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Selection of competent oocytes by morphological criteria for assisted reproductive technologies

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Abstract

Invasive and noninvasive methods are commonly used to select developmentally competent oocytes that can improve the take-home baby rates in assisted reproductive technology (ART) centers. One of the noninvasive methods conventionally utilized to determine competent oocytes is the morphological analysis of cumulus complex, first polar body, zona pellucida, perivitelline space, meiotic spindle, and ooplasm. Successful fertilization, early embryo development, uterine implantation, and healthy pregnancy depend on the quality of oocytes, and morphological evaluation is one of the options used to predict quality levels. In this review, the morphological criteria being utilized in certain ART centers are comprehensively evaluated with special references to their predictive values and potential contributions to selecting high-quality oocytes.

KEYWORDS
assisted reproductive technology, embryo quality, oocyte morphology, oocyte selection

1 | INTRODUCTION

Infertility rate among couples gradually increases worldwide due to delaying childbearing, which is the result of various social and economic factors (Balasch & Gratacos, 2012). Thus, assisted reproductive technologies (ARTs) acquire more and more importance each day. For the purpose of obtaining metaphase II (MII) oocytes in ART centers, recombinant follicle-stimulating hormone (rFSH) or its analogs are routinely used to induce gonadotropin-dependent follicular development (Schoot et al., 1992). Then, human chronic gonadotropin (hCG) is used to resume meiotic progression from prophase I (PI) to MII stages only after observing follicles greater than 17 or 18 mm in diameter. Eventually, cumulus–oocyte complexes (COCs) including MII oocytes are collected from antral follicles 36 h after injection of hCG. As is known, in vitro maturation (IVM; Cha et al., 1991; Pincus & Enzmann, 1935) is an alternative treatment to conventional ARTs to retrieve MII oocytes from poor responders and patients with polycystic ovary syndrome (PCOS). To this end, immature germinal vesicle (GV; at the PI stage of first meiosis) oocytes are recovered from growing small follicles less than 14 mm in diameter following stimulated with rFSH or its analogs (Junk & Yeap, 2012). Then, GV oocytes undergo nuclear and cytoplasmic maturation to produce MII oocytes during routine IVM procedure (Hatirnaz et al., 2018). After obtaining MII oocytes from either conventional ARTs or IVM, the cumulus cells surrounding these oocytes can be removed by gentle pipetting to readily manipulate denuded oocytes during intracytoplasmic sperm injection (ICSI) application. The most difficult and valuable part before applying these ART procedures such as ICSI and in vitro fertilization (IVF) is to select the best oocytes that enable the production of early embryos with high quality. Thus, the birth of healthy offspring can be increased.

As the success rate of ART applications remains at ~35% (pregnancy rate per embryo transfer based on the ESHRE annual report 2016), selection criteria for human oocytes as well as sperm cells and early embryos are crucially important. Selecting high-quality oocytes based on morphological analysis may increase success rates of fertilization and subsequent embryo development (Gilchrist, Lane, & Thompson, 2008). The main challenge related to selecting better oocytes is that developmentally incompetent oocytes may exhibit the same morphologies as the good ones. In addition to morphological analysis, there are molecular biological

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techniques such as proteomic and genomic applications requiring samples obtainable only by invasive methods that might influence the developmental potential of early embryos and subsequent terms. The first and second polar bodies can also be analyzed by these molecular biological techniques; however, they do not precisely reflect the developmental potential of relevant oocytes (Cimadomo et al., 2016; Geraedts et al., 2010). In recent years, as single-embryo transfer has become an extensively accepted way in many ART centers to decrease the rate of multiple pregnancies that may lead to medical and perinatal complications, selection of competent oocytes as well as early embryos is also vital for preventing undesirable outcomes. It is worth noting that since there are legal, social, and/or religious restrictions for producing supernumerary embryos in some countries, oocytes selection can further increase the general efficiency of ARTs in these countries. Moreover, in women of advancing ages, oocytes quality gradually declines due to deteriorating physiological microenvironment around oocytes; therefore, good-quality oocytes should be selected to increase the chance of conceiving in these women having a limited reproductive window. As a result, morphological analysis for selecting competent oocytes can be utilized in ART applications in spite of its limited contributions.

2 | MORPHOLOGICAL EVALUATION ON HUMAN OOCYTES

It is generally accepted that morphological evaluation of oocytes can be performed without damaging their initial developmental potential and subsequent embryonic development. Also, this evaluation is cost-effective, noninvasive, compatible with the workflow of ART, and may contribute to reducing the generation of surplus fertilized oocytes and early embryos. A great number of morphological characteristics of cumulus complex (CC), zona pellucida (ZP), perivitelline space (PS), shape and volume of oocytes, first polar body (PB), ooplasm, meiotic spindle, and multiple properties can be assessed to select the oocytes with high quality in certain ART centers (Lasiene, Vitkus, Valanciute, & Lasys, 2009; Figure 1).

2.1 | Cumulus complex

As MII oocytes and surrounding cumulus cells communicate with each other by using gap junctions for exchanging glucose metabolites and ions, or with paracrine signaling (Tanghe, Van Soom, Nauwynck, Coryn, & de Kruif, 2002), the morphological features of CC may help to determine the oocyte quality. It was noted that the presence of blood clots in CC may adversely affect the rates of fertilization and cleavage of early embryos derived from corresponding oocytes (Daya, Kohut, Gunby, & Younglai, 1990), and decreases these oocytes quality and blastocyst formation rate (Ebner et al., 2008a; Table 1). Blood clots also lead to decreasing the number of harvested oocytes when compared with the oocytes number whose CC exhibits normal appearance (Ebner et al., 2008a). Intriguingly, removing blood clots from CC could not rescue the defects observed in the early embryos (Ebner et al., 2008a), suggesting that blood clots may execute undesirable effect(s) in the antral follicles. The adverse effects of blood clots seem to originate from the production of reactive oxygen species (ROS) at high levels from existing blood components (Attaran et al., 2000). Taken together, the oocytes having blood clots in CC might not be used for ART applications at least in the first attempt if there are more oocytes appearing normal.

A few studies also examined whether the expansion status of cumulus layers influences the oocytes' quality, early embryos development, and pregnancy rates (Table 1). To this end, Rattanachaiyanont, Leader, and Leveille (1999) evaluated the expansion grades of the corona–cumulus layers in COCs using an inverted microscope. These researchers revealed that no correlation exists between corona–cumulus expansion grades and the parameters such as nuclear maturity, fertilization, embryonic cleavage, and pregnancy rates (Rattanachaiyanont et al., 1999). Likewise, Ebner et al. (2008) observed that the expansion of corona radiata and cumulus matrix of COCs could not allow for the prediction of nuclear maturity and quality of the oocytes undergoing ICSI.

In addition to cumulus expansion, the apoptotic index of cumulus cells was analyzed to predict the nuclear maturity of oocytes. The rate of apoptosis in the cumulus cells of immature oocytes at GV or metaphase I (MI) stage is at higher levels than in the cumulus cells from mature oocytes (Host, Gabrielsen, Lindenberg, & Smidt-Jensen, 2002). Moreover, a higher rate of apoptosis in the cumulus cells of mature oocytes correlates with the lower fertilization rate in comparison with the control group having a lower apoptosis rate (Host et al., 2002).

Consistently, Faramarzi, Khalili, and Omidi (2019) revealed that there is a close relationship between apoptosis gene expression including *BAX*, *Caspase3*, and *BCL2* in the cumulus cells and embryo morphokinetics (Table 1). The *BAX* and *Caspase3* messenger RNAs expression were at higher levels in the cumulus cells of ovulated oocytes that produced embryos with uneven blastomeres than in the cumulus cells of oocytes that produced embryos without uneven blastomeres. Also, there were predominantly higher *BAX*, *Caspase3*, and *BAX/BCL2* expression, but a lower *BCL2* expression, in the cumulus cells of oocytes that generated embryos with either cell fusion or trichotomous mitosis when compared with the cumulus cells of oocytes that generated embryos not having cell fusion or trichotomous mitosis. These studies suggest that oocytes showing better developmental potential are the ones whose cumulus cells have apoptosis rates at lower levels, and lower expression of the apoptosis-related genes (like *BAX* and *Caspase3*) independent of cumulus expansion.

