Review

An oocentric view of folliculogenesis and embryogenesis

Karla J Hutt conducted her graduate studies, which focused on receptor-ligand interactions and female gamete biology, at the Australian National University in Canberra. She was awarded her PhD degree in 2006 and is now at the University of Kansas Medical Center where she is a post-doctoral fellow under the supervision of David F Albertini. Dr Hutt's research interests include using confocal imaging techniques to understand the characteristics of meiotic spindle assembly, dynamics and integrity of in-vitro matured and cryopreserved oocytes.

Karla J Hutt, David F Albertini[1]
The Center for Reproductive Sciences, Molecular and Integrative Physiology, University of Kansas Medical Centre, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA
[1]Correspondence: Tel: +11 913 588 0412; Fax: +11 913 588 0456; e-mail: dalbertini@kumc.edu

Abstract

The mammalian oocyte undertakes a highly complex journey to maturity during which it successively acquires a series of characteristics necessary for fertilization and the development of a healthy embryo. While the contribution of granulosa cells to oocyte development has been studied for many years, it has recently become apparent that the oocyte itself plays a key role in directing its own fate as well as the growth and differentiation of the follicle. This regulatory capacity is achieved through the synthesis and secretion of oocyte-specific factors, such as growth and differentiation factor 9 and bone morphogenetic protein 15, which act on granulosa cells to modify their proliferation, function and differentiation, as well as through direct physical contacts that occur at the granulosa cell–oocyte interface. This review describes key mechanisms by which the oocyte manipulates its own environment in order to achieve meiotic and developmental competence. The potential consequences of assisted reproductive technologies, such as in-vitro maturation and cryopreservation, on oocyte–granulosa cell interactions are also discussed, along with the impact of impaired oocyte development on early embryogenesis.

Keywords: *assisted reproductive technology, BMP-15, cumulus cells, GDF-9, oocyte, signalling*

Introduction

There is an ever increasing awareness within the field of human and animal assisted reproductive technologies that establishment of pregnancy and offspring health are invariably linked to properties exhibited by oocytes at the time of fertilization. While specific determinants of oocyte quality have eluded rigorous physiological definition, compelling evidence now favours the idea that the follicular environment both prior to and during ovulation ultimately dictates the developmental competence of the preimplantation conceptus. This review will fashion an oocentric perspective in addressing critical phases of folliculogenesis that impart the molecular, organellar, and organizational determinants needed to successfully journey through the preimplantation stages of embryogenesis. Two phases of folliculogenesis are evaluated that encompass the growth or vegetative phase of oogenesis, typically restricted to the protracted pre-antral follicle and the maturative phase that is associated with ovulation of the Graafian follicle in response to the LH surge. By no means is omission of the generative or proliferative phase of oogenesis intended to suggest that it is of less importance than later stages in the ontogeny of a mature oocyte. Rather, the intention is to emphasize what is and what is not known about how the non-quiescent oocyte commencing its growth influences the microenvironment of the follicle to assure, somewhat selfishly, that it acquires the necessary ingredients to support the placental and fetal fates of the conceptus. As stated by Anderson, 'it is obvious that while one of the goals of oogenesis is to commence embryogenesis, an early objective of this phenomenon is the production of a mature egg' (Anderson, 1974).

The production of a mature egg is best characterized as a symbiotic process (**Figure 1**). The germ line first engages the metabolic support of somatic cells to achieve the mass equivalence of an embryo. In so doing, the oocyte in many mammalian forms

