A Case for the Oocyte: Why Grading of Oocyte Morphology Should Be Implemented in the IVF Laboratory and How

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In Vitro Fertilization (IVF) is generally accepted as the most effective treatment for infertility. Its success depends on the correct and meticulous implementation of each stage in the procedure. The process of systematically examining embryos is standardized through the use of internationally recognized criteria. On the other hand, the evaluation of oocyte quality continues to be conducted more arbitrarily. A morphologically good quality mature human oocyte is universally described as one that shows a homogeneous cytoplasm, has a single polar body (PB), an appropriate zona pellucida (ZP) thickness and a proper perivitelline space (PVS). An abnormality in one or more of these features are very common in IVF cycles and may be related to several factors that are extrinsic and intrinsic to the patient. There has been extensive speculation over whether specific anomalies in the structure of oocytes can suggest a reduced developmental capacity. The most notable among the dysmorphisms of oocytes are the severe morphological deviations, such as smooth endoplasmic reticulum clusters, cytoplasm granularity, and giant oocytes that are related to genetic abnormalities, and extra-cytoplasmic parameters such as PB morphology, the PVS and ZP abnormalities that may indicate oocyte ageing. This paper acknowledges the significance of oocyte morphology grading as an important and practical predictor of a successful IVF outcome and it can serve as a supplementary measure to embryonic assessment in order to optimize efficacy of assisted reproductive technology (ART). It discusses the fundamental knowledge that infertility specialists and embryologists should possess to enable its routine application in the ART laboratory.

Key words: oocyte quality, oocyte morphology, oocyte grading, oocyte maturity, In vitro fertilization, intracytoplasmic sperm injection, assisted reproductive technology, dysmorphism, metaphase II oocyte, embryo development, meiotic spindle, ooplasm

Introduction

In Vitro Fertilization (IVF) is generally considered to be the most effective treatment for infertility. There are several factors that affect IVF success rates, but the most important of which is embryo quality.[1] This is the rationale behind the routine grading of embryos in the laboratory, that is, to identify the ones that have the highest implantation potential that will result in a healthy pregnancy.[2] Considering the vital role played by the oocyte in the developmental process of the embryo, selection criteria involving the stage preceding fertilization would be extremely useful in selecting embryos for transfer.[3] For this reason, it may be prudent to consider evaluating the quality of oocytes, particularly with regard to their morphology, as a preferable or potentially optimal approach.

In the context of oocyte cryopreservation, it becomes even more crucial to assess the quality of the oocytes thoroughly. By including morphological assessment in the evaluation of oocytes for

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cryopreservation, fertility specialists can make more informed decisions about the suitability of oocytes for freezing. This information is more relevant now that many women are wishing to delay childbearing for social reasons. Given the development of more effective techniques for freezing embryos and oocytes, and the recent recognition of egg freezing as a non-experimental procedure by the American Society for Reproductive Medicine, it may be advantageous to utilize morphology grading as a practical and non-invasive indicator of oocyte quality.

The selection of oocytes with the greatest potential for development, as determined by their morphological characteristics, is a topic of significant interest. Numerous studies and reviews have been conducted to establish a correlation between oocyte morphology and the outcome of intracytoplasmic sperm injection (ICSI). There has been much speculation regarding the potential of certain morphological irregularities to indicate a compromised developmental capacity of oocytes.[4,5,6] Oocyte morphology grading is thought to serve as a possible valuable means of identifying competent oocytes before the process of fertilization.

Although there's a prevailing opinion that dysmorphisms of oocytes often fail to predict its fertilizing ability and developmental competence, most researchers would agree that some detectable features of metaphase II (MII) oocytes indicate seriously compromised developmental capacity.[7] The Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology convened a workshop (2011)[8] addressing the morphological assessment of oocytes and embryos. According to this group, extracytoplasmic abnormalities such as first polar body morphology (PB1), perivitelline space size (PVS), and the appearance of the zona pellucida (ZP) are simply phenotypic variations that are often related to in vitro culture and/or oocyte ageing. On the other hand, a special deviation in the cytoplasmic texture, such as the presence of aggregations of smooth endoplasmic reticulum (SER), is potentially lethal and developmental competence of these oocytes should be interpreted with caution.

The IVF specialist, who is in charge of stimulating the woman's ovaries in an effort to harvest an adequate number of eggs, typically has no clue as to the quality of those oocytes. The quantity and maturity of the oocytes are usually the sole information communicated by the embryologist on the day of retrieval. The details that are then provided following this are the number of zygotes that were obtained the next day, the cleavage rate two days later, and the number of embryos that reached the blastocyst stage on day 5 or day 6 prior to freezing or transfer. Because documentation of oocyte morphology is not a routine practice in the IVF facility, the opportunity to gauge oocyte quality by assessing morphology in relation to the patient's features and stimulation regimen is lost permanently.

Aside from the stimulation regimen, other extrinsic factors such as the culture and laboratory conditions, and intrinsic factors such as the woman's age, ovarian reserve, body weight, and presence of gynecologic conditions such as pelvic endometriosis can potentially affect oocyte quality.[4] The information regarding oocyte abnormalities in relation to these factors is potentially useful in cases of failed IVF because it can aid in the counselling of patients and provide guidance on how to proceed as it can offer a window of opportunity to see if there are conditions that affect oocyte quality that may be corrected in a subsequent cycle and therefore help improve IVF success rates.

