INTRODUCTION

The oocyte is known to be a unique and highly specialized cell responsible for creating, activating, and controlling the embryonic genome, as well as supporting basic processes, such as cellular homeostasis, metabolism, and cell cycle progression in the early embryo. An oocyte is formed in the ovarian follicle and is the largest single cell. Meiosis in the mammalian oocyte is initiated during fetal development and is arrested at the diplotene stage of the first meiotic prophase. After stimulation by endogenous luteinizing hormone surge, oocyte meiosis resumes and progresses to the second meiotic phase with a dynamic change. Ovulation leads to the release of an oocyte into the oviduct where meiosis stops at metaphase II (MII) until fertilization. Ovulated oocytes are presumed to have acquired fertilization and developmental competence, which is related to the ability to undergo meiotic maturation, fertilization, embryonic development, and successful pregnancy. Developmental competence is gradually acquired during oogenesis, and the final stage is important for optimal development prior to ovulation because the synchronization between nuclear and cytoplasmic maturation in the oocyte is completed at this stage.

Usually, for in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), oocyte selection is based on morphological
parameters related to the cumulus cells, polar body, and cytoplasm.\textsuperscript{6,7} It has been speculated that some morphological irregularities that are easily assessed under light microscopy may reflect a compromised developmental ability and could therefore be useful for selecting competent oocytes prior to fertilization.\textsuperscript{8} The first polar body (PB1) is the easiest indicator for judging nuclear maturation. However, studies using polarized light microscopy have shown that oocytes displaying a polar body may still be immature.\textsuperscript{9} In addition, just after the extrusion of PB1, oocytes do not acquire sufficient developmental competence, despite exhibiting the morphologic features of the MII stage.\textsuperscript{10} Furthermore, if the structure of the mitotic spindle collapses due to overmaturatiion, developmental competence decreases.\textsuperscript{10} These previous findings indicate that developmental competence changes, even in the MII oocyte.

Accurate sorting of mature oocytes that are healthy and have a high developmental competence will improve the pregnancy rate. However, the morphological criteria that are currently used for the grading and screening of oocytes are subjective and controversial, and they may not be related to the intrinsic competence of the oocyte.\textsuperscript{11,12} The identification of objective and noninvasive molecular markers that predict oocyte ability is a major research goal. Factors related to the quality of oocytes are being elucidated at the molecular level, but it is technically difficult to develop an index based on the visualization of these factors. Accordingly, new indices that reflect intracellular conditions are necessary. In this review, an overall summary of morphological factors related to oocyte quality is provided, recent studies of molecular markers are reviewed, and intracellular temperature is introduced as a potentially effective marker.

2 | FACTORS INFLUENCING OOCYTE QUALITY

Fresh matured oocytes with intact PB1 are enclosed within the zona pellucida, made up of glycoprotein, and consist of meiotic spindle with aligned chromosomes, microtubule-organizing centers (pericentriolar materials, PCMs) located at the spindle poles, mitochondria, microfilaments, and regularly aligned cortical granules underneath the oocyte cortex in the cytoplasm. The zona pellucida is covered with abundant cumulus cells. Aging or overmaturatiion of oocytes is associated with numerous morphological and cellular alterations, including changes in the structure of the plasma membrane, zona pellucida, cytoskeleton, and mitochondria. It is also associated with displacement of the spindle, misalignment of chromosomes, and displacement of PB1 and cortical granules.\textsuperscript{4} The presence of clear PB1 and the attachment level of cumulus cells are major indicators used to determine the quality of oocytes because these are easy to observe using a microscope.

Many studies have reported that changes in oocyte constituents are linked to oocyte quality. We provide an overview of factors contributing to oocyte quality, focusing on the PB1, meiotic spindle, cumulus cells, mitochondria, and oxygen consumption.

