# Oocyte competency is the key to embryo potential

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The oocyte is the major determinant of embryo developmental competence in women. It delivers half the chromosomal complement to the embryo, but the maternal and paternal genomes are neither symmetrical nor equal in their contributions to embryo fate. Unlike the paternal genome, the maternal genome carries a heavy footprint of parental aging. Indeed, age is the single best predictor of reproductive outcome in women, and the oocyte is the locus of reproductive aging in women. The oocyte transmits not only the mother's nuclear but also her mitochondrial genome to the embryo, and mitochondrial DNA is known to be especially susceptible to aging. Morphological studies of the oocyte and its associated cumulus corona cells provide only marginal value in the assessment of embryo developmental potential. A number of novel technologies, however, have improved the noninvasive assessment of oocyte quality. Moreover, during maturation, the oocyte ejects half its homologous chromosomes into the first polar body and half its chromatids into the second polar body. Polar body DNA is remarkably similar to that of the oocyte, so analysis of polar body DNA provides a window into the oocyte's genome and telomeres, which may enhance prediction of embryo developmental competence. (Fertil Steril® 2015;103:317–22. ©2015 by American Society for Reproductive Medicine.)

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*Embryogenesis begins during oogenesis*
–E. B. Wilson, 1918

Reproduction in women declines well before other organ systems begin to fail (1, 2). Fecundity begins to decrease at least 10 years before menopause, while thousands of primary oocytes remain within the ovary and menstrual cycles continue. By age 40, half of women are sterile, and the other half have decreased fecundity compared with their younger years (2). The locus of reproductive aging in women must reside within the oocyte because donation from young to older women nearly completely abrogates the effects of aging on fertility (1–3). Maternal age provides the best predictor of oocyte quality and embryo developmental capacity (3), but the extent of reproductive aging among women of the same age varies widely, so clinically useful biomarkers of oocyte competency are needed. The localization of reproductive aging to a single cell provides a unique opportunity because profusion of new technologies now enables study of the structure and function of individual cells (4). At the same time, the need for biomarkers of oocyte competency has become urgent as women increasingly delay attempts at childbearing (5). The proportion of women attempting to conceive after age 35, when rates of fertility decrease and miscarriage and nondisjunction increase logarithmically, is at an all-time high (5). Clinically useful biomarkers of oocyte function most likely will derive from studies of the biology underlying oocyte aging and from new technologies to assess single cells. This review provides an overview of age-related oocyte dysfunction and the technologies available to assess it noninvasively.

## DEVELOPMENT OF THE OOCYTE

The reproductive life span in women is influenced by the number of primordial follicles and their survival throughout the life of the woman. A fetus starts life with a finite population of primordial follicles, which undergo marked attrition during in utero development (6). By the time of birth, only 20% of the original cohort remains to sustain her reproductive life span. Between birth and puberty, oocytes enter a protracted phase of prophase arrest. Some cells remain in the state of meiotic prophase arrest for up to 50 years. During this time, cells proliferate to form the supporting cumulus-corona and resulting primordial follicles. Each month, a cohort of follicles is recruited, but only a small fraction of these reach maturity because most undergo atresia. This continued depletion of germ cells chips away at oocyte reserve until

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menopause ensues, when fewer than a thousand oocytes remain. It is not known how the fate of germ cells is established in the ovary (6). Questions remain as to whether survival results from a vetting process that allows only the fittest cells to develop or whether the selection of oocytes to survive is stochastic.

During fetal life, germ cells do not exit the proliferative phase of oogenesis and enter meiosis all at once. Rather they enter meiosis in a production line (7). Pulse-labeling oocyte DNA during early and again during late fetal oogenesis demonstrates that the first germ cells to enter meiosis during fetal oogenesis are the first to ovulate in the adult and that the last to enter meiosis are the last to ovulate in the adult (8). This means that oocytes ovulating from older women have traversed more cell cycles during fetal oogenesis than those ovulating from young women. The cellular basis of this production line of germ cell production in fetal development and subsequent aging in adult life remains poorly understood, but we have argued that attrition of telomeres could provide a cellular timekeeper of replication cycles during oogenesis (1, 9, 10).