In a different work, Wolf (1988) graded COCs based on the number of corona radiata layers and the expansion state of cumulus cells. Grade 1 is characterized by absent to sparse cumulus cells and 1–3 layers of corona cells; Grade 2 exhibits dense cumulus cells and tightly packed corona cells; Grade 3 has expanded, fluffy cumulus cells and expanded corona cells; Grade 4 is defined as expanded, scanty cumulus cells and partially lost corona cells. Ng, Chang, and Wu (1999)

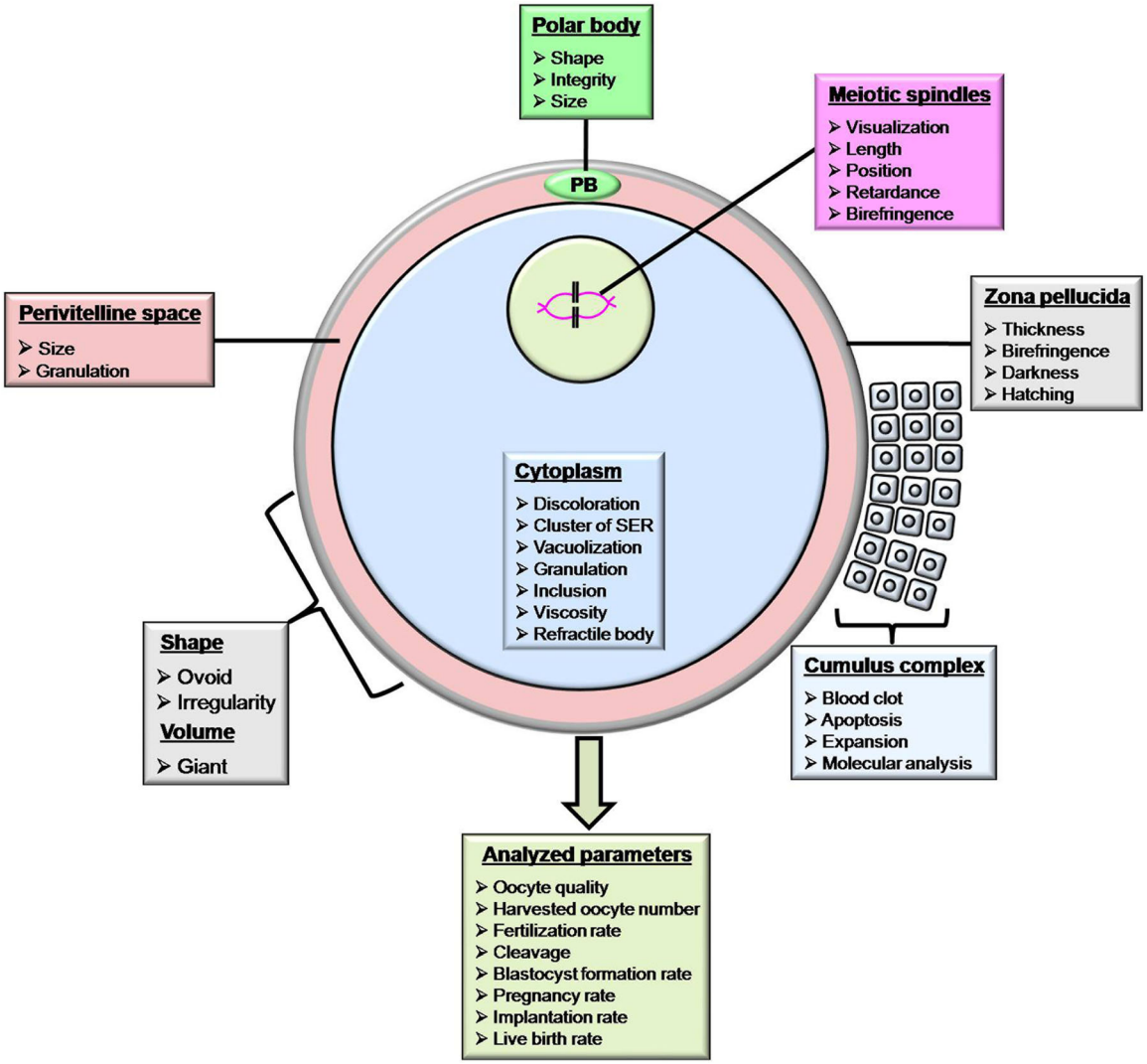


FIGURE 1 Morphological properties used to assess oocyte competency. SER, smooth endoplasmic reticulum

reported that the oocytes from grade 3 COCs showed significantly higher fertilization rates than the Grades 1, 2, and 4. Although there were no differences related to cleavage and polyspermy rates, the pregnancy success rates displayed a higher positive correlation with the Grade 3 COCs when compared with other grades (Ng et al., 1999). This higher success rate was interpreted as resulting from better cytoplasmic maturation of the oocytes producing better fertilization and pregnancy rates (Ng et al., 1999). On the other hand, Dal Canto et al., (2012) documented that morphological evaluation of COCs follows expanded (EC) and compact cumulus (CC) masses both of which involve immature oocytes and expanded cumulus mass enclosing mature oocytes (EC–MII) at the time of collection. These COCs were analyzed to predict the competence of oocytes. Although there was no difference in the fertilization rates among groups, both implantation and pregnancy rates increased progressively and significantly from EC to EC–MII groups (Dal Canto et al., 2012; Table 1). As a result, there is a positive correlation between cumulus expansion and implantation and pregnancy rates of the relative oocytes. How cumulus cell expansion affects the quality of oocytes and which molecular mechanisms are involved should be evaluated in future studies.

As there is bidirectional signaling and exchange of small molecules between cumulus cells and oocytes, transcriptome analysis of cumulus cells may help to predict the competence of oocytes and subsequent early embryos development (Table 1). For this purpose, a more detailed molecular biological analysis of cumulus cells was performed to identify the oocytes with high quality. The expression levels of cyclooxygenase 2 (*COX2*, also known as *PTGS2*), hyaluronic acid synthase 2 (*HAS2*), and gremlin 1 (*GREM1*) genes were significantly higher in the cumulus cells of oocytes that generated the early embryos with high quality (Grades 3, 4, and 5) when compared with the low-quality embryos (Grades 1 and 2; McKenzie et al., 2004). Moreover, *HAS2* and *GREM1* were expressed at higher levels in the cumulus cells obtained from oocytes that produced high-quality embryos on Day 3 than in the cumulus cells from oocytes that were not fertilized or produced poor-quality embryos (Cillo et al., 2007). However, there was no significant difference detected for the

TABLE 1 The potential outcomes of morphological and molecular changes in the cumulus complex of human oocytes

Cumulus complex	Outcome
• Presence of blood clot	↓ Harvested oocyte number (Ebner et al., 2008) ↓ oocyte quality (Ebner et al., 2008) ↓ fertilization rate (Daya et al., 1990) ↓ cleavage rate (Daya et al., 1990) ↓ blastocyst formation rate (Ebner et al., 2008)
• Higher apoptosis rate	↑ Immaturity (Host et al., 2002) ↓ fertilization (Host et al., 2002)
• Expanded, fluffy cumulus cells and expanded corona cells	↑ Fertilization rate (Ng et al., 1999) ↑ pregnancy rate (Ng et al., 1999)
• Expanded cumulus mass	↑ Implantation rate (Dal Canto et al., 2012) ↑ pregnancy rate (Dal Canto et al., 2012)
• Higher expression of *COX2*, *HAS2*, and *GREM1* genes	↑ Embryo quality (McKenzie et al., 2004)
• Higher expression *HAS2* and *GREM1* genes	↑ Day 3 embryo quality (Cillo et al., 2007) ↑ fertilization rate (Cillo et al., 2007)
• Higher expression of *BCL2L11* and *PCK1* genes, and lower *NFIB* expression	↑ Embryo potential (Assou et al., 2008) ↑ successful pregnancy rate (Assou et al., 2008)
• Higher *PTGS2* and lower *BDNF* genes expression	↑ Normal fertilization (Anderson et al., 2009)
• Higher expression of *DPP8*, *HIST1H4C*, *UBQLN1*, *CALM1*, *NRP1*, and *PSMD6* genes	↑ Pregnancy rate (Assidi et al., 2011) ↑ implantation rate (Assidi et al., 2011)
• Downregulation of *SPSB2* and *TP53I3* genes expression	↑ Chromosomally abnormal oocytes (Fragouli et al., 2012)
• Relative ranking levels of *HAS2*, *FSHR*, *VCAN*, and *PR* genes	↑ Oocyte quality (Ekart, McNatty, Hutton, & Pitman, 2013) ↑ blastocyst formation rate (Ekart et al., 2013) ↑ live birth rate (Ekart et al., 2013)
• Higher expression of *lncRNA* and *PSMD6* genes	↑ Pregnancy rate (Li et al., 2015)
• Lower expression of *AMHR2* and *LIF* genes	↑ Embryo quality (Devjak et al., 2016)
• Higher expression of *BAX* and *Caspase3*	↑ Embryos with uneven blastomere, cell fusion, and trichotomous mitoses (Faramarzi et al., 2019)
• Higher expression of *CAMK1D*	↑ Blastocyst formation (Scarica et al., 2019)