The objective of this review was to summarize the existing literature on oocyte morphology grading for the purpose of exploring its role as a prognostic factor for embryo development and implantation. By emphasizing the necessity for embryologists to initiate the examination and documentation of the morphology of a cohort of oocytes obtained during an IVF or egg freezing cycle, along with regular reporting of this information to the clinician in a systematic manner using standard nomenclature, and with the inclusion of this morphologic oocyte evaluation in IVF scoring systems, potential correlations with clinical aspects can be gathered in order to further contribute to the existing knowledge on this extremely important subject matter.

Morphological Assessment of the Human Oocyte

Morphological assessment of oocytes can be carried out under an inverted microscope without compromising their development into embryos. Despite the subjectivity associated with this method,

it offers advantages due to its affordability, non-invasiveness, and alignment with the workflow of the IVF laboratory.[9] To select the best oocytes, a range of characteristics can be assessed, such as the cumulus oocyte complex (COC), zona pellucida (ZP), perivitelline space (PS), oocyte shape and size, first polar body (PB1), the ooplasm, and the meiotic spindle.[10] An ideal mature human oocyte, based on morphological characteristics, should have a 'normal-looking' cytoplasm, a single polar body, an appropriate ZP thickness and proper PVS.[11]

Abnormalities of human oocytes can be classified into: extracytoplasmic abnormalities (dark ZP and large PVS), intracytoplasmic abnormalities (dark or granular cytoplasm, clusters, vacuoles), refractile bodies (lipid bodies, vacuoles and lipofuscin bodies); size and shape abnormalities, and multiple abnormalities (Balaban, et al., 1998). Some studies have revealed that the highest implantation rates were from embryos derived from oocytes exhibiting normal structure. However, it is also well-established that even apparently normal oocytes may actually not be competent.[4]

The preliminary evaluation of oocytes can be conducted utilizing a stereo microscope to observe the organization of cumulus cells and the morphology of the PB1. In preparation for ICSI, oocyte denudation is performed via enzymatic action of hyaluronidase and mechanical pipetting, allowing for the detailed morphological evaluation of the nuclear and cytoplasmic maturation status, as well as the intra and extra-cytoplasmic structures. After denudation, oocytes are categorized according to the presence or absence of polar body and germinal vesicle (GV) with metaphase II (MII) oocytes having only the first polar body without GV, metaphase 1 (MI) oocytes having no polar body and no GV, and prophase I oocytes having germinal vesicle present and no polar body.[12] Different levels of meiotic maturity are visible in oocytes recovered from patients after controlled ovarian hyperstimulation. Only the oocytes that are in MII are appropriate for ICSI. Oocytes exhibiting a germinal vesicle at prophase I, as they have a diploid chromosomal set, and metaphase I where the first polar body is not yet extruded, cannot be used for ICSI.[9]

I. The Cumulus Oocyte Complex

The cumulus-oocyte complex comprises the oocyte and its surrounding cells. COC are categorized based on their level of compactness and clarity. The observed clarity pertains to the accumulation of lipids in the ooplasm, which could potentially be linked to favorable developmental outcomes.[9] The cumulus cells play a vital role in the production of energy inside the COC and also serve as a protective barrier against reactive oxygen species.[13] Some studies have indicated that the quality of oocytes improves with an increased number of surrounding cell layers.[5]

The cumulus cells' active secretion of hyaluronic acid is responsible for its expanded configuration and mucinous matrix. This extracellular molecule is situated between the cumulus cells, separating them and giving the cumulus–corona mass a fluffy 'cloud-like' appearance. Oocytes are classified as 'mature' or in metaphase II of maturation when they possess an expanded and luteinized cumulus matrix and a radiant or sunburst appearance. An intermediate stage of maturity, which corresponds to metaphase I is denoted by a less expanded cumulus-corona complex. And an absence of an expanded cumulus generally correlates with prophase I maturation or germinal vesicle stage.[12] It is important to note however, that the appearance of the COC may not always be indicative of a mature oocyte. In stimulated cycles, there may be asynchrony between the nuclear maturation status of the oocyte and the expansion of the cumulus–corona cell mass possibly due to the altered sensitivity of these cells to the stimulating agents.[14] Some authors also believe that there does not seem to be any correlation between COC morphology and fertilization or embryo cleavage.[15] Whether it is beneficial to perform late denudation to allow further contact between granulosa cells and the oocyte is still controversial. According to the Alpha-ESHRE consensus document, there is limited verified evidence to establish a connection between the characteristics of the cumulus-oocyte complex and the developmental competence of embryos. Nevertheless, it is recommended to document a COC that exhibits an expanded cumulus and a radiating corona as a criterion for a "good" COC.

