news and views

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Channels of communication in the ovary

In mammals, unfertilized eggs and corpora lutea originate from Graafian follicles in the ovary. Two recent reports support the view that to produce Graafian follicles, oocytes and surrounding granulosa cells must communicate with each other. However, there is evidence to suggest that oocyte development can take place in the absence of granulosa cells.

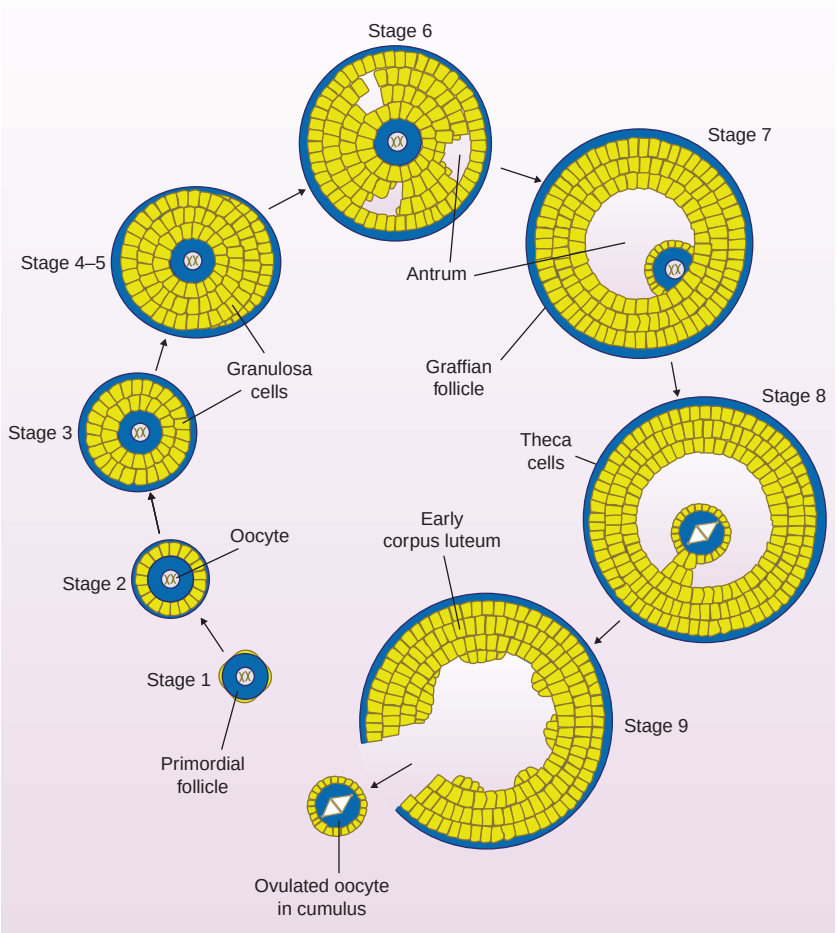

Figure 1 **Stages of follicular growth in mammals.** Follicular growth begins with stage-1 primordial follicles in the ovary, which consist of a non-growing oocyte surrounded by a few epithelial-like somatic cells. As growth is initiated in stage-2 follicles, the somatic cells or granulosa cells become cuboidal. During stages 3–5, the granulosa cells proliferate while the oocyte continues to increase in diameter and lays down a zona pellucida. In stage 6, a fluid-filled cavity (the antrum) begins to form and by stage 8 the antrum is complete. Surrounded by a thick zona pellucida, the fully-grown oocyte sits at the end of a stalk of granulosa cells, which is in turn surrounded by several layers of cumulus cells. At stage 9, the oocyte, which has arrested at metaphase II of meiosis, is ovulated into the oviduct. The follicle that is left behind becomes a corpus luteum. In mice, it takes 2–3 weeks for this developmental process to be completed. This figure is adapted from ref. 11 with permission from Cambridge University Press.

The Graafian follicle of the mammalian ovary is a thing of beauty. It consists of two cellular compartments (Fig. 1). At the centre of the follicle is a fully grown oocyte arrested in diplotene of the first meiotic prophase. The oocyte is positioned at the end of a stalk of granulosa cells surrounded by several layers of cumulus cells and a large, fluid-filled cavity (the antrum). Lining the wall of the mature follicle are

tens-of-thousands of additional granulosa cells (mural granulosa cells) arrayed in many layers. In mice, a fully developed follicle (~600 μm in diameter), consists of one oocyte (~80 μm in diameter) and ~60,000 granulosa cells. At ovulation, in response to hormones, the oocyte is expelled from the Graafian follicle. At this stage, the oocyte (still surrounded by cumulus cells) moves into the oviduct and becomes an unfertilized egg arrested at metaphase II. Simultaneously, the group of somatic follicular cells left behind becomes an endocrine gland, the corpus luteum, prepared to support pregnancy after implantation of an embryo (expanded blastocyst) into the uterus.

