Portrait of an oocyte: our obscure origin

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Oocytes play a pivotal role in the cycle of human life. As we discuss here, after emerging from germline stem cells in the fetus, they grow in a follicular niche in which development is harmonized for timely ovulation and hormone secretion after puberty. Most human oocytes have poor developmental competence and are peculiarly vulnerable to chromosomal malsegregation, especially as women pass the optimal years of fertility and may begin to turn to assisted reproductive technologies (ARTs) and egg donation. Research needs to focus on the molecular factors involved and the environmental niche required for optimal development of oocytes, with the aim of increasing their numbers and quality for ARTs, since these are the factors that so often limit human fertility.

Eggs have fascinated philosophers and scientists ever since Aristotle pondered the mysteries of chick development, but the minute mammalian egg remained elusive until von Baer's discovery in 1826 (1). Subsequently, the creation of haploid gametes through meiosis and their union by fertilization were observed and understood by successive generations of biologists (2). While the spermatozoon makes a complementary genetic contribution to the zygote, the oocyte is obviously its major cytoplasmic donor, contributing nearly all the organelles and nonchromosomal molecules needed for development. Although fertilization marks the debut of a new genetic entity, it is abundantly clear that embryogenesis is deeply rooted in oogenesis.

Oocytes are highly specialized to undergo the unique processes of meiosis and fertilization and to execute a molecular program for development. Upon fertilization, their reproductive role is achieved through formation of blastomeres, the cells produced by the first cleavage divisions of the fertilized oocyte that are the precursors of all cell lineages in the fetus and its membranes. Oocytes are therefore highly differentiated and the mothers of totipotent cells at the same time, a paradox we address later (see *Molecular program for development* below). But it is not just their amazing biology that drives scientific curiosity — they set limits on the reproductive lifespan of a woman, are very common causes of infertility, and contribute to major birth defects, including Down syndrome. Unfortunately, research is hindered by their extreme rarity and by greater bioethical constraints than with any other cell type, except products of their fertilization.

Until oocytes were harvested routinely for in vitro fertilization (IVF), beginning in the early 1980s, few gynecologists had ever seen a living female gamete from our species. Although IVF procedures are common and ovarian stimulation is standard practice, oocytes remain very scarce and precious. Moreover, the great majority of oocytes donated to research come from IVF programs in which they failed to be fertilized or were immature, and only rarely do freshly harvested cells presumed to be mature and fertile become available for study. Such drawbacks are compounded by a postovulatory lifespan of only one day at most, and by the inability to propagate oocytes, as can be done with cell lines. Progress has therefore depended on animal models, including nonmammalian species, despite differences in the size and polarity of eggs. The cytoplasm of human oocytes appears relatively uniform, as we shall explain, and this has huge implications for invasive assisted reproductive technology (ARTs) such as intracytoplasmic sperm injection (ICSI), in which a single sperm is injected into the cytoplasm of an oocyte to treat most forms of male infertility, and embryo biopsy for preimplantation genetic diagnosis, in which one cell is removed for genetic diagnosis and/or screening for aneuploidy. Materials deposited during oogenesis visibly polarize the eggs of amphibians, flies, and many other animals, determine the plane of the first cleavage division, and become sequestered in specific early lineages of the embryo. Another notable difference between species is the absence of germline stem cells in mammals, or at least active ones, after birth, and ovaries are generally believed to eke out a dwindling endowment of nonrenewable oocytes during adulthood. The fecundity of human ovaries is more constrained than in most other species because the store of oocytes is exhausted by mid-life (i.e., menopause). Moreover, oocyte fertility starts to decline precipitately early in mid-life (beginning when women are approximately 30 years old), when aneuploidy becomes remarkably prevalent (3). Since rapid ovarian aging is universal in our species, an evolutionary explanation is called for.

Oocytes harvested for IVF or at ovulation are arrested at metaphase II in a cloud of "cumulus" cells, a subpopulation of granulosa cells in the follicle whose main functions are to support oocyte development and to contribute to hormone and growth factor production. This is the final stage of a long, complex history beginning with primordial germ cells in the epiblast of an implanting embryo (4). After multiplying and migrating to the gonadal anlagen, they continue to undergo mitosis until entering meiotic prophase, so that at birth virtually the entire 1–2 million germ cells, now called oocytes, have reached the diplotene stage of prophase I (5). They have therefore already undergone meiotic recombination, so that each oocyte has a unique genetic constitution, and they become enveloped in a layer of pregranulosa cells to form primordial follicles. But they cannot ovulate, resume meiosis, or undergo fertilization until after several weeks of growth in the follicle, which enlarges from the primordial stage (approximately 35 microns diameter) to the grape-sized Graafian stage that is ripe for ovulation. In this Review we consider oocyte development across the entire span of follicle growth.

Developmental niche

Oocytes cannot be understood in isolation from their follicles, which provide the environment in which they become competent to undergo fertilization and produce a viable embryo. Follicles (specifically granulosa cells) create an essential niche for oocyte

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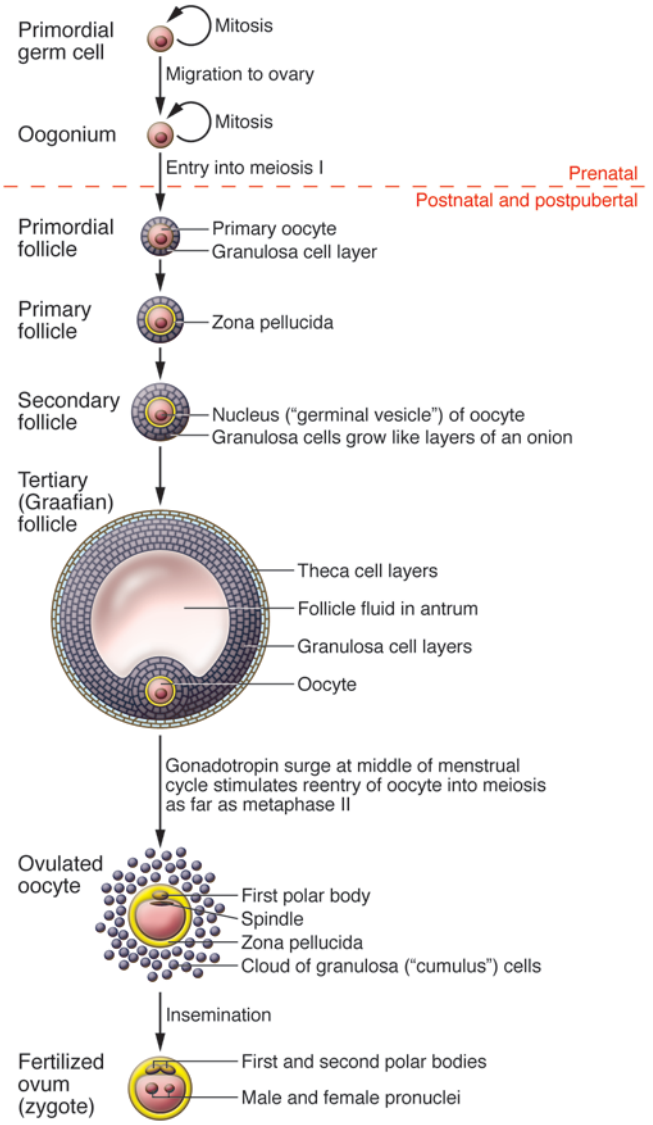

Figure 1
Schematic diagram of follicle maturation in the ovary. Prior to birth, primordial germ cells migrate to the developing ovary and, after a period of proliferation, they enter meiosis I. At birth the ovary contains many primordial-stage follicles, which contain small, non-growing oocytes surrounded by a single granulosa cell layer. These follicles develop in the step-wise manner depicted to become preovulatory, Graafian-stage follicles, which contain a fully grown oocyte. Oocytes reach metaphase II of meiosis after the ovulatory surge of gonadotropins that occurs mid–menstrual cycle. Meiosis is only completed upon oocyte fertilization.

survival, nourishment, and regulation (Figure 1). After remaining quiescent in the ovary for one to more than fifty years, primordial follicles initiate growth of their oocyte and pregranulosa cells, a process that involves the PI3K signaling pathway (6) but is independent of follicle-stimulating hormone (FSH) from the pituitary gland (7). Only a small fraction of the total oocyte population (apparently chosen at random) grows at one time; otherwise, the ovary would be rapidly depleted. Oocyte growth is coordinated with that of granulosa cells, which become progressively more active in steroid and inhibin production under the influence of gonadotropin stimulation and which, in turn, provide feedback on the hypothalamo-pituitary unit. The granulosa and theca cells have somewhat complementary roles in the production of estrogens and androgens (and progesterone, beginning shortly before ovulation), but although oocytes contain steroid receptors, they are not known to be affected by these hormones, except in frogs, in which progesterone triggers meiosis. Anti-Mullerian hormone is also secreted by granulosa cells, but at an earlier stage and as a paracrine factor affecting small follicle commitment to growth (8).

At one time, oocytes were regarded as passengers in follicles, passive and dependent, but the metaphor has changed to that of oocytes being like captains of their vessels (9). Strong evidence that they direct follicle development was obtained when mid-growth oocytes were shown to accelerate follicle development to the Graafian stage (Figure 1) after being combined with granulosa cells from an earlier stage of follicle development (10).