2.1 | First polar body (PB1)

The removal of cumulus cells from oocytes allows a detailed observation of the morphological characteristics. Extrusion of PB1 is a cellular landmark of meiotic maturation. Recent studies have investigated the correlation between PB1 morphology and oocyte competence; although PB1 does not participate in the developmental process, studies of mouse oocytes have supported this connection.\textsuperscript{7} PB1 morphology is frequently used to evaluate oocyte quality. Oocytes with an intact PB1 have high fertilization rates and a high oocyte quality, whereas those displaying a PB1 characterized by large size, irregular shape, rough surface, or fragmentation are developmentally less competent after IVF, yielding low pregnancy rates after embryo transfer.\textsuperscript{13,14} PB1 degeneration occurs within a few hours after extrusion, and it is associated with oocyte aging.\textsuperscript{15–17} Some PB1 shows displacement from the MII spindle at nuclear maturation, and the distance between PB1 and MII spindle increases overtime during oocyte aging. Moreover, the perivitelline space increases over time and facilitates the lateral displacement of the degenerating PB1.\textsuperscript{17}

2.2 | Meiotic spindle

The meiotic spindle is a chromosome distribution cytoskeletal structure, critically important for the accurate distribution of chromosomes to the dividing blastomeres, thereby ensuring accurate embryonic development.\textsuperscript{4} In fresh oocytes, spindles display a vertical orientation with respect to the oolemma and spindle poles associate with PCMs to create a compact bipolar spindle. This morphology changes during aging at the MII stage; the spindle becomes elongated and/or loses tension in its microtubules and becomes weak.\textsuperscript{18–21} The shape of the spindle is determined by the position of the spindle pole and changes over time after spindle formation. Oocytes exhibiting a reduction in the distance between the PCMs at the spindle pole have a higher developmental potential than those exhibiting an increase in the distance between the PCMs.\textsuperscript{10} When the distance between PCMs is short, a small rhomboid spindle body is formed, and as the distance increases, a large spindle is formed. The morphology of the mitotic spindle is an important indicator of oocyte condition. Conventionally, spindles were mainly visualized by confocal microscopy, which requires cell fixation and hence cannot be applied to live cells. Alternatively, meiotic spindles can be observed directly using a polarization microscope.\textsuperscript{22}

The molecular mechanisms underlying meiotic spindle in fresh oocytes have shown the importance of meiotic spindles in fertilization and embryonic development.\textsuperscript{23,24} The meiotic spindle is essential for the accurate separation of homologous chromosomes or two sets of chromatids during germ cell division.\textsuperscript{25} Oocyte aging results in significant increases in premature chromosome separation, which is strongly associated with aneuploidy.\textsuperscript{26,27} Aneuploidy is involved in inheriting too many or too few of any of the chromosomes. Most aneuploid embryos that inherit only one copy of an autosome develop severe abnormalities and die.
before pregnancy. In contrast, inheriting an extra copy of an auto-
some is also associated with severe developmental abnormalities
and miscarriages. Chromosome 21 trisomy, the cause of Down’s
syndrome, is by far the most frequent aneuploidy affecting live
births.28–30 Chromosomes in 2-day-old oocytes are no longer
aligned at the spindle equator but are scattered within the degen-
erating spindle. In oocytes aged 3–4 days, chromosomes become
more decondensed and display nuclear alterations. Chromosome
loss, fragmentation, or the clumping of chromosomes and chroma-
tid separation have been observed in aged oocytes.18,31,32

2.3 | Cumulus cells

Cumulus cells are critical for oocyte maturation, ovulation, and fer-
tilization,33 and are a determinant of oocyte quality.34 Cumulus cells
support energy production in the cumulus-oocyte complex.35,36
Additionally, cumulus cells that surround oocytes may protect
against the damaging effects of reactive oxygen species (ROS).37
Recent studies suggest that the mitochondrial function of cumu-
lus cells can directly influence the ability to achieve a successful
pregnancy.38,39 The identification of surrogate markers of oocyte
competence and favorable reproductive outcomes in assisted re-
productive technology is a goal of many transcriptome, proteome,
and metabolome studies. The analysis of granulosa and cumulus
cells is considered one of the best noninvasive strategies available
today.40

2.4 | Mitochondria

The mitochondrion is directly involved in many essential cellular
functions, including energy production, management of ROS lev-
els, and regulation of apoptosis. Mitochondria play an extremely
important role in supplying the energy that is consumed during the
maturation process.41,42 The primary function of mitochondria is
to synthesize adenosine triphosphate (ATP), the preferred energy
source of cells. Synthesis of ATP in adequate amounts is critical for
cell survival, and severe ATP deficiency often leads to apoptosis.43
Although several metabolic pathways of ATP production have been
identified, most of the ATP generated from glucose is produced via
mitochondrial oxidative phosphorylation (OXPHOS).44 All the com-
plex processes that occur in the oocyte prior to ovulation and fertili-
ization require energy, which is derived mainly from ATP production
via OXPHOS.45 Moreover, higher ATP content in oocytes and em-
byros has been correlated with better reproductive results among
infertile patients.46 In contrast, mitochondrial dysfunction has been
implicated in decreased oocyte quality, and clinical and experimental
data have suggested decreased oocyte quality as the main factor
in the age-related deterioration of reproductive capacity. However,
the molecular mechanisms underlying this mitochondrion-related
decline in oocyte quality remain poorly understood.47,48