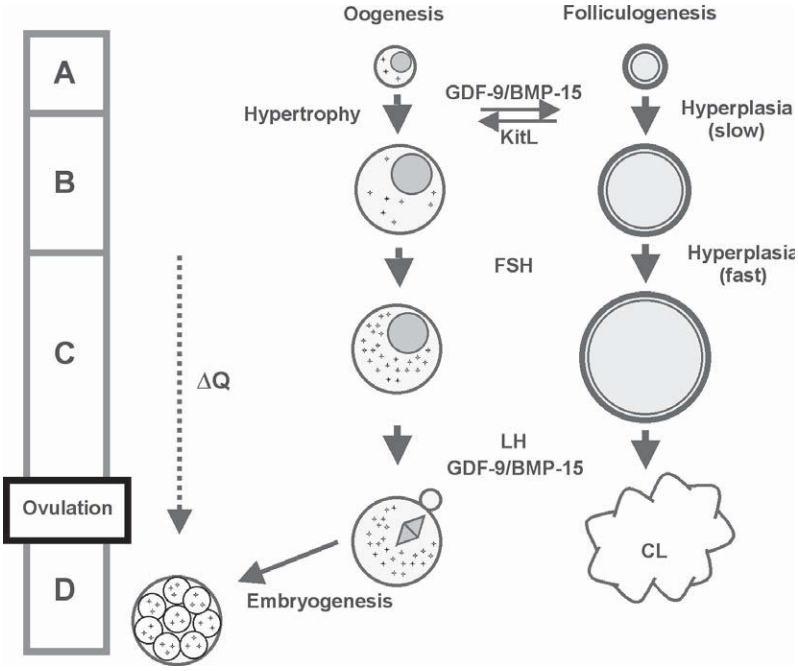

Figure 1. Schematic representation of growth and maturation stages of oogenesis illustrating the relative sequence of factors co-ordinating folliculogenesis and embryogenesis. Oocytes entering the growth phase exit from quiescence in primordial follicles (top) and under the influence of growth and differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP-15) establish a feedback loop that condones germ cell hypertrophy while limiting somatic cell hyperplasia. Acquisition of FSH sensitivity, typically near the end of the oocyte growth phase in rodents, launches somatic cell hyperplasia and specification of mural and cumulus lineages. While a fully-grown oocyte prior to exposure to FSH has the mass equivalence to form a compacted embryo, FSH and LH follicular exposure is necessary to complete acquisition of developmental competence. Physical separation of the germ line from the mural lineage encourages and supports transformation of the Graafian follicle into the corpus luteum (CL). Note that the acquisition of developmental competence (ΔQ) is gradual and may be apportioned to different stages of this developmental progression depending on the species. (A) Primordial follicle activation and initial growth; (B) pre-antral development; (C) antral development; (D) early embryogenesis; KitL = kit ligand.

acquires a molecular and organellar quota either just before or during the stage when the follicle differentiates into an oestrogen-producing gland. The consumer identity of the oocyte continues through the shorter FSH-dependent phase of antral follicle growth while preventing 'muralization' of the cumulus lineage of granulosa cells. The peri-antral phase also coincides with the time during which meiotic cell cycle competence is achieved. Oocyte dominance continues through ovulation as factors of germ cell origin facilitate cumulus expansion and meiotic progression through complex feedback mechanisms modulated by LH. How the oocyte impacts follicle growth and differentiation is first discussed, especially with reference to the establishment and maintenance of bidirectional feedback loops at the somatic germ cell interface.

Growth phase of folliculogenesis

Female gametes are stored within the ovary in the form of primordial follicles, which are comprised of small, non-growing functionally immature oocytes surrounded by a single layer of squamous granulosa cells. Throughout the reproductive lifespan of most mammals, a continuous trickle of primordial follicles are released from dormancy and enter the growing follicle pool. Once growth is initiated, the follicle embarks on a complex path of development during which the oocyte progresses through a series of highly co-ordinated phases of development that are necessary for its successful ovulation and fertilization. This process begins as soon as the pool of primordial follicles is established in the ovary and continues until the pool is exhausted and folliculogenesis ceases, a time corresponding to the transition to menopause in humans. It has been known for many years that the granulosa and thecal cells of the follicle support the oocyte on this journey, through the provision of essential nutrients, information molecules, metabolic precursors, growth factors and hormones (Biggers *et al.*, 1967; Donahue and Stern, 1968; Brower and Schultz, 1982; Haghighat and Van Winkle, 1990). However, it is also becoming apparent that the oocyte itself plays an active and dominant role in directing follicle growth by synthesizing factors that regulate the proliferation, function, survival and differentiation of granulosa cells, the recruitment of theca cells, and the secretion of extracellular matrix components (Buccione *et al.*, 1990; Salustri *et al.*, 1990; Canipari *et al.*, 1995; Eppig *et al.*, 2002; Hussein *et al.*, 2005).

In this regard, the oocyte manipulates its own environment to ensure it is adequately supported throughout pre-antral, antral and pre-ovulatory development.

The pre-antral phase of folliculogenesis is characterized by zona pellucida formation, granulosa cell proliferation, which is at first slow, the recruitment of thecal cells to the follicular basal lamina and a dramatic increase in oocyte volume (Pedersen, 1969). Pre-antral follicle growth occurs independently of extra-ovarian hormonal stimuli (Halpin *et al*., 1986; Kumar *et al*., 1997) and its regulation predominantly involves direct interactions between granulosa cells and oocytes and the local production of growth factors. In particular, two oocyte-specific members of the transforming growth factor (TGF)β super family, growth differentiation factor 9 (GDF-9) and bone morphogenetic factor 15 (BMP-15), have been shown to play important regulatory roles during pre-antral follicle development (Hanrahan *et al*., 2004).