TGF-β family members are some of the most important secretory products of growing oocytes, notably growth differentiation factor 9 (GDF9) and the closely related bone morphogenetic protein 15 (BMP15), which have synergistic roles in follicle growth (11, 12). BMP15 stimulates granulosa cell expression of KIT ligand, which in turn acts via the KIT receptor on oocytes to inhibit BMP15 expression, although the physiology is undoubtedly more complex, involving also the theca cell layer (13–15). This local feedback loop helps to explain why differentiation goes awry in oocytectomized follicles. In the presence of the oocyte, granulosa cells are stimulated to multiply and synthesize hyaluronic acid, while plasminogen activator and progesterone secretion are inhibited, in part by the actions of GDF9 and BMP15. These interactions thus coordinate compartments of the follicle to prevent premature ovulation and luteinization, the process whereby a postovulatory ovarian follicle transforms into a corpus luteum (16–19).

Despite the oocytes' commanding role, communication is bidirectional, and follicular cells are interdependent. Metabolic cooperativity is needed for the very survival of oocytes, which are deficient in some metabolic pathways. For example, they are unable to use glycolysis to generate energy, instead depending on pyruvate as a principal energy source (20). GDF9, BMP15, and FGF8 secreted by oocytes approaching maturity promote expression in granulosa cells of genes encoding enzymes that stimulate production of donor metabolites for oxidative metabolism (21). Likewise, they promote expression of genes encoding amino acid transporters and enzymes and outsource cholesterol biosynthesis to granulosa cells for membrane growth and other functions (22, 23). Nutrient and informational molecules diffuse across the porous zona pellucida, which is the thick glycoprotein shell secreted by growing oocytes that persists until shortly before embryos implant, but cytoplasmic continuity created by heterologous gap junctions is also vital, allowing bidirectional transport of small molecules between the granulosa cells and oocytes. These gap junctions, composed of connexin 37 hexamers, form at contact points between transzonal projections from granulosa cells and the oocyte membrane or microvilli (24, 25). When connexin 37 is genetically deleted, follicle and oocyte development halt in mid-growth (Figure 1) (26). FSH, a master survival factor for granulosa cells, affects the density of transzonal projections by an unknown mechanism, implying that ovarian stimulation protocols might influence oocyte quality (27). During conventional stimulation for harvesting multiple

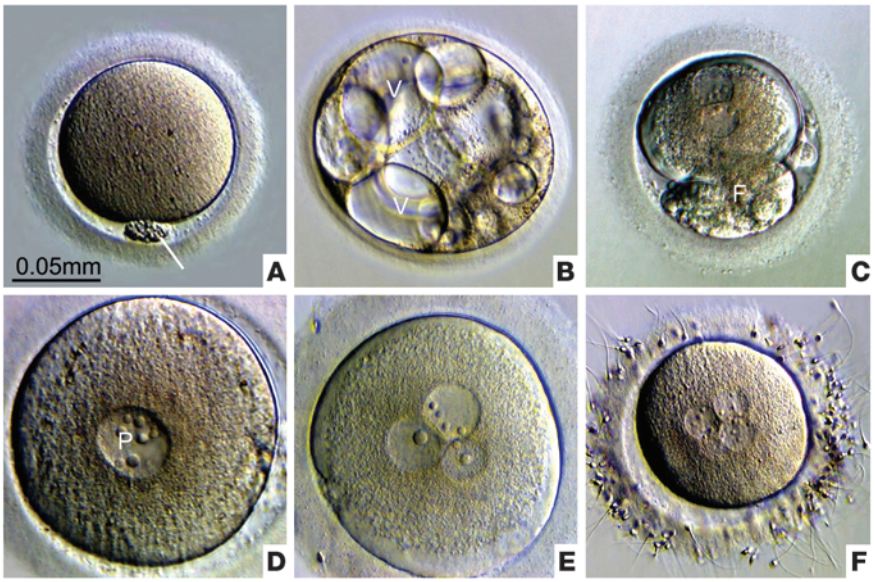

Figure 2
A panel showing 6 human oocytes harvested after ovarian stimulation for assisted conception that are either pre- or post-fertilization and vary in quality and developmental potential. (**A**) Normal, metaphase II stage with first polar body (indicated by white line). (**B**) Unfertilized oocyte containing many large vacuoles (V). (**C**) Pronuclear stage with extensive cytoplasmic fragmentation (F). (**D**) Oocyte with a single pronucleus (P) after fertilization by ICSI (haploid). (**E**) Triploid zygote with 2 large and 1 small pronucleus, suggesting that fertilization was dispermic. (**F**) Tetraploid zygote with four pronuclei after presumptive trispermic fertilization. **A**, **B**, **D**, and **F** reproduced with permission from *An Atlas of Human Gametes and Conceptuses* (50). **C** and **E** reproduced with permission from *An Atlas of Human Blastocysts* (141).

oocytes for IVF treatment, endogenous FSH is pharmacologically suppressed to gain greater control of the ovarian response to FSH administration. If oocyte quality is somewhat lower in stimulated than natural cycles, as is widely assumed, this is not necessarily due to any adverse effects of FSH per se, but likely a result of recruiting a wider range of follicle stages than the narrow window of selection for the single, dominant follicle in spontaneous cycles.

Harmonious oocyte and granulosa cell growth and differentiation, so important for timely maturation, ovulation, and steroidogenesis, are safeguarded by cellular interactions and interdependency within follicles, the fundamental developmental units of the ovary (28). Hence, research that aims to culture small oocytes, either as an alternative technology for ovarian stimulation in vivo or after frozen banking for fertility preservation, must heed the physiological requirements of granulosa cells in which they are nurtured. The follicular structure also helps to explain why oocyte (and therefore embryo) quality is heterogeneous within a cohort, since each oocyte has a unique follicular microenvironment and developmental trajectory. Finally, as knowledge advances, we may see renewal of the old teleological debate about whether the chance of a given follicle ovulating is affected by the intrinsic quality of its oocyte.

Growth and differentiation

Although mammalian eggs are comparatively tiny, they are far more voluminous than any somatic cell, expanding to 120 μm in diameter in humans and growing 100-fold in volume and in abundance of organelles and structural and soluble components (29, 30). The enlarged nucleus is called a "germinal vesicle" because its diffuse chromosomes stain weakly with basic dyes. They only faintly resemble the "lampbrush" chromosomes of the much larger frog egg, in which chromatin forms brush-like loops serving as factories for RNA synthesis (31). Nevertheless, mammalian oocytes are transcriptionally superactive, as the large nucleolus indicates ribosomal RNA production. When transcription is halted, a hood of heterochromatin forms around the nucleolus in fully grown oocytes competent to resume meiosis (32).

The small oocytes of primordial follicles contain a dense cloud of organelles of unknown significance comprising mitochondria, Golgi elements, and ER. This structure, reminiscent of the so-called "Balbiani body" in non-mammalian eggs, disperses after growth commences (33). The Golgi apparatus breaks into flattened sacs in the cortex, where zona proteins are processed and cortical granules are packaged to prepare for their role in hardening the zona pellucida against the entry of additional sperm after fertilization (34). The cytoplasm contains a honeycomb of ER, and the widely distributed polyribosomes increase four-fold during oocyte growth to meet the demands of protein synthesis. In humans, the dominant centrosomal material for aster formation (i.e., formation of the star-shaped microtubule cluster surrounding the centrosome for the first embryonic division) is not maternally inherited (as in mice) but comes from the fertilizing spermatozoon (35). As a result, male infertility due to defects in aster formation is unlikely to be overcome by ICSI (35). Oocytes have only small reserves of glycogen and lipids, and specific yolk proteins comparable to vitellogenins in non-mammals are absent (except in the platypus), having disappeared during the evolution of internal fertilization, placentation, and lactation (36).

Lattice-like structures in rodent oocytes were at one time thought to be yolk proteins because they occupy more than 5% of the cytoplasm at maturity and progressively disappear after fertilization, until they are lost in blastocysts (37). Recent studies of peptidylarginine deiminase 6 (PADI6) support an alternative hypothesis propounded many years ago, namely that the cytoplasmic lattices represent storage of mRNAs, ribosomes, and the rest of the translational machinery (38, 39). Consistent with this view, the lattices contain PADI6 and, if the *Padi6* gene is knocked out, they are completely absent in eggs, which exhibit defective ribosomal function and embryonic genome activation and never develop beyond the 2-cell stage (40, 41). Such findings imply the lattices are involved with the translational machinery, and the presence of a human ortholog of *Padi6* points to similar mechanisms in human eggs.

