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Markers of Oocyte Quality to Enhance Human IVF Outcomes: A Bibliographic Review

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Abstract

The markers of oocyte quality have remained a major controversy in the field of embryology due to the subjectivity of the different methods of oocyte assessment. Various scholars use *oocyte quality* and *oocyte competence* interchangeably. *Oocyte quality* can be defined as the overall health of an oocyte whereas oocyte competence refers to the ability of an oocyte to be fertilized and develop into a healthy embryo. Diminished oocyte quality is believed to be a result of alterations in oocyte growth and maturation processes that stem from several pelvic and systemic factors before and after oocyte retrieval. In this review, we focus on the morphological and nonmorphological markers of oocyte quality. Strict restrictions that limit the number of oocytes fertilized in various countries have triggered researchers around the world to come up with the most appropriate and noninvasive markers that enhance oocyte selection and optimize IVF outcomes. PubMed, Google Scholar, and the Cochrane Library were used to search for peer-reviewed, original articles about oocyte quality markers. The review was written in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Morphological markers are commonly used, but they are subjective, and no single marker can be used exclusively to predict oocyte competence and subsequent embryonic development potential. Furthermore, transcriptomics of differentially expressed genes in cumulus cells and assessment of metabolomics and other contents of follicular fluid have shown greater precision. However, their specificity to the different quality determinants needs further research.

Keywords

Oocyte Quality, Oocyte Competence, Metabolomics, Transcriptomics, Oocyte Morphology

1. Introduction

Oocyte quality can be defined as the overall health of an oocyte. It encompasses chromosomal integrity, cytoplasmic maturation, mitochondrial function and developmental potential. Oocyte competence is another term various scholars use interchangeably with oocyte quality however it refers to the ability of oocyte to be fertilized and develop into a healthy embryo [1]. High oocyte quality correlates with increased competence [2]. Oocyte quality is a result of oogenesis, a preceding downstream process that takes place throughout oocyte development.

Oogenesis begins at 6 - 8 weeks of gestation with a rapid mitotic multiplication of germ cells to 6,000,000 to 7,000,000 oogonia by 16 - 20 weeks [3]. This is the point at which the highest Oogonal content of the gonad occurs. This number irreversibly decreases throughout the life of a female due to cellular apoptosis and oocyte/follicular atresia phenomena. These oogonia enter the first meiotic division, transform into oocytes, and arrest as a germinal vesicle at the diplotene stage of prophase 1. This process occurs throughout pregnancy and is completed at birth [3]. Inhibiting factors produced by the granulosa cells perhaps sustain the arrest of meiosis. Studies in mice suggest that retinoic acid derived from the mesonephros may act as a functional meiosis-inducing factor in the female germ cells [4]. Oocytes are then prepared for meiosis resumption by the action of follicle-stimulating hormone, which requires gap-junction networks to enhance the communication between granulosa cells and the oocyte. In response to gonadotropins, granulosa cells apparently secret a group of sterols into the follicular fluid (FF) that activates oocyte meiotic resumption and maturation [5].

During its maturation, an oocyte goes through several ultrastructural changes at both cytoplasmic and nuclear levels. At the cytoplasmic level, cortical granules relocate below the Oolemma, mitochondria redistribute homogeneously, the big vesicles of the Golgi apparatus disappear, and reunion of the smooth endoplasmic reticulum (SER) vesicles occurs. Additionally, at the nuclear level, the nuclear envelope breaks down, chromatin condenses, and the meiotic plate is configured in metaphase I and II [6]. Cytoplasmic competence is attained through the mobilization of mRNA for their translation into specific proteins of the mature oocyte, increased sensitivity to calcium-dependent signaling pathways, alterations in the membrane-transport system, and post-translation modifications such as the removal of the proteins required for the arrest at prophase 1 [1].

Alterations in the oocyte growth and maturation process will yield oocytes with diminished quality, which will generate less competent embryos for transfer. Besides these alterations, various studies have established several other fac-

tors that could influence oocyte quality before and after retrieval [7]. The factors that may influence oocyte quality before retrieval are mainly due to various systemic and pelvic conditions such as endometriosis, polycystic ovary syndrome, obesity, diabetes, and pelvic infections [8]. Zhang *et al.* (2020) showed advanced maternal age has also been considered a determinant of oocyte quality because it increases mitochondrial dysfunction and alters the expression of maternal effect genes that are essential for human oocyte quality. Increasing evidence from various animal and human studies shows that exogenous hormones compromise oocyte quality and subsequent embryonic development [9]. After oocyte retrieval, the environment in the laboratory and the handling of the FF and cumulus-oocyte complex may alter the FF contents and greatly affect oocyte quality [8]. Air quality in the laboratory, expertise of the embryologist, handling media and culture conditions, time between oocyte retrieval and insemination, and processing temperature may distort the chromosomal alignment on the meiotic spindle and the general morphological appearance of the oocyte [10]. In summary, when observing an oocyte under a microscope at its present stage, it is pertinent for the embryologist to always bear in mind its possible developmental history and align it with the morphological appearance to enhance better selection.