II. The Zona Pellucida

The zona pellucida is an extracellular matrix that surrounds the oocyte. It is secreted by the oocyte at

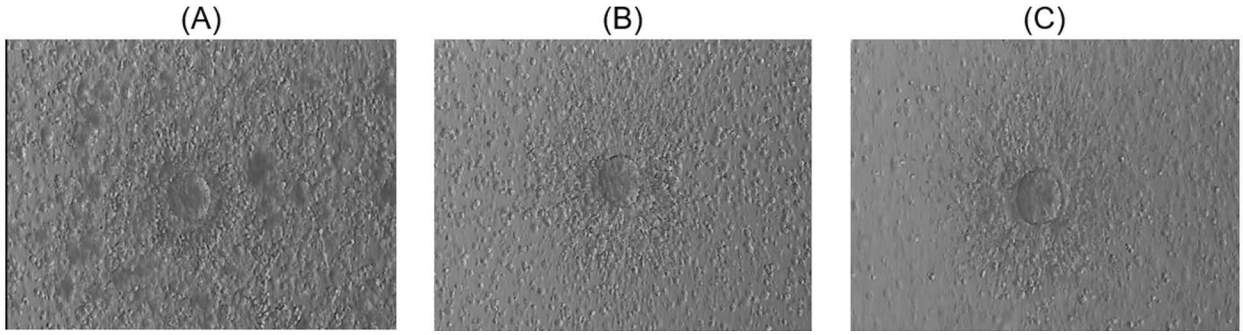

Figure 1. Normal Cumulus oocyte complex COC: (A) COC of Germinal Vesicle (GV); (B) COC of Metaphase I (MI) oocyte; (C) COC of Metaphase II (MII) oocyte

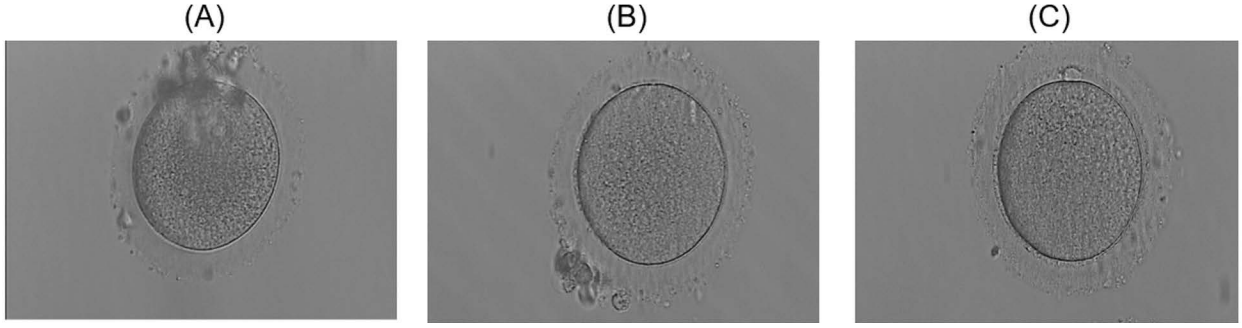

Figure 2. Normal oocytes: (A) GV; (B) MI; (C) MII

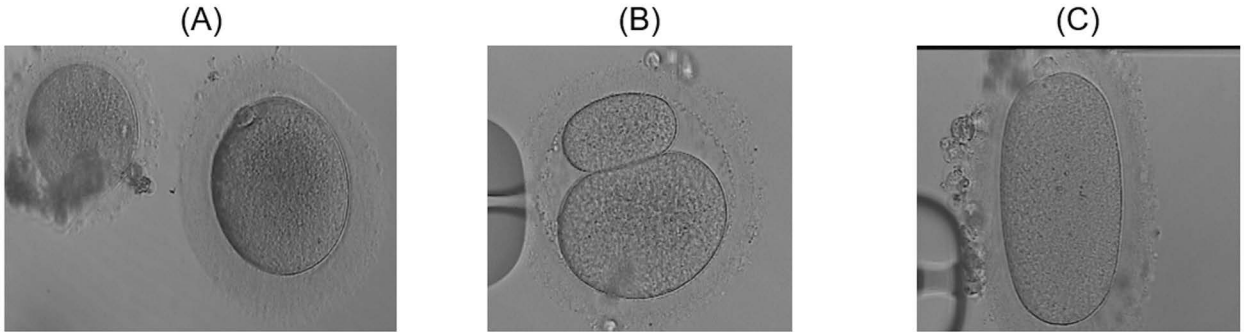

Figure 3. Abnormal size and shape: (A) giant oocyte next to a normal sized oocyte; (B) oocyte with giant polar body; (C) Ovoid oocyte

the pre-antral follicle stage, and the peri-oocyte cells of the corona radiata.[5] According to the findings of Pelletier, et al. (2004)[16], it was determined that the ZP is composed of three distinct layers. The overall thickness of the zona pellucida has a range of 10 to 31 µm, typically falling within the range of 15 to 20 µm.[17] It has been observed that immature human oocytes had a thicker zona pellucida (measuring approximately 20.4±2.4 µm) compared to mature oocytes (measuring approximately 19.5±2.2 µm) and day 3 embryos (measuring approximately 15.2±2.8 µm). The thickness of the ZP has an impact on the ability of sperm to penetrate it. The oocytes exhibited the best fertilization rate when the thickness of the zona pellucida was below 18.6 µm. The presence of a thick ZP, measuring 22 µm or more in thickness, (Figure 4) may suggest the need for intracytoplasmic sperm injection, as suggested by Bertrand, et al. (1995).[18]