Note: ↑, increased; ↓, decreased.

pentraxin 3 (*PTX3*) expression in the same groups (Cillo et al., 2007). The expression levels of *BCL2L11* (BCL-like protein 11, at the higher level), *PCK1* (phosphoenolpyruvate carboxykinase 1, at the higher level), and *NFIB* (nuclear factor IB, at the lower level) genes in the cumulus cells contributed to the prediction of the early embryos with higher potential and successful pregnancy outcomes (Assou et al., 2008). Furthermore, *PTGS2* expression in the cumulus cells was at higher levels in the mature oocytes, and the brain-derived neurotrophic factor (*BDNF*) expression was at lower levels in the cumulus cells whose oocytes underwent normal fertilization (Anderson et al., 2009). And, the *GREM1* expression level exhibited a weak positive correlation with embryo quality, but *BDNF* expression level was found to be the negative predictor of embryo quality (Anderson et al., 2009). Consequently, the oocytes whose cumulus cells have higher *GREM1* expression should be transferred or cryopreserved. Also, the detectable levels of soluble human leukocyte antigen-G (sHLA-G) molecule ranging from 300 to 800 pg/ml in the medium of in vitro matured COCs can also be used as a biomarker for predicting oocyte maturity (Rizzo et al., 2009). Overall, higher or lower expression profiles of the gene(s) mentioned above could be employed as a biomarker for determining maturity, fertilization success, embryo quality, or pregnancy outcome of the oocytes.

The expression of a number of genes in the cumulus cells before ICSI was explored by using microarray technology. The six genes whose expression in the cumulus cells was predominantly decreased in unsuccessful pregnancies may be related to a successful pregnancy and implantation rates (Assidi, Montag, Van der Ven, & Sirard, 2011). These genes were dipeptidyl peptidase 8 (*DPP8*; playing a role in protein folding and/or apoptosis); histone cluster 1, H4c (*HIST1H4C*; implicating in the regulation of gene expression and cell differentiation); ubiquilin 1 (*UBQLN1*; functioning in intercellular signaling and cell cycle, and also in ubiquitination machinery); calmodulin 1 (*CALM1*; involving in calcium-dependent pathways); neuropilin 1 (*NRP1*; playing roles in cell assembly and interactions); and proteasome 26S subunit, non-ATPase, 6 (*PSMD6*; involved in protein folding and/or apoptosis; Assidi et al., 2011). In addition, Fragouli, Wells, Iager, Kayisli, and Patrizio (2012) reported that downregulated expression of two genes, splA/ryanodine receptor domain and suppressor of cytokine signaling (SOCS) box containing 2 (*SPSB2*) and tumor protein p53 inducible protein 3 (*TP53I3*), in the cumulus cells were significantly associated with chromosomal abnormalities in the oocytes (Fragouli et al., 2012; Table 1). While SPSB2 implicates in intracellular signaling and homeostasis, TP53I3 is involved in carbohydrate metabolism and apoptosis (Fragouli et al., 2012).

A similarly designed study by Ekart et al. (2013) indicated that the relative ranking levels of candidate genes, including *HAS2*, follicle-stimulating hormone receptor (*FSHR*), versican (*VCAN*), and progesterone receptor (*PR*) in the cumulus cells from young women (<38 years old), contributed to selecting the oocytes with higher quality, resulted in higher blastocyst formation and live birth rates when compared with the cumulus cells of randomly selected oocytes. The expression of both long noncoding RNAs (*lncRNAs*, also known as *AK124742*) and *PSMD6* (natural antisense of *AK124742*) was significantly higher in the cumulus cells of a pregnancy group in comparison with the cumulus cells from a nonpregnancy group (Li et al., 2015). The high or low expression levels of anti-Mullerian hormone receptor type 2 (*AMHR2*) and leukemia inhibitory factor (*LIF*) genes in the cumulus cells may facilitate the prediction of high or low-quality embryo (Devjak et al., 2016; Table 1). Although a limited number of cumulus cells from three women were analyzed by using microarray technology, numerous differentially expressed genes involved in sugar metabolism, ribosome biogenesis, and the JAK-STAT signaling pathway were proposed as useful markers for choosing competent embryos (Xu, Chen, Zhang, Wei, & Cao, 2015). On the other hand, it was demonstrated that RNAseq profiles of cumulus cells could not predict subsequent early embryo development potential (Green et al., 2018). Also, there were no significant differences in the transcriptome of cumulus cells of the oocytes whose early embryos resulted in live births when compared with the cumulus cells of sibling oocytes whose early embryos could not be successfully implanted (Green et al., 2018). In a recently published research, Scarica et al. (2019) reported that *CAMK1D* (calcium/calmodulin-dependent protein kinase type 1d) expression levels in cumulus cells can be employed for predicting the developmental potential of the oocytes in blastocyst formation. In a conclusion, although a significant relationship between certain genes expression at a higher or lower levels in cumulus cells and developmental features of the oocytes and early embryos has been revealed, there is no consensus on which particular genes can be precisely used and easily analyzed. Therefore, new studies especially related to transcriptomic and proteomic evaluations in cumulus cells are required for the purpose of accurately and expediently predicting which oocytes are competent.

2.2 | Zona pellucida

It is well known that oocytes in primary follicles and onward are surrounded by ZP that is generated both by oocytes and enclosing granulosa cells. Bertrand, Van den Bergh, and Englert (1995) reported that the oocytes having thinner ZP (the mean of 16.6 ± 3.2 μm) exhibited a higher fertilization rate. This was most likely because sperm penetration was facilitated in these circumstances when compared with the oocytes whose ZP thickness was at the mean of 18.9 ± 4.0 μm (Bertrand et al., 1995). In the same study, no correlation was detected between ZP thickness and ooplasm diameter. However, there was a positive correlation between ZP thickness and embryo quality scores (Host et al., 2002). It is worth noting that for these analyses polarized microscopes (PolScope) were commonly utilized to visualize oocyte structural components at higher magnification by using polarized light. The studies using PolScope revealed that the thickness of the inner layer of ZP seems to associate with oocytes' developmental potential (Shen, Stalf, Mehnert, Eichenlaub-Ritter, & Tinneberg, 2005). Analogously, the oocytes with an inner layer of ZP at 10–12 nm thickness measured by using PolScope exhibited better development than the oocytes having a thickness of 8–10 nm or less than 8 nm (Rama Raju, Prakash, Krishna, & Madan, 2007; Table 2). These studies propose that oocytes whose ZP or its inner layer thickness is thinner than others may be initially used.