One of the preconditions for obtaining a healthy embryo is first obtaining a healthy oocyte. However, a lack of clear understanding of the vital markers of oocyte quality remains. This review will therefore discuss the morphological and non-morphological markers of oocyte quality that can be the basis of oocyte selection for assisted reproduction procedures.

2. Morphological Markers of Oocyte Quality

An ideal oocyte morphology should demonstrate a spherical structure bounded by a uniform zona pellucida (ZP), with a uniform translucent cytoplasm free of inclusions and a size-appropriate polar body. It is also appropriate that the oocytes should have attained both nuclear and cytoplasmic maturation [11]. Despite several studies evaluating the different aspects of oocyte morphology, the ideal oocyte morphology has remained controversial due to its subjectivity [12].

Oocyte morphology has long been assessed via grading and classification of the oocyte corona cumulus complex (OCCC), polar body, spindle, and cytoplasm, which is further classified based on granularity, inclusions, coloration, vacuoles, and regions of organelle clustering [7]. Other oocyte morphological markers include the perivitelline space (PVS), oocyte size, and ZP dysmorphism.

2.1. Oocyte Corona Cumulus Complex

Cumulus cells are follicular cells that surround and nourish the oocyte throughout its development in the ovary. After oocyte retrieval, the OCCC is the first component of the oocyte that the embryologist will observe under a stereo microscope. The communication between the oocyte and the cumulus cell is either

paracrine or via gap junctions that aid its nutrition and maturation from diplotene to the metaphase 2 stage [10].

During the meiotic arrest, cumulus cells generate cyclic guanosine monophosphate (cGMP) that transfers to the oocyte via gap junctions and hinders the hydrolysis of cyclic adenosine monophosphate (cAMP) by the phosphodiesterase PDE3A. This hindrance retains cAMP in high concentrations in the oocyte and prevents meiotic resumption [13]. The luteinizing hormone (LH) surge reverses the effects of cGMP by reducing their levels in the cumulus cells through the closure of the gap junctions between the oocyte and cumulus cells. The hindrance of the hydrolysis of cAMP by PDE3A is removed due to the decrease in oocyte cGMP, which leads to a decrease in oocyte cAMP and results in meiosis resumption [13].

The OCCC has mainly been used in the assessment of oocyte quality based on its first appearance in the IVF laboratory, and scholars have used various grading and scoring systems [14]. The grading and scoring systems have been based on the expansion degree of the cumulus cells, compactness of the corona cells, and characteristics of the ooplasm (clear or dark), but limited evidence correlates these features to embryo developmental competence [11].

In one of the schemes for evaluation of the OCCC, grades 1 - 4 were used. Grade 1 was categorized by immature OCCCs that demonstrated corona radiata cells and meagre cumulus cells. Grade 2 was slightly mature OCCCs that had tightly packed corona cells and compact cumulus cells. Grade 3 was also classified as mature OCCCs displaying expanded corona cells and expanded fluffy cumulus cells proposedly due to active secretion of hyaluronic acid. Grade 4 was postmature or overgrown OCCCs; dark, disintegrated corona cells; and expanded scanty cumulus cells [10].

Another grading system in bovine oocytes based on the compactness of the cumulus mass and ooplasm characteristics graded OCCC-A as having compact cumulus mass and translucent ooplasm, OCCC-B as having less compact cumulus mass and dark ooplasm, and OCCC-C as having expanded cumulus cells and dark ooplasm (**Figure 1**). However, various studies have reported that OCCC-B has better developmental competence followed by OCC-A and then OCCC-C [12].

OCCCs were graded from 0 - 4, depending on the expansion degree of the corona cells, where 0 has a dense layer with no visible oocyte, 1 has the least expansion, and 4 has the maximum expansion. Various studies observed no correlation between OCCC morphologic grade and oocyte maturity, fertilization rate, and embryo cleavage, which has rendered OCCC morphology a poor marker of oocyte quality. Another study using a similar grading system reached a comparable conclusion but deduced that the presence of blood clots denoted low oocyte competence [14]. Other studies correlated OCCC morphology positively with fertilization and pregnancy rate but not with embryo cleavage [15].