Oocytes have radial symmetry apart from a slightly eccentric nucleus, but the meiotic spindle forming before ovulation migrates to the cortex to determine the cleavage plane for shedding the first polar body (the daughter cell generated by asymmetric cell division of the oocyte during meiosis I that contains very little cytoplasm)

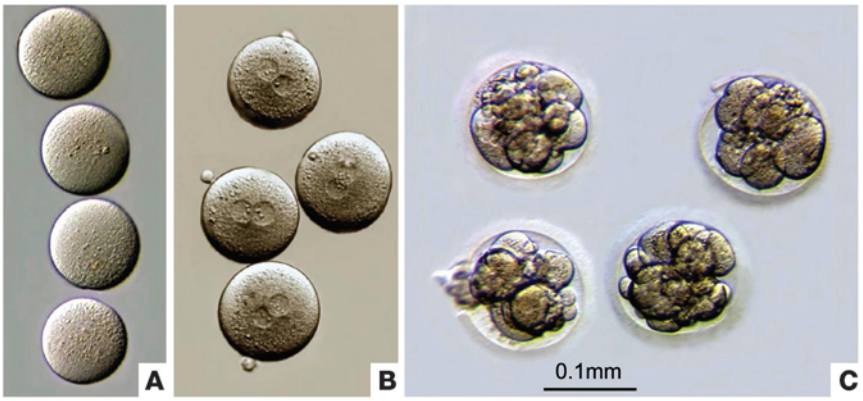

Figure 3
A rare case of mature human oocytes uniformly zona-free. (**A**) The zona pellucida was completely absent in all oocytes harvested from a patient with infertility. (**B** and **C**) The eggs formed pronuclei after ICSI and cleaved to the 2–4 cell stage in vitro (**B**), although they never generated an ongoing pregnancy when replaced in the uterus (**C**), even after transfer to evacuated zonae. Reproduced with permission from *An Atlas of Human Gametes and Conceptuses* (50).

(Figure 2A). This defines what is sometimes called the "animal pole", strictly speaking a misnomer because, in contrast to those in frogs, human cytoplasmic domains fated to form specific cell lineages after fertilization do not exist. Despite the continuing debate about a molecular program for forming an embryonic axis, mammalian eggs are definitely not constrained, if any poles exist at all, because they have a regulative character (42). This is reassuring because loss of a blastomere(s) by fragmentation, cryoinjury, or biopsy (e.g., for preimplantation genetic diagnosis) is unlikely to cause maldevelopment, although a larger reduction in the endowment of maternal cytoplasm certainly reduces viability (43).

The zona pellucida accounts for more than 5% of peak protein synthesis. Because it contains the primary sperm receptor (zona pellucida 3 [Zp3]) (44), its specificity and function made it an attractive target for immunocontraception research in the past. The mouse zona pellucida consists of three highly glycosylated proteins organized as Zp2–Zp3 fibrils noncovalently cross-linked by Zp1, with expression coordinated by the transcription factor FIGLA (factor in the germline α), which is also needed for establishing germ cells (45) and which is present in humans (46). Human oocytes possess a fourth zona protein, ZP4, which is only represented in mice as a truncated pseudogene (47).

When the three zona pellucida proteins are knocked out in turn, different effects are observed in mice (48). Without Zp1, the zona pellucida is weaker than normal and the females are less fertile. Without Zp2, the zona matrix is merely a thin membrane of Zp1 and Zp3 fibrils that disappears before ovulation, and the eggs fail to develop afterward. The most severe phenotype is caused by lack of Zp3, which completely inhibits zona pellucida formation and fertility. This phenotype can be rescued by inserting into the cells the coding sequence for human *ZP3*, although the oocytes are unable to bind human sperm because they need either ZP4 or species-specific glycosylation (49). The corresponding mutations have not been reported in humans and are likely to be rare causes of infertility. Among thousands of patients, our center has encountered only a single case in which every oocyte was naked in recurring stimulation cycles and a natural cycle (50) (Figure 3). The oocytes were delicate, requiring reduced suction pressure for successful follicular retrieval and gentle injection for ICSI, but after fertilization they cleaved to the 8-cell stage, at which point they were transferred to empty donor zonae before being placed in the uterus. However, implantation failed in every cycle except for one, which resulted in a short-lived pregnancy, although the woman subsequently had a viable twin pregnancy from donor eggs. This case confirms the critical importance of a normal zona pellucida for human fertility.

Apart from a few obvious examples, morphology is unreliable for judging the competence of living oocytes, and there are no visible indicators of aneuploidy (51). The degree of cytoplasmic granulation probably has little significance, although large cytoplasmic inclusions, central granularity (resembling a bull's eye), and ER clusters are claimed to be detrimental (52–54). Occasionally, the cytoplasm is highly vacuolated (possibly from obstructed ER tubes) or it breaks up into fragments during meiotic maturation, both of which predict a poor outcome before or at least after implantation (Figure 2, B and C). Abnormal ploidy is common and has many causes (Figure 2, D–F). But the morphology of eggs is unclear until their cumulus cells have been dispersed for ICSI or after fertilization and, in general, the appearance and growth rate of embryos are better indicators of implantation potential than is oocyte morphology (55).

Molecular program for development

Synthesis of mRNAs is strongly upregulated in growing oocytes, reaching a plateau at full size before falling silent when the germinal vesicle breaks down, which is a sign that the nuclear membrane has disappeared as meiosis resumes after a prolonged stopover at prophase I (56, 57). Nascent mRNAs are usually processed and translated quickly, but many are converted to storage forms in oocytes, and regulation of gene expression becomes completely dominated by translation instead of transcription. Hence, a critical period exists from when the oocyte reenters meiosis until embryo cleavage, when the embryonic genome becomes activated, implying that the first days of development depend on protein synthesis from the repository of maternal transcripts. Consequently, the health of early embryos depends on materials and biosynthetic activity laid down in the egg. As implied earlier, individual differences in mRNA profile and abundance within a cohort of oocytes or with age might predict their viability and potential (58), and there is some evidence that deficiency of the spindle checkpoint assembly regulator MAD2 and related molecules contribute to aneuploidy (59).

On economic grounds, it might be expected that gene products would appear in a timely manner and in quantities closely matching physiological need, but this is not necessarily the case. Components of maturation-promoting factor (MPF), part of the complex molecular machinery driving the cell cycle, are expressed long before oocytes become competent for meiosis. Moreover, lactate dehydrogenase is a superabundant protein, greatly exceeding

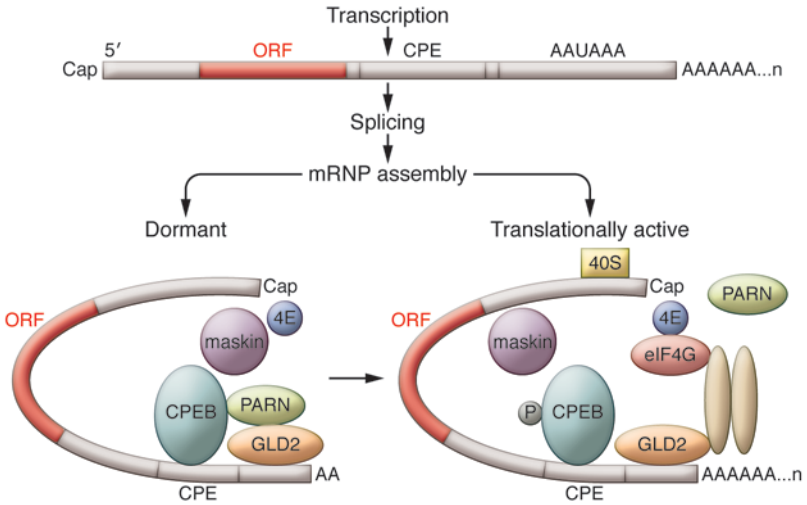

Figure 4
Diagrammatic representation of the suppression of mRNA translation in RNP complexes in oocytes. After the freshly transcribed and spliced transcript is incorporated into a RNP complex, it can either be translated directly or enter a prolonged period of translational dormancy. Transcripts that can be stored contain a CPE at the 3′-untranslated end, to which the protein CPEB binds. Phosphorylation of the latter causes release of PARN from the complex, enabling GLD2 to elongate the poly(A) and hence facilitate translation by interaction with other proteins. In dormant mRNAs, another CPEB-associated protein, maskin, inhibits an initiation factor (eIF4G) required for recruiting the 40S ribosomal subunit to the 5′ end of the mRNA by binding the cap-binding eukaryotic initiation factor (4E). 40S, 40S ribosomal subunit.

the needs for carbohydrate metabolism. Such inconsistencies may eventually be explained by more pleiotropic roles.

Since the oocyte population forming prenatally has to serve the entire reproductive lifespan, some germ cells have to survive for decades, during which time they probably deteriorate from accumulated effects of aging. Once released from their follicular environment at ovulation, they deteriorate rapidly. Between the primordial stage and ovulation, they do not have a rigidly fixed schedule for development. Some develop faster than others, depending on the follicle trajectory, and there is flexibility in their timetable before ovulation until activated by the surge of luteinizing hormone. For example, in patients with polycystic ovaries who develop ovarian hyperstimulation syndrome (OHSS) from FSH stimulation, it is possible to "coast" follicles (delay ripening) for up to three days by withdrawing stimulation without seriously compromising oocyte quality (60). Presumably mRNA and protein stability are unaffected.

The half-lives of mRNAs in oocytes are measured in days or weeks, not hours, as in most somatic cells. After they are recruited from storage for oocyte maturation, fertilization, and cleavage, more than 90% of transcripts disappear by the first cleavage division, and the rest have disappeared by the blastocyst stage (61). How do these molecules achieve such stability, and what determines their degradation? A freshly transcribed mRNA undergoes splicing to make a mature molecule that is exported to the cytoplasm where, in a ribonucleoprotein (RNP) complex and after incorporation of initiation factors, nascent polypeptide chains form. Translation depends on polyadenylation at the 3′ end of the molecule, but stored mRNAs have had their poly(A) tails shortened to 20–40 nucleotides and are "masked" (62). Translational control is complex, but it involves three main elements. First, transcripts for storage in oocytes have U-rich, cis-acting cytoplasmic polyadenylation elements (CPEs) upstream of the processing signal (AAUAAA) in the 3′ untranslated region (63, 64). Second, CPE-binding protein (CPEB) plays a key role in translational repression/activation by recognizing the CPE in association with other factors (which is the third element) (65). Figure 4 provides a simplified illustration of interactions between the molecular players involved in the transition from translational dormancy to expression, induced by the Aurora A kinase in frog eggs. When poly(A) ribonuclease (PARN) is expelled from the complex, germ-line development factor 2 (GLD2) elongates the tails by 200–250 nucleotides, a precondition for translation. In addition, maskin, a CPEB-associated protein, inhibits translation by competing with other factors that enable recruitment of the 40S ribosomal subunit to the AUG start codon. Details are easier to study in species with large eggs, such as frogs, but are likely to apply broadly to mammals.