The ZP birefringence, a refractive index obtained from polarization and propagation direction of light, can be analyzed to predict the developmental potential of oocytes and early embryos (Table 2). High birefringence of the inner layer of ZP in the oocytes resulted in higher implantation, pregnancy, and live birth rates than that of ones with low birefringence of the inner layer of ZP (Madaschi et al., 2009; Montag et al., 2008). The miscarriage rate was also higher in the transferred embryos obtained from oocytes showing low birefringence when compared with the ones having high ZP birefringence (Madaschi et al., 2009). In addition, Madaschi et al. (2009) observed a positive correlation between ZP birefringence and meiotic spindle visualization. The fertilization rate of oocytes showing high birefringence was at a similar level with the oocytes having low birefringence (Madaschi et al., 2009). On the other hand, Ebner et al. (2010) demonstrated that the birefringence of the inner layer of ZP in the oocytes correlated with predicting blastocyst formation, but there was no relationship observed with embryo quality or pregnancy proportion. In addition, there were significant positive correlations observed between ZP birefringence and the rates of fertilization, embryo quality, and conception cycles (Gonzalez-Ortega et al., 2016). By contrast, no correlation was found between the expression levels of potential biomarker genes (*Calm1*, *Dpp8*, *Hist1h4c*, *Nrp1*, *Psmd6*, *Tom1*, and *Ubqln1*) in the cumulus cells playing roles in the oocytes development and ZP birefringence scores (Assidi, Montag, & Sirard, 2015). It is noteworthy that ZP birefringence scores can also be compared with other oocyte-development-related genes to understand the molecular biological background of ZP birefringence. Consistent with the previous findings in this field mentioned above, ZP birefringence in the oocytes decreased with increasing age over 35 years old (Gonzalez-Ortega et al., 2016). Importantly, there was no significant correlation detected between the embryo morphokinetic features (pronuclear fading timing, cleavage, and multinucleation) and high/low ZP birefringence in patients with PCOS (Tabibnejad, Soleimani, & Aflatoonian, 2018). Taken together, although there are contradicting findings related to ZP birefringence and early development parameters, more studies suggest that the oocytes whose ZP or its inner layer at higher birefringence can be selected. Also, the molecular background of ZP birefringence and its relevance with development potential should be analyzed in surplus oocytes and early embryos in

TABLE 2 Extracytoplasmic parameters of human oocytes related to zona pellucida, perivitelline space, shape, and giantess

Extracytoplasmic factor	Property	Outcome
Zona pellucida	• Thin	↑ Fertilization (Bertrand et al., 1995)
	• Thick	↑ Oocyte development (Rama Raju et al., 2007; Shen et al., 2005) ↑ embryo quality (Host et al., 2002)
	• High birefringence	↑ Meiotic spindle visualization (Madaschi et al., 2009) ↑ fertilization rate (Gonzalez-Ortega et al., 2016) ↑ embryo quality (Gonzalez-Ortega et al., 2016) ↑ blastocysts formation (Ebner et al., 2010) ↑ implantation (Madaschi et al., 2009; Montag et al., 2008) ↑ pregnancy (Madaschi et al., 2009; Montag et al., 2008) ↑ live birth (Madaschi et al., 2009; Montag et al., 2008)
	• Low birefringence	↑ Miscarriage rate (Madaschi et al., 2009)
Perivitelline space	• Large	↓ Fertilization rate (Xia, 1997) ↓ embryo quality (Xia, 1997); ↑ embryo quality (Ten et al., 2007)
	• Presence of granules	↑ Oocyte maturity (Hassan-Ali et al., 1998) ↓ implantation rate (Farhi et al., 2002) ↓ pregnancy rate (Farhi et al., 2002)
Shape	• Ovoid shape	↓ Cleavage (Ebner et al., 2008) ↑ delayed compaction (Ebner et al., 2008) ↑ delayed blastocyst formation (Ebner et al., 2008)
	• Peripheral defect	↑ Degeneration (Ebner et al., 2001)
Giantess	• Presence of giant oocyte	↑ Estradiol level (Balakier et al., 2002; Lehner et al., 2015) ↑ obtained oocyte number (Balakier et al., 2002; Lehner et al., 2015) ↑ abnormal embryonic cleavage (Machtinger et al., 2011)

Note: ↑, increased; ↓, decreased.

large groups after providing a consensus on the technical applications and sampling homogeneity.

In addition, the darkness and hatching of ZP in oocytes was evaluated in a limited number of studies. The darkness of ZP did not exhibit any association with fertilization and implantation rates, or embryo quality (De Sutter, Dozortsev, Qian, & Dhont, 1996; Ten, Mendiola, Vioque, de Juan, & Bernabeu, 2007). And, Balaban, Ata, Isiklar, Yakin, and Urman (2008) reported that ZP hatching did not affect either cryosurvival of embryos or blastocyst formation. To determine the potential relationship of darkness and ZP hatching with oocytes quality and subsequent embryo development features, more studies are required.

2.3 | Meiotic spindles

Visualization and measuring the length of meiotic spindles were examined to determine whether they can be used to predict the early development-related features (Table 3). For this purpose, the microscope images of oocytes were captured before ICSI by using PolScope. Then, the length and size of spindles were measured with software such as the Oosight imaging system (Tomari et al., 2018). The visualization of spindles in the oocytes was found to be associated with higher ZP birefringence (Madaschi et al., 2009), fertilization rate (Braga et al., 2008; Garcia-Oro et al., 2017; Rama Raju et al., 2007), and progression to the blastocyst stage (Braga et al., 2008; Rama Raju et al., 2007). Furthermore, the presence of visible spindles in the oocytes correlated with increased implantation (Garcia-Oro et al., 2017; Madaschi et al., 2008) and pregnancy rates of the embryos derived from these oocytes (Konc, Kanyo, & Cseh, 2004; Madaschi et al., 2008) when compared to the embryos produced from oocytes not having detectable spindles. Petersen et al. (2009) revealed that the oocytes having visible spindles exhibited significantly higher developmental parameters including normal pronuclei appearance, Day 3 embryo quality, the rates of fertilization, cleavage, and blastocyst formation in comparison with nonvisible ones. There was, however, no correlation in the implantation and pregnancy rates when compared with the oocytes whose spindles were at undetectable levels (Petersen et al., 2009). In addition to a positive relation with the rates of fertilization and embryo quality, the conception cycle was also at a significantly higher proportion in the oocytes with visible spindles than that of nonvisible ones (Gonzalez-Ortega et al., 2016). In contrast, Chamayou et al. (2006) reported that there was no observed correlation regarding the presence or absence of spindles and the embryo quality and clinical pregnancy rates. In the patients with PCOS, although there was no significant relationship between the mean timing of embryo morphokinetics and meiotic spindle visualization, the rates of implantation, live birth, chemical, and clinical pregnancy were found to be higher in the transferred embryos derived from oocytes having visible spindles than those not having visible spindles (Tabibnejad et al., 2018). It is important to note that the visualization of spindles also decreased with increasing age (Gonzalez-Ortega et al., 2016). It seems reasonable to conclude that analyzing spindles visualization in oocytes will increase the fertilization, implantation, and pregnancy rates, though there are some studies that found no correlation. The contradicting findings may be the result of using microscopes having different technical attachments/software programs and varying

TABLE 3 The potential outcomes of morphological and structural changes in the meiotic spindles of human oocytes