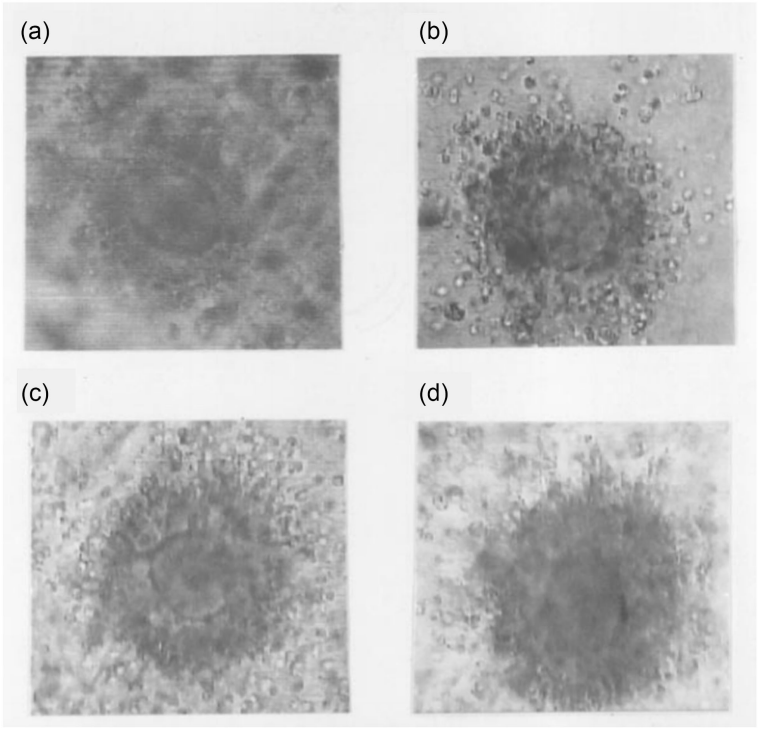

Figure 1. An illustration of the grading of OCCCs where grades 1, 2, 3, and 4 correspond to letters (a), (b), (c), and (d) respectively [15].

In most of the studies reviewed, OCCC grading showed greater importance for oocytes that were to be treated by means of conventional IVF technique, this is because these oocytes never require removal of the cumulus cells which facilitate sperm penetration. To observe other intra- and extra-cytoplasmic morphological markers, removing the cumulus cells (denudation) prior to intracytoplasmic sperm injection (ICSI) is essential [10].

2.2. Extra Cytoplasmic Markers

After denudation, the embryologist will observe the intra- and extra-cytoplasmic features of the oocyte under a light microscope. These markers provide useful information about the possible history of the oocyte, although their effectiveness in the prediction of oocyte quality is still uncertain [12].

2.3. Zona Pellucida

The Zona Pellucida (ZP) is an extracellular matrix surrounding the oocyte and initial embryo and composed of three to four glycoproteins arranged in a subtle filamentous matrix that is crucial for normal fertilization and preimplantation development [16]. The ZP is measured in terms of thickness variation, which is visualized under an inverted light microscope at a considerable magnification. Although some studies suggest the ZP thickness has no additional benefit in oocyte quality assessment, a decreased fertilization rate even in the presence of normal sperm parameters is a possibility [17]. Zonal thickness has been asso-

ciated with estradiol levels during ovulation induction. Li *et al.* (2020) showed ZP thinning has been correlated to type 1 diabetes but with no effect on zona hardening [18]. Lewis *et al.*'s (2017) study in mice observed vitrification to increase zona hardening in oocytes and embryos. However measures of assisted hatching have been taken to counter this effect, and the association between zona hardening or thickness and implantation did not vary [19].

2.4. Perivitelline Space

The Perivitelline Space (PVS) is the area between the Oolemma and the zona pellucida. It has a considerable variation in size at different oocyte development stages. An oocyte with a germinal vesicle has a considerably small PVS, which is at times difficult to visualize under a light microscope. When the first polar body is extruded, the PVS becomes uneven and distended around it. There is no significant difference after fertilization until cleavage where it follows the outlines of the blastomeres [20].

An anomalous PVS is determined by its distention and fragmentation.

Anomalous PVSs have proven to be among the most important extra cytoplasmic markers of oocyte quality, although their mechanism is not clearly understood [21]. Hassa *et al.* (2014) established a significant association between PVS abnormalities and the quality of Day 3 embryos and stated that the PVS granules are fragments of coronal cell processes withdrawn as the oocyte endures meiosis resumption (**Figure 2**). They observed a positive correlation between large PVS and low fertilization rate and pronuclear morphology, whereas the PVS granularity had no correlation with fertilization, cleavage rates, and subsequent embryo quality [21]. Other studies showed no correlation between PVS abnormalities and fertilization rates, embryo quality on Day 3, and blastocyst formation but a negative effect on implantation and ongoing pregnancy rates was observed [22].

