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Abstract

Assessment of oocyte morphology is a difficult task, since underlying mechanisms that change the appearance of the oocyte are multifactorial and complex. Significant morphological variations are known to exist among oocytes that may affect the developmental competence and implantation potential of the derived embryo. Morphological variations of the oocyte may result from intrinsic factors such as age and genetic defects or extrinsic factors such as stimulation protocols, culture conditions, and nutrition. The effect of these morphological variations of the oocyte on embryo development and implantation, however, is not conclusively defined because of methodological flaws inherent to most of the studies in the literature. This review will mainly discuss morphological markers of oocyte quality/viability in relation to the oocyte morphology and attempt to clarify whether morphological evaluation of the oocyte can be utilized for predicting the implantation potential of the derived embryo.

Keywords: embryo quality, implantation, IVF, oocyte morphology

Introduction

The cause of fertilization failure in IVF is most often due to the absence of sperm penetration (Dyban et al., 1992), but the reason why fertilization does not occur when intracytoplasmic sperm injection (ICSI) is applied is not clear. Lack of fertilization with ICSI may be attributed to the deficiency of sperm-associated oocyte activating factor (Dozortsev et al., 1995). However, some intrinsic oocyte problems may also be responsible for fertilization failure. Oocyte abnormalities that can be assessed at the light microscopy level, such as refractile bodies, may be associated with failure of fertilization in the presence of apparently normal spermatozoa.

It has been reported that 13% of unfertilized oocytes after IVF show morphological abnormalities (Van Blerkom and Henry,
maturity because it is highly dependant on the experience of the embryologist. Although a high (28%) rate of asynchrony was reported between nuclear and cumulus–corona mass maturity, recent studies promoted the utilization of the latter, as it was shown to have a close relationship with fertilization and pregnancy rates (Ng et al., 1999) as well as blastocyst development and quality (Lin et al., 2003).

Ng et al. (1999), who used a modified grading system adapted by Wolf (1988) (Table 1), showed that mature grade 3 cumulus–oocyte complexes (COC) were associated with higher fertilization rates. The pregnancy rate was higher in cycles when >50% of retrieved COC were grade 3 compared with cycles where ≤50% of COC were similarly graded. Correlation between the percentage of grade 3 COC and the pregnancy rate was independent of patient age and the number of COC retrieved.

Why and how oocyte morphology should be used as a prognostic factor for embryo development and implantation is unfortunately not clear in the literature. Whether oocyte morphology assessment should be taken into account for embryo selection is debatable. The aim of the current review is to summarize the data in the literature that studied the factors affecting oocyte morphology, criteria for assessment, and the effect of oocyte morphology on embryo development and implantation.

**Cumulus–corona morphology and its relation to the quality and the maturity of the oocyte**

Oocyte maturity at the time of retrieval is difficult to assess as it is obscured by a large cumulus mass infiltrated with abundant hyaluronic acid. Earlier studies by Hammit et al. (1992, 1993) showed that cumulus–corona grading is a poor marker of oocyte maturity. When cumulus cells were denuded for ICSI, 60–70% of all oocytes retrieved showed abnormal morphological characteristics. Denudation of cumulus cells creates an opportunity to observe morphological deviations from the so-called normal. A good quality metaphase II oocyte is defined as an oocyte with clear, moderately granular cytoplasm, small perivitelline space, and a clear to colourless zona pellucida (Veeck, 1988). Most frequently observed morphological variations of the oocyte are cytoplasmic and include changes in colour, granularity and homogeneity of the cytoplasm, and cytoplasmic incorporations. Extracytoplasmic variations are deviations from normal of perivitelline space, zona pellucida colour and oocyte shape (Van Blerkom, 1990).

It has been suggested that varying degrees of asynchrony exist between cumulus–coronal cell morphology and nuclear maturity in stimulated cycles (Testart et al., 1983; Laufer et al., 1984). This may be due to either a different sensitivity of the cumulus–coronal cells and the oocyte nuclei to gonadotrophin-induced maturation, or to other intrafollicular factor(s) (Laufer et al., 1984; Bar-Ami et al., 1989).