GDF-9 is expressed by the oocyte throughout folliculogenesis and is required for progression beyond the primary stage of development (Dong *et al*., 1996; Carabatsos *et al*., 1998; Elvin *et al*., 1999a). Female mice that are homozygous for a targeted deletion of exon 2 of the *gdf-9* gene are infertile and their ovaries contain oocytes that grow rapidly and undergo nuclear remodelling consistent with oocyte differentiation, though granulosa cells only undergo limited proliferation and theca cells fail to assemble around the follicle (Carabatsos *et al*., 1998; Elvin *et al*., 1999b). The oocytes of these defective follicles eventually degenerate. These observations, along with other studies, suggest that GDF-9 is important for both granulosa cell proliferation and theca cell recruitment (Vitt *et al*., 2000; Gilchrist *et al*., 2004). Based on the accelerated growth rate of oocytes in GDF-9-deficient animals, it has also been postulated that GDF-9 may participate in a negative feedback pathway to temper oocyte growth (Combelles and Albertini, 2003). While the mechanism by which GDF-9 exerts this effect is yet to be elucidated, there is some evidence to suggest that GDF-9 may restrain oocyte growth by down-regulating granulosa cell expression of the oocyte growth factor kit ligand (KitL) (Joyce *et al*., 2000). This work emphasizes the necessity for a complex intra-follicular signalling pathway at the oocyte–granulosa cell interface. The physiological and structural determinants that mediate the dialogue remain to be fully established.

BMP-15 is a sequence homologue of GDF-9 that is expressed by oocytes throughout folliculogenesis. In sheep that are homozygous for inactivating mutations in the *bmp-15* gene, follicles fail to develop beyond the primary stage and oocytes are soon lost from within activated follicles, though granulosa cells remain in an empty cluster (Braw-Tal *et al*., 1993; Galloway *et al*., 2000, Hanrahan *et al*., 2004). Additionally, Juengel *et al*. (2002) have shown using in-vivo immunoneutralization studies that both GDF-9 and BMP-15 are essential for follicle development in sheep. Collectively, these observations suggest that BMP-15 is required for follicle progression, granulosa cell proliferation and for their sensitivity to apoptosis in this species. Interestingly, inactivating mutations in the *bmp-15* gene of mice has little effect on the progression of folliculogenesis, suggesting that the relative importance of BMP-15 during early folliculogenesis varies between species. Though BMP-15 may not be required for the earliest stages of follicle development in mice, important roles for BMP-15 have been established for this molecule in this species. Such roles include the promotion of granulosa cell proliferation and modification of granulosa cell differentiation by suppressing FSH receptor expression. In contrast to GDF-9, BMP-15 stimulates KitL expression, while KitL down-regulates BMP-15 expression, creating a paracrine negative feedback loop between granulosa cells and the oocyte. Otsuka and Shimasaki found that signalling via the KitL receptor Kit, located on oolemma, is important, though not essential, for BMP-15 mediated granulosa cell mitosis *in vitro* (Otsuka and Shimasaki, 2002). Studies in mice have also shown that the addition of partly grown oocytes to granulosa cell cultures increases KitL expression, while fully grown oocytes suppress granulosa cell KitL production, and this activity is thought to be regulated by temporal changes in the relative oocyte expression levels of BMP-15 and GDF-9 (Joyce *et al*., 1999, 2000). While little is known about the role of BMP-15 during human folliculogenesis, one recent study has linked a mutation in the *bmp-15* gene with ovarian failure in women (Di Pasquale *et al*., 2004). Thus, it seems likely that that BMP-15 is highly important for human fertility.

In addition to the local production of soluble factors that act in an autocrine and paracrine fashion during pre-antral follicle development, oocytes communicate with and modify their surroundings via direct physical contacts with granulosa cells (Hertig and Adams, 1967; Anderson and Albertini, 1976; Albertini and Barrett, 2003; Albertini, 2004). Though a variety of different somatic cells can support oocyte survival, only granulosa cells provide the appropriate heterocellular interaction required for the generation of a fully mature and developmentally competent oocyte (Cecconi and Colonna, 1996). The foundation of this relationship lies partly in highly specialized oocyte–somatic cell contacts called trans-zonal projections (TZP) that are established at the onset of folliculogenesis and which are dynamically modified throughout the course of follicular development (Albertini and Anderson, 1974; Anderson and Albertini, 1976). TZP are cytoplasmic processes comprised of microtubules, microfilaments and intermediate filaments that extend from granulosa cells and pass through the zona pellucida to directly interact with the oolemma (**Figure 2**) (Suzuki *et al*., 2000; Albertini *et al*., 2001). TZP mediate gap junction-based communication that facilitates the transport of nutrients and small molecules, such as biosynthetic substrates and meiosis-arresting signals, between the cytoplasm of the granulosa cell and oocyte (Biggers *et al*., 1967; Donahue and Stern, 1968; Haghighat and Van Winkle, 1990). The importance of this interaction is emphasized by studies demonstrating that deletion of the gene for the oocyte-specific gap junctional subunit Cx37 causes female sterility associated with a failure in follicle development at the pre-antral–antral transition (Simon *et al*., 1997; Carabatsos *et al*., 2000).