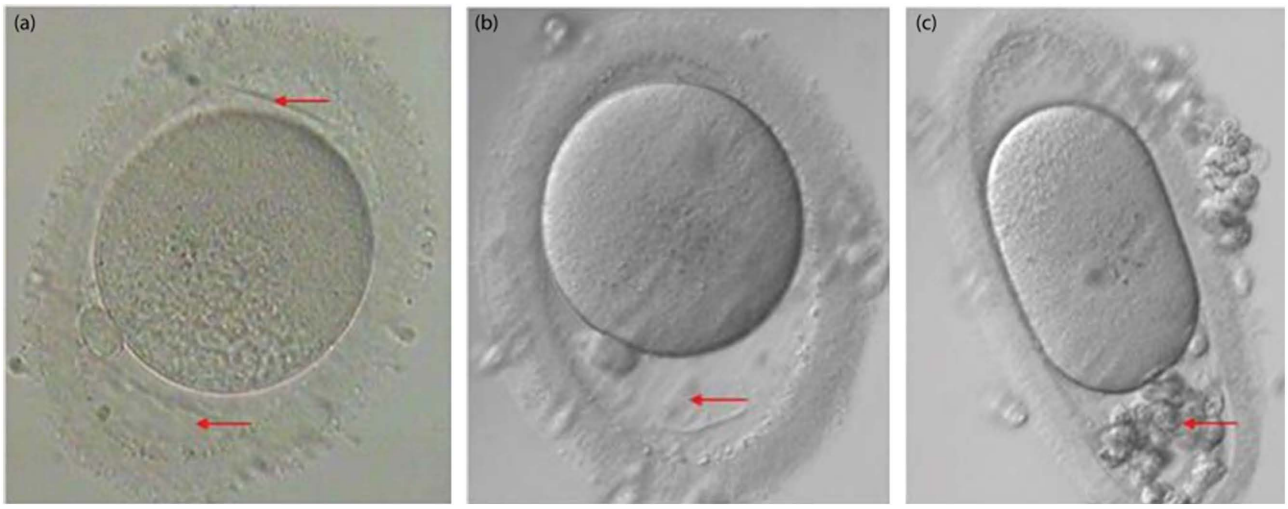

Figure 2. Illustration of perivitelline space abnormalities: (a) shows distended PVS in two areas, (b) shows distended PVS in one area, and (c) shows an oval oocyte with a distended PVS containing fragments at magnification 400X [10].

2.5. First Polar Body Morphology

Unlike the spermatozoa, the two subsequent meiotic divisions in the oocyte yield only one mature ovum and extrude the excess genetic material as one polar body at each meiotic division. The first polar body (PB1) is the first and best distinctive feature of a mature metaphase II (MII) oocyte [3]. PB1 is visualized under a light microscope after denudation of the oocyte cumulus complex. The absence of the polar body indicates incomplete maturation and further categorization as metaphase 1 or germinal vesicle (GV) depends on the presence or absence of a nucleus with nucleolus. Apart from indicating the post-ovulatory age of the oocyte, the morphology of the PB1 considering size, shape, texture, and integrity has proven a useful predictor of oocyte quality [12].

PB1 morphology is usually graded from 1 - 4, depending on its appearance, with 1 being the best and 4 the worst grade. PB1 grade 1 is ovoid or round with a smooth surface, grade 2 is ovoid or round with a rough surface, grade 3 is fragmented PB1, and 4 is large PB1 [23]. In the same study, considerably lower fertilization rates in oocytes with grades 3 and 4 were observed. The authors also deduced that grade 4 oocytes were associated with an enlarged PVS, multiple morphological abnormalities, and high frequency of aneuploidy; thus, they should not be selected for transfer. Despite other authors drawing similar conclusions on the fertilization rate and subsequent embryo quality, other studies have shown divergent outcomes that exhibited no significant correlation between PB1 morphology, fertilization rates, and subsequent embryo quality [24]. In conclusion, most embryologists have related PB1 size to oocyte ploidy, but this has proven to be speculative because most studies have shown no significant correlation. However, it is suggested that oocytes with extremely large PB1 should not be considered for transfer because they pose a potential risk of oocyte aneuploidy [11]. Nevertheless, a need still exists for more studies to justify the relationship between PB1 size and oocyte ploidy.

2.6. Oocyte Size and Shape

The most common and significant anomalous sizes and shapes of oocytes are giant oocytes and oval oocytes, although their occurrence is rare at only 0.3% or 0.12% [25]. The giant oocytes have double the cytoplasmic volume of a normal sized oocyte whose mean diameter is estimated to be 112.21 μm. They are a result of a failure in cytokinesis during mitotic division of oogonia [25]. One can mainly identify them by comparing them with sibling oocytes in the same culture dish under a light microscope. Micro-oocytes may also be observed. However, a need still exists for more research regarding their existence and significance in relation to oocyte quality. The mechanism behind the existence of oval oocytes is not so clear. However, they are associated with mechanical stress upon the oocyte during follicular puncture and oocyte denudation procedures that likely alter its shape [26].

Various scholars have established no difference in fertilization in both oocyte

cohorts with giant oocytes and those without by ICSI and IVF treatment cycles [25]. Oocyte cohorts with giant oocytes showed no considerable difference in embryo quality and development to blastocysts after successful fertilization. However, a considerable reduction in implantation and clinical pregnancy rate coupled with an increase in miscarriage rates due to a possible risk of chromosomal abnormalities associated with giant oocytes was reported [25]. To sum up, no significant difference exists between fertilization rates and embryo quality between oval-shaped oocytes and normal oocytes [26].

2.7. Intracytoplasmic Markers

Visualization of the ooplasm is done under an inverted light microscope after denudation of the oocyte-cumulus complex, which makes it possible to observe the cytoplasmic inclusions such as SER, vacuoles, refractile bodies, and cytoplasmic granularity. Incorporation of polarized light to the microscope enables visualization of the meiotic spindle, and new innovations such as fluorescence lifetime imaging microscopy (FLIM) are also beginning to assess the function of cytoplasmic organelles such as mitochondria, which is essential for oocyte viability [27].