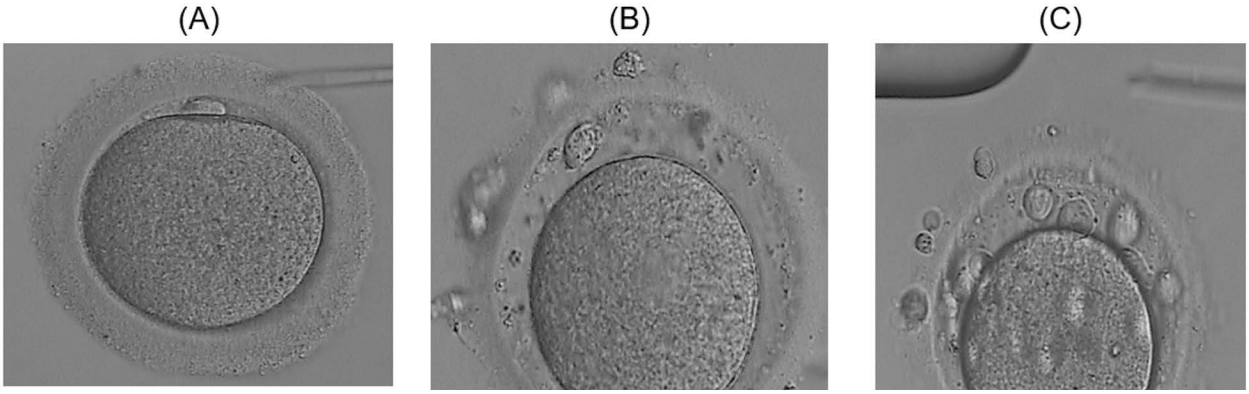

Figure 4. Extracytoplasmic abnormalities: (A) oocyte with thick zona pellucida; (B) oocyte with wide perivitelline space; (C) oocyte with fragmented polar body

The zona pellucida can be either homogeneous or non-homogeneous. Shi et al., (2016)[19] noted that defects of the ZP, including its composition, color, shape, and thickness, have been extensively associated with ultrastructural alterations in oocytes, lower fertilization, implantation, and clinical pregnancy rates. The zona may display duplication of the inner layer, or the presence of a tear between the layers resulting in the formation of an intra-zonal space.[14] Shen et al.,(2005)[20] observed that oocytes with zona splitting are probably caused by mechanical stress during retrieval or denudation, and were exclusively associated with non-conception cycles. Another additional explanation would be that in these cases the patterning of proteins may be temporarily interrupted during formation of the extracellular coat.[21] Though these types of ova usually show an ovoid shape it has to be noted, that the zona is responsible for the dysmorphism, the oocyte, however, maintains a spherical shape. If both zona pellucida and oocyte are involved in distortion, corresponding embryos run the risk of developmental incompetence.[21]

III. The Perivitelline Space

The perivitelline space refers to the space containing the polar body, situated between the zona pellucida and the membrane of the oocyte. The PVS may vary in volume and content with the presence or absence of the granules or fragments.[22] According to some studies, a large perivitelline space correlated with low fertilization rates and compromised pronuclear morphology, but had no effect on the embryo quality after ICSI.[7,22] While other studies showed that oocytes, which had large granules in the PVS developed less after fertilization than those without granules.[23] Some researchers on the other hand, believe that although this type of dysmorphism does not influence fertilization, cleavage behavior and pregnancy outcome,[24] it is associated with gonadotropin impregnation, indicating a potential physiological aspect of maturation.[15] The nature and origin of such granules still remains to be determined, but there is some evidence that this debris may derive either from coronal cell process remnants or from an extracellular matrix.[9] In some instances, the oocyte may extrude a large portion of cytoplasm during formation of the PB1, leading to a large and granulated PVS with debris.[25] A large PVS may also be associated with overmature eggs, wherein the egg has shrunk in relation to the ZP. (Figure 5) These studies on PVS, though subjective and non-standardized, show that is preferable to use oocytes with a thin perivitelline space and devoid of granulation.[9] Nevertheless, it is not advisable to utilize the assessment of the PVS as a determinant of oocyte quality.[5]

IV. The First Polar Body

The polar body is a small cell that is expelled during the maturation of the oocyte and contains 23 chromosomes. The presence and appearance of the first polar body is important because it is considered a marker of the transition of the immature oocyte to the metaphase II stage. Some authors suggested that morphological criteria of the PB1 such as the shape (round or ovoid), size (large or small), surface (smooth or rough) and integrity of cytoplasm (intact

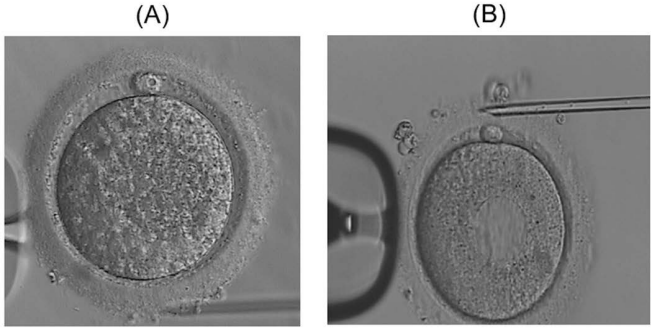

Figure 5. Cytoplasmic abnormalities: (A) oocyte with diffusely granulated cytoplasm; (B) oocyte with large vacuole

or fragmented) can be used to predict the oocyte quality.[26,27]