A more recent study graded oocyte maturity on a scale from 1 to 5 based on the morphology of the ooplasm, cumulus mass, corona radiata, and membrana granulosa cells (Lin et al., 2003) (Table 2). Grade 1 (mature) oocytes were included in the first group, whereas the second group consisted of oocytes graded from 2 to 5 (immature). This study showed that mature oocytes yielded higher fertilization rates. Although cleavage rates were similar in both groups, the percentage of poor morphology day

### Table 1. Grading system for cumulus–oocyte complexes adapted from Wolf et al. (1988).

| Grade 1 (A) | Absent to sparse cumulus cells and 1–3 layers of corona cells |
| Grade 2 (B) | Dense cumulus cells and tightly packed corona cells |
| Grade 3 (C) | Expanded, fluffy cumulus cells and expanded corona cells |
| Grade 4 (D) | Expanded, scanty cumulus cells and expanded, often partially lost corona cells |

### Table 2. Oocyte maturity grading system by Lin et al. (2003).

| Grade 1 (mature or pre-ovulatory): expanded cumulus, very radiant corona, distinct zona pellucida, clear ooplasm, expanded well aggregated membrana granulosa cells |
| Grade 2 (approximately mature): expanded cumulus mass, slightly compact corona radiata, expanded well aggregated membrana granulosa cells |
| Grade 3 (immature): dense compact cumulus if present, very adherent compact layer of corona cells, ooplasm if visible with the presence of the germinal vesicle, compact and non-aggregated membrana granulosa cells |
| Grade 4 (post-mature): much expanded cumulus with clumps, radiant corona radiata yet often clumped, irregular or incomplete, very visible zona, slightly granular or dark ooplasm, small and relatively non-aggregated membrana granulosa cells |
| Grade 5 (atretic): rarely with associated cumulus mass, clumped and very irregular corona radiata if present, very visible zona, dark and frequently misshapen ooplasm, membrana granulosa cells with very small clumps of cells |
3 embryos from the immature group was significantly higher than from the mature group.

When IVF is to be undertaken for insemination, oocyte maturity may be scored indirectly according to the morphological appearance of the oocyte–corona–cumulus complex (OCCC); however, inferences regarding oocyte maturity using such a scoring system are not necessary in ICSI, since the oocytes are denuded prior to insemination. Lending credence to the latter, Rattanachayanon et al. (1999) scored cumulus–corona layers separately according to the degree of cellular expansion in each layer in patients scheduled to undergo ICSI and found no correlation between the OCCC morphological grade and nuclear maturity, fertilization rate and embryo cleavage.

It may be concluded that in women undergoing ICSI for fertilization assessment of OCCC morphology is pointless, since there is no correlation between OCCC morphology and fertilization and cleavage. Whether late denudation to allow further contact between the granulosa cells and the oocyte might be beneficial for OCCC deemed to be immature is debatable. For conventional IVF, OCCC morphology may be useful for gamete selection if the couple wants to electively limit the number of oocytes to avoid creating surplus embryos for cryopreservation (Engman et al., 2005).

Evaluation of first polar body morphology of the metaphase II oocyte

First polar body (PB) morphology assessment can be used to determine the post-ovulatory age of the oocyte (Rütter-Eichenlaub et al., 1995). To this end, it may serve as a useful marker to predict embryo development and implantation potential. Ebner et al. (1999) described five different morphological appearances of the first polar body. Grade 1 first polar bodies (round or ovoid and intact) differed from grade 2 first polar bodies (also round or ovoid and intact) in that the former has a smooth surface. Grade 3 (more than two fragments) and grade 4 (broken into two) first polar bodies were defined as fragmented, whereas grade 5 first polar bodies were characterized by their huge appearance being extruded to a similarly large perivitelline space. Classification based on this grading system revealed a significant correlation with the appearance of the first polar body and fertilization rate and embryo quality. ICSI to oocytes showing grade 1 and 2 first polar bodies resulted in higher fertilization rates and gave rise to higher quality embryos (Ebner et al., 2000). This notwithstanding, elective transfer of embryos selected on the basis of first polar body morphology assessment was associated with higher implantation and pregnancy rates (Ebner et al., 1999; Balaban et al., 2001). More recently, Ebner et al. (2002) showed that embryos with an intact first polar body group were associated with an increased rate of blastocyst formation when compared with the fragmented first polar body group. The authors indicated that preselection at such an early stage may be helpful in identifying a subgroup of preimplantation embryos with a good prognosis to form blastocysts and consequently to implant. 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 (Verlinsky et al., 2003).