2.8. Meiotic Spindle Morphology

The meiotic spindle is the main oocyte component that conducts the segregation of chromosomes during meiosis I and meiosis II. Correct chromosomal segregation leads to the formation of a haploid oocyte, which is critical for accurate embryonic development. An oocyte becomes haploid after the elimination of half of the chromosomal content into the PB1, which may be referred to as *meiotic maturation*. The second meiotic division is completed after fertilization, when half of the remaining sister chromatids are extruded into the second polar body [28].

The evaluation of oocyte quality based on the meiotic spindle depends on its presence, length, and location. The mechanism for central positioning of the meiotic spindle is determined by the combination of pulling and pushing forces generated by dyneins and kinesins, respectively. Although mechanical forces on microtubules can orient the spindle along the long axis of the cell, more recent studies have affiliated the spindle orientation to only the pulling forces plus the cell size and shape [29]. Some studies have attached the establishment of the orientation of the first cleavage plane to the location of the meiotic spindle [29]. In the past, the visualization of the meiotic spindle was done by confocal microscopy, which required cell fixation and thus could not be applied to the study of living cells. The invention of polarized light microscopy has made it possible to visualize the meiotic spindle in oocytes without altering their viability.

Korkmaz *et al.* (2011) elaborated that the chances of damaging the spindle during ICSI with the polar body positioned at 6 - 12 o'clock were as low as 14.7% since 62.6% of the oocytes retrieved had their spindles in proximity with the polar body [30]. The authors evaluated the different angles of deviation between

the polar body and meiotic spindle and deduced that the oocytes with a small angle of deviation (0˚ - 30˚) had higher fertilization rates (67.6%) compared to those with angle of deviation of 45˚ - 90˚ and >90˚ but with no effect on embryo quality. They also compared the outcomes of oocytes with visible and invisible spindles. The authors found that fertilization rates in oocytes with visualized spindles (65.6%) were higher than those in which spindles could not be visualized (47.3%) and with a significant difference in the quality of Day 2 embryos but no significant correlation in the quality of Day 3 and 5 embryos [30].

Rienzi *et al.*, (2003) showed similar findings of no correlation between angle of deviation and fertilization outcome when the angle was less than 90˚, but angles >90˚ showed abnormal and failed fertilization (tripronuclear zygotes) due to possible displacement of the spindle leading to failure in extrusion of the second polar body [31]. Furthermore, the presence or absence of the meiotic spindle also proved to be a possible predictor of fertilization and subsequent embryo quality. Tilia *et al.*, (2020) also supported the outcomes of the previously reviewed studies regarding meiotic spindle positioning and fertilization but made further categorization of the oocyte meiotic spindle as dysmorphic, translucent, telophase, and nonvisible, and then correlated them with blastocyst formation and ploidy [32]. Variable prospects of blastocyst euploidy were revealed where oocytes with a nonvisible or translucent spindle were significantly less likely to form euploid blastocysts compared to normal or dysmorphic spindles with all factors kept constant. The spindles in telophase resulted in embryos with low implantation potential.

2.9. Cytoplasmic Inclusions

Prior to ICSI, denuded oocytes were observed under an inverted light microscope for the potential presence of the different cytoplasmic inclusions because these may be vital predictors of the potential success of the ICSI procedure outcome (**Figure 3**). Discs or aggregates of SER, vacuoles, and refractile bodies are the most easily and commonly recognized inclusions, although dense central granularities may also be visible [17].

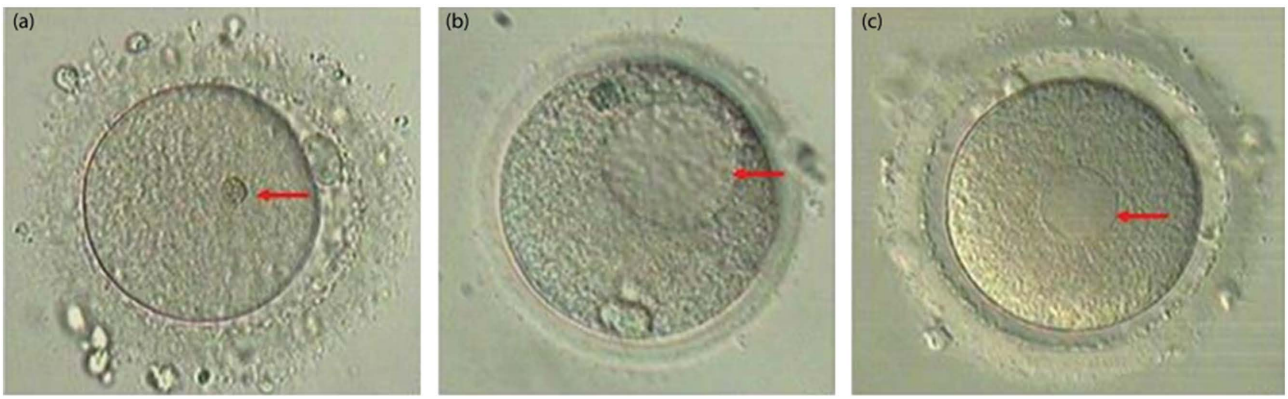

Figure 3. Illustration of some of the cytoplasmic inclusions in a denuded oocyte prior to ICSI as indicated by the arrows. (a) refractile body, (b) vacuole, and (c) smooth endoplasmic reticulum at 40x magnification [10].