The distribution and organization of mitochondria during oocyte
maturation are dynamic, and these changes may be related to mito-
ochondrial function. Oocytes with higher concentrations of ATP have
significantly higher fertilization and blastocyst rates.42,49 Lower ATP
content in oocytes is at least partially responsible for positive spindle
formation in in vitro maturation mammalian oocytes.50,51 Decreasing
the ATP content in mouse oocytes by treatment with carbonyl cyan-
ide p-trifluoromethoxyphenylhydrazone, an inhibitor of OXPHOS,
leads to a reduction in the percentage of oocytes with nuclear matu-
ration, normal spindle formation, and chromosome alignment, evenly
distributed mitochondria, and the ability to form blastocysts.52 ATP
is extremely important for nuclear and cytoplasmic maturation
events. Spindle formation and chromosome movements depend on
the expression and activity of motor proteins, which use ATP as their
energy source. Due to the critical role of energy metabolism in oo-
cyte maturation, ATP content has been proposed as an indicator of
the developmental potential of oocytes.53–55

Oxidative stress (OS) results from an imbalance between the
production of ROS and neutralizing antioxidant molecules.56 In
mammalian mature oocytes, OS causes substantial mitochondrial
dysfunction, impacting both mitochondrial ATP synthesis and the
activation of mitochondrial-mediated apoptotic mechanisms.57,58
Enhanced and unbalanced ROS production may be a predominant
cause of impaired mitochondrial OXPHOS.59 External factors con-
tribute to the higher OS observed in vitro, including exposure to
visible light, non-ideal pH and temperature, centrifugation, cryo-
preservation, culture medium composition, oxygen concentra-
tions, and oocyte and embryo manipulation processes.60 mtDNA
is particularly susceptible to several elements causing OS, and
mtDNA disruption leads to critical loss of function and, ultimately,
diminished capacity to generate ATP.58,59 Oocyte mtDNA content
increases until the stage that immediately precedes fertilization.
In healthy embryos, the accumulated mtDNA is divided equally
among all cells during embryogenesis.61–64 Recent studies have
proposed quantification of mtDNA in cumulus, granulosa, and tro-
phoectoderm cells as a promising strategy for predicting embryo
quality and viability.62,65 Since mtDNA content in cumulus cells
is correlated with that in oocytes for each cumulus-oocyte com-
plex, it is suggested that the mitochondrial characteristics of the
cells may serve as a marker of the oocyte quality.60 Furthermore,
mutations or deletions in mtDNA have been correlated with or-
ganelle dysfunction, low ATP levels, and embryonic developmental
arrest.66 With aging, mtDNA deficiency of luteinizing granulosa
cells and cumulus cells increases, which leads to a decrease in
pregnancy rate.66,67 These findings corroborate the understanding
that mitochondrial function of granulosa and cumulus cells directly
influences embryonic development, as well as the maturation and
fertilization of oocytes.68

Various mitochondrial anomalies have been linked to the age-
related deterioration of oocyte quality, and at least some of these
may be reflective of the changes in specific mitochondrial subpop-
ulations.69 The most prominent of these defects are atypical mito-
ochondrial localization and aggregation, reduced mtDNA content,
reduced membrane potential (consequently, bioenergetic capacity),
increased OS, and increased frequency of mtDNA mutations and
deletions.47,50,70–79
3 | MOLECULAR MARKERS RELATED TO OOCYTE QUALITY

3.1 | Meta-analysis of microarray studies

Multiple microarray studies have been performed to identify markers associated with oocyte quality and developmental competence in oocytes or both oocytes and cumulus cells. Based on a meta-analysis of previously published microarray data for various models of oocyte and embryo quality, 63 candidate genes associated with oocyte quality across several species were identified. Biological networks and transcription factor regulation associated with oocyte quality were also identified. If factors that control oocyte quality and molecular mechanisms are clarified, it might provide a basis for objectively evaluating oocyte quality.