More recent data, however, challenged this notion by showing that first polar body morphology assessment is not predictive of oocyte quality (Verlinsky et al., 2003; Ciotti et al., 2004; De Santis et al., 2005). Ciotti et al. reported that there was no significant relationship between the first polar body morphology and the fertilization and cleavage rates, embryo quality, and pregnancy and implantation rates. It was interesting to note that in this study, a subgroup of oocytes that were checked twice for polar body fragmentation showed different degrees of fragmentation at the first and second observations (11.1 and 22.8% respectively). Furthermore, a significantly positive relationship was found between the frequency of the first polar body fragmentation and the time elapsed between denudation and ICSI. The fragmentation rate of the oocytes where the time interval was >3.5 h was 26.7%. Verlinsky et al. (2003) also detected first polar body morphology grading changes in terms of fragmentation in more than one-third of the oocytes studied. It appears that morphology 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. Therefore, first polar body morphology assessment may not serve as a reliable marker of oocyte quality and competence.

Morphological dysmorphisms of the MII oocytes

Morphological abnormalities of a metaphase II (MII) oocyte after retrieval and after denudation of the corona–cumulus layer may be examined under two subgroups.

Extracytoplasmic abnormalities include: shape abnormalities (irregular shape of the MII oocyte); zona pellucida abnormalities (dark or thick ZP); and perivitelline space abnormalities (large PVS and PVS granularity).

Cytoplasmic abnormalities include: different types and degrees of cytoplasmic granulations, and variations in colour (dark coloured cytoplasm, slightly, or excessive whole/centrally located granulation); and appearance of refractile bodies, smooth endoplasmic reticulum clusters, or vacuolization in the ooplasm

Extracytoplasmic abnormalities

It is difficult to understand the effect of extracytoplasmic abnormalities (zona, shape and PVS irregularities of the oocyte) on embryo development and implantation potential, since most of the studies published so far combine extracytoplasmic with cytoplasmic abnormalities. One of the earlier studies showed that extracytoplasmic abnormalities such as dark zona pellucida, large perivitelline space or irregular shape of the oocyte are not related to fertilization rate and embryo quality after ICSI and commented that the oocytes showing such morphological deviations should be regarded as normal (De Sutter et al., 1996).

Xia et al. (1997) graded oocytes according to two extracytoplasmic (first polar body: fragmented/intact, perivitelline space: large/normal morphology) and one
cytoplasmic (presence of inclusions) characteristic and assessed the effect of these deviations on fertilization rates and embryo quality. Although it is difficult to comment on only the effect of the extracytoplasmic abnormalities, since the grading system was based on numerous factors including a cytoplasmic abnormality, the results indicated that the main factor affecting fertilization and embryo quality was the characteristic of the cytoplasm which highly significantly decreased fertilization rates and embryo quality for the group of oocytes with cytoplasmic inclusions even without any extracytoplasmic abnormality. In contrast, there was a slight decrease in the fertilization rate and embryo quality in the group of oocytes without cytoplasmic inclusions but with extracytoplasmic abnormalities (fragmented first polar body, large perivitelline space). Therefore, the main determinant for embryo developmental potential was found to be the cytoplasmic properties rather than extracytoplasmic deviations. In line with these observations, Balaban et al. (1998) showed that extracytoplasmic abnormalities of the oocyte such as dark zona pellucida, large perivitelline space or shape abnormalities were not associated with a decreased fertilization rate or unfavourable embryo quality after ICSI.

Perivitelline space granularity, considered to be an extracytoplasmic abnormality, may be a sign of gonadotrophin overdose, since the percentage of oocytes with perivitelline granules was significantly higher in the high dose (>45 ampoules) stimulated group compared with the low dose (<30 ampoules) stimulated group (Hassan-Ali et al., 1998). Fertilization and cleavage rates, embryo quality, pregnancy outcome as well as implantation rates were similar between the group of patients after the transfer of embryos generated from oocytes with >50% and ≤50% perivitelline granules. Perivitelline space granularity may be a physiological maturation-related phenomenon that has no effect on fertilization and cleavage rates, embryo quality, and the clinical outcome of assisted reproduction.