The importance of polyadenylation is revealed for *Mos* RNA, which encodes a regulator of meiosis. In the absence of the protein, oocytes fail to progress from meiosis I to meiosis II, but the cells are rescued by injecting the mRNA, provided it contains the proper CPE to enable polyadenylation (66). Stem loop–binding protein (SLBP) is another translational regulator that is abundant in oocytes, where it binds to the 3′ region of histone mRNAs for regulating translation (67). Histones are needed to replace acidic protamines in the male pronucleus by insertion into newly synthesized DNA in growing embryos (67). Without SLBP, histones H3 and H4 fail to accumulate and embryos stop dividing at the 2-cell stage, although they can be rescued by replacing the protein (68). Despite examples of successfully reversed deficiency of a gene product, microinjection of mRNAs or proteins into oocytes is unlikely to become a general method for improving their viability in the near future because many genes are active at earlier, less accessible stages of oogenesis, and the extent to which deficiencies in transcription during oocyte growth or translation afterward contribute to poor oocyte quality is far from clear.

Recently, small RNAs have been implicated as translational controllers, many of them shadowing the timing of appearance and disappearance of mRNAs encoding proteins. When Dicer, an enzyme that processes precursor molecules into microRNAs (miRNAs), is knocked out in oocytes, virtually the entire, heterogeneous population of miRNAs is eliminated, spindle defects appear, and development never progresses to the 2-cell stage (69). Furthermore, as a protective mechanism for the germ line, siRNAs from pseudogenes, also processed by Dicer and acting with Piwi-interacting RNAs, serve through RNA interference pathways to suppress mobile genetic elements from transposons (70, 71). Such discoveries are surely the beginning of a new chapter for understanding the fine control mechanisms for translation in oocytes.

We now return to the conundrum mentioned earlier of how oocytes can be differentiated and totipotent at the same time. The most plausible explanation is that they are RNA-centric, meaning

Table 1
Maternal effect genes expressed in mouse oocytes

Gene	Expression	Function	Stage at which development arrests	Ref.
Bnc1	Oocyte; sperm; early embryo	Transcription factor	1- to 2-cell stage embryo	86
Smarca4	Ubiquitous	Chromatin remodeling; EGA	2-cell stage embryo	87
Dnmt1o	Oocyte through to implanting embryo	DNA methylation	Post-implantation	88
Filia	Oocyte	Spindle integrity	Pre-implantation	84
Ooep	Oocyte	Cortical complex with Mater	2-cell stage embryo	85
Hsf1	Oocyte to blastocyst; adult tissue	Molecular chaperone	1- to 2-cell stage embryo	89
Nlrp5	Oocyte to blastocyst	Translational control	2-cell stage embryo	90
Npm2	Oocyte to blastocyst	Chromatin remodeling	1- to 2-cell stage embryo	91
Padi6	Oocyte to blastocyst	Translation control	2-cell stage embryo	41
Dppa3	Germ cell to blastocyst; stem cells; germ cell tumors	RNA/DNA binding	Blastocyst	92
Zar1	Oocyte to 2-cell stage embryo; testis	Chromatin remodeling	2-cell stage embryo	93

Bnc1, basonuclin 1; *Dnmt1o*, the oocyte form of DNA methyltransferase (cytosine-5) 1; *Dppa3*, developmental pluripotency–associated 3 (also known as *Stella*); *Hsf1*, heat shock factor 1; *Nlrp5*, NLR family, pyrin domain containing 5 (also known as *Mater*); *Ooep*, oocyte-expressed protein homolog (also known as *Floped*); *Smarca4*, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (also known as BRM/SWI2-related gene 1 [*Brg1*]); *Zar1*, zygote arrest 1.

that developmental control has been transferred from transcription to translation. The brief divorce between the genome, whose synthetic activity pauses, and the cytoplasm, whose stored program is translated, confers developmental plasticity on the cell so that transcripts can be recruited for highly specific functions, while the chromatin is at liberty to be remodeled for embryogenesis (72). This theme encompasses the evolutionary spectrum, although the timing of embryonic genome activation varies between species. In human embryos this activation has been pinpointed by inhibiting RNA polymerase activity to the 4- to 8-cell stage (73), whereas it occurs 1–2 cleavage divisions earlier in mice. In either case it is remarkably early compared with frogs, in which transcription is not reinitiated until the tadpole stage, although this is reached within hours, not days, of fertilization because cell division is so rapid.

Imprinted genes are developmentally important and expressed mono-allelically according to the parent of origin. The inactive allele is silenced by epigenetic modification, specifically DNA methylation of CpG islands in the 5′ region. Taking complementary examples, *H19* is unmethylated (and expressed) in mouse oocytes, whereas the maternally imprinted genes small nuclear ribonucleoprotein N (*Snrpn*) and insulin-like growth factor 2 receptor are progressively methylated (and silent) during oocyte growth (74). Although *H19* and *Snrpn* expression is normal in mouse embryos generated after superovulation, surprising methylation errors are observed transgenerationally in sperm from the resulting male offspring (75). Moreover, the normally silent paternal *H19* allele is expressed in mouse embryos cultured in a suboptimal medium (76). This is a cautionary signal for in vitro maturation (IVM) of oocytes harvested from preovulatory follicles (an ART that eliminates or substantially reduces the need for the gonadotropin-induced ovarian stimulation step of IVF and its associated health risks, in particular OHSS), especially if culture starts at early stages of oocyte maturation when epigenetic reprogramming is occurring. Although there have been only 1,000–2,000 births so far from IVM worldwide, the health as a whole of the several million babies born from ARTs has been reassuring, and imprinting abnormalities are very rare (77).

One of the problems of assessing ART safety is the high biological incidence of aneuploidy in oocytes, which rises so much faster with age than in spermatozoa or lymphocytes (78). Weak checkpoint control and chromatid presegregation at metaphase due to meiosis-specific cohesin deficiency appear to be proximate causes, providing some molecular foundations for the enigmatic vulnerability of eggs (79, 80). Human oocytes are also moderately sensitive to ionizing radiation, especially at primordial follicle stages, which can be completely eradicated by apoptosis after exposure to therapeutic doses of either radiotherapy or high-dose chemotherapy (81). Interestingly, when mice were treated with ethyl nitrosourea, a potent mutagen, the great majority of infertility phenotypes were in males rather than females, in which sperm production was either partially impaired or completely absent (http://reproductivegenomics.jax.org) (82). More reassuringly, the effects are apparently all-or-nothing, since young cancer survivors of either sex who remain fertile after gonadotoxic treatment do not have an excess risk of having children with major congenital malformations (83).

While few mutations specifically affecting oocyte and embryo quality have been described, genetic polymorphisms could account for occasional cases of infertility in which meiotic maturation, fertilization, or cleavage is blocked. Maternal effect genes are principal candidates because mutant females are infertile, though healthy, and males are completely unaffected because there is no significant carryover of gene products stored in spermatozoa to the embryo (Table 1). The proteins encoded by maternal effect genes are some of the most abundant in oocytes, most are involved with transcription and translation, although some are found in cortical complexes (84). In mammals, about a dozen maternal effect genes have been identified so far, depending on definition, with the aforementioned *Padi6* an example. Mutations of maternal effect genes block mouse oocyte/embryo development between late oogenesis and the blastocyst stage (Table 1; refs. 41, 84–93), as does mutation of some genes expressed more widely, but only the former have effects specific to fertility. As examples of other oocyte-expressed genes, CCCTC-binding factor and postmeiotic segregation increased 2 (*Pms2*) are translated from mouse oocyte mRNAs needed for cleavage, but they are not considered products of maternal effect genes because they also have somatic functions including epigenome regulation (94) and DNA mismatch repair (95), respectively. Genetic defects in orthologs of maternal effect genes in humans are likely to present a