Meiotic spindle	Outcome
• Visualization	↑ ZP birefringence (Madaschi et al., 2009) ↑ fertilization rate (Braga et al., 2008; Garcia-Oro et al., 2017; Gonzalez-Ortega et al., 2016; Petersen et al., 2009; Rama Raju et al., 2007) ↑ normal pronuclei appearance (Petersen et al., 2009) ↑ cleavage rate (Petersen et al., 2009) ↑ Day 3 embryo quality (Petersen et al., 2009) ↑ embryo quality (Gonzalez-Ortega et al., 2016) ↑ blastocyst development (Braga et al., 2008; Garcia-Oro et al., 2017; Petersen et al., 2009; Rama Raju et al., 2007) ↑ implantation rate (Garcia-Oro et al., 2017; Madaschi et al., 2008) ↑ conception cycle (Gonzalez-Ortega et al., 2016) ↑ pregnancy rate (Konc et al., 2004; Madaschi et al., 2008)
• Longer spindle length	↑ Fertilization success (Korkmaz et al., 2015) ↑ blastocyst formation (Rama Raju et al., 2007)
• Misaligned meiotic spindle position	↑ Fertilization defect (Rienzi et al., 2003)
• Localization to beneath or adjacent to PB	↑ Fertilization rate (Fang et al., 2007)
• Higher retardance	↑ Better pronuclear score (Shen et al., 2006) ↑ blastocyst formation (Rama Raju et al., 2007) ↑ pregnancy rate (Shen et al., 2006)
• Higher density	↑ Pregnancy rate (Kilani et al., 2009)
• Normally appearing spindles	↑ Fertilization rate (Kilani et al., 2009; Tilia et al., 2020) ↑ Day 3 embryo development (Tilia et al., 2020) ↑ blastocyst quality (Tilia et al., 2020) ↑ euploid blastocyst number (Tilia et al., 2020) ↑ implantation rate (Kilani et al., 2009) ↑ pregnancy rate (Kilani et al., 2009) ↑ live birth rate (Kilani et al., 2009)
• Fine aligned	↑ Embryo cleavage (Asa et al., 2017) ↑ embryo quality (Asa et al., 2017) ↓ embryo fragmentation (Asa et al., 2017) ↑ implantation rate (Asa et al., 2017)
• Birefringent	↑ Pre-embryo number (Shen et al., 2006) ↑ fertilization rate (Madaschi et al., 2008) ↑ good quality pronuclei (Shen et al., 2006) ↑ early embryo cleavage (Madaschi et al., 2008)

Note: ↑, increased; ↓, decreased.

levels of embryologists' experience in evaluating meiotic spindles as well as developmental heterogeneity of the oocytes at the time of analyzing.

The oocytes having meiotic spindle lengths of more than 12 μm possessed higher chances of producing blastocysts when compared to the oocytes with meiotic spindle lengths of 10–12 μm or less than 10 μm (Rama Raju et al., 2007). Similarly, Korkmaz, Tekin, Sakinci, and Ercan (2015) found a correlation between the length of spindles and fertilization success of women older than 35 years old (Korkmaz et al., 2015). In a recent study by Tomari et al. (2018) reported that the oocytes whose meiotic spindle sizes were between 90 and 120 μm^2 had higher fertilization, blastocyst formation, and clinical pregnancy rates when compared with the oocytes having larger or smaller spindle sizes. The spindle location with regard to PB also indicated the first cleavage plane and early embryo development parameters (Cooke, Tyler, & Driscoll, 2003). Indeed, the misalignment of spindles according to PB resulted in fertilization defects, but there was no correlation with the cleavage potential of the embryos derived from these oocytes (Rienzi et al., 2003). The oocytes whose spindles localized beneath or adjacent to the PB had significantly higher fertilization rates when compared with the differently localized ones (Fang et al., 2007; Table 3). In contrast, Moon et al. (2003) demonstrated that no relationship existed between spindle localization in the oocytes and fertilization rate as well as normal early embryo development (Moon et al., 2003). Based on these studies, we conclude that localization, size, and length of meiotic spindles may be used in oocytes selection for the purpose of increasing fertilization success rates and early development potential.

The relationship between spindle retardance (order and density of spindles) and embryo development parameters was also evaluated. Although it was not statistically significant, there was a weak correlation found between low retardance and poor embryo quality (De Santis et al., 2005). On the other hand, Shen et al. (2006) reported that high retardance of spindles in oocytes was associated with better pronuclear scores as well as higher pregnancy (Shen et al., 2006) and blastocyst formation (Rama Raju et al., 2007) rates. Moreover, the oocytes resulting in pregnancy possessed notably higher meiotic spindle densities in comparison with the ones not resulting in pregnancy (Kilani, Cooke, Kan, & Chapman, 2009). In the same study, the oocytes having a significantly higher number of normally appearing spindles at 39–40 h following hCG treatment resulted in pregnancy when compared with those that did not result in successful pregnancies (Kilani et al., 2009). The fertilization rate was also significantly higher in the oocytes with normal spindles in comparison with the oocytes having abnormal spindles (Kilani, Cooke, Tilia, & Chapman, 2011). The implantation and live birth rates of the embryos derived from oocytes with normal spindles were likewise higher than the embryos produced from the oocytes having abnormal spindles (Kilani et al., 2011). In addition, the fine spindle-aligned subgroup exhibited positive correlations with embryo

cleavage and implantation rates when compared with the average and poorly aligned subgroups which showed lower embryonic quality and more fragmentation (Asa, Tabatabaee, Farrokhi, & Nejatbakhsh, 2017; Table 3). In conclusion, the retardance, appearance, and alignment of spindles can be used as biomarkers to predict the developmental potential of the oocytes for subsequent developmental processes such as fertilization, implantation, and pregnancy. Determining the molecular biological relationship between meiotic spindles and early developmental processes is ideal subjects for future investigations.

For the spindle configuration, there was no significant difference detected between fresh and frozen- and thawed-mature oocytes when analyzed by using a confocal microscopy (Coticchio et al., 2010). The oocytes with high birefringent spindles produced a significantly higher number of pre-embryos with good pronuclei than the oocytes with low birefringent spindles generated pre-embryos having low quality (Shen et al., 2006). The rates of fertilization and early embryo cleavage derived from oocytes with birefringent spindles were at higher levels when compared with the spindle-non-detected oocytes (Madaschi et al., 2008). The birefringent spindles in oocytes could be used in predicting their quality, fertilization, and developmental abilities (Rienzi et al., 2005; Table 3). It should be kept in mind that various types of defects in spindles may lead to aneuploidies or maturation arrests in the oocytes (Eichenlaub-Ritter, Shen, & Tinneberg, 2002). Therefore, as reported in a recent study, spindle morphologies of oocytes may be utilized in the prediction of euploid blastocysts (Tilia, Chapman, Kilani, Cooke, & Venetis, 2020). In the same study, it was displayed that, based on the intention-to-treat (ITT) analysis, oocytes having normal spindles exhibited higher rates of fertilization, Day 3 embryo development and blastocyst quality when compared with the oocytes with nonvisible spindles or having a translucent or telophase appearance (Tilia et al., 2020; Table 3).

As a result, the analysis of spindles by using PolScope or other microscope types which do not seem to damage the developmental potential of the oocytes may be an easy way to predict early development features from fertilization to pregnancy. However, the molecular mechanism(s) underlying the correlations between spindle morphologies and oocyte quality should be identified in new studies to reach precise, simple, and practical prediction method(s).

2.4 | Perivitelline space

The two morphologies involving size (enlarged or not; Xia, 1997) and content (presence of granulation; Hassan-Ali et al., 1998) in PS might be used in assessing oocyte quality (Table 2). Generally, it is accepted that normal MII oocytes have a small PS including PB and no granulation (Ubaldi & Rienzi, 2008). Xia (1997) revealed that large PS in the oocytes without PB fragmentation and inclusions led to significantly diminished fertilization and 3–8 cell embryo rates when compared with the oocytes in normal appearance. On the other hand, De Sutter et al. (1996) and Balaban et al. (1998) reported that there was no correlation observed between fertilization/embryo quality rates and large PS. Similarly, no relationship was found between PS size and the following morphokinetic values: second PB extrusion timing, pronuclear morphology, pronuclei fading, 2–8 cell embryo formation, and irregular cleavage pattern (Faramarzi et al., 2019) as well as oocyte yield (Weghofer et al., 2019). Interestingly, Ten et al. (2007) documented that large PS caused 1.8 times higher embryo quality when compared with the oocytes having normal PS. Most studies explored this subject suggest that PS size does not seem to affect the subsequent developmental potential of oocytes.

When the impact of granules in PS was analyzed, the coarse granules decreased the implantation and pregnancy rates of the oocytes in comparison to control ones (Farhi et al., 2002). Furthermore, Hassan-Ali et al. (1998) revealed that the incidence of granules in PS enhanced due to high dosages of human menopausal gonadotropin (HMG) treatment, and it was positively associated with the oocytes maturity. However, there was no correlation observed between granulation in PS and the parameters such as fertilization, cleavage, and pregnancy rates (Hassan-Ali et al., 1998). Based on the studies aimed to determine the possible association between PS size/granulation and oocytes developmental potential: although there is no consensus on these evaluated parameters, it seems reasonable to prefer the oocytes having small PS size and no granulation. It is important to emphasize that this does not mean that oocytes having large and granular PS cannot be used for producing embryos.