Loutridis et al. (1999) studied the effect of different types of oocyte morphological abnormalities in three subgroups of patients. Patients were grouped according to the quality of the embryos obtained. Although the group of oocytes with severe triple cytoplasmic defects gave rise to poor quality embryos and lower pregnancy rates, oocytes with zona pellucida abnormalities (extracytoplasmic defects) were associated with normal fertilization rates, and better embryo quality.

It has been shown that the repetition of specific oocyte dysmorphisms from cycle to cycle is a negative predictor of pregnancy and implantation rates in ICSI (Meriano et al., 2001). However, none of the extracytoplasmic abnormalities such as large or granulated PVS or zonal abnormalities was found to be repetitive from one cycle to another.

Similar to MII oocytes retrieved from stimulated cycles, in-vitro matured oocytes from patients with normal or polycystic ovaries may have some morphological deviations from the ideal looking MII oocyte. Mikkelsen and Lindenberg (2001) showed that extracytoplasmic abnormalities of in-vitro matured oocytes had similar fertilization and cleavage rates and resulted in good quality embryos when compared with in-vitro matured ideal looking oocytes. Furthermore, there was no difference in morphology between in-vitro matured oocytes obtained from normal ovaries and in-vitro matured oocytes obtained from polycystic ovaries.

One of the important effects of extracytoplasmic abnormalities is the increased rate of degeneration after ICSI. Although Ebner et al. (2001) and Plachot et al. (2002) showed that extracytoplasmic abnormalities of the oocyte did not affect fertilization and embryo quality, both studies reported higher lysis rates after ICSI in the group of oocytes with outer layer abnormalities (fragile oolemma, dark zona pellucida, large perivitelline space, and shape irregularity). Outer layer abnormalities may be somehow related to sudden oolemma breakage pattern described by Palermo et al. (1996).

It may be concluded in view of the published studies that embryo developmental rate and implantation potential is not affected by the extracytoplasmic abnormalities of the oocyte. Oocyte dysmorphism to a certain degree seems to be a normal occurrence, much like the phenotypic heterogeneity of male gametes.

**Cytoplasmic abnormalities**

It is generally assumed that severe cytoplasmic dysmorphisms reflect a reduced developmental competence of the oocyte. De Sutter et al. (1996) studied the developmental outcome of oocytes with cytoplasmic abnormalities such as dark cytoplasm, refractile bodies, dark incorporations, spots, and single or multiple vacuolization in the cytoplasm and found that none of these cytoplasmic abnormalities were related to fertilization or embryo quality. Although there was an increased degeneration rate after ICSI of oocytes with multiple vacuoles, the difference was not significant.

Serhal et al. (1997) showed normal fertilization and embryo development in oocytes with abnormal cytoplasmic morphology, such as granulated cytoplasm or cytoplasm with inclusions, but the resulting embryos failed to demonstrate the same implantation potential as those derived from oocytes with normal cytoplasm. Xia et al. (1997) showed that the cytoplasmic morphology of the oocyte was related to fertilization rate and embryo quality after ICSI. Significantly lower fertilization rates, embryo cleavage rates, and lower embryo quality was reported for the group of oocytes with cytoplasmic inclusions when compared with the group of oocytes with normal cytoplasm. The incidence of the oocytes with cytoplasmic inclusions was significantly higher for female factor infertility compared with male factor infertility patients. The appearance of cytoplasmic inclusions significantly increased in women aged >35 when compared with women aged <35.

In contrast with the studies of Serhal and Xia, Balaban et al. (1998) showed that in couples undergoing ICSI, abnormal oocyte morphology (both extracytoplasmic and cytoplasmic) was not associated with decreased fertilization rates or unfavourable embryo quality. Furthermore, transfer of embryos derived from normal oocytes yielded similar clinical pregnancy and implantation rates compared with embryos derived from normal oocytes. However, oocytes with severe cytoplasmic detects such as different types of vacuolizations and excessive central or whole granulations were not studied. Ebner et al. (2001) similarly showed that cytoplasmic abnormalities as well as the extracytoplasmic abnormalities did not affect the fertilization rate, mean number of blastomeres, and fragments on day 2. Although oocytes with cytoplasmic abnormalities were defined...
as a group of oocytes with dark cytoplasm, refractile bodies, dark incorporations or vacuoles, the severity of the cytoplasmic defects were not clearly defined in this study.