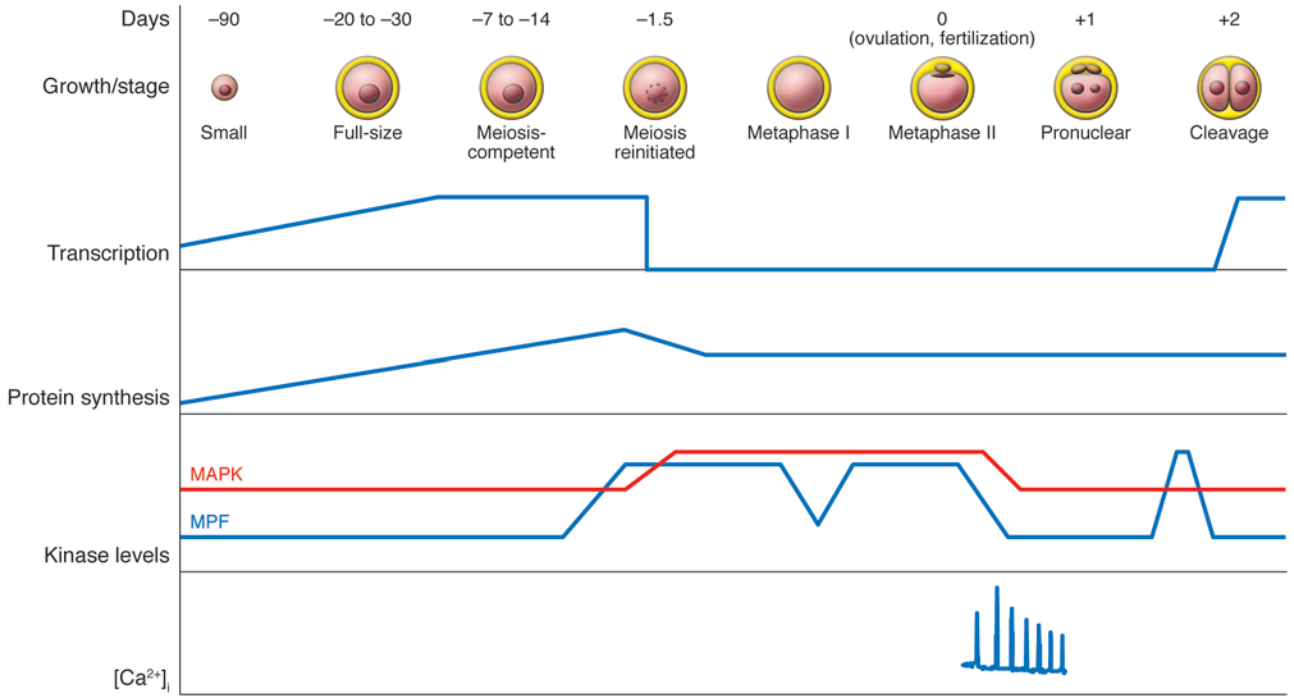

Figure 5
Schematic illustration of molecular and cellular maturation of human oocytes. Stages of development from initiation of oocyte growth in small follicles through meiotic maturation and fertilization to cleavage. Competence to resume meiosis is reached in fully grown oocytes several days before ovulation. Transcription becomes silent after the resumption of meiosis until the 4- to 8-cell stage, and gene expression is dependent on translation of stored mRNAs until embryonic genome activation occurs 3–4 days later. Protein synthesis increases to a plateau when the oocyte is fully grown. Progression through stages of meiosis depends on phosphorylation changes in MPF and MAPK. When the oocyte is cytoplasmically mature, oscillations of $[Ca^{2+}]_i$ released from stores in the ER are triggered by the fertilizing sperm.

similar phenotype and might explain why so many human embryos are of poor quality. Perhaps hypomorphic proteins from partially penetrating polymorphisms only have mild effects on fertility unless, when more than one is present, their impact is additive, because several of the abundant maternal effect proteins interact in the subcortical complex (85).

Oocyte maturation

Oocyte maturation is the process that creates the fully mature gamete whose fate hangs on timely fertilization after ovulation. The cell is suspended between mortality and the possibility of producing virtually unlimited numbers of progeny. Maturation is triggered by the gonadotropin surge that occurs in the middle of a woman's menstrual cycle and, after polar body emission, the cell cycle continues without interruption or pausing for DNA synthesis to the next meiotic division. The division halts at metaphase II approximately 36 hours after the surge (Figure 5). While the first meiotic reduction division uniquely involves genetic recombination and extends over many years, the second is brief, similar to mitosis, and its completion depends on fertilization. It has long been known that germinal vesicle oocytes can spontaneously resume maturation in culture without hormones, a process that apparently involves protein phosphorylation and withdrawal from inhibitory influences within the follicle. Since mature oocytes were always rare, some of the early pioneers of IVF could only obtain immature oocytes that had to be matured in vitro (96). IVM is being adopted again as a strategy for avoiding the risks of OHSS in patients with polycystic ovaries. It produces respectable pregnancy rates, although oocyte competence is lower than in standard IVF cycles, presumably because a premature harvest yields oocytes that are not yet cytoplasmically mature even though they readily undergo nuclear maturation (97).

Extensive reorganization of intracellular organelles occurs during oocyte maturation, notably reorganization of mitochondria to provide a local energy supply, cortical granule migration to prepare for blocking polyspermy, and ER movement anticipating the phasic release of Ca^{2+} from ER stores after fertilization (98–100). Maturation is driven by cell cycle kinases, mostly those also responsible for regulating mitotic cycles (101, 102). MPF and MAPK are key molecules regulating downstream targets and contributing to spindle assembly (103–105). Their activation coincides with germinal vesicle breakdown, and activity increases during maturation, apart from a transient decline in MPF activity after metaphase I, until they peak in fully mature oocytes (103, 106, 107) (Figure 5). They represent the cytostatic factor long known to maintain metaphase II arrest (108).

Maturation is essential for normal development and ploidy because premature fertilization at metaphase I can cause triploidy. However, most immature oocytes cannot elicit the repeated Ca^{2+} responses needed for successful activation by a fertilizing spermatozoon (109–111). This capacity is not fully acquired until metaphase II, and it wanes during postovulatory aging, when the intracellular calcium signal converts from an activating signal to one that induces apoptosis (109, 112, 113). After an initial burst

of intracellular Ca^{2+} ($[Ca^{2+}]_i$) oscillations follow with a periodicity of 20–30 minutes, triggering a cascade of cortical granule exocytosis, resumption of meiosis, recruitment of mRNAs, pronuclear formation, and progression to the first mitotic division (114). The key importance of $[Ca^{2+}]_i$ oscillations was confirmed by experiments in which injections of Ca^{2+} stimulated parthenogenesis in unfertilized oocytes (115), whereas a Ca^{2+} chelator inhibited fertilization (116, 117). When electrical pulses triggered Ca^{2+} release from intracellular stores, the extent of parthenogenetic development varied with the number of $[Ca^{2+}]_i$ oscillations elicited. Cortical granule release (an early response) needed fewer $[Ca^{2+}]_i$ transients than pronuclear formation and entry into mitosis (late events) (118, 119), and a high frequency was needed for survival to post-implantation stages (120). Failure of Ca^{2+} signaling could be responsible for cases in which a patient's oocytes fail completely in repeated IVF treatment cycles to activate after ICSI. A Ca^{2+} ionophore has been used to rescue fertilization (121), although a more physiological stimulus is desirable, as we describe next.

Fertilization-induced $[Ca^{2+}]_i$ oscillations are driven by the phosphoinositide signaling pathway in which phosphatidylinositol 4,5-bisphosphate anchored in the plasma membrane is cleaved by PLC into two second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (122). IP_3 binds to IP_3 receptors (IP_3Rs) located on the ER membrane and induces release of Ca^{2+} into the cytoplasm from ER stores (123), whereas DAG regulates Ca^{2+} influx through activation of PKC (124). In mammalian oocytes, fertilization-induced $[Ca^{2+}]_i$ oscillations are mediated predominantly by the type 1 IP_3R (IP_3R-1) (125, 126), since antibodies specific for IP_3R-1 block both the Ca^{2+} response and activation (117, 127). Abnormalities in abundance or localization or posttranslational modifications of IP_3R-1 during oocyte maturation are all potential causes of fertilization failure (111, 128, 129). Overcoming abnormalities in the signaling pathway will be challenging, but it might be possible to reverse deficiency of the "sperm factor" responsible for triggering $[Ca^{2+}]_i$ transients by injecting the sperm-specific isoform of PLC (PLCζ) to activate eggs if ICSI fails in male factor infertility cases (130, 131).

After the oocyte is activated, the sperm nucleus undergoes decondensation and histone substitution to form the male pronucleus, which acquires latent transcriptional potential. Decondensation is achieved by reduction of the disulfide bonds in protamines (132), probably involving glutathione accumulated during oogenesis and the product of a maternal effect gene, nucleoplasmin 2 (*NPM2*) (91). Fertilization arrest from failure of decondensation can theoretically be caused by either an incompetent oocyte or a resistant sperm nucleus.

Future developments

Human oocytes have moved toward the center stage of modern biology from behind a curtain of obscurity. Yet, despite the dramatic growth in the use of ARTs, the ratio of oocytes harvested to live-born babies has remained stubbornly high at approximately 25:1 for young women, with wide variation between treatment centers, numbers harvested, cryopreservation, and maternal age (133). This low efficiency is mainly due to poor oocyte quality, particularly when reproductive age is advanced, and better screening methods are needed to identify competent gametes. Single cells present a difficult challenge for screening, and non-invasive methods (such as genetic/epigenetic/molecular analyses of polar bodies, cumulus cells, and spent culture medium), although attractive in theory, have limited applications or are still under development. Selection of embryos for implantation potential is likely to remain more practicable than screening eggs because there is more time for abnormalities to become manifest after fertilization. Improving oocyte quality (oomedicine) is another strategy that is attractive in theory but difficult in practice. As for boosting the number of oocytes for treatment and research, current ovarian stimulation protocols have probably reached the biological limit. Yet, there are urgent needs to produce more and better-quality oocytes. A breakthrough would relieve pressures on egg donation services and facilitate single-embryo transfer to avoid pregnancy with multiples. Oocytes are also needed for research into the causes of aneuploidy and for somatic cell cloning in regenerative medicine.

