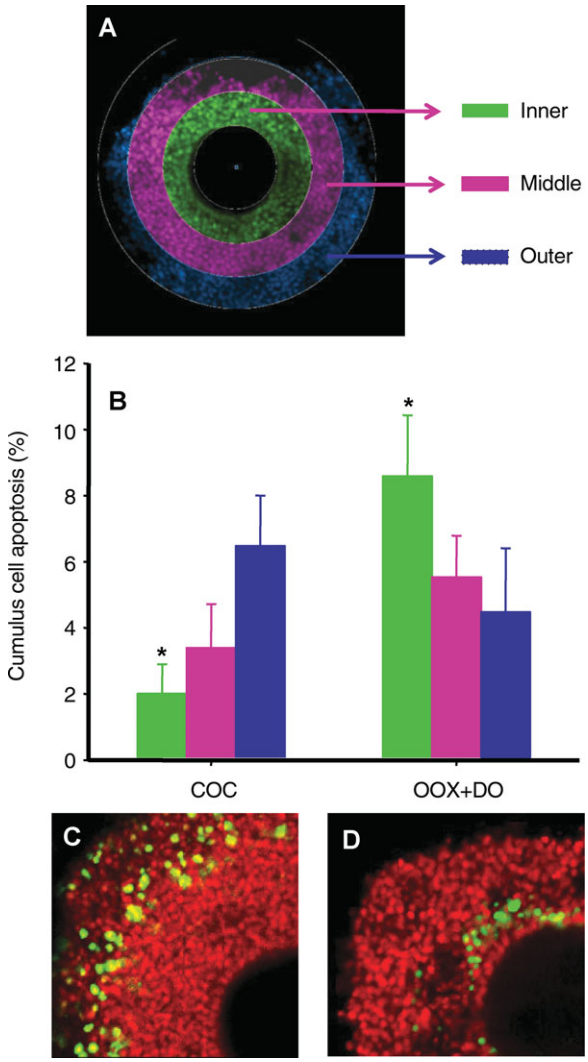

Figure 5: OSFs maintain a morphogenic gradient across the COC
Pattern of apoptosis (green cells) in a bovine COC compared with OOX+DO, where the source of OSFs differ, i.e. inside or outside the complex, respectively. Apoptosis was higher in the outer layer of CCs in the case of the intact COC (**B** and **C**), and in the inner layers in the OOX+DO where the DOs were on the outside of the complex (B and **D**). The incidence of CC apoptosis increased with increasing distance from the oocyte, demonstrating a gradient of anti-apoptotic factors emanating from the oocyte. Diameters of unexpanded COCs and OOXs were measured after culture without FSH using confocal microscopy and divided into three layers; inner, middle and outer layers, each layer representing 33% of the radius (**A**). The proportion of apoptotic cells was quantified in each layer. CCs, cumulus cells; COC, cumulus–oocyte complex; OOX, oocytectomized complex; DO, denuded oocyte; OSF, oocyte-secreted factor. Reproduced from Hussein *et al*. (2005) with permission of the Company of Biologists

Oocyte-secreted factor regulation of oocyte quality

Oocyte–somatic cell communication is clearly a bidirectional process involving gap-junctional and paracrine signaling, and so far this review has focused in detail on oocyte paracrine signaling to GCs/CCs, as currently this communication axis is the least understood. It is abundantly clear, however, that CCs play an indispensable role, first, in the appropriate development of the oocyte within the follicle for the oocyte to acquire developmental competence (see 'The follicular microenvironment determines oocyte developmental potential') and, secondly, in the process of ovulation (Russell and Robker, 2007). Given that we now know that the oocyte governs exquisite control of CC function via OSFs, it seems reasonable to propose that oocyte paracrine signaling to CCs and thereby appropriate maintenance of the COC microenvironment must be a critical function of oocytes that is required for its own development.

We have recently tested this hypothesis by exposing COCs to additional exogenous OSFs during IVM, using bovine and murine oocytes as two disparate experimental models of mammalian oogenesis (Hussein *et al*., 2006; Yeo *et al*., 2007). In the bovine model, we treated immature COCs with exogenous OSFs using two different methods: (i) we exposed COCs to an uncharacterized mix of native OSFs by co-culturing intact COCs with DOs during IVM (Fig. 6A) or (ii) we treated COCs during IVM with recombinant GDF9 or BMP15 (Fig. 6B) (Hussein *et al*., 2006). Following maturation, oocytes underwent conventional IVF and embryo culture as a measure of oocyte developmental competence. The results in Fig. 6 demonstrate that the capacity of IVM oocytes to proceed to the blastocyst stage is substantially improved by treating COCs during IVM with either source of OSF (Hussein *et al*., 2006). Exposure of COCs to OSFs also improved subsequent embryo quality as evidenced by increased total and trophectoderm cell numbers (Hussein *et al*., 2006). Likewise, in the mouse model, compared with control COCs, those treated with recombinant GDF9, during IVM, went on to produce embryos that developed faster *in vitro* and produced blastocysts containing more total cells due to a larger inner cell mass (Yeo *et al*., 2007). Upon transfer of embryos to pseudopregnant females, there was no difference in implantation rates; however, embryos derived from GDF9-treated COCs had almost double the rate of fetal survival (Fig. 6C; Yeo *et al*., 2007).