Cytoplasmic viscosity is another important characteristic of the oocyte that should be taken into consideration when assessing quality and the developmental capacity of the oocyte (Ebner et al., 2003). It has been shown that embryo quality, blastocyst formation rate, blastocyst quality, as well as the pregnancy and implantation rates are decreased in the group of oocytes with increased cytoplasmic viscosity. Loutradis et al. (1999) demonstrated that it is not the type but the severity of the cytoplasmic defect that affects fertilization rates, embryo quality and its developmental potential. Although oocytes with dark cytoplasm, or many vacuoles, or fragments in the cytoplasm, showed similar fertilization capacity and yielded embryos of similar quality, when triple cytoplasmic defects such as dark cytoplasm, many vacuoles and fragments in the cytoplasm were combined in the same oocyte, embryo quality was significantly impaired.

Meriano et al. (2001) showed that when >50% of the retrieved oocytes were dysmorphic and these dysmorphisms were repetitive implantation rates were lower compared with the groups where >50% of the oocytes were dysmorphic but not repetitive and when <30% of the oocytes were dysmorphic. The type of cytoplasmic dysmorphisms studied were the appearance of smooth endoplasmic reticulum (smooth or slightly elliptical flat disc) organelle clustering (central distinct area of dark indented granulation of the cytoplasm), and fluid filled vacuoles (round reflective fluid filled cavities). Intracytoplasmic organelle clustering was the only significant repetitive abnormality that was found to be a negative predictor of pregnancy and implantation after ICSI. However, fertilization and embryo cleavage rates, and embryo quality did not appear to be negatively affected. Other oocyte dysmorphisms were not associated with adverse ICSI outcome, were unlikely to be repetitive, and were found with equal frequency between all groups of patients studied. More research is needed to define the molecular and cellular mechanisms of organelle clustering.

Kahraman et al. (2000) studied the fertilization rate, and implantation of embryos derived from oocytes with centrally located granular cytoplasm. Significantly decreased pregnancy and implantation rates were observed following the transfer of embryos derived from granulated oocytes compared with non-granulated oocytes.

Otsuki et al. (2004) studied the relationship between pregnancy outcome and smooth endoplasmic reticulum clusters (sERC) in MH human oocytes. Smooth endoplasmic reticulum clusters were defined as translucent vacuoles that could be clearly distinguished from fluid filled vacuoles. Unlike sERC, vacuoles are defined as membrane-bound cytoplasmic inclusions filled with fluid that is virtually identical with perivitelline fluid (Van Blerkom, 1990). Patient characteristics and stimulation protocols of the two study groups: sERC(+) and sERC(−) were similar. Although serum progesterone concentrations were similar between the groups, serum oestradiol concentrations were significantly higher in the group of patients with sERC(+). The presence of sERC in the cytoplasm significantly impaired embryo quality, and was associated with a lower chance of conception even in sERC(−) oocyte derived embryos from the same cohort that were transferred along with embryos derived from sERC(+) oocytes. Because Ca2+ release from sER plays a pivotal role in oocyte maturation, fertilization, and early embryonic development (Tesarik, 1997), studies of Ca2+ signalling in sERC (+) oocytes may contribute to understanding of the cause and the effect of sERC.

Ebner et al. (2005) recently analysed the actual influence of vacuolization (time of first appearance, number, and size of vacuoles) on preimplantation embryo development up to the blastocyst stage. It was assumed that vacuoles arose either spontaneously (Van Blerkom, 1990) or by fusion of pre-existing vesicles derived from the smooth endoplasmic reticulum and/or Golgi apparatus (El Shafie et al., 2000). In this study three types of vacuoles could be identified: (i) those already present at oocyte collection (day 0); (ii) those that are artificially created by ICSI (day 1); and (iii) those accompanied with developmental arrest (day 4). The later the vacuoles arose, the more detrimental was their effect on blastocyst formation. There was a significant relationship between the size of vacuoles (cut-off value 14 μm) and fertilization. The mean diameter of vacuoles in fertilized oocytes was 9.8 μm, compared with 17.6 μm for non-fertilized oocytes. The fertilization rate was significantly lower for the group of oocytes with multiple vacuoles when compared with the oocytes with a single vacuole in the cytoplasm.