The SER is one of the most abundant organelles in the ooplasm. It is essential for the storage and redistribution of ca^{2+} following sperm entry into the ooplasm. The aggregation of SER clusters appears circular and smooth within the ooplasm surrounded by mitochondria and compact granules comprising tiny vesicles [33]. The mechanism for the existence of the SER clusters is not clear even though they are believed to arise by dilatation and fusion of SER during oocyte maturation and disappear after pronuclei appearance. The studies of ca^{2+} signaling in oocytes containing SER clusters may also be pivotal in elucidating how these clusters are formed [34]. Vacuoles can be clearly distinguished from SER clusters because they appear as fluid-filled structures of varying sizes. Vacuoles that measure 5 - 10 μm in diameter have shown no serious concerns, whereas those >14 μm are associated with fertilization failures [35]. It has also been noted that even for oocytes that fertilize, the vacuoles that persist past syngamy may hinder the cleavage planes, leading to a reduced blastocyst rate [11].

Considering various reports from experts, SER clusters have been considered the worst cytoplasmic dysmorphism and hence researchers recommend that oocytes with this anomaly should not be considered for injection during ICSI [11]. Other studies have shown that the exclusion of oocytes with SER clusters from treatment via ICSI would increase the incidence of cycle cancellations, especially in patients with a small number of oocytes. However, if they are to be treated, then caution should be taken and patients should receive adequate counseling about the possible risk of fetal aberrations and low success rates following embryo transfer [36].

Evidence from various studies has shown that oocytes with SER clusters have no significant effect on fertilization, cleavage, and pregnancy rates, although one of the babies born from oocytes with SER clusters was diagnosed with Beckwith Wiedemann syndrome, which is a typical imprinting disorder because of gene mutations [34]. In a more in-depth study that compared multiple outcomes, oocytes with SER clusters and those without, but each collected from different patients, showed comparable fertilization outcomes, whereas from fraternal oocytes, those with SER clusters had lower fertilization rates compared to those without clusters. Oocytes with SER clusters of large diameter (51.2 ± 18.5 μm) had lower fertilization and high oocyte degeneration rates after ICSI compared to those of smaller diameters (22.6 ± 10.1 μm). Regarding embryo cleavage, no significant correlation was observed, although a lower blastocyst rate was reported for oocytes that had SER clusters. No significance was reported between clinical pregnancy and implantation rates in either oocyte cohort. However, high miscarriage rates and low take-home baby rates occurred in oocytes with SER clusters [35].

Fancsovits 2012 focused on oocyte granularity, outcomes were compared between oocytes that had one or more refractile body and dense central granularity. There was no significant correlation between oocyte granularity and fertilization rates, zygote morphology, cleavage and blastocyst rates. However, there was a significant correlation between oocytes with refractile bodies of a darker color

than the ooplasm and the degree of fragmentation of the subsequent embryo [37].

2.10. Mitochondria

Mitochondria are the most abundant organelles in the ooplasm. Their activity generates 90% of the cell's energy requirements in the form of adenosine triphosphate (ATP). They also play a major role in cellular apoptosis processes and in calcium homeostasis [38]. Mitochondria have their own genetic material (mitochondrial deoxyribonucleic acid (mtDNA), and its replication is independent of the cell cycle. The inheritance of mtDNA is maternal and does not follow Mendelian genetics [39]. The assessment of mitochondria for oocyte competence and subsequent embryonic development is highly dependent on its distribution throughout the ooplasm, as well as the quantity of mitochondrial DNA and ATP in the cell cytoplasm. Various studies have illustrated that insufficient mitochondrial distribution in the ooplasm may be indicative of cytoplasmic immaturity [12].

Mounting evidence demonstrates that advanced maternal age is one of the major causes of mitochondrial DNA mutations due to 5-kb mitochondrial deletion [40]. These mutations lead to mitochondrial dysfunction that impairs oxidative phosphorylation and thus leads to a reduction in total ATP production. This causes an increase in reactive oxygen species (ROS), which, in turn, increases the mtDNA copies as a compensatory mechanism for dealing with inefficient ATP production. This further justifies why increased mtDNA in the aneuploid blastocysts of old women is associated with implantation failure [38].

In summary, mitochondrial distribution, the quantity of mtDNA, and the quantity of ATP are potential predictors of oocyte quality. However, due to the invasiveness of the process of assessing them, the practical use of these tools remains controversial. The future assessment of mitochondrial function in relation to oocyte quality looks brighter with the discovery of noninvasive methods, such as metabolic imaging with the use of fluorescence lifetime imaging microscopy technology [27].