2.5 | Shape

A study by De Sutter et al. (1996) documented that there was no correlation between fertilization rate/embryo quality score and irregular oocyte shape. Similarly, the shapes of oocytes were not found to be associated with cryosurvival (Rienzi et al., 2008), aneuploidy (Yakin, Balaban, Isiklar, & Urman, 2007), and implantation or clinical pregnancy rate (Chamayou et al., 2006). Although the oocytes with ovoid shape did not lose their fertilizability, they exhibited abnormalities in cleavage as well as delayed compaction and blastocyst formation (Ebner, Shebl, Moser, Sommergruber, & Tews, 2008). There was no effect, however, on the blastocyst quality. The morphological defects in the periphery of oocytes involving fragile oolemma and irregular shape were related to degeneration rather than cytoplasmic abnormalities, and did not correlate with the development of the early embryo (Ebner et al., 2001; Table 2). Overall, the shapes of oocytes except for ovoid ones do not seem to influence the fertilization, early embryo development, and the rates of implantation and clinical pregnancy.

2.6 | Giant oocytes

A giant oocyte is characterized by having twice the volume of a normal oocyte (~200 μm in diameter; Balakier, Bouman, Sojecki, Librach, & Squire, 2002) and by having tetraploidy that is possibly derived either from the absence of cytogenesis following a nuclear

division or fusion of two oogonia during early development (Austin, 1960). It is worth noting that women producing giant oocytes possessed higher levels of estradiol and an increased number of obtained oocytes, suggesting that this issue may also originate from ovarian stimulation protocols (Balakier et al., 2002). The incidence of giant oocytes is normally at a frequency of 0.26–0.31% among recovered oocytes (Balakier et al., 2002; Rosenbusch, Schneider, Glaser, & Brucker, 2002). If binucleated giant MII oocytes were fertilized by normal spermatozoa, tripronuclear digynic zygotes emerged (Rosenbusch & Schneider, 1998). Interestingly, these fertilized giant oocytes are capable of undergoing normal cleavage and blastocyst formation; however, they should be discarded even if they have normally appeared two pronuclei (Ebner, Moser, & Tews, 2006), due to the possibility of subsequent production of abnormal embryos (Balakier et al., 2002; Table 2).

A study by Machtinger, Politch, Hornstein, Ginsburg, and Racowsky (2011) documented that the presence of giant oocytes in a cohort of retrieved oocytes led to abnormal embryonic cleavage (increased percentage of 1- and 10-cell embryos and reduced percentage of 6- and 7-cell embryos; Table 2), but there were no differences in the rates of embryos with perfect symmetry, fragmentation and implantation, as well as clinical or ongoing/delivered pregnancy rates when compared with the cohort of oocytes without a giant oocyte. In a different study, researchers created two groups: a giant group involved cycles with at least one giant oocyte in the cohort of obtained oocytes, and a normal group included cycles with no giant oocytes (Lehner et al., 2015). The giant group had significantly higher estradiol levels and a higher number of retrieved oocytes compared with the normal group. By contrast, there were no dramatic changes in the embryo quality and clinical pregnancy rate between the two groups (Lehner et al., 2015). It was reported in the same study that ovarian stimulation cannot influence the incidence of giant oocytes. As a result, the presence of giant oocytes in the cohort of recovered oocytes does not appear to influence the developmental features of the sibling oocytes.

2.7 | First polar body

The extrusion of PB indicates successful meiotic maturation of the oocytes (Eichenlaub-Ritter, Schmiady, Kentenich, & Soewarto, 1995). Therefore, PB can be used as a biomarker of quality and even aging status of the oocytes because in vivo aged MII oocytes had a degenerated PB due to overmaturity, which resulted in developmental disabilities (Eichenlaub-Ritter et al., 1995). Analogously, Xia (1997) revealed that intact PB significantly correlated with the fertilization rate and embryo quality. The embryos obtained from oocytes with PB having well-shaped morphology had higher implantation and pregnancy rates, and a decreased multiple pregnancy, when compared with the control embryos, included embryo fragmentation (Ebner et al., 1999). Similarly, Ebner et al. (2000) showed that PB either in intact or well-shaped morphology correlated with higher fertilization and embryo quality rates. Moreover, PB with intact integrity and having a smooth surface was found to be positively correlated with the fertilization rate and embryo quality as well as with the implantation and pregnancy rates (Ebner et al., 2002; Table 4).

An interesting study by Fancsovits et al. (2006) reported that the oocytes having fragmented PB exhibited higher developmental progress than those with intact PB. On the other hand, the oocytes with large PB showed lower viability than the normal ones (Fancsovits et al., 2006). Although degeneration or largeness in PB was found to be correlated with reduced fertilization rates, no relationship was detected with the pronuclear morphology or embryo quality (Rienzi et al., 2008). And, Navarro et al. (2009) documented a significant association between large PB in the oocytes and the rates of decreased fertilization, cleavage, and embryo quality following ICSI application. In the same study, no correlation was observed for PB fragmentation. Also, it was reported that the oocytes with intact PB produced significantly higher rates of Day 3 good-quality embryos and blastocysts, and more embryos in comparison to the fragmented ones (Zhou, Fu, Sha, Chu, & Li, 2016). However, there were no

TABLE 4 The potential outcomes of morphological changes in the first polar body of human oocytes

First polar body	Outcome
• Intact	↑ Fertilization rate (Xia, 1997) ↑ embryo quality (Xia, 1997) ↑ Day 3 good quality embryo (Zhou et al., 2016) ↑ blastocyst quality (Zhou et al., 2016) ↑ available embryo number (Zhou et al., 2016)
• Well-shaped morphology	↑ Implantation rate (Ebner et al., 1999) ↑ pregnancy rate (Ebner et al., 1999) ↓ multiple pregnancy rate (Ebner et al., 1999)
• Intact and well-shaped morphology	↑ Fertilization rate (Ebner et al., 2000) ↑ embryo quality (Ebner et al., 2000)
• Intact and smooth surface	↑ Fertilization rate (Ebner et al., 2002) ↑ embryo quality (Ebner et al., 2002) ↑ implantation rate (Ebner et al., 2002) ↑ pregnancy rate (Ebner et al., 2002)
• Large	↓ Viability (Fancsovits et al., 2006) ↓ fertilization rate (Rienzi et al., 2006; Navarro et al., 2009) ↓ cleavage (Navarro et al., 2009) ↓ embryo quality (Navarro et al., 2009)
• Degenerated	↓ Fertilization rate (Rienzi et al., 2006)
• Fragmented	↑ Developmental progress (Fancsovits et al., 2006

Note: ↑, increased; ↓, decreased.

differences for pregnancy or implantation rates between the two groups. In addition to PB in oocytes, the second PB angle below 24.25° in zygotes contributed to the prediction of good-quality embryos when compared with the zygotes whose PB exhibited a greater angle between them (Zhang, Guo, & Li, 2016; Table 4). In light of these investigations, the oocytes with intact structure and well-shaped PB seem to have a higher developmental potential than that of the ones displaying the opposite features. Consequently, these oocytes may be selected above all others to improve the rate of take-home babies.

In contrast to the studies mentioned above, Verlinsky et al. (2003) revealed that the abnormal PB morphologies involving fragmentation and irregular shape did not correlate with fertilization rate, embryo quality, embryo survival to blastocyst stage, embryo transfer outcome, and aneuploidies. In parallel with this study, no relationship was found between fragmentation in PB and the following parameters: fertilization and cleavage rates, embryo quality, pregnancy and implantation rates (Ciotti et al., 2004). Similarly, De Santis et al. (2005) evaluated the following parameters such as size (normal and large) and shape (smooth, fragmented and rough) of PB, and determined that there were no significant differences between fertilization rate or embryo quality and the PB morphologies. The contradicting findings for the PB morphologies may derive from the differences in (a) the criteria and technical equipment being used for defining PB morphologies; (b) the experience of laboratory technician analyzing PB; (c) the numbers of cases enrolled in each study; and (d) the timing of PB morphology evaluation; for example, the frequency of PB fragmentation increases during the interval from 2 h 31 min to 3 h 30 min, that is, the time of passage from oocyte denudation to ICSI (Ciotti et al., 2004).