For the foreseeable future, the number and quality of oocytes will remain strictly limited for biological and bioethical reasons. There is therefore tremendous hope, mingled with caution, of progress through new culture technologies, either by generating gametes grown from the more abundant small-follicle stages (134) or from germline stem cells persisting after birth (135, 136). A successful culture environment will need to be sophisticated enough to mimic the follicular niche and incorporate stage-specific signals to control growth, differentiation, epigenetic modifications, and meiosis. Standard culture conditions are unlikely to suffice, and advances in microfluidics may help to engineer a more physiological microenvironment. Oocyte-like cells were recently derived in culture from ES cells and various somatic cells, including the pancreas, fetal skin, and surfaces of human postmenopausal ovaries (137). While there is no doubt that germ cells can form in embryoid bodies from ES cells, culture conditions are still unable to recapitulate the specific and evolving conditions needed to generate a fertile oocyte, defined by the ability to produce a healthy baby.

Likewise, there are widespread doubts about attempts to improve oocyte quality by transferring cytoplasm from young donor oocytes, especially given the small amounts that can be injected and limited understanding of the causes of poor quality and aging. Although a number of babies have been conceived after this procedure (138), concerns about genetic safety suspended practices in the USA. Germinal vesicle transfer to an enucleated donor oocyte enables a more complete replacement of cytoplasm but is limited by the effectiveness of protocols for IVM and qualms about mitochondrial heteroplasmy (in which mitochondria from more than one cellular origin are present in a single cell). Transfer of the spindle–metaphase II chromosome complex to enucleated donor oocytes has, however, proved successful, with three healthy monkeys born so far (139). Since mitochondria are only inherited maternally, the risk of transmitting mitochondria from an egg containing mitochondrial DNA mutations for a child-to-be is virtually eliminated. But all these strategies depend on limited supplies of donor oocytes.

Despite many obstacles, there is reason to be optimistic about the future of this field. Exquisitely sensitive molecular tools now enable single oocytes to be analyzed, something that is extremely important when studying rare and heterogeneous cells. A national egg bank could facilitate progress, since low-temperature preservation is now more effective, so that researchers need not be attached to IVF clinics to obtain material and precious cells are not wasted. Eventually, a deeper understanding of oocyte competence and aging will emerge, giving leverage for new technologies for culturing oocytes and even repairing defects by molecular medicine. Additionally, if the promise of induced pluripotent stem cells (iPS

cells) is sustained, they may provide a new source of oocytes and create opportunities for correcting genetic abnormalities at an early stage. iPS cells closely resemble ES cells but are generated from transfected somatic cells, and healthy mice created from them have fertile germ cells (140). Thus, we anticipate human iPS cells will eventually become a source of germ cells for making oocytes. Though we cannot predict the pace of progress, nor whether the gametes will be safe for treating infertility, the discovery process will surely bring the benefits of a deeper understanding of the biology and pathology of oocytes.

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1. Von Baer CE. *De ovi mammalium et hominis genesi.* Leipzig: Sumptibus Leopoldi Vossii; 1827.
2. Lopata A. History of the egg in embryology. *Journal of Mammalian Ova Research.* 2009;26:2–9.
3. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet.* 2001;2(4):280–291.
4. Saitou M, Barton SC, Surani MA. A molecular programme for the specification of germ cell fate in mice. *Nature.* 2002;418(6895):282–283.
5. Baker TG. A quantitative and cytological study of germ cells in human ovaries. *Proc R Soc Lond B Biol Sci.* 1963;158:417–433.
6. Reddy P, et al. Oocyte-specific deletion of *Pten* causes premature activation of the primordial follicle pool. *Science.* 2008;319(5863):611–613.
7. Oktay K, Briggs D, Gosden RG. Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. *J Clin Endocrinol Metab.* 1997;82:3748–3751.
8. Carlsson IB, et al. Anti-Müllerian hormone inhibits initiation of growth of human primordial ovarian follicles in vitro. *Hum Reprod.* 2006;21(9):2223–2227.
9. Gosden R. The vocabulary of the egg. *Nature.* 1996; 383(6600):485–486.
10. Matzuk MM, Burns KH, Viveiros MM, Eppig JJ. Intercellular communication in the mammalian ovary: oocytes carry the conversation. *Science.* 2002; 296(5576):2178–2180.
11. Dong J, et al. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature.* 1996;383(6600):531–535.
12. Yan C, et al. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol Endocrinol.* 2001;15(6):854–866.
13. Otsuka F Shimasaki S. A negative feedback system between oocyte bone morphogenetic protein 15 and granulosa cell kit ligand: its role in regulating granulosa cell mitosis. *Proc Natl Acad Sci U S A.* 2002; 99(12):8060–8065.
14. Thomas FH, Ethier JF, Shimasaki S, Vanderhyden BC. Follicle-stimulating hormone regulates oocyte growth by modulation of expression of oocyte and granulosa cell factors. *Endocrinology.* 2005; 146(2):941–949.
15. Elvin JA, Yan C, Wang P, Nishimori K, Matzuk MM. 1999. Molecular characterization of the follicle defects in the growth differentiation factor 9-deficient ovary. *Mol. Endocrinol.* 1999;13(6):1018–1034.
16. Buccione R, Vanderhyden BC, Caron PJ, Eppig JJ. FSH-induced expansion of the mouse cumulus oophorus *in vitro* is dependent upon a specific factor(s) secreted by the oocyte. *Dev Biol.* 1990; 138(1):16–25.
17. Vanderhyden BC, Telfer EE, Eppig JJ. Mouse oocytes promote proliferation of granulosa cells from preantral and antral follicles *in vitro*. *Biol Reprod.* 1992;46(6):1196–1204.
18. Vanderhyden BC, Cohen JN, Morley P. Mouse oocytes regulate granulosa cell steroidogenesis. *Endocrinology.* 1993;133(1):423–426.
19. Canipari R, Epifano O, Siracusa G, Salustri A. Mouse oocytes inhibit plasminogen activator production by ovarian cumulus cells. *Dev Biol.* 1995; 167(1):371–378.
20. Harris SE, Leese HJ, Gosden RG, Picton HM. Pyruvate and oxygen consumption throughout the growth and development of murine oocytes. *Mol Reprod Dev.* 2008;76(3):231–238.
21. Sugiura K, et al. Oocyte-derived BMP15 and FGFs cooperate to promote glycolysis in cumulus cells. *Development.* 2007;134(14):2593–2603.
22. Haghighat N, van Winkle LJ. Developmental change in follicular cell-enhanced amino acid uptake into mouse oocytes that depends on intact gap junctions and transport system Gly. *J Exp Zool.* 1990; 253(1):71–82.
23. Su YQ, Sugiura K, Eppig JJ. Mouse oocyte control of granulosa cell development and function: paracrine regulation of cumulus cell metabolism. *Semin Reprod Med.* 2009;27(1):32–42.
24. Anderson E, Albertini DF. Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. *J Cell Biol.* 1976;71(2):680–686.
25. Rodrigues P, Limback D, McGinnis LK, Plancha CE, Albertini DF. Oogenesis: prospects and challenges for the future. *J Cell Physiol.* 2008;216(2):355–365.
26. Simon AM, Goodenough DA, Li E, Paul DL. Female infertility in mice lacking connexin 37. *Nature.* 1997;385(6616):525–529.
27. Combelles CM, Carabatsos MJ, Kumar TR, Matzuk MM, Albertini DF. Hormonal control of somatic cell oocyte interactions during ovarian follicle development. *Mol Reprod Dev.* 2004;69(3):347–355.
28. Edson MA, Nagaraja AK, Matzuk MM. The mammalian ovary from genesis to revelation. *Endocr Rev.* 2009;30(6):624–712.
29. Wassarman PM, Josefowicz WJ. Oocytes development in the mouse: an ultrastructural comparison of oocytes isolated at various stages of growth and meiotic competence. *J Morph.* 1978;156(2):209–236.
30. Gosden R, Krapez J, Briggs D. Growth and development of the mammalian oocyte. *BioEssays.* 1997; 19(10):875–882.
31. Davidson EH. Gene activity during oogenesis. In: *Gene activity in early development.* 3rd ed. Orlando, FL: Academic Press; 1986:305–407.
32. Mattson BA, Albertini DF. Oogenesis: chromatin and microtubule dynamics during meiotic prophase. *Mol Reprod Dev.* 1990;25(4):374–383.
33. Pepling ME, Wilheilm JE, O'Hara AL, Gephardt GW, Spradling AC. Mouse oocytes within germ cell cysts and primordial follicles contain a Balbiani body. *Proc Natl Acad Sci U S A.* 2007;104(1):187–192.
34. Sun QY. Cellular and molecular mechanisms leading to cortical reaction and polyspermy block in mammalian eggs. *Microsc Res Tech.* 2003;61(4):342–348.
35. Schatten H, Sun QY. The role of centrosomes in mammalian fertilization and its significance for ICSI. *Mol Hum Reprod.* 2009;15(9):531–538.
36. Brawand D, Wahli W, Kaessmann H. Loss of egg yolk genes in mammals and the origin of lactation and placentation. *PLoS Biol.* 2005;6(3):e63.
37. Szollosi D. Changes of some cell organelles during oogenesis in mammals. In: Biggers JD, Schuetz AW, eds. *Oogenesis.* Baltimore: University Park Press; 1972:47–64.
38. Bachvarova R, De Leon V, Spiegelman I. Mouse egg ribosomes: evidence for storage in lattices. *J Embryol Exp Morphol.* 1981;62:153–164.
39. Vitale AM, et al. Proteomic profiling of murine oocyte maturation. *Mol Reprod Dev.* 2007;74(5):608–616.
40. Esposito G, et al. Peptidylarginine Deiminase (PAD) 6 is essential for oocyte cytoskeletal formation and female fertility. *Mol Cell Endocrinol.* 2007; 273(1–2):25–31.
41. Yurttas P, et al. Role for PADI6 and the cytoplasmic lattices in ribosomal storage in oocytes and translational control in the early mouse embryo. *Development.* 2008;135(15):2627–2636.
42. Johnson MH. From mouse egg to mouse embryo: polarities, axes, and tissues. *Annu Rev Cell Dev Biol.* 2009;25:483–512.
43. Wakayama T, Yanagimachi R. Fertilisability and developmental ability of mouse oocytes with reduced amounts of cytoplasm. *Zygote.* 1998;6(4):341–346.
44. Wassarman PM. Zona pellucida glycoproteins. *J Biol Chem.* 2008;283(36):24285–24289.
45. Liang L, Soyal SM, Dean J. FIGalpha, a germ cell specific transcription factor involved in the coordinate expression of the zona pellucida genes. *Development.* 1997;124(24):4939–4947.
46. Huntriss J, et al. Isolation, characterization and expression of the human Factor In the Germline alpha (*FIGLA*) gene in ovarian follicles and oocytes. *Mol Hum Reprod.* 2002;8(12):1087–1095.
47. Conner SJ, Lefièvre L, Hughes DC, Barratt CLR. Cracking the egg: increased complexity in the zona pellucida. *Hum Reprod.* 2005;20(5):1148–1152.
48. Dean J. Oocyte-specific gene expression: roles in folliculogenesis, fertilization and early development. In *Biology and Pathology of the Ooctye.* Trounson AO, Gosden RG, eds. Cambridge, United Kingdom: Cambridge University Press; 2003:67–80.
49. Rankin T, et al. Human *ZP3* restores fertility in *Zp3* null mice without affecting order-specific sperm binding. *Development.* 1998;125(13):2415–2424.
50. Veeck LL. *An atlas of human gametes and conceptuses.* London: Parthenon; 1999.
51. Balaban B, et al. Oocyte morphology does not affect fertilization rate, embryo quality and implantation rate intracytoplasmic sperm injection. *Hum Reprod.* 1998;13(12):3431–3433.
52. Serhal PF, et al. Oocyte morphology predicts outcome of intracytoplasmic sperm injection. *Hum Reprod.* 1997;12(6):1267–1270.
53. Kahraman S, et al. Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. *Hum Reprod.* 2000;15(11):2390–2393.
54. Otsuki J, Okada A, Morimoto K, Nagai Y, Kubo H. The relationship between pregnancy outcome and smooth endoplasmic reticulum clusters in MII human oocytes. *Hum Reprod.* 2004;19(7):1591–1597.
55. Ebner T, Moser M, Sommergruber M, Tews G. Selection based on morphological assessment of oocytes and embryos at different stages of preimplantation development: a review. *Hum Reprod Update.* 2003;9(3):251–262.
56. Piko L, Clegg KB. Quantitative changes in total RNA, total poly(A), and ribosomes in early mouse embryos. *Dev Biol.* 1982;89(2):362–378.