Extrusion of a large PB (see figure) is due to the dislocation of the meiotic spindle. The rates of fertilization, cleavage and formation of good quality embryos were found to be significantly lower when the oocytes with an enlarged first polar body were used for ICSI than those with an intact first polar body of normal size or a fragmented first polar body.[28] More recent data, however, showed that the appearance of the first polar body changes after a few hours of in-vitro culture, and it can vary according to the time during which the observation is carried out. Thus, first polar body morphology assessment cannot be used as a predictor of oocyte quality but can be predictive of post-ovulatory ageing status.[27,29] Furthermore, although it may be speculated that chromosomal aberrations may be one of the reasons for a significantly diminished percentage of blastocysts found in the fragmented first polar body group, recent data showed that aneuploidy rates in oocytes are unrelated to the status of the first polar body.[29] The diversity of studies and their conclusions on the morphology of the polar body suggests that this is still a controversial criterion for assessing oocyte quality.

V. The Meiotic Spindle (Visualization Using Polarized Light Microscope)

The indication of nuclear maturity in oocytes is traditionally associated with the presence of the first polar body. However, new research has suggested that the existence of the meiotic spindle is a more accurate marker for determining the maturity of the oocyte at the metaphase II stage. With the use of polarized light microscopy, the birefringence of meiotic spindle can be studied without loss of oocytes. Some researchers estimated that the oocytes with visible spindles had statistically significantly higher fertilization and cleavage rates; and percentage of top quality embryos after IVF or ICSI, compared with those in which meiotic spindle was not observed.[30]

Generally, for fertilization to occur, the spindle and the PB1 are closely apposed. If there is a displacement of >90° between the two structures, the fertilization is impaired.[31] An oocyte may still be immature if it is in telophase of the first meiosis, and the spindle is seen to be present between the ooplasm and the separating PB1.[32] Using the polarized light microscope, the location of the meiotic spindle could be noted to vary in oocytes suggesting that there is a potential to damage the oocytes in "blind" intracytoplasmic sperm injections. This dislocation of the meiotic spindle from the polar body may occur during denudation of oocytes prior to ICSI. Some studies showed a higher proportion of high quality embryos were obtained from oocytes injected with the polar body oriented at 11 o'clock that resulted in a higher clinical pregnancy rate. Therefore, the precise analysis of oocyte morphology, in combination with spindle visualization using the polarized microscopy, could be a more informative, non-invasive and reliable method of oocyte evaluation for predicting subsequent embryonic quality and developmental competence.[10]

VI. The Oocyte Size and Shape

The pre-ovulatory oocyte's diameter is around 150 μm including the zona pellucida and periviteline space.[12] There is a team that aimed to correlate mean oocyte diameter (MOD) with the quality of developing good-quality embryos. This team showed that oocytes with MOD between 105.96 and 118.69 μm had higher rates of good day five blastocyst.[33] Although slight variations in the size of the oocyte is not found to be associated with fertilization or the quality of development, the giant oocyte, which is approximately double the size of a normal oocyte (Figure) is deemed inappropriate for transfer. These oocytes demonstrate nuclear division, while cytoplasmic division is not observed. They are tetraploid which may result in the development

of digynic triploidy. Nevertheless, the presence of giant oocytes in a cohort does not appear to affect the developmental potential of sibling oocytes.[14]

Rarely, two oocytes can be found within the one follicular complex. Each oocyte is usually surrounded by a ZP but the ZP immediately between the two oocytes is commonly shared rather than duplicated. It is not uncommon for these conjoined oocytes to show different nuclear maturational states. It has been suggested that such oocytes may play a role in producing dizygotic twins; however, even when both of the conjoined oocytes are mature it is rare that both fertilize and no pregnancies have been reported from such oocytes.[34]

As opposed to deviations in oocyte size, oocytes with abnormal shape, such as ovoid and elongated oocytes, are usually normally fertilized and do not correlate with compromised embryo quality.[10] Oocytes that have acquired ovoid zonae may exhibit a flattened arrangement of blastomeres that may lead to a deviation from normal morphology of human 4-cell embryos, which is characterized by a 'crosswise' appearance of the blastomeres (see figure). This may subsequently cause a reduction in cell-to-cell contact which is a prerequisite for an optimal development of the embryo. This decrease in the number of available tight junctions could cause delayed formation of the blastocoele and full expansion of the blastocyst not before day 6.[35] Nevertheless, there is no correlation between the dimensions of the form anomaly and either fertilization or embryo quality. Studies have shown that oocytes exhibiting pronounced shape anomalies possess the capacity for fertilization and have the ability to result in the birth of viable offspring.[36]