2.8 | Cytoplasmic changes

Cytoplasmic changes such as vacuolization and various types of incorporations including refractile bodies, granulation, inclusion, discoloration, and clustering of the smooth endoplasmic reticulum (SER) in the oocytes were evaluated for the purpose of predicting their potential effects on developmental competence (Ebner et al., 2001; Table 5). De Sutter et al. (1996) reported that there was no observed correlation between the fertilization rate/embryo quality score and the presence of vacuoles in the ooplasm. In contrast, it was documented that the vacuoles derived from tubular-type SER clusters were associated with an increased biochemical pregnancy rate and serum estradiol level at the day of hCG treatment, and with the reduced clinical pregnancy rate (Otsuki, Okada, Morimoto, Nagai, & Kubo, 2004). Also, the oocytes with vacuolar cytoplasm or central granulation exhibited declined cryosurvival, and were unable to

TABLE 5 Cytoplasmic changes and their potential outcomes in the human oocytes

Cytoplasmic changes	Outcome
• Presence of vacuole	↑ Serum estradiol level (Otsuki et al., 2004) ↓ oocyte cryosurvival (Balaban et al., 2008) ↓ good quality blastocyst (Balaban et al., 2008) ↓ zona pellucida hatching (Balaban et al., 2008) ↑ biochemical pregnancy (Otsuki et al., 2004) ↓ clinical pregnancy (Otsuki et al., 2004)
• Presence of granulation	↓ Oocyte cryosurvival (Balaban et al., 2008) ↑ fertilization rate (Wilding et al., 2007) ↑ compromised pronuclear morphology (Rienzi et al., 2008) ↓ good quality blastocyst (Balaban et al., 2008) ↓ zona pellucida hatching (Balaban et al., 2008) ↓ pregnancy rate (Serhal et al., 1997)
• Presence of inclusion	↓ Fertilization rate (Xia, 1997) ↓ embryo quality (Xia, 1997) ↓ implantation rate (Serhal et al., 1997) ↓ pregnancy rate (Serhal et al., 1997)
• Presence of SER aggregate	↓ Oocyte maturation (Setti et al. 2016) ↓ fertilization rate (Sa et al., 2011) ↓ embryo quality (Ebner et al. 2008b; Sa et al. 2011) ↑ embryo transfer cancellation (Restelli, et al., 2015) ↓ implantation rate (Setti et al. 2016) ↓ pregnancy rate (Otsuki et al. 2004) ↑ miscarriage rate (Braga et al. 2013) ↓ transcriptome level (Stigliani et al., 2018)
• High viscosity	↓ Fertilization rate (Ebner et al., 2003) ↓ embryo quality (Ebner et al., 2003) ↓ blastocyst formation (Ebner et al., 2003)
• Presence of refractile body	↓ Fertilization rate (De Sutter et al., 1996; Otsuki et al., 2007) ↓ blastocyst formation (De Sutter et al., 1996; Otsuki et al., 2007)
• Presence of darkness	↓ Embryo quality (Ten et al., 2007)
• Presence of multiple cytoplasmic dysmorphisms	↓ Embryo quality (Chamayou et al., 2006) ↓ implantation rate (Serhal et al., 1997) ↓ pregnancy rate (Serhal et al., 1997)
• Texture, inclusion, vacuole and central granulation	↓ Embryo quality (Chamayou et al., 2006)
• Granulation, inclusion, SER cluster and refractile body	↓ Implantation rate (Serhal et al., 1997) ↓ pregnancy rate (Serhal et al., 1997)

Note: ↑, increased; ↓, decreased.

develop into good quality blastocysts able to reach hatching stage (Balaban et al., 2008). Accordingly, although the transferred embryos derived from normal oocytes reached 24% pregnancy rate, it was only 3% in the embryos produced from oocytes having homogeneous granular cytoplasm (Serhal et al., 1997) and 12.8% in the oocytes with granular cytoplasm (Kahraman et al., 2000). On the other hand, there was no relationship found between oocytes with centrally located granular cytoplasm and fertilization, embryo development, and pregnancy rates (Kahraman et al., 2000).

In other similarly designed studies, no correlation was detected between cytoplasmic inclusions and the development-related parameters such as implantation, fertilization, and embryo quality rates (Balaban et al., 1998; De Sutter et al., 1996). Interestingly, Wilding et al. (2007) revealed that the oocytes having cytoplasmic granulation possessed a higher fertilization rate than the oocytes not having any type of granularity. On the other hand, Rienzi et al. (2008) reported that diffuse peripheral granulation in the MII oocytes resulted in compromised pronuclear morphology. It is important to note that approximately 90% of the oocytes exhibited circular waves of cytoplasmic granulation at the time period of 20–53 min after ICSI (Payne, Flaherty, Barry, & Matthews, 1997). To establish accurate correlations of presence and localization of the vacuoles and cytoplasmic granulation with the oocytes developmental potential, more studies in large groups are needed to reach a consensus on how to predict competent oocytes.

The potential effects of cytoplasmic inclusion on the early developmental progress were also analyzed. Xia (1997) reported that cytoplasmic inclusion in the oocytes was at higher levels in the patients older than 35 years old when compared with the patients younger than 35 years old, and is correlated with a decreased fertilization rate and embryo quality. Although there were no pregnancy and implantation observed upon transfer of the embryos derived from the oocytes with cytoplasmic inclusions, the fertilization, and early embryo development were at similar rates between the two groups (Serhal et al., 1997). This study suggests that cytoplasmic inclusions might exhibit their adverse effect(s) at the later terms of prenatal development.

According to the Alpha/EHSRE consensus, the percentage of embryo transfer cancellation of the oocytes with aggregates of SER was significantly higher when compared with the embryos resulting from oocytes without aggregates (Restelli et al., 2015). Indeed, SER aggregates were associated with lower oocyte maturation (Setti et al., 2016) and embryo quality (Ebner, Moser, Shebl, Sommerguber, & Tews, 2008b; Sa et al., 2011), and reduction of fertilization (Sa et al., 2011), implantation (Setti et al., 2016), and pregnancy rates (Otsuki et al., 2004), even increased miscarriage rates (Braga et al., 2013) when compared with the oocytes lacking of SER aggregates (Ferreux et al., 2019). The potential impact of SER aggregates on the transcriptome of MII oocytes was also evaluated by using microarray (Stigliani et al., 2018). The oocytes with these aggregates showed significantly decreased expression of the genes being involved in certain biological activities such as cellular division, spindle organization, chromosome segregation, G2/M mitotic cell cycle transition, organization of cytoskeleton and microtubules, and mitochondrial function (Stigliani et al., 2018). Although these aggregates seem to be related to the altered genes expression in the oocytes, it remains elusive whether this type of oocytes should be used in ART centers.

In a limited number of studies, other cytoplasmic changes such as cytoplasmic viscosity and refractile bodies were explored. As is known, cytoplasmic viscosity is briefly defined as the persistence of injection funnel after withdrawing into ICSI pipette (Ebner et al., 2003). It influences the movement of cellular organelles and microtubule organization. Therefore, high cytoplasmic viscosity in the oocytes adversely affected the fertilization, embryo quality, and blastocyst formation rates (Ebner et al., 2003). Also, the presence of refractile bodies derived from lipofuscin inclusions correlated with the decreased fertilization rate and defective blastocyst formation (De Sutter et al., 1996; Otsuki, Nagai, & Chiba, 2007). The darkness of ooplasm was found to be related to the reduced possibility of obtaining good-quality embryos (Ten et al., 2007; Table 5). Overall, although oocytes showing granulation, cytoplasmic inclusions, SER clusters, and refractile bodies exhibit normal fertilization and early embryo development, the resultant embryos have lower implantation and pregnancy rates when compared with the embryos derived from oocytes with normally appearing cytoplasm as documented in the study by (Serhal et al., 1997). Also, multiple cytoplasmic dysmorphisms in the oocytes such as texture, inclusions, vacuoles, and central granulation adversely affected the embryo quality (Table 5); however, there was no relationship with implantation and pregnancy rates (Chamayou et al., 2006). As a result, the oocytes having no inclusions and aggregates in the cytoplasm and lower cytoplasmic viscosity should be chosen to achieve higher pregnancy rates.