Adhesion junctions involving anchoring integral membrane proteins located at the interface of the oocyte and granulosa cells facilitate the prolonged interaction of these two cell types. Adhesive contact sites such as these may serve as active signalling domains for the interaction of receptor kinases with growth factors and it has been proposed that TZP enable the processing, activation and delivery of certain oocyte- and granulosa cell-derived paracrine factors, such as GDF-9 and KitL respectively, to the appropriate receptor targets (Fagotto and Gumbiner, 1996; Albertini *et al*., 2001). Whether oocyte-derived factors in turn regulate TZP structure and stability

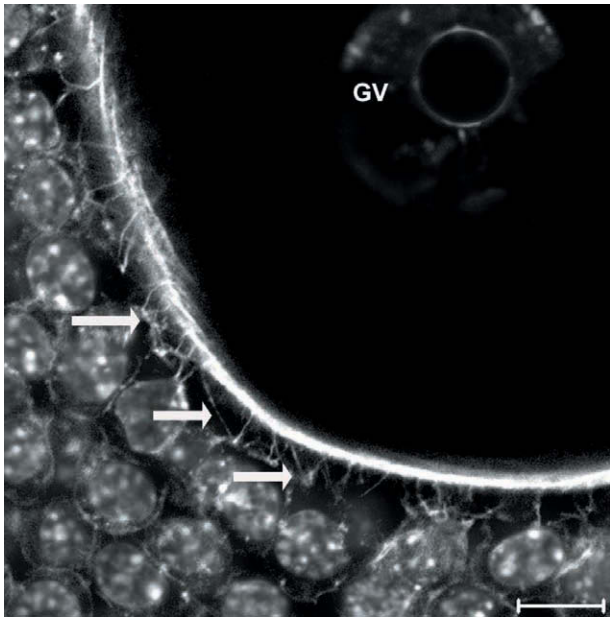

Figure 2. Confocal micrograph depicting trans-zonal projections (arrows) emanating from cumulus cells and contacting the oolemma of a germinal vesicle (GV)-stage oocyte. Scale bar represents 10 μm.

during pre-antral follicle development is yet to be determined. However, the observation that follicles from GDF-9 deficient mice have reduced TZP density and altered structural integrity suggests that GDF-9 is important for maintaining appropriate interactions between somatic cells and the oocyte (Carabatsos *et al*., 1998).

Antral and pre-ovulatory follicle development

Whereas the pre-antral phase of folliculogenesis is primarily reliant on the supply of local growth factors, follicles become dependent on the cyclical secretion of pituitary hormones, FSH and LH, for progression through the antral and pre-ovulatory phases of development. Progression through antral follicle development is promoted by FSH and coincides with cessation of oocyte growth, acquisition of competence to complete meiosis, continued granulosa cell proliferation and the differentiation of cumulus and mural granulosa cells upon formation of the follicular antrum. The phenotypic differentiation of these two granulosa cell populations is regulated by the oocyte, which suppresses the expression of the LH receptor and promotes the cumulus cell phenotype in those cells closely associated with it (murine: Eppig *et al*., 1997a; bovine: Li *et al*., 2000). Fully grown oocytes also induce cumulus cells to provide them with metabolic support. For instance, oocytes secrete factors that promote cumulus cell uptake of amino acids that are then transported to oocytes via gap junctions (Eppig *et al*., 2005).

FSH is a key regulator of oocyte–cumulus cell interactions during antral follicular development and the density and organization of the highly dynamic TZP network changes dramatically in response to this stimulus. Studies in which FSH was injected into mutant mice unable to produce functional endogenous FSH demonstrate that FSH causes TZP retraction coincident with changes in oocyte transcriptional activity and acquisition of meiotic competence (Combelles *et al*., 2004). Moreover, the spatial and temporal regulation of TZP by FSH appears to be crucially important for the production of oocytes of high quality (Combelles *et al*., 2004).

The final phase of folliculogenesis is precipitated by a surge of LH that results in the generation of a metaphase II-arrested oocyte capable of being fertilized and able to support embryonic development. Pre-ovulatory follicle development involves resumption of meiosis, cytoplasmic maturation, the termination of TZP-mediated gap junction communication and cumulus cell expansion (Eppig *et al*., 1993). The oocyte continues to direct the fate of the follicle by producing factors that regulate the production of steroids (Vanderhyden *et al*., 1993), plasminogen activator (Canipari *et al*. 1995), LH receptors (Eppig *et al*., 1997b), hyaluronic acid (Tirone *et al*., 1997), cumulus expansion (Eppig *et al*., 1993) and cumulus survival (Hussein *et al*., 2005). The oocyte also produces factors that inhibit luteinization of mural granulosa cells until it is released at ovulation, by suppressing progesterone production and enhancing granulosa cell proliferation (Shimasaki *et al*., 1999; Otsuka *et al*., 2001; Gilchrist *et al*., 2004; McNatty *et al*., 2005). Many of these activities are supported by GDF-9 and BMP-15 *in vitro*. Moreover, the differential expression of GDF-9 and BMP-15 has been implicated in the selective sensitization of follicles to FSH, which ultimately leads to selection of the dominant follicle (Otsuka *et al*., 2001).