VII. The Ooplasm

The presence and proper function of the organelles within the ooplasm are critical for the normal development and subsequent implantation of the embryo. Consequently, the occurrence of cytoplasmic abnormalities can have adverse effects on pronuclear development due to impaired functionality of the cytoskeleton and potential displacement of the MII spindle from its polar location. Furthermore, after the continuation of the initial meiotic division, the synchronous cytoplasmic maturation process is distinguished by the zonal capacity to release calcium and cortical granules, alterations in mitochondrial structure, protein synthesis, and modifications in the cytoskeleton. Hence, cytoplasmic abnormalities are capable of hindering these vital processes even in the presence of normal genetic material.[37]

One of the abnormal morphological features in human oocytes is the dark granular appearance of the cytoplasm, with or without inclusions, and a thick ZP, commonly called "brown eggs". Ten, et al. (2007)[38] and Loutradis et al. (1999)[39] reported on the compromised quality of embryos derived from these oocytes with dark cytoplasm. Other studies on the other hand, found that fertilization, embryo development, and successful pregnancy can be achieved after transfer of these brown embryos with the same rate as embryos from normal morphology oocytes and believe that brown eggs are probably normal and this morphological criterion does not seem to be indicative of any adverse outcome in IVF. Some authors[15,40] believe the dark cytoplasm to be an unreliable predictor in the majority of the in vitro or in vivo characteristics that were studied.

The presence and appearance of cytoplasmic granularity in oocytes (see figure) can be indicative of the oocyte's health and maturity. However, the interpretation may vary depending on the specific context and the methods used for assessment. Centrally located cytoplasmic granulation (CLCG) is a common cytoplasmic dysmorphism in human oocytes retrieved after controlled ovarian hyperstimulation (COH). Fancsovits, et al., (2012)[41] stated that the occurrence of cytoplasmic granularity was influenced by the patient's age and characteristics of stimulation and that the type of granulation had no effect on fertilization rate and zygote morphology. However, some type of granulation resulted in a lower cleavage rate and more fragmented embryos. Yi, et al., (2019)[42] found that the central granulated area size did not affect fertilization rate, cleavage rate, embryo utilization rate, and high quality embryo rate. Their results suggested CLCG might be a normal morphology of oocyte. The oocytes from patients with or without CLCG had no significant difference in their developmental potentials. Sun, et al., (2022)[43] proposed that partial granulation may reflect a specific population of patients, and that the central granulation structure is sensitive to cryopreservation. According to Rienzi et al.

(2008)[7], diffuse peripheral granulation is linked to altered pronuclear morphology. However, any kind of cytoplasmic granulation was linked to higher rates of fertilization than in oocytes that had no granularity at all.[44]

A common oocyte dysmorphism is cytoplasmic vacuolization. They can be observed in 5–12% of oocytes and vary in size as well as in number.[45] Vacuoles are membrane-bound cytoplasmic inclusions filled with fluid that is virtually identical with perivitelline fluid. They arise either spontaneously or by fusion of preexisting vesicles derived from Golgi apparatus or smooth endoplasmic reticulum.[46] It has been shown that vacuolated oocytes have significantly reduced fertilization rates and developmental ability.7 Moreover, a vacuole >14 μm in diameter can completely block fertilization.[21]

A smooth endoplasmic reticulum (SER) cluster is another intracytoplasmic dysmorphism that has been suggested to interfere with calcium stores and oscillations during fertilization, and may have a negative effect on embryo development and implantation.[47] SER clusters can be easily distinguished from vacuoles as they are not filled with fluid and are translucent when observed and are seen as plaques in higher magnification under the microscope. Studies have shown that compromised fertilization, embryo development, pregnancy rates, and obstetric and neonatal outcomes result when oocytes presenting with SER clusters are injected and the embryo thus derived is transferred.[21,48] The Alpha Scientists in Reproductive Medicine and European Society of Human Reproduction and Embryology (ESHRE) Special Interest Group of Embryology recommended not inseminating oocytes that presented with SER clusters because they might be associated with an increased risk of abnormal outcomes. However, more recently, a study showed that healthy babies could be obtained using oocytes presenting with SER clusters.[49]

A refractile body (RF) is also one of the main morphological abnormalities which can be observed in the cytoplasm of human oocytes.[50] RFs consist of a mixture of lipids and dense granular materials and have a yellow autofluorescence, which is consistent with lipofuscin.[51] Oocytes containing refractile bodies were usually fertilized normally by ICSI (De Sutter, et al., 1996) but not with IVF.[51] Lower embryo development rates of embryos with the presence of RFs have also been consistently reported.[52] Various authors consider these incorporations as minor dysmorphisms and unlikely to have any impact on fertilization rate and embryo quality in ICSI patients.[15,52,53] Other authors, on the other hand, ascertained that oocytes with intracytoplasmic abnormalities fertilized poorly and a high frequency of aneuploidy was found in many cells of the developing embryos.[15,54]

Multiple oocyte anomalies) were also related to decreased fertilization and pregnancy rate.[39] Both SER and CLCG oocytes may reflect cytoplasmic alterations, with further implications in spindle size, chromosome misalignment, and cortical actin disorganizations.[55]

Based on the published data, it is very clear that severe cytoplasmic deviations of the oocyte (such as organelle clustering, centrally severe granulation, excessive vacuolization) do impair the developmental and implantation potential of the embryo. Therefore, it may be speculated that only these types of severe deviations from cytoplasmic normality should be considered as abnormal, and thus should be taken into consideration for the selection of the viable oocyte that would result in an embryo with a higher implantation potential.[14]