2.9 | Multiple morphological parameters

Some studies were examined the probable effects of multiple morphological changes on the developmental potential of oocytes (Table 6). Xia (1997) demonstrated that oocytes with large PS, fragmented PB, and cytoplasmic inclusions exhibited significantly decreased fertilization rate and embryo quality. Moreover, the oocytes showing dark cytoplasm with many vacuoles or fragments produced poor quality embryos, resulted in lower pregnancy rates when compared with the oocytes with normal morphology (5.5% vs. 29.4%; Loutradis et al., 1999). A significant relationship was discovered between oocyte morphologies (presence of vacuoles, abnormal PB, large PS, diffused cytoplasmic granularity, and centrally located granular area) and the clinical pregnancy rate, a number of retrieved oocytes, female ages, and basal FSH levels on Day 3 (Rienzi et al., 2008). Yakin et al. (2007) found similar results and reported that the morphological changes in oocytes related to the meiotic spindle, ZP, PS, PB as well as the presence of vacuoles or granulations in the ooplasm remarkably decreased the blastocyst formation. As expected, high-quality oocytes based on the morphological characteristics (granularity, vacuoles, and inclusions in the ooplasm, and the resistance and fragility of the oocytes plasma membrane at

TABLE 6 Multiple morphological, structural, and metabolic parameters in the human oocytes

Multiple morphologies	Outcome
• Large PS, fragmented PB, and cytoplasmic inclusion	↓ Fertilization rate (Xia, 1997) ↓ embryo quality (Xia, 1997)
• Dark cytoplasm, many vacuoles or fragments in cytoplasm	↓ Embryo quality (Loutradis et al., 1999) ↓ Pregnancy rate (Loutradis et al., 1999)
• Vacuoles, abnormal PB, large PS, diffused cytoplasmic granularity	↓ Oocyte number (Rienzi et al., 2008) ↓ clinical pregnancy (Rienzi et al., 2008) ↑ basal FSH (Rienzi et al., 2008)
• Single PB, normally appearing cytoplasm, proper ZP thickness, and PS	↑ Transferable embryo number (Swain et al., 2008)
• Dismorphologies in meiotic spindle, ZP, PS, PB as well as the presence of vacuoles or granulations in the ooplasm	↓ Blastocyst formation (Yakin et al., 2007)
• Abnormal PB, existing vacuoles, and large PS	↓ Fertilization rate (Rienzi et al., 2008)
• Granularity, vacuoles, and inclusions in the ooplasm, and the resistance and fragility of the oocyte plasma membrane	↓ Fertilization rate (Wilding et al., 2007) ↓ embryo quality (Wilding et al., 2007) ↓ clinical pregnancy rate (Wilding et al., 2007)
• Large PS, diffused/central cytoplasmic granulation	↓ Pronuclear quality (Rienzi et al., 2008) ↓ Day 2 embryo quality (Rienzi et al., 2008)
• Lower apoptotic index in cumulus cells and higher oxygen consumption of oocytes	↑ Fertilization (Ruvolo et al., 2013) ↑ embryo cleavage (Ruvolo et al., 2013)

Note: ↑, increased; ↓, decreased.

Abbreviations: FSH, follicle-stimulating hormone; PB, polar body; PS, perivitelline space; ZP, zona pellucida.

ICSI) possessed higher fertilization, embryo quality, and clinical pregnancy rates than the low-quality group (Wilding et al., 2007). Also, it was documented that abnormal PB, existing vacuoles, and large PS in the oocytes led to lower fertilization rates (Rienzi et al., 2008). In the same study, large PS and diffused/central cytoplasmic granulation in the oocytes were found to affect the pronuclear morphology and Day 2 embryo quality. In addition to the morphological analysis, lower apoptotic index (<25%) in the cumulus cells and higher oxygen consumption of individual oocytes seemed to be an indicator of better fertilization and embryo cleavage abilities (Ruvolo, Fattouh, Bosco, Brucculeri, & Cittadini, 2013). As a result, the oocytes having single and intact PB, normally appearing cytoplasm and meiotic spindle, proper ZP thickness, and PS exhibit a higher potential for producing transferable embryos, as elegantly reviewed by (Swain & Pool, 2008; Table 6).

On the other hand, the morphologies such as extracytoplasmic changes (dark ZP and enlarged PS), cytoplasmic abnormalities (dark cytoplasm, granular appearance, and refractile bodies), and shape defects in the oocytes did not influence fertilization and clinical pregnancy rates as well as implantation potential and embryo quality when compared with the normal ones (Balaban et al., 1998). Correspondingly, Chamayou et al. (2006) observed no correlation between the clinical pregnancy/implantation rates and the oocyte morphological parameters (ooplasm texture, PS size/granulation, PB shape, and meiotic spindle visualization). Also, no association was detected between the oocyte dysmorphisms related to PS content, shape, PB, cytoplasmic granularity, cytoplasmic vacuoles as well as with semen source, donor age, number of days of stimulation, plasma estradiol concentration, and the development parameters including fertilization rate and embryo quality (Ten et al., 2007). In agreement with the previous studies, morphological changes in the oocytes associated with the meiotic spindle, ZP, PS, PB, vacuolization, or granulation did not influence the rates of fertilization, cleavage, and aneuploidy (Yakin et al., 2007). Likewise, there was no correlation determined between the morphological changes in COC, ZP, PS, PB, vacuolization or granulation of the oocytes, and the rates of fertilization, implantation, pregnancy, take-home baby, and multiple pregnancy (La Sala et al., 2009).

Based on the studies presented in this section, there are contradictory findings regarding the ability of multiple morphological analyses to predict oocytes with high quality. The opposite findings for similar multiple morphologies suggest that distinct molecular biological mechanisms having different effects on the developmental potential of oocytes may exhibit a correlation with the same morphologies. Therefore, to reach a consensus on the multiple morphologies, more studies are required to elicit the signaling pathways showing direct or indirect interaction(s) with these morphologies. Until these investigations are carried out, multiple morphologic analysis can be used to predict competent oocytes by employing a mathematical formula based on the prediction value of each morphology revealed in these studies.

3 | CONCLUSION

The morphological criteria being evaluated in some ART centers by using light or polarized microscopy may contribute to successfully selecting good quality oocytes for the purpose of increasing the rate of take-home baby. The most commonly analyzed morphologies in the oocytes are related to the meiotic spindle, CC, ZP, PS, vacuoles

or refractile bodies, shape, granulation, and viscosity of ooplasm. There is, however, still no consensus on which of these morphologies is the best one for oocyte selection. Therefore, multiple morphological analyses seem to be a good way of predicting the oocytes having a higher potential for producing competent early embryos.

The oocytes having a clear or moderate granular cytoplasm, narrow PS, PB in an intact appearance, normally appearing meiotic spindles and CC, and colorless and birefringent ZP should be selected for the initial ARTs application if there are no restrictive factors such as patient's age, harvested oocyte numbers, and previous ART failures. It is noteworthy that these morphological criteria must be evaluated in a large sample of homogenous cases with the participation of multiple ART centers. Together with morphological analysis, more sophisticated technologies such as genomics, transcriptomics, proteomics, and metabolomics can be used in more accurately predicting the oocytes with high quality.

In addition to choosing oocytes, the selection of competent embryos is also a predominant strategy in infertility treatment for the purpose of increasing the success rates and decreasing multiple pregnancy rates. if there are no limitations of obtaining oocytes fertilized by IVF or ICSI, the produced embryos can be selected based on their morphological parameters and preimplantation genetic testing for aneuploidy analysis in some cases. In addition, morphokinetic, metabolomic, proteomic, epigenetic, and genomic evaluations on the early embryos are being investigated for selecting the ones having higher quality. In the cases generating a limited number of oocytes due to low fertility characteristics or legal restrictions, the morphological criteria for determining competent oocytes can be used despite its clinical value lower than expected.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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