3. Non-Morphological Markers of Oocyte Quality

Due to the subjectivity of morphological markers of oocyte quality, different scientists have come up with other objective noninvasive measures for assessing oocyte quality. The OMIC (metabolomics, proteomics, transcriptomics, epigenomics, and genomics) sciences have emerged to enhance the knowledge of the existing and imminent perspectives of embryology practice. These sciences provide adequate evidence pertaining to the success of reproductive processes through the assessment of cellular biological systems [41]. The evaluation of the contents of the FF (follicular fluid) is also important because oocytes require an adequate follicular environment to acquire full developmental competence [8].

The analysis of biochemical markers and the metabolomics profile of biofluids, such as FF, are the most practical and applicable ways of assessing oocyte

quality in recent times [42]. The metabolomics profile of FF can be assessed by use of nuclear magnetic resonance (NMR) and liquid chromatography coupled with the use of a mass spectrometer (LC-MS) [43]. The observation of some of the biomarkers and biochemical markers in FF has also shown promising outcomes.

3.1. Follicular Fluid Hormones, Cytokine, and Growth Factors

The use of hormones, cytokines, and growth factors in FF is another advancement in the assessment of oocyte maturity and developmental competence, although their use is still filled with many controversies [12]. The determination of the concentrations of these substances in FF is made using commercial enzyme immunoassay kits. These include estradiol (E2), LH, follicle-stimulating hormone, progesterone, prolactin, growth hormones, anti-mullerian hormone, leptin, interleukin 1 beta, and necrosis tumor factor alpha, among others [14].

The analysis of further findings from this study showed that oocytes that were generated from follicles with high FF concentrations of LH and GH had high developmental competence. High concentrations of insulin growth factor 1(IGF-1) showed similar outcomes, but IGF-1 was low in the follicles of low responders [12]. This showed that the intra-follicular action of GH could be mediated by IGF-1. In contrast to LH and GH, the concentrations of IL-1 were lower in cases of pregnancy as opposed to fertilization. This may be because IL-1 supports oocyte cytoplasmic maturation proceedings, thus leading to optimal fertilization but not post-fertilization embryo vitality. The elevated E2 levels in FF may stem from high LH and GH, which promote steroidogenesis in theca and granulosa cells [44].

The serum anti-mullerian hormone (AMH) is known to be an excellent predictor of ovarian reserves. It is entirely produced by granulosa cells of the ovarian follicle [45]. Some studies have also established a positive correlation between this hormone and oocyte quality [46]. In a study on AMH levels in FF, no difference was observed in FF AMH levels between follicles that yielded mature metaphase II oocytes and those that yielded immature or atretic oocytes, with other factors being kept constant. An insignificant difference was also found in FF AMH levels between both fertilized and unfertilized MII oocytes (with no relationship to oocyte and subsequent embryo morphology [47]. In a more recent study, a combined analysis of FF AMH and progesterone showed that high levels of these two hormones were positively correlated with oocyte developmental competence and deduced their use as noninvasive predictive biomarkers of oocyte quality [45]. In conclusion, although high levels of FF necrosis tumor factor alpha have been negatively correlated with oocyte quality and reduced fertilization [8], they can be used as predictors of patients who are at risk of developing moderate to severe ovarian hyperstimulation [48].

3.2. Oxidative Stress Markers in Follicular Fluid

Oxidative stress (OS) may literally refer to an imbalance between the systemic

manifestation of ROS or free radicals and antioxidants in each biological system [49]. At the follicular level, high levels of OS alter the function of granulosa cells, which may induce their apoptosis. The apoptosis of granulosa cells affects the biosynthesis of estradiol 17β in the ovary, thus reducing the growth and development of follicular oocytes. This, in turn, leads to oocyte apoptosis and diminished oocyte quality [50].

In some studies, various biomarkers of OS—such as lipid peroxidase and 8-hydroxy-2-deoxyguanosine in granulosa cells, and total antioxidant capacity and ROS in FF—have been correlated with oocyte competence [12]. Aging accompanied with a deficient antioxidant mechanism may increase the production of ROS and reactive nitrogen species. These promote free radical formation, which may alter macromolecules and lead to mutations in mitochondria. This may, in turn, lower ATP production, which damages the oocyte. One of the reviewed studies carried out on lipid peroxidase and total antioxidant capacity showed no correlation with oocyte competence [49].

3.3. Metabolomics of Follicular Fluid

The invention of the metabolic profiling of FF has played a vibrant role in oocyte selection in the recent past. Metabolomics refers to the quantitative and qualitative analysis of metabolites of low molecular weight produced by a concrete biological system in a determined physiological state [51]. In most of the reviewed studies, metabolic profiling was conducted using proton nuclear magnetic resonance (NMR) and LC-MS [43]. Some of the metabolites that various investigators analyzed in FF include amino acids, fatty acids, lipids, ion composition, presence of glucose, Urea, lactate and some proteins [52].

The comparison of amino acid content in FF and serum has shown lower levels in FF, which is perhaps an indicator of the fact that oocytes use some amino acid content during their development. Various research studies have also shown that oocytes undergo anaerobic metabolism in follicles. Furthermore, oocytes that attained successful fertilization were correlated with the glycolytic pathway and with fatty acid metabolism, but no single metabolite had a correlation with oocyte quality [52]. Another study showed that the fatty acid composition of FF might be a better biomarker of oocyte competence compared with other metabolites. This was evidenced by a significant difference in FF fatty acid content and in the FF collected from follicles when comparing oocytes that fertilized and cleaved, and oocytes that fertilized but failed to cleave [43]. In summary, the need still exists for further research in this area to establish specific metabolic markers of oocyte quality.