Together these studies provide evidence toward a new paradigm in oocyte biology that OSFs play a role in the regulation of oocyte quality (Fig. 7). Conceptually, these studies are likely to be important because they demonstrate that the secretion of these growth factors by oocytes, and appropriate regulation of CC function, is a crucial function the oocyte must undertake for its future development. Supplementation of OSFs during the short window of oocyte maturation appears to have a profound effect on developmental programming of the oocyte, a legacy that persists through late pre-implantation development and into fetal development (Yeo *et al*., 2007). Hence, it appears that the capacity of an oocyte to regulate its own microenvironment via OSFs constitutes an important component of oocyte 'cytoplasmic maturation' or the acquisition of oocyte developmental competence. The molecular mechanisms that underpin OSF-enhancement of oocyte developmental potential require further studies, which are likely to provide important new insights into our fundamental understanding of the regulation of oocyte quality.

From a practical perspective, these studies are the first to demonstrate the concept and the validity of OSFs as IVM media additives to improve oocyte quality and subsequent embryo and fetal developmental potential (Hussein *et al*., 2006; Yeo *et al*., 2007). A substantial increase in embryo production efficiency (in the bovine, from 40% to ~60% in a completely serum-free, defined IVM system) clearly has significant clinical and commercial applications (Gilchrist and Thompson, 2007). This has immediate applications in domestic animals and rodents, where oocyte IVM is already in widespread use as a platform technology for many different applications. The studies also support the concept of developing diagnostic markers of oocyte developmental potential based on specific CC functions under the control of OSFs (Table II). Such markers would also have an invaluable role in current clinical IVF procedures as laboratories strive to select the best oocyte to inseminate and which embryo to transfer

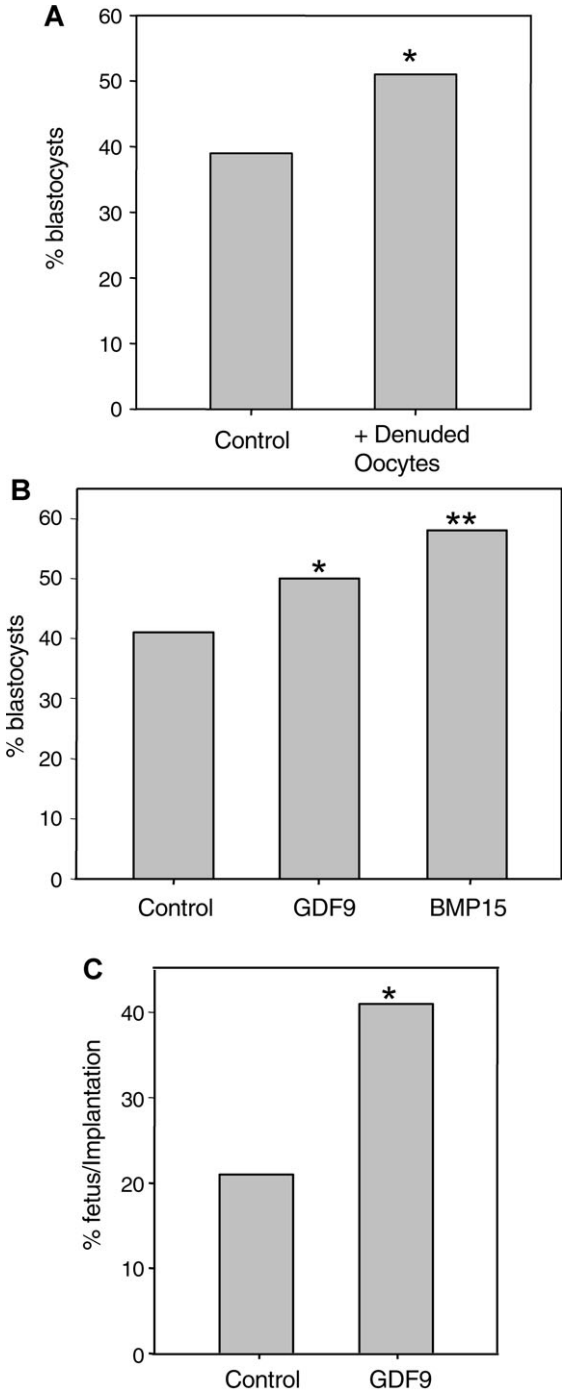

Figure 6: OSFs enhance oocyte developmental potential
Exposure of COCs during IVM to OSFs enhances bovine pre-implantation embryo development (**A** and **B**) and post-implantation embryo survival in the mouse (**C**). (A) To expose bovine COCs to additional native OSFs, COCs were co-cultured with denuded oocytes (0.5 DOs/μl) prior to IVF. (B) Bovine COCs were treated during IVM with recombinant murine GDF9 or ovine BMP15. (C) Murine COCs were treated with GDF9 during IVM, followed by culture to the blastocyst stage *in vitro*, then transferred to pseudopregnant mothers and assessed for number of fetuses per implantation site. In all experiments the controls are COCs matured in serum-free IVM media supplemented with FSH (A, B) or with FSH and EGF (C).COC, cumulus-oocyte complex; IVM, *in vitro* maturation; OSF, oocyte-secreted factor; DO, denuded oocyte; GDF9, growth-differentiation factor 9; BMP15; bone morphogenetic protein 15; EGF, epidermal growth factor. Data from Hussein *et al*. (2006) and Yeo *et al*. (2007)