The preovulatory LH surge, triggered by the rising levels of $E_2$ produced by the growing follicles acting on the pituitary and hypothalamus, initiates resumption of meiosis in the oocyte. The meiotic spindle forms when kinetochores on each homologous chromosome nucleate microtubule polymerization. At metaphase, homologous chromosomes employ pushing and pulling forces from microtubules attached to each kinetochore to congress to the metaphase plate of the meiotic spindle. At anaphase, one chromosome from each pair of homologues migrates to the first polar body (PB). The oocyte progresses through the second meiotic cell cycle and arrests at metaphase of meiosis II. Fertilization triggers resumption of meiosis and final separation of sister chromatids (11).

In addition to their nuclear genome, oocytes transmit mitochondria (12). Mitochondria contain their own DNA (mtDNA), and mtDNA is transmitted exclusively through the maternal germ line (12). Primary oocytes contain only a limited number of mtDNA copies. Later, during oogenesis, the mtDNA copy number expands to populate the oocyte. As the germ cell passes from oogonia to oocyte, the mtDNA copy number increases from 200 to hundreds of thousands (12, 13). Presumably, initially limiting inheritance to a few copies followed by expansion during later oogenesis allows selection of high-quality mtDNA during oogenesis (12, 13).

A number of these processes have been implicated in age-related oocyte dysfunction (2) including mitochondrial dysfunction, meiotic spindle dysfunction, free radical production, cohesins, genomic instability, and telomere attrition. Intriguingly, experimental telomere attrition recapitulates each of these reproductive aging phenotypes in mice, a species that normally does not exhibit appreciable age-related oocyte dysfunction (1, 9, 10, 14, 15).

## MITOCHONDRIA, FREE RADICALS, AND OOCYTE COMPETENCY

Mitochondria have been linked to aging in a number of long-lived, post mitotic cells (16). The mitochondrial genome is highly susceptible to reactive oxygen species (ROS) and genotoxic damage. It lacks nucleosomes and receives minimal protection from the DNA damage repair pathways that protect the nuclear genome (16). MtDNA is localized within the organelle that generates the most oxygen free radicals, which in turn promotes DNA mutations and oxidative phosphorylation (OXPHOS) uncoupling and exacerbates free radical production. Accumulation of damage during the oocyte's prolonged arrest in prophase I provides a compelling rationale for age-related changes in the oocyte. The mtDNA from eggs of older women harbor the most common age-related mutation, a 5-kb deletion flanked by direct repeats (17), although the proportion of mtDNA affected by this or other mutations actually is quite low relative to the total number of mtDNA copies. Chronic exposure to ROS and the inherent genomic instability of mtDNA render it susceptible to a wide variety of mtDNA rearrangements.

Pathology of mtDNA as a cause of reproductive aging is attractive in theory. It has been hypothesized that even low levels of mtDNA mutations impair OXPHOS and increase ROS. These in turn increase mtDNA copy number as a compensatory response. Deficiencies in ATP, so the argument goes, impair chromosome separation and produce aneuploidy (18). Oocytes with low total mtDNA content demonstrate poor development, women with low oocyte mtDNA copy number have low ovarian reserve, and the morphology of preimplantation embryos correlates with oocyte ATP content (19–23). Modeling mitochondrial dysfunction by disrupting OXPHOS increases ROS, disrupts meiotic spindles, leads to chromosome misalignment, and increases oocyte and embryo death (24).

Despite the appeal of this hypothesis, results from a number of recent studies call into question the role of mitochondria in oocyte aging. Measurement of mtDNA copy number in oocytes and PBs shows both decreased and increased mtDNA in oocytes. Moreover, engineering mouse oocytes to contain even very low copies of mtDNA has minimal effect on oocyte and embryo development, until mtDNA copy numbers reach levels below those reported in human oocytes (22). Similarly, female mice (25) and women (26) with levels of mtDNA mutations in their oocytes high enough to transmit mitochondrial diseases to their offspring do not themselves typically experience infertility.

The lack of effect of mitochondrial disruption on fertility in mice and women may stem from the fact that the metabolism in oocytes and embryos differs from that in somatic cells (27). Early embryos derive ATP not from OXPHOS but rather from aerobic glycolysis, the so-called Warburg effect. Consistent with a reduced role in generating ATP, mitochondria in oocytes and early embryos contain few cristae, and consumption of oxygen by oocytes and cleavage-stage embryos remains exceptionally low until the blastocyst stage of development (28). A recent study in bovine oocytes demonstrates a unique method of generating ATP (29), the adenosine salvage pathway. Resumption of meiosis is triggered by destruction of cycle AMP by phosphodiesterases. This reaction produces adenosine monophosphate that is converted to ATP by adenylate kinases and creatine kinases.