57. Bachvarova R, De Leon V, Johnson A, Kaplan G, Paynton BV. Changes in total RNA, polyadenylated RNA and Actin RNA during meiotic maturation of mouse oocytes. *Dev Biol*. 1985;108(2):325–331.
58. Pan H, Ma P, Zhu W, Schultz RM. Age-associated increase in aneuploidy and changes in gene expression in mouse eggs. *Dev Biol*. 2008;316(2):397–407.
59. Steuerwald N, Cohen J, Herrera RJ, Sandalinas M, Brenner CA. Association between spindle assembly checkpoint expression and maternal age in human oocytes. *Mol Hum Reprod*. 2001;7(1):49–55.
60. Levinson-Tavor O, Friedler S, Schachter M, Raziel A, Strassburger D. Coasting-what is the best formula? *Hum Reprod*. 2003;18(5):937–940.
61. Hamatani T, Carter MG, Sharov AA, Ko MSH. Dynamics of global gene expression changes during mouse preimplantation development. *Dev Cell*. 2004; 6:117–131.
62. Meric F, Searfoss AM, Wormington M, Wolffe AP. Masking and unmasking maternal mRNA. The role of polyadenylation, transcription, splicing, and nuclear history. *J Biol Chem*. 1996;271(48):30804–30810.
63. Huarte J, et al. Transient translational silencing by reversible mRNA deadenylation. *Cell*. 1992; 69(6):1021–1030.
64. Verrotti AC, Thompson SR, Wrernden C, Strickland S, Wickens M. Evolutionary conservation of sequence elements controlling cytoplasmic polyadenylation. *Proc Natl Acad Sci U S A*. 1996;93(17):9027–9032.
65. Richter JD CPEB: a life in translation. *Trends Biochem Sci*. 2007;32(6):279–285.
66. Gebauer F, Xu W, Cooper GM, Richter JD. Translational control by cytoplasmic polyadenylation of c-*mos* mRNA is necessary for oocyte maturation in the mouse. *EMBO J*. 1994;13(23):5712–5720.
67. Allard P, Yang Q, Marzluff WF, Clarke HJ. The stem-loop binding protein regulates translation of histone mRNA during mammalian oogenesis. *Dev Biol*. 2005;286(1):195–206.
68. Arnold DR, Françon P, Zhang J, Martin K, Clarke HJ. Stem-loop binding protein expressed in growing oocytes is required for accumulation of mRNAs encoding histones H3 and H4 and for early embryonic development in the mouse. *Dev Biol*. 2008; 313(1):347–358.
69. Tang F, et al. Maternal microRNAs are essential for mouse zygotic development. *Genes Dev*. 2007; 21(6):644–648.
70. Tam OH, et al. Pseudogene-derived small interfering RNAs regulate gene expression in mouse oocytes. *Nature*. 2008;453(7194):534–539.
71. Watanabe T, et al. Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. *Nature*. 2008;453(7194):539–543.
72. Seydoux G, Braun RE. Pathway to totipotency: lessons from germ cells. *Cell*. 2006;127(5):891–904.
73. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature*. 1988;332(6163):459–461.
74. Lucifero D, Mertineit C, Clarke HJ, Bestor TH, Trasler JM. Methylation dynamics of imprinted genes in mouse germ cells. *Genomics*. 2002;79(4):530–538.
75. Stouder C, Deutsch S, Paoloni-Giacobino A. Superovulation in mice alters the methylation pattern of imprinted genes in the sperm of the offspring. *Reprod Toxicol*. 2009;28(4):536–541.
76. Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol Reprod*. 2000;62:1526–1535.
77. Buckett WM, et al. Obstetric outcomes and congenital abnormalities after in vitro maturation, in vitro fertilization, and intracytoplasmic sperm injection. *Obstet Gynecol*. 2007;110(4):885–891.
78. Pellestor F, Andréo B, Arnal F, Humeau C, Demaille J. Maternal aging and chromosomal abnormalities: new data drawn from in vitro unfertilized human oocytes. *Hum Genet*. 2003;112(2):195–203.
79. Hodges CA, Revenkova E, Jessberger R, Hassold TJ, Hunt PA. SMC1beta-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction. *Nat Genet*. 2005;37(12):1351–1355.
80. Liu L, Keefe DL. Defective cohesion is associated with age-dependent misaligned chromosomes in oocytes. *Reprod Biomed Online*. 2008;16(1):103–112.
81. Wallace WH, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. *Hum Reprod*. 2003; 18(1):117–121.
82. The Jackson Laboratory. Reproductive genomics: mutant mouse models of infertility. Available at: http://reproductivegenomics.jax.org.
83. FossÅ SD, et al. Parenthood in survivors after adulthood cancer and perinatal health in their offspring: a preliminary report. *J Natl Cancer Inst Monogr*. 2005; 34(34):77–82.
84. Zheng P, Dean J. Role of Filia, a maternal effect gene, in maintaining euploidy during cleavage-stage mouse embryogenesis. *Proc Natl Acad Sci U S A*. 2009;106(18):7473–7478.
85. Li L, Baibakov B, Dean J. A subcortical maternal complex essential for preimplantation mouse embryogenesis. *Dev Cell*. 2008;15(3):416–425.
86. Ma J, Zeng F, Schultz RM, Tseng H. Basonuclin: a novel mammalian maternal-effect gene. *Development*. 2006;133(10):2053–2062.
87. Buttman SJ, et al. Maternal BRG1 regulates zygotic genome activation in the mouse. *Genes Dev*. 2006; 20(13):1744–1754.
88. Howell CY, et al. Genomic imprinting disrupted by a maternal effect mutation in the Dnmt1 gene. *Cell*. 2001;104(6):829–838.
89. Metchat A, et al. Mammalian heat shock factor 1 is essential for oocyte meiosis and directly regulates Hsp90alpha expression. *J Biol Chem*. 2009; 284(14):9521–9528.
90. Tong ZB, Bondy CA, Zhou J, Nelson LM. A human homologue of mouse *Mater*, a maternal effect gene essential for early embryonic development. *Hum Reprod*. 2002;17(4):903–911.
91. Burns KH, et al. Roles of NPM2 in chromatin and nucleolar organization in oocytes and embryos. *Science*. 2003;300(5619):633–636.
92. Payer B, et al. Stella is a maternal effect gene required for normal early development in mice. *Curr Biol*. 2003;13(23):2110–2117.
93. Wu X, et al. Zygote arrest 1 (Zar1) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. *Nat Genet*. 2003;33(2):187–191.
94. Wan LB, et al. Maternal depletion of CTCF reveals multiple functions during oocyte and preimplantation embryo development. *Development*. 2008;135(16):2729–2738.
95. Gurtu VE, et al. Maternal effect for DNA mismatch repair in the mouse. *Genetics*. 2002;160(1):271–277.
96. Edwards RG, Bavister BD, Steptoe PC. Early stages of fertilization in vitro of human oocytes matured in vitro. *Nature*. 1969;221(5181):632–635.
97. Chian RC, Lim JH, Tan SL. State of the art in in-vitro oocyte maturation. *Curr Opin Obstet Gynecol*. 2004; 16(3):211–219.
98. Liu XY, et al. Cortical granules behave differently in mouse oocytes matured under different conditions. *Hum Reprod*. 2005;20(12):3402–3413.
99. FitzHarris G, Marangos P, Carroll J. Changes in endoplasmic reticulum structure during mouse oocyte maturation are controlled by the cytoskeleton and cytoplasmic dynein. *Dev Biol*. 2007;305(1):133–144.
100. Liu S, Li Y, Gao X, Yan JH, Chen ZJ. Changes in the distribution of mitochondria before and after in vitro maturation of human oocytes and the effect of in vitro maturation on mitochondrial distribution [published online ahead of print May 5, 2009]. *Fertil Steril*. doi:10.1016/j.fertnstert.2009.03.050.
101. Jones KT. Turning it on and off: M-phase promoting factor during meiotic maturation and fertilization. *Mol Hum Reprod*. 2004;10(1):1–5.
102. Brunet S, Maro B. Cytoskeleton and cell cycle control during meiotic maturation of the mouse oocyte: integrating time and space. *Reproduction*. 2005; 130(6):801–811.
103. Polanski Z, et al. Cyclin synthesis controls the progression of meiotic maturation in mouse oocytes. *Development*. 1998;125(24):4989–4997.
104. Fan HY, Sun QY. Involvement of mitogen-activated protein kinase cascade during oocyte maturation and fertilization in mammals. *Biol Reprod*. 2004; 70:535–547.
105. Yu LZ, et al. MEK1/2 regulates microtubule organization, spindle pole tethering and asymmetric division during mouse oocyte meiotic maturation. *Cell Cycle*. 2007;6(3):330–338.
106. Kubiak JZ, Weber M, Geraud G, Maro B. Cell cycle modifications during the transitions between meiotic M-phases in mouse oocytes. *J Cell Sci*. 1992; 102:457–467.
107. Hampl A, Eppig JJ. Analysis of the mechanism(s) of metaphase I arrest in maturing mouse oocytes. *Development*. 1995;121(4):925–933.
108. Masui Y, Markert CL. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J Exp Zool*. 1971;177:129–145.
109. Mehlmann LM, Kline D. Regulation of intracellular calcium in the mouse egg: calcium release in response to sperm or inositol trisphosphate is enhanced after meiotic maturation. *Biol Reprod*. 1994; 51(6):1088–1098.
110. Jones KT, Carroll J, Merriman JA, Whittingham DG, Kono T. Repetitive sperm-induced Ca2+ transients in mouse oocytes are cell cycle dependent. *Development*. 1995;121(10):3259–3266.
111. Lee B, Yoon SY, Fissore RA. Regulation of fertilization-initiated [Ca2+]i oscillations in mammalian eggs: a multi-pronged approach. *Semin Cell Dev Biol*. 2006;17(2):274–284.
112. Fujiwara T, Nakada K, Shirakawa H, Miyazaki S. Development of inositol trisphosphate-induced calcium release mechanism during maturation of hamster oocytes. *Dev Biol*. 1993;156(1):69–79.
113. Gordo AC, et al. Intracellular calcium oscillations signal apoptosis rather than activation in in vitro aged mouse eggs. *Biol Reprod*. 2002;66:1828–1837.
114. Schultz RM, Kopf GS. Molecular basis of mammalian egg activation. *Curr Top Dev Biol*. 1995;30:21–62.
115. Fulton BP, Whittingham DG. Activation of mammalian oocytes by intracellular injection of calcium. *Nature*. 1978;273(5658):149–151.
116. Kline D, Kline J. Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse egg. *Dev Biol*. 1992;149(1):80–89.
117. Xu Z, Kopf G, Schultz R. Involvement of inositol 1,4,5-trisphosphate-mediated Ca2+ release in early and late events of mouse egg activation. *Development*. 1994;120(7):1851–1859.
118. Ozil JP, Huneau D. Activation of rabbit oocytes: the impact of the Ca2+ signal regime on development. *Development*. 2001;128(6):917–928.
119. Ducibella T, et al. Egg-to-embryo transition is driven by differential responses to Ca2+ oscillation number. *Dev Biol*. 2002;250(2):280–291.
120. Ozil JP, Banrezes B, Toth S, Pan H, Schultz RM. Ca2+ oscillatory pattern in fertilized mouse eggs affects gene expression and development to term. *Dev Biol*. 2006;300(2):534–544.
121. Heindryckx B, De Gheselle S, Gerris J, Dhont M, De Sutter P. Efficiency of assisted oocyte activation as a solution for failed intracytoplasmic sperm injection. *Reprod Biomed Online*. 2008;17(5):662–668.
122. Berridge MJ. Inositol trisphosphate and calcium signaling. *Nature*. 1993;361(6410):315–325.
123. Zhu CC, Wojcikiewicz RJ. Ligand binding directly stimulates ubiquitination of the inositol 1,4,5-trisphosphate receptor. *Biochem J*. 2000;348:551–556.
124. Oancea E, Meyer T. Protein kinase C as a molecular