The relationship between oocyte and embryo quality

Thus far, the two essential processes required to produce a developmentally competent oocyte have been discussed: the vegetative or growth phase of oogenesis and the maturative phase of oogenesis. While classic studies referred to the

generative or proliferative phase anteceding the growth phase of oogenesis, more and more emphasis has been placed on the final peri-ovulatory maturation of the oocyte for several reasons, as summarized in **Figure 3**. First, achieving a state of maturity that allows the oocyte to complete maturation of both the cytoplasm and the nucleus engenders a complex and rather poorly understood series of modifications in the bidirectional signalling that occurs between the oocyte and the surrounding cumulus oophorus (Eppig , 1991). Thus, even though meiotic competence is known to arise at earlier stages of follicle development in most mammalian species, acquisition of embryonic competence appears to involve metabolic, molecular, and structural changes prior to and following the LH surge (Eppig *et al*., 1994). This basic principle, of course, guides all assisted reproduction programmes by attempting to elicit maturation through ovulation induction prior to egg retrieval and presumably recapitulates the cascade of signalling events that lead to the production of a developmentally competent ovum (**Figure 3**). Second, the longstanding observation that oocytes undergo spontaneous resumption and completion of meiosis upon removal from the Graafian follicle has reinforced the importance of the timing of meiotic progression in oocytes matured under in-vivo or in-vitro conditions (Sanfins *et al*., 2004). This fact again illustrates the importance of co-ordinating the final stages of oocyte and follicle maturation and in recent years has instructed investigators seeking to optimize conditions for the use of in-vitro maturation in clinical and experimental settings, particularly as related to both the time and extent of gonadotrophin exposure (Combelles *et al*., 2002). Finally, evidence is accumulating to suggest that factors synthesized, processed and secreted by oocytes within the cumulus impact on the developmental potential of zygotes (Hussein *et al*., 2006). Whether these factors modify the zona and enhance binding to the pericellular moieties associated with the oocyte and preimplantation conceptus is at present unknown. It is clear, however, that many of the practices commonly used in human assisted reproductive technologies modify cumulus cell and/or zona integrity directly and therefore alter the immediate microenvironment of the oocyte normally deployed to co-ordinate the early events of development. In this context, and as discussed below, maintaining and/or re-establishing this microenvironment will be essential in the design and implementation of human oocyte in-vitro maturation and cryopreservation protocols.

Expanding the repertoire of assisted reproductive technologies into the realm of ovarian grafting, follicle culture, in-vitro maturation and oocyte cryopreservation has necessitated refocusing experimental approaches to the interface between oocytes and granulosa cells both before and during ovulation. For example, while oocyte cryopreservation strategies have generally been restricted to the use of mature metaphase II oocytes, efforts to restore functional interactions between cumulus and oocytes, especially in attempts to achieve cryopreservation of