Of particular significance was the identification of gap junctions between growing oocytes and the surrounding innermost cumulus cells…

In females, the production of unfertilized eggs and corpora lutea from primordial follicles is vital for propagation of mammalian species. Unfertilized eggs develop from non-growing oocytes present in primordial follicles in the ovary at birth (Fig. 1). By not dividing, growing oocytes increase markedly in size and simultaneously become competent to undergo the first meiotic reductive division (that is, meiotic maturation). Corpora lutea develop from just the few epithelial-like somatic cells that surround each non-growing oocyte within primordial follicles. The few somatic cells that encompass oocytes undergo both differentiation and marked proliferation (mitotic divisions) until tens-of-thousands are found in Graafian follicles. One important question concerns what factors regulate these vital events.

For several decades, researchers have investigated potential relationships between mammalian oocytes and somatic follicular cells during the development of Graafian follicles[1,2]. Of particular significance was the identification of gap junctions between growing oocytes and the surrounding innermost cumulus cells, as well as between all other granulosa cells. The narrow channels (pore size, ~15 Å) present at these junctions, which permit the passage of relatively small molecules (relative molecular mass (M_r) <1,000; for example, amino acids, nucleotides, metabolites and cyclic AMP), could provide routes of intercommunication between all cells of the developing follicle. This means that the ovarian follicle could represent a functional syncytium. In fact, female mice that are homozygous null for either connexin-37 (a protein associated with gap junctions between oocytes and innermost cumulus cells) or connexin-43 (a protein associated with gap junctions between granulosa cells) are deficient in both multilayered follicles and the normal growing oocyte, and are infertile[3,4].

In addition to gap junctions, several other factors have been identified that significantly influence development of the Graafian follicle[2]. Among these are members of the transforming growth factor-β (TGF-β) superfamily, such as growth differentiation factor-9 (GDF-9), bone morphogenetic factor-15 (BMP-15), and the receptor kinase c-Kit and its ligand, stem cell factor (SCF). For example, in female mice that are homozygous nulls for GDF-9 (a product of oocytes in wild-type mice), follicular development beyond the one-layer follicle stage fails to take place and the animals are infertile[5]. Interestingly, oocytes present in these null mice undergo growth and chromatin remodelling consistent with both normal oocyte development and the acquisition of meiotic competence[6].

Two recent studies present new evidence that neighbouring cell types, oocytes and granulosa cells may indeed communicate with each other, perhaps through gap junctions, and as a result, may affect each others development. Eppig *et al.* have used reaggregated oocyte-follicular cell complexes to determine whether growing oocytes influence the rate of follicular development[7]. To perform these experiments, oocytes at the mid-stage of growth (~50 μm in diameter) were isolated from 12-day-old mice and mixed with ovarian somatic cells obtained from newborn mice (that is, from primordial follicles). The reconstituted cellular complexes were then implanted beneath the renal capsule of ovariectomized host females. As a control, non-growing oocytes (~12 μm in diameter) from newborn mice were combined with ovarian somatic cells (also from newborn mice) and implanted in an identical manner.

In mice, under normal conditions, it takes approximately 21 days after birth for some of the pool of primordial follicles to develop into Graafian follicles. In their experiments, Eppig *et al.* asked whether introducing mid-growth stage oocytes to somatic cells from primordial follicles would enhance the rate of follicle development. Remarkably, it did. Only nine days after the complexes were implanted, they had primarily become large antral follicles. This is in contrast to control complexes containing non-growing oocytes and somatic cells from newborn mice, which took 19–20 days to exhibit large antral follicles (the normal timetable for antral follicle development). The increased rate of follicle development caused by mid-growth stage oocytes was accompanied by several other changes normally associated with the production of antral follicles. The granulosa cells lining the follicular wall expressed receptors for luteinizing hormone (LH), cumulus cells surrounding the oocyte underwent mucification in response to follicle stimulating hormone (FSH) and a large proportion of oocytes released from the antral follicles resumed meiosis *in vitro*. Furthermore, more than half of the oocytes could be fertilized and cleaved to the two-cell stage, and many completed preimplantation development to the blastocyst stage. Collectively, these results demonstrate that mid-growth stage oocytes accelerate follicular development. This is attributable to a developmental programme intrinsic to the oocyte. In other words, if there is a 'folliculogenesis clock', it is apparently set by the oocyte[2].