Oocyte Morphology in Relation to Stimulation Protocol

For the purpose of obtaining an adequate number of MII oocytes for IVF, gonadotropins with or without GnRH analogues and/or other oral medications such as anti-estrogens, aromatase inhibitors and progestins are commonly used to induce multiple follicular development while preventing a spontaneous LH surge. GnRH agonist and/or human chorionic gonadotropin (hCG) is/are then used to resume meiotic progression from prophase I to MII stage after observing follicles greater than 17 or 18 mm in diameter followed by collection of COCs from the ovaries after 36 hours from injection. In stimulated cycles, the administration of pharmacologic doses of gonadotropins results in the elevation of hormone levels beyond the normal physiological range. This elevation induces the development of a cohort of follicles that, under natural circumstances, would typically undergo atresia and regression.

Consequently, some of the oocytes that are retrieved may be derived from slower-developing follicles, and therefore the cytoplasm would be at a different maturation stage upon the resumption of meiosis resulting in desynchronization of nuclear and cytoplasmic maturity.[56]

Figueira et al., (2010)[57] has shown that between 60-70% of the oocytes recovered after COH will exhibit at least one dysmorphic oocyte. They also showed that excessive ovarian response, characterized by an increased number of aspirated follicles and retrieved oocytes, and the total dose of administered FSH has a detrimental effect on oocyte quality, resulting in a higher incidence of intra and extra-cytoplasmic defects. Earlier studies indicate that in about 15% of meiotically mature human oocytes, an incomplete and premature exocytosis of cortical granules can occur[46] and that PVS granularity may be a sign of gonadotropin overdose.[24] According to Otsuki et al., (2004)[48], the risk of producing ova with an aggregation of the SER is increased in patients with high levels of serum estradiol on the day of hCG administration. They also stated that application of a short protocol seems to favor formation of SER clusters. The presence of dark cytoplasm has been related to increased risk of obtaining poor quality embryos in cycles with donor oocytes.[38] A prospective study reported similar fertilization and embryo quality rates in GnRH agonist cycles with or without dark cytoplasm. Murber et al.,(2009)[58] found a significantly higher incidence of cytoplasmic changes in the GnRH antagonist regime when compared with the agonist, while Cota et al., (2012)[59] did not find any difference in terms of oocyte morphology when the two protocols were compared. Lai et al., (2013)[60] compared agonist and antagonist protocols and concluded that there was no difference in fertilization and early embryo development, although a slightly higher embryo quality in the antagonist group was observed. Ng et al., (2001)[61] studied the effect of HMG versus FSH stimulation on oocyte maturity as well as on extra-cytoplasmic and cytoplasmic morphology of the oocyte and found similar percentage of mature oocytes between the groups. The incidence of oocytes with extra-cytoplasmic abnormalities such as zonal abnormality or first polar body abnormality, as well as oocytes with cytoplasmic abnormalities was also similar. Rashidi et al., (2005)[62] also showed that nuclear maturity, oocytes with abnormal zona, polar body or cytoplasmic morphology were similar between the group of patients stimulated with either HMG or recombinant FSH.

On the whole, there appears to be no reliable evidence in the literature regarding the effect of different stimulation protocols, different gonadotropin preparations and dosage, duration of ovarian stimulation and estradiol concentrations on the maturity and quality of oocytes. It is interesting to note however that some studies show that the frequency of some dysmorphisms changed substantially between cycles, suggesting that COH protocols may affect the quality of retrieved oocytes.[63] While on the other hand, others reported that serious oocyte dysmorphisms, such as clusters and vacuoles in the cytoplasm, can be a recurrent phenomenon in consecutive IVF treatments for the same patient making it difficult in these cases to counsel patients as to how to proceed.

Oocyte Morphology in Relation to Other Factors

The origin of morphological abnormalities of oocytes is most probably multifactorial. Aside from the possible effects of ovarian stimulation regimens, laboratory procedures and handling (egg retrieval, denudation, cryopreservation, insemination), culture conditions (pH, temperature, pO2), environmental conditions (light, air humidity, and quality), and culture medium can all have an important impact on its quality. The oocyte can also be negatively impacted by endocrine disruptors that arise from exposure to unhealthy conditions or chemicals. Furthermore, oocyte quality can also be influenced by factors inherent to the patient, such as advanced age, obesity, lifestyle issues as smoking and alcohol consumption, presence of gynecologic pathologies such as endometriosis and polycystic ovary syndrome, in addition to genetic factors, such as hormone receptor polymorphisms. Research has demonstrated that these factors can all have enduring consequences on the process of implantation, the growth of the fetus, and the subsequent health of the progeny.[65]

A recent study by Robin et al., (2021)[66] concluded that endometriosis does not have a negative impact on oocyte morphology. On the contrary, Shebl et al., (2016)[67] observed a lower number of oocytes with

normal morphology in women with endometriosis. Older studies show that oocytes from women with endometriosis were found to have a significant increase in morphological abnormalities of the cytoplasm and the ZP compared to controls. Oocytes were frequently misshapen and had intracytoplasmic vacuoles but the first polar body was less often fragmented. Interestingly as well, some research has indicated that maternal age does not have an impact on the presence of oocyte abnormalities, this is despite the expectation of reduced oocyte quality in older women, considering that the mechanism of follicular selection becomes less stringent with advancing age, and follicular atresia is assumed to be more severe.[57]

Given the current body of evidence, it appears that the assessment of oocyte quality in relation to potential causative factors, such as age, cannot be sufficiently evaluated solely based on morphologic parameters. Therefore, it may be appropriate to employ more advanced albeit expensive or invasive methods.