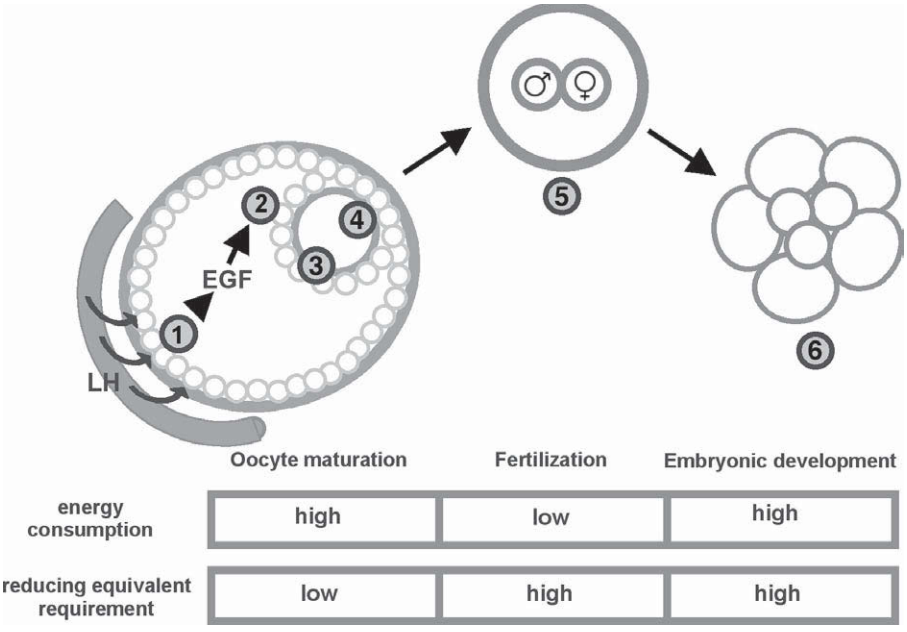

Figure 3. Peri-ovulatory signal transduction events culminate in oocyte maturation, fertilization and embryogenesis in rodents. (1) Circulating LH induces a cascade of gene expression in mural granulosa cells, including the expression of epidermal growth factor (EGF)-like ligands, which act on cumulus granulosa cells (2). Cumulus cells in turn modify their physical interactions with the oocyte and secrete factors that promote nuclear and cytoplasmic maturation of the oocyte (3). (4) The oocyte resumes meiosis and accumulates cytoplasmic factors required to support pronuclear (5) and early embryonic development (6). Oocyte cytoplasmic factors known to be important for embryonic development include energy stores in the form of ATP and reducing equivalents of glutathione for protection from oxidative stress; note the divergent patterns of resource utilization.

immature germinal vesicle-stage oocytes, will require a better understanding of the cryobiological properties of both cell types. The consequences of cryoprotectants and cooling on the metabolic capacity of both cell types is another area that demands the deployment of new molecular and cell biological strategies so that impacts on specific targets such as microtubules and mitochondria can be established prior to and following recovery from freezing. The effects that these manipulations have on the connections between oocyte and granulosa cells remain completely unstudied (Younis *et al*., 1996).

With respect to the specific case of in-vitro maturation, there is a growing sense that upon removal from the follicle, a rapid and irreversible modification in TZP occurs that would be likely to affect developmental competence of oocytes subjected to culture even in the presence of the cumulus (Moor *et al*., 1998). For example, oocyte-specific factors, as alluded to above, are now recognized to mediate cumulus expansion and deposition of the hyaluronate matrix. The impact of BMP-15 and GDF-9 may then be mutually beneficial, as inducers of cumulus differentiation and embryo modifiers after fertilization has taken place, if their lifespan and restricted diffusion are involved with early signalling events. Certainly the implementation of volume-restrictive and nanotechnology-based microfluidics will be essential for future developments in this field should such parameters be associated with enhancements in oocyte and embryo quality.

Concluding remarks

In conclusion, by taking an oocentric view of folliculogenesis and embryogenesis, the realization cannot be avoided that advances in understanding of the interface established between the somatic and germ cells of the ovary are required for the future development of human assisted reproductive technologies. Use of both animal and human models, in conjunction with evolving technologies, will lead to the wide scale application of in-vitro maturation and cryopreservation methodologies. In the end, the benefits afforded to the reproductive health status of patients, who need to protect their germ plasm at a young age in order to bear children after undergoing fertility-threatening disease conditions and treatments, will be a laudable goal and one that may also reduce the risks of multiple gestations following the more conventional use of assisted reproductive technologies.

Acknowledgements

DA was supported by ESHE Fund, Hall Family Foundation and NIH HD 42076. KH was supported by KUMC Biomedical Research Training Grant.

References

Albertini DF 2004 Oocyte-Granulosa Cell Interactions. In: Van Blerkom J and Gregory L (eds) *Essential IVF: Basic Research and Cinical Applications*. Kluwer Academic Publishers, USA, pp. 43–58.

Albertini DF, Anderson E 1974 The appearance and structure of intercellular connections during the ontogeny of the rabbit ovarian follicle with particular reference to gap junctions. *Journal of Cell Science* **63**, 234–250.

Albertini DF, Barrett SL 2003 Oocyte–somatic cell communication. *Reproduction Supplement* **61**, 49–54.

Albertini DF, Combelles CM, Benecchi E *et al*. 2001 Cellular basis for paracrine regulation of ovarian follicle development. *Reproduction* **121**, 647–653.

Anderson E 1974 Comparative aspects of the ultrastructure of the female gamete. *International Review of Cytology* **Supplement 4**, 1–70.

Anderson E, Albertini DF 1976 Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. *Journal of Cell Biology* **71**, 680–686.

Biggers JD, Whittingham DG, Donahue RP 1967 The pattern of energy metabolism in the mouse oocyte and zygote. *Proceedings of the National Academy of Sciences of the United States of America* **58**, 560–567.

Braw-Tal R, McNatty KP, Smith P *et al*. 1993 Ovaries of ewes homozygous for the X-linked Inverdale gene (*FecXI*) are devoid of secondary and tertiary follicles but contain many abnormal structures. *Biology of Reproduction* **49**, 895–907.

Brower PT, Schultz RM 1982 Intercellular communication between granulosa cells and mouse oocytes: existence and possible nutritional role during oocyte growth. *Developmental Biology* **90**, 144–153.

Buccione R, Schroeder AC, Eppig JJ 1990 Interactions between somatic cells and germ cells throughout mammalian oogenesis. *Biology of Reproduction* **43**, 543–547.

Canipari R, Epifano O, Siracusa G *et al*. 1995 Mouse oocytes inhibit plasminogen activator production by ovarian cumulus and granulosa cells. *Developmental Biology* **167**, 371–378.