In the second study, Klinger and De Felici employed a multistep *in vitro* culture system to examine factors involved in the initiation and maintenance of mouse oocyte growth and the acquisition of meiotic competence[8]. In their experiments, the investigators used non-growing oocytes recovered from the primordial follicles of embryos (15.5–16.5 days after fertilization) and granulosa cells from pre-antral follicles of ovaries (10–12 days old). When non-growing oocytes were cultured for 4 days in the absence of follicle cells but in the presence of SCF, the diameter of the oocytes increased from 10 ± 2 μm (–SCF) to 19 ± 2 μm (+SCF). The oocytes failed to increase further in size after day 4 of culture. However, when these oocytes were seeded onto granulosa cells under conditions known to result in the formation of functional gap junctions between oocytes and granulosa cells, they underwent growth, increasing to 42 ± 4.5 μm in diameter after three additional days of culture. These growing oocytes exhibited a thin zona pellucida, the extracellular coat characteristic of growing oocytes in ovaries. It was noted that reseeding of these oocytes on fresh granulosa cell monolayers in the absence of SCF resulted in a resumption of growth to a mean diameter of 50 ± 2.5 μm after a few days of culture. The authors conclude that there are three stages to mouse oocyte growth: an initial stage induced by SCF and independent of gap junctions with follicle cells; a second stage that is dependent on both SCF and follicle cell contacts; and a third stage independent of SCF and dependent on follicle cell contacts.

Results reported by Zamboni and Upadhyay twenty years ago[9,10] are of considerable interest in connection with the two studies mentioned above. These investigators used light and electron microscopy to examine the morphology of mouse germ cells located in adrenal glands, an ectopic site outside the gonads. Apparently, migration of primordial germ cells to different organs, rather than to the genital ridges, is not unusual in mice. Surprisingly, the cells progressed from primordial germ cells to mitotic oogonia (day-14 and -15) and from oogonia to non-growing, meiotic oocytes (day-17 onwards) in both male and female foetuses. In doing so, the ectopic germ cells followed the same timetable of meiotic events that are characteristic of ovarian germ cells. What happened next in postnatal animals was even more provocative. In the complete absence of follicle cells, non-growing oocytes in the adrenal glands began to grow, and in 14–21-day-old animals they resembled oocytes that are normally associated with large antral follicles; this included the presence of a zona pellucida around the oocytes and appropriate changes in cellular organelles. Again, the growing oocytes followed a timetable characteristic of ovarian oocytes. It was noted that oocytes disappeared from the adrenal glands of animals older than 21 days. These observations strongly suggest that in the presence of foreign somatic cells of the adrenal cortex and medulla, oocytes can undergo normal growth and other changes that are usually associated with ovarian oocytes.

There is considerable evidence that growing oocytes affect the differentiation and proliferation of somatic follicular cells. However, it is tempting to suggest that oocyte growth, perhaps accompanied by the acquisition of meiotic competence, may not be wholly dependent on the nature of the surrounding somatic cells. A variety of other cell types, such as cells of the adrenal cortex and medulla, may also be able to support oocyte growth. For example, it would be interesting to examine whether cells, other than granulosa cells, could provide a feeder layer in stages 2 and 3 of oocyte growth, as described by Klinger and DeFelici[8]. In this context, Zamboni and Upadhyay found that adrenal cells surrounding oocytes were in intimate contact with oocytes, creating an arrangement reminiscent of unilaminar ovarian follicles[9,10]. Indeed, growing oocytes may depend on channels of communication with surrounding somatic cells, but they may not require bona fide granulosa cells as their partners. ❑

1. Pedersen, T. in *Oogenesis* (eds, Biggers, J. D. & Schuetz, A. W.) 361–376 (University Park Press, Baltimore, 1972).
2. Matzuk, M. M., Burns, K. H., Viveiros, M. M. & Eppig, J. J. *Science* **296,** 2178–2180 (2002).
3. Simon, A. M., Goodenough, D. A., Li, B. Y. & Paul, D. L. *Nature* **385,** 525–529 (1997).
4. Ackert, C. L., Gittens, J. E., O'Brien, M. J., Eppig, J. J. & Kidder, G. M. *Dev. Biol.* **233,** 258–270 (2001).
5. Dong, J., Albertini, D. F., Nishimori, K., Kumar, T. R., Lu, N. & Matzuk, M. *Nature* **383,** 531–535 (1996).
6. Carabatsos, M. J., Elvin, J., Matzuk, M. M. & Albertini, D. F. *Dev. Biol.* **204,** 373–384 (1998).
7. Eppig, J. J., Wigglesworth, K. & Pendola, F. L. *Proc. Natl Acad. Sci. USA* **99,** 2890–2894 (2002).
8. Klinger, F. G. & De Felici, M. *Dev. Biol.* **244,** 85–95 (2002).
9. Upadhyay, S. & Zamboni, L. *Proc. Natl Acad. Sci. USA* **79,** 6584–6588 (1982).
10. Zamboni, L. & Upadhyay, S. *J. Expl. Zool.* **228,** 173–193 (1983).
11. Baker, T. G. in *Reproduction in mammals: 1 Germ cells and fertilization* (eds Austin, C. R. & Short, R. V.) 17–45 (Cambridge University Press, Cambridge, 1982).