Other Methods to Evaluate Oocyte Quality

Morphological criteria are currently the primary method employed to evaluate oocyte quality because of their non-invasive nature. Nevertheless, the amount of detail provided by light microscopy is typically inadequate for observing organelles such mitochondria, endoplasmic reticulum, Golgi apparatus, and the spindle apparatus with the necessary precision for thorough analysis. To gain a deeper understanding of the intricate components within cells, advanced imaging methods like electron microscopy and confocal microscopy are utilized. These techniques allow researchers to examine the ultrastructure of cells, providing valuable insights into the finer features of organelles and other cellular components to offer supplementary data beyond basic morphological features and contribute to a more thorough evaluation of oocyte quality.[5]

Analysis of the gene expression or metabolism of cumulus cells is used to identify markers associated with oocyte developmental competence. Several intrinsic markers (such as mitochondrial status and glucose-6-phosphate dehydrogenase 1 activity) and extrinsic markers (such as apoptosis of follicular cells and levels of the transforming growth factor-beta superfamily in follicular fluid or serum) have been reported as useful indicators of oocyte competence and embryo quality.[68]

Polar body (PB) analysis can be used to detect chromosomal abnormalities to provide information about the chromosomal content of the oocyte. As PBs do not contribute to normal fertilization or embryonic development, their removal has no detrimental effects. In countries where genetic testing should be finished before syngamy, PB biopsy is the only legal option. The special benefit of PB biopsy, compared to biopsy of the embryo during pre-implantation genetic testing is its exclusive assessment of the maternal genetic contribution.[69]

Evaluation of mitochondrial function and content in oocyte can be performed using fluorescent probes and imaging techniques which can impact energy production and overall developmental competence. As oocyte maturation requires a large amount of ATP for continuous transcription and translation, the availability of the right number of functional mitochondria is crucial. There is a correlation between the quality of oocytes and both the amount of mtDNA and the amount of ATP. Dysfunctional mitochondria have a lower ability to counteract reactive oxygen species production which leads to oxidative stress. Kirillova et al. (2021)[70] studied the recent observations on oocytes' intracellular mitochondrial distribution and on mitochondrial genomes during their maturation, both in vivo and in vitro.

Artificial intelligence techniques, particularly the use of machine learning algorithms is an exciting and emerging field of science that has been applied to analyze various aspects of oocyte morphology and its application in assessment of oocyte quality. Training a convolutional neural network (CNN) to predict fertilization potential from oocyte images and to identify oocytes with the highest fertilization potential is one possible approach.[71] This technology allows for the development of novel quality assurance tools used to monitor oocyte stimulation regimens, assess ICSI performance, maintain optimal fertilization and embryo culture conditions, and evaluate oocyte vitrification and warming procedures. Sacha et al. (2021)[72] found this oocyte quality algorithm to be helpful in identifying an association between oocyte morphology and subsequent embryo development.

Dickinson, et al. (2020)[73] used deep CNN to locate the first extruded polar body, which allowed them to distinguish mature, MII oocytes from MI and GV stage oocytes. Pinpointing the location of the extruded polar body also allowed this algorithm to identify the correct location on the oocyte to inject spermatozoa for ICSI. The deep learning CNN was able to correctly identify the location of the polar body and the corresponding location for sperm injection.[73]

These are just some of the other methods that complement traditional morphological assessments that can potentially contribute to a more comprehensive understanding of oocyte quality. Integrating multiple techniques can enhance the accuracy of predicting development potential in the context of ART. Although these methods are quantitative and objective, the main disadvantage is that they can be difficult to use and implement in clinical practice.

Conclusions and Recommendations

Without a question, the quality of oocytes is the single most significant factor that affects fertilization rates, the subsequent development of high-quality embryos, and eventually, the occurrence of clinical pregnancy and live birth. However, due to the challenges that face scientists in doing research on this precious and unique cell, and despite the significant interest in this topic within the scientific community, the literature still contains wide disagreement regarding the correlation between oocyte morphology and the likelihood of a healthy pregnancy. Thus, in order to gain as much information from oocyte structure in the IVF laboratory, the practice of documenting oocyte abnormalities as they are observed is encouraged. This will allow for their immediate association with other characteristics such as the patient's features, the laboratory procedures followed, and the stimulation protocols. Furthermore, additional predictive value could be obtained by integrating the assessment of oocyte grading with evaluation of embryonic preimplantation development. Moreover, large scale studies are recommended to confirm the validity of oocyte morphology in relation to molecular and cellular markers, as well as newer technologies such as artificial intelligence, to offer additional insights into the function of the oocyte. The importance of these forthcoming investigations is underscored to achieve a consensus and maximize the predictive potential of morphological analysis in human oocytes.

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