machine for decoding calcium and diacylglycerol signals. *Cell*. 1998;95(3):307–318.
125. Parrington J, et al. Expression of inositol 1,4,5-trisphosphate receptors in mouse oocytes and early embryos: the type I isoform is upregulated in oocytes and downregulated after fertilization. *Dev Biol*. 1998;203(2):451–61.
126. Fissore RA, Longo FJ, Anderson E, Parys JB, Ducibella T. Differential distribution of inositol trisphosphate receptor isoforms in mouse oocytes. *Biol Reprod*. 1999;60(1):49–57.
127. Miyazaki S, et al. Block of Ca2+ wave and Ca2+ oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. *Science*. 1992;257(5067):251–255.
128. Jones KT, Carroll J, Whittingham DG. Ionomycin, thapsigargin, ryanodine, and sperm induced Ca2+ release increase during meiotic maturation of mouse oocytes. *J Biol Chem*. 1995;270(12):6671–6677.
129. Mehlmann LM, Mikoshiba K, Kline D. Redistribution and increase in cortical inositol 1,4,5-trisphosphate receptors after meiotic maturation of the mouse oocyte. *Dev Biol*. 1996;180(2):489–498.
130. Yoon SY, et al. Human sperm devoid of PLC, zeta 1 fail to induce Ca(2+) release and are unable to initiate the first step of embryo development. *J Clin Invest*. 2008;118(11):3671–3681.
131. Heytens E, et al. Reduced amounts and abnormal forms of phospholipase C zeta (PLC zeta) in spermatozoa from infertile men. *Hum Reprod*. 2009; 24(10):2417–2428.
132. Perreault SD, Wolff RA, Zinkin BR. The role of disulfide bond reduction during mammalian sperm nuclear decondensation in vivo. *Dev Biol*. 1984; 101(1):160–167.
133. Inge GB, Brinsden PR, Elder KT. Oocyte number per live birth in IVF: were Steptoe and Edwards less wasteful? *Hum Reprod*. 2005;20(3):588–592.
134. Gosden RG, Mullan J, Picton HM, Yin H, Tan SL. Current perspective on primordial follicle cryopreservation and culture for reproductive medicine. *Hum Reprod Update*. 2002;8(2):105–110.
135. Bukovsky A, et al. Oogenesis in adult mammals, including humans: a review. *Endocrine*. 2005;26(3):301–316.
136. Zou K, et al. Production of offspring from a germline stem cell line derived from neonatal ovaries. *Nat Cell Biol*. 2009;11(5):631–636.
137. Marques-Mari AI, Lacham-Kaplan O, Medrano JV, Pellicer A, Simon C. Differentiation of germ cells and gametes from stem cells. *Hum Reprod Update*. 2009;15(3):379–390.
138. Barritt JA, Brenner CA, Malter HE, Cohen J. Mitochondria in human offspring derived from ooplasmic transplantation. *Hum Reprod*. 2001;16(3):513–516.
139. Tachibana M, et al. Mitochondrial gene replacement in primate offspring and embryonic stem cells. *Nature*. 2009;461(7262):367–372.
140. Zhao X-Y, et al. iPS cells produce viable mice through tetraploid complementation. *Nature*. 2009; 461:86–94.
141. Veeck LL, Zaninovic N. *An atlas of human blastocysts*. London: Parthenon; 2003.