Carabatsos MJ, Sellitto C, Goodenough DA *et al*. 2000 Oocyte–granulosa cell heterologous gap junctions are required for the coordination of nuclear and cytoplasmic meiotic competence. *Developmental Biology* **226**, 167–179.

Carabatsos MJ, Elvin J, Matzuk MM *et al*. 1998 Characterization of oocyte and follicle development in growth differentiation factor-9-deficient mice. *Developmental Biology* **204**, 373–384.

Cecconi S, Colonna R 1996 Influence of granulosa cells and of different somatic cell types on mammalian oocyte development *in vitro*. *Zygote* **4**, 305–307.

Combelles CM, Albertini DF 2003 Assessment of oocyte quality following repeated gonadotropin stimulation in the mouse *Biology of Reproduction* **68**, 812–821.

Combelles CM, Carabatsos MJ, Kumar TR *et al*. 2004 Hormonal control of somatic cell oocyte interactions during ovarian follicle development. *Molecular Reproduction and Development* **69**, 347–355.

Combelles CM, Cekleniak NA, Racowsky C *et al*. 2002 Assessment of nuclear and cytoplasmic maturation in in-vitro matured human oocytes. *Human Reproduction* **17**, 1006–1016.

Di Pasquale E, Beck-Peccoz P, Persani L 2004 Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (*BMP15*) gene. *American Journal of Human Genetics* **75**, 106–111.

Donahue RP, Stern S 1968 Follicular cell support of oocyte maturation: production of pyruvate *in vitro*. *Journal of Reproduction and Fertility* **17**, 395–398.

Dong J, Albertini DF, Nishimori K *et al*. 1996 Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* **383**, 531–535.

Elvin JA, Clark AT, Wang P *et al*. 1999a Paracrine actions of growth differentiation factor-9 in the mammalian ovary. *Molecular Endocrinology* **13**, 1035–1048.

Elvin JA, Yan C, Wang P *et al*. 1999b Molecular characterization of the follicle defects in the growth differentiation factor 9-deficient ovary. *Molecular Endocrinology* **13**, 1018–1034.

Eppig JJ 1991 Maintenance of meiotic arrest and the induction of oocyte maturation in mouse oocyte-granulosa cell complexes developed *in vitro* from preantral follicles. *Biology of Reproduction* **45**, 824–830.

Eppig JJ, Pendola FL, Wigglesworth K *et al*. 2005 Mouse oocytes regulate metabolic co-operativity between granulosa cells and oocytes: amino acid transport. *Biology of Reproduction* **73**, 351–357.

Eppig JJ, Wigglesworth K, Pendola FL 2002 The mammalian oocyte orchestrates the rate of ovarian follicular development. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 2890–2894.

Eppig JJ, Wigglesworth K, Pendola F, Hirao Y 1997a Murine oocytes suppress expression of luteinizing hormone receptor messenger ribonucleic acid by granulosa cells *Biology of Reproduction* **56**, 976–984.

Eppig JJ, Chesnel F, Hirao Y *et al*. 1997b Oocyte control of granulosa cell development: how and why. *Human Reproduction* **12**, 127–132.

Eppig JJ, Schultz RM, O'Brien M *et al*. 1994 Relationship between the developmental programs controlling nuclear and cytoplasmic maturation of mouse oocytes. *Developmental Biology* **164**, 1–9.

Eppig JJ, Wigglesworth K, Chesnel F 1993 Secretion of cumulus expansion enabling factor by mouse oocytes: relationship to oocyte growth and competence to resume meiosis. *Developmental Biology* **158**, 400–409.

Fagotto F, Gumbiner BM 1996 Cell contact-dependent signaling. *Developmental Biology* **180**, 445–454.

Galloway SM, McNatty KP, Cambridge LM *et al*. 2000 Mutations in an oocyte-derived growth factor gene (*BMP15*) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature Genetics* **25**, 279–283.

Gilchrist RB, Ritter LJ, Cranfield M *et al*. 2004 Immunoneutralization of growth differentiation factor 9 reveals it partially accounts for mouse oocyte mitogenic activity. *Biology of Reproduction* **71**, 732–739.

Haghighat N, Van Winkle LJ 1990 Developmental change in follicular cell-enhanced amino acid uptake into mouse oocytes that depends on intact gap junctions and transport system Gly. *Journal of Experimental Zoology* **253**, 71–82.

Halpin DM, Charlton HM, Faddy MJ 1986 Effects of gonadotrophin deficiency on follicular development in hypogonadal (hpg) mice. *Journal of Reproduction and Fertility* **78**, 119–125.

Hanrahan JP, Gregan SM, Mulsant P *et al*. 2004 Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biology of Reproduction* **70**, 900–909.