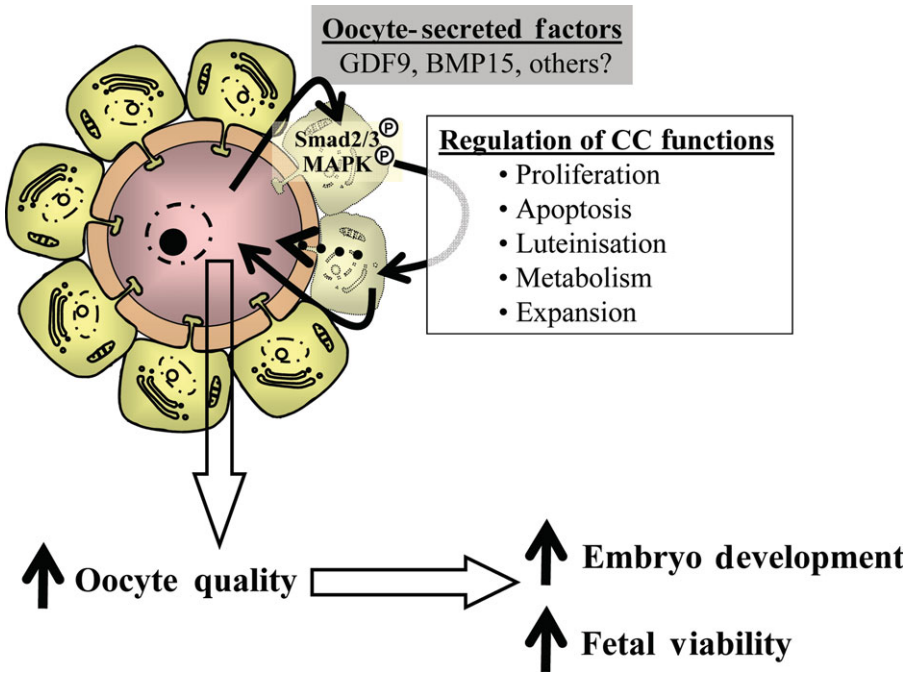

Figure 7: OSF regulation of CC function and oocyte quality
Model depicting oocyte–CC interactions and their impact on subsequent oocyte developmental potential. An oocyte–somatic cell regulatory loop exists, whereby oocytes and CCs regulate each others functions by paracrine and gap-junctional communication. Oocytes secrete soluble growth factors, notably GDF9 and BMP15 and probably others, that lead to the activation of SMAD two-three and MAPK signaling in CCs, which in turn regulate a multitude of CC gene expression and key CC functions. Appropriate CC function and maintenance of the COC microenvironment is dependent on OSFs. In conjunction with maternal signals such as FSH and EGF, CCs pass regulatory growth factors and small metabolites back to the oocyte via paracrine and gap-junctional signaling. This bidirectional CC–oocyte communication loop appears to regulate unknown processes in the oocyte that improves its quality, as assessed by improved developmental potential. CC, cumulus cell; COC, cumulus–oocyte complex; OSF, oocyte-secreted factor; GDF9, growth-differentiation factor 9; BMP15, bone morphogenetic protein 15; EGF, epidermal growth factor. MAPK, mitogen-activated protein kinase

in a treatment cycle. Currently, the relatively poor success rate of IVM in humans (as defined by poor embryo development post-IVF and poor pregnancy rates compared with conventional IVF) is the primary factor limiting its widespread clinical implementation. Validation of the efficacy of adding OSFs to human IVM oocytes is required, and if this also leads to improved oocyte developmental potential, this could have significant implications for the widespread application of IVM to clinical practice and hence for the way human infertility is treated (Edwards, 2007).

Conclusions

Over the past decade, we have gained significant new insight into the nature of the oocyte–somatic cell communication axis. The most important concept to emerge is that the oocyte is not passive in the ovarian follicle, but rather is a fundamental regulator of somatic cell differentiation and function and that the oocyte plays a central role in the regulation of folliculogenesis and thereby its own development. Although some of the molecular events mediating oocyte–CC paracrine signaling are emerging, this brings a significant new challenge, which is the integration of this critical new axis into a holistic model of processes governing oocyte quality, incorporating CC–oocyte gap-junctional signaling, CC–oocyte bidirectional paracrine signaling and the interaction of these processes with maternal signals in a constantly dynamic follicular microenvironment. Many questions remain unanswered; however, as our knowledge of processes regulating mammalian oocyte quality improves, this will provide new opportunities for the management of human infertility.

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