3.4. Transcriptomics of Cumulus Cells

Cumulus cells (CCs) surround the oocyte and play an integral role in its maturation. CCs and oocyte intracellular communication are facilitated by the gap junction network, which aids in the transportation of metabolites, growth fac-

tors, and other substances that are essential for oocyte growth and maturation [10]. In recent years, interest has been growing among researchers in the study of the expression of genes in the CCs of oocytes that have attained full maturity and those that are immature at retrieval. The possible identification of differentially expressed genes in these CCs could present a potential noninvasive biomarker of oocyte competence [42].

The reverse transcriptase real-time polymerase chain reaction (RT-PCR) has proved to be the technique of choice for aiding in the sequencing of these genes. CCs are isolated after follicular puncture and are washed in phosphate-buffered saline. These cells are randomly separated from oocytes and are transferred to a tube containing lysis buffers to obtain the ribonucleic acid (RNA) for RNA sequencing (RNA-Seq) [9]. In the same study by Liu and team, six genes were found to be differentially expressed in the CCs of oocytes that yielded low-quality embryos. They also used RT-qPCR to verify some differentially expressed genes identified via RNA-Seq. These include MSTN, CTGF, NDUFA1, VCAN, SCD5, and STAR as potential markers of oocyte quality. However, the use of a small sample size limited the study [9].

In a more recent study using next-generation sequencing (NGS), 42 differentially expressed genes have been found to be previously linked with CC expansion and oocyte maturation. Downregulated genes in MII-CC were found to be enriched in the processes of nuclear maturation, chromatin remodeling and replication initiation, apoptosis, accurate chromosome segregation, and inflammation. Meanwhile, processes that were enriched through upregulated genes involved the use of extracellular matrix (ECM) components, remodeling enzymes, and steroid metabolism [42]. Oocyte selection via CC transcriptomics has shown great accuracy, and it could be recommended in countries whose regulations strictly limit the number of oocytes to be inseminated. However, its limitation may lie in the cost of its application and an extended time between collection, analysis and delivery of results which may not permit an embryo transfer in the ongoing cycle [53].

4. Oocyte Quality Improvement

Delayed maternity has become an increasing lifestyle trend in recent years because many females today consider pursuing their careers first before starting families. The advancement of maternal age because of delayed maternity comes with an associated deterioration in oocyte quality and an increase in aneuploid embryos [54]. Mitochondria dysfunction in old oocytes greatly alters cell energy requirements, thus distorting the spindle assembly, which increases the probability of meiotic errors. Cytoplasm transfer has proved to be one of the best inventions regarding oocyte rejuvenation [39]. This rejuvenation can be a partial process, where a portion of donor oocyte cytoplasm is used to revamp recipient oocyte competence. Depending on the damage done to the oocyte, a total transfer can also be done through the total replacement of deficient mitochondria

using nuclear transfer technologies [55]. In-vitro maturation is among other methods of oocyte quality improvement practiced for oocytes that have not attained complete in-vivo maturation at the time of oocyte retrieval. The germinal vesicle and metaphase 1 oocytes are matured and later treated through ICSI after the extrusion of the first polar body [10].

5. Search Methods

PubMed, Google Scholar, and the Cochrane Library were used to search for peer-reviewed original articles on oocyte quality markers. This review was written in line with the PRISMA guidelines. Studies of 15 years and below were given priority except for a few older studies. Additionally, only papers in the English language were considered. Searches were performed using such keywords as oocyte quality, oocyte morphological markers, mitochondria, cumulus cells, metabolomics, FF, and meiosis. Keywords were joined to other phrases related to the research topic.

6. Conclusion

An oocyte has a broad range of markers ranging from its morphology to the contents of its in vivo and in vitro environments. As individual morphological markers are highly subjective, the use of a combination of morphological markers to determine oocyte quality yields better precision in the determination of oocyte quality and therefore would be recommended. Mitochondria are a good determinant of oocyte competence but may not be easily applied for selection due to their invasiveness and the limited time required for oocyte exposure to the external environment. Metabolomics are great predictors of oocyte competence, although more research is still needed in this area to identify a single metabolite to target a given oocyte distortion. The use of transcriptomics of CCs to determine differentially expressed genes has proved to be the most accurate way of determining oocyte competence. However, the cost of its applicability, high technological advancements required and ethical approval for use in determination of oocyte quality and competence remains a major limiting factor to its use in most IVF centers. The future of oocyte selection looks brighter with the invention of new markers, such as oocyte intracellular temperature, fluorescence lifetime-imaging microscopy technology for the assessment of mitochondrial function, and more, which remains a subject of research.

Author's Contributions

M.K. conceived the study and wrote the paper, TM, NB, KF, MM and DR performed critical review during writing of the paper.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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