3.2 | microRNAs

Gamete maturation requires extensive signaling between germ cells and their surrounding somatic cells. In the ovary, from the theca cells, mural granulosa cells, cumulus cells, and oocyte secrete factors that are critical for ovulation of high-quality oocyte, throughout follicle growth and oocyte maturation. Recent studies of a variety of species have uncovered the presence of cell-secreted vesicles in follicular fluid. These cell-secreted vesicles contain small non-coding regulatory RNAs called microRNAs, which can be shuttled between maturing gametes and surrounding somatic cells. In humans, it is known that extracellular microRNAs of follicular fluid are associated with fertilization ability and early embryo quality, although little is known about the exact mechanism by which microRNAs are loaded into these cell-secreted vesicles or are transferred and modulate gene expression and function. However, recent studies suggest that microRNAs in cell-secreted vesicles are involved in oocyte maturation. These microRNAs involved in gamete maturation are potential therapeutic targets and diagnostic markers associated with fertility.
the formation and maintenance of the spindle, and this repetition might be responsible for heat generation.

Furthermore, heat production by mitochondria is considered a factor influencing intracellular temperature. Substantial heat generation in the mitochondria was observed when HeLa cells expressing tsGFP1-mito, a genetically encoded thermosensor specifically targeting the mitochondria, were treated with carbonyl cyanide 3-chloro-phenylhydrazone. Simultaneous visualization of tsGFP1-targeting the mitochondria, were treated with carbonyl cyanide 3-chloro-phenylhydrazone. 102 Simultaneous visualization of tsGFP1-mito in HeLa cells using JC-1, a dye that visualizes high mitochondrial membrane potential, and ATeam, a genetically encoded ATP sensor, revealed high temperature in mitochondria with high membrane potential and that there was a positive correlation between ATP levels and membrane potential.103,104 This result demonstrates that constitutive thermogenesis occurs via the respiratory chain or OXPHOS in a subpopulation of mitochondria in HeLa cells.101 Interestingly, even in oocytes collected under the same conditions, the intracellular temperature and temperature distribution differed among oocytes, suggesting that the temperature represents the state of each oocyte well. Taken together, these reports indicate that the intracellular temperature may be affected by the function of organelles such as microtubules and mitochondria. Thus, the intracellular temperature of oocytes can be a strong predictor of oocyte quality and developmental competence.

5 | CONCLUSION

Individuals with identical morphological features can differ with respect to developmental competence. It is well known that oocyte quality determines the developmental potential of embryos after fertilization.6 Oocytes arrested at the MII stage are normally fertilized within a few hours after ovulation or PB1 emission. If fertilization does not occur within the proper time, the unfertilized oocyte undergoes a time-dependent deterioration in quality, resulting in oocyte aging, a cause of fertilization failure. Sakai et al reported that the optimal period of fertilization can be specified based on the distance between the PCMs of the meiotic spindle.59 It is important to accurately determine the generation capacity of individual oocytes, but it is also necessary to perform IVF/ICSI according to the fertilization period.

Factors related to oocyte quality are becoming evident at the molecular level. However, it is not easy to accurately evaluate the developmental competence of mature oocytes based on morphology or molecular activity without damage. Typically, oocyte selection is based on microscopically determined morphological properties; however, all morphological criteria that are currently used for the grading and screening of oocytes are sometimes subjective and may not reflect the intrinsic competence of the oocyte. Moreover, morphological evaluation depends greatly on the experience and subjectivity of the observer and lacks quantitativeness. A major advantage of using intracellular temperature as a predictor is that it can be evaluated objectively and quantitatively using a temperature imaging system. If we can definitively prove that the temperature accurately reflects phenomena in the cell, it could be an indicator of oocyte quality. We are working toward elucidating the mechanism by which temperature influences cellular processes. In this study, FPT has been injected into oocytes to measure intracellular temperature, but in the future, noninvasive methods for temperature measurement should be developed for visualization.

DISCLOSURES

Conflict of interest: The author declares no conflict of interest. Human rights statement and informed consent: This article does not contain any experiment performed with human subjects. Animal studies: All institutional and national guidelines for the care and use of laboratory animals were followed. All the experiments were approved and conducted in accordance with the guidelines of the Committee of Animal Experiments of Hiroshima University, Hiroshima, Japan.

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