Hertig AT, Adams EC 1967 Studies on the human oocyte and its follicle. I. Ultrastructural and histochemical observations on the primordial follicle stage. *Journal of Cell Biology* **34**, 647–675.

Hussein TS, Thompson JG, Gilchrist RB 2006 Oocyte-secreted factors enhance oocyte developmental competence. *Developmental Biology* **296**, 514–521.

Hussein TS, Froiland DA, Amato F *et al*. RB 2005 Oocytes prevent cumulus cell apoptosis by maintaining a morphogenic paracrine gradient of bone morphogenetic proteins. *Journal of Cell Science* **118**, 5257–5268.

Joyce IM, Clark AT, Pendola FL *et al*. 2000 Comparison of recombinant growth differentiation factor-9 and oocyte regulation of KIT ligand messenger ribonucleic acid expression in mouse ovarian follicles. *Biology of Reproduction* **63**, 1669–1675.

Joyce IM, Pendola FL, Wigglesworth K *et al*. 1999 Oocyte regulation of kit ligand expression in mouse ovarian follicles. *Developmental Biology* **214**, 342–453.

Juengel JL, Hudson NL, Heath DA *et al*. KP 2002 Growth differentiation factor 9 and bone morphogenetic protein 15 are essential for ovarian follicular development in sheep. *Biology of Reproduction* **67**, 1777–1789.

Kumar TR, Wang Y, Lu N *et al*. 1997 Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. *Nature Genetics* **15**, 201–204.

Li R, Norman RJ, Armstrong DT, Gilchrist RB 2000 Oocyte-secreted factor(s) determine functional differences between bovine mural granulosa cells and cumulus cells. *Biology of Reproduction* **63**, 839–845.

McNatty KP, Juengel JL, Reader KL *et al*. 2005 Bone morphogenetic protein 15 and growth differentiation factor 9 co-operate to regulate granulosa cell function. *Reproduction* **129**, 473–480.

Moor RM, Dai Y, Lee C *et al*. 1998 Oocyte maturation and embryonic failure. *Human Reproduction Update* **4**, 223–236.

Otsuka F, Shimasaki S 2002 A negative feedback system between oocyte bone morphogenetic protein 15 and granulosa cell kit ligand: its role in regulating granulosa cell mitosis. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 8060–8065.

Otsuka F, Yamamoto S, Erickson GF *et al*. 2001 Bone morphogenetic protein-15 inhibits follicle-stimulating hormone (FSH) action by suppressing FSH receptor expression. *Journal of Biological Chemistry* **276**, 11387–11392.

Pedersen T 1969 Follicle growth in the immature mouse ovary. *Acta Endocrinologica (Copenh)* **62**, 117–132.

Salustri A, Ulisse S, Yanagishita M *et al*. 1990 Hyaluronic acid synthesis by mural granulosa cells and cumulus cells *in vitro* is selectively stimulated by a factor produced by oocytes and by transforming growth factor-beta. *Journal of Biological Chemistry* **265**, 19517–19523.

Sanfins A, Plancha CE, Overstrom EW *et al*. 2004 Meiotic spindle morphogenesis in in-vivo and in-vitro matured mouse oocytes: insights into the relationship between nuclear and cytoplasmic quality. *Human Reproduction* **19**, 2889–2899.

Shimasaki S, Zachow RJ, Li D *et al*. 1999 A functional bone morphogenetic protein system in the ovary. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 7282–7287.

Simon AM, Goodenough DA, Li E *et al*. 1997 Female infertility in mice lacking connexin 37. *Nature* **385**, 525–529.

Suzuki H, Jeong BS, Yang X 2000 Dynamic changes of cumulus–oocyte cell communication during in-vitro maturation of porcine oocytes. *Biology of Reproduction* **63**, 723–729.

Tirone E, D'Alessandris C, Hascall VC *et al*. A 1997 Hyaluronan synthesis by mouse cumulus cells is regulated by interactions between follicle-stimulating hormone (or epidermal growth factor) and a soluble oocyte factor (or transforming growth factor beta1). *Journal of Biological Chemistry* **272**, 4787–4794.

Vanderhyden BC, Cohen JN, Morley P 1993 Mouse oocytes regulate granulosa cell steroidogenesis. *Endocrinology* **133**, 423–426.

Vitt UA, McGee EA, Hayashi M *et al*. 2000 In-vivo treatment with GDF-9 stimulates primordial and primary follicle progression and theca cell marker CYP17 in ovaries of immature rats. *Endocrinology* **141**, 3814–3820.

Younis AI, Toner M, Albertini DF *et al*. 1996 Cryobiology of non-human primate oocytes. *Human Reproduction* **11**, 156–165.

Paper based on contribution presented at the Tecnobios Procreazione Symposium 2006 and 2nd International Conference on the Cryopreservation of the Human Oocyte in Bologna, Italy, 5–7 October 2006.

Received 1 February 2007; refereed 15 March 2007; accepted 11 April 2007.