Given the data in the literature it may be concluded that only severe cytoplasmic defects such as organelle clustering/centrally located granulation, appearance of smooth endoplasmic reticulum clusters, and certain types of fluid-filled vacuoles should be considered as abnormalities; whereas slight deviations from the normal cytoplasmic structure should be accepted as normal oocytes with a phenotypically heterogeneous cytoplasm. It may be questioned whether oocytes with severe cytoplasmic defects be discarded and not subjected to fertilization in vitro. There has been a published case report by Esfandiari et al. (2005) that reported a successful pregnancy and delivery following fertilization by ICSI of oocytes showing severe cytoplasmic and extracytoplasmic abnormalities.

Effect of number of morphological abnormalities on developmental competence of the oocyte

The effect of the number of morphological abnormalities in the oocyte on quality and implantation potential of the embryo is unknown. De Sutter et al. (1996) showed that the fertilization rate and embryo quality of the oocytes with no abnormality, one or two, or more abnormalities were similar. In a later study, Balaban et al. (1998) demonstrated that the incidence of two abnormalities in a group of oocytes with morphological deviations was 26%, whereas this rate decreased to 6% for three abnormalities. Fertilization rate and embryo quality did not differ in the group of oocytes with no abnormality or one, two, or three morphological abnormalities. In contrast to the above, Loutradis et al. (1999) showed a deleterious effect of the type and the number of only cytoplasmic abnormalities on the developmental potential of the oocyte. Fertilization rate was similar for all groups of oocytes regardless of the type and number of abnormalities. Derived embryo quality was significantly decreased in the group of oocytes showing a triple
cytoplasmic abnormality. It can be concluded that the severity and the number of cytoplasmic defects have a deleterious effect on embryo quality. A more recent study on in-vitro matured oocytes also showed that embryo quality was decreased in parallel to the number of the morphological abnormalities of the oocyte from which it was derived (Mikkelsen et al., 2001).

Genetic constitution of oocytes with morphological abnormalities

Based on the published data, it appears that the developmental potential of oocytes with severe cytoplasmic defects is significantly impaired. However, it is still not known for certain if these cytoplasmic dysmorphisms are a reflection of a developmental defect in the oocyte or if the dysmorphism itself is inhibitory to the eventual development of the oocyte and subsequent embryos. One of the possible reasons shown is the defective genetic constitution of these oocytes. Van Blerkom and Henry (1992) showed that as many as half of the oocytes with dysmorphic phenotypes that arise early in meiotic maturation are aneuploid, with hypohaploidy being predominant. In contrast, cytoplasmic defects which occur at or after metaphase-I are associated with a relatively low frequency (<15%) of aneuploidy which is comparable to that reported for oocytes with a normal cytoplasmic appearance (Van Blerkom and Henry, 1988).

Alikani et al. (1995) analysed the relationship between variations found in oocyte morphology and aneuploidy as well as abnormal fertilization, embryo development and pregnancy and implantation rates. Contrary to previous findings linking some dysmorphisms to non-assisted fertilization failure, in this study no single abnormality led to a reduction in the fertilization rate, nor was fertilization compromised in oocytes with multiple abnormalities.

More recently Kahraman et al. (2000) showed that 53% of the embryos derived from the group of oocytes with centrally granulated cytoplasm were aneuploid. Although the genetic constitution of the oocytes displaying cytoplasmic abnormalities was studied, the fate of the oocytes with extracytoplasmic abnormalities is unknown. It was recently demonstrated that, although the aneuploidy rate of the group of embryos derived from oocytes with extracytoplasmic abnormalities (46.7%) was similar to the group of embryos derived from oocytes with normal morphology (41.8%), the aneuploidy rate for the group of embryos derived from oocytes with cytoplasmic abnormality was higher (60.0%) when compared with the group of embryos derived from oocytes with extracytoplasmic abnormality, and the group of embryos derived from oocytes with normal morphology (Balaban et al., 2003).

High aneuploidy rates were reported even for the group of embryos derived from oocytes with normal morphology (Van Blerkom and Henry, 1988). This, however, may be attributed to advanced female age and/or repeated implantation failures in the couples included in the study.

Otsuki et al. (2004) reported a born baby that was diagnosed with Beckwith–Wiedemann syndrome after the transfer of embryos derived from oocytes with smooth endoplasmic reticulum clusters. At present there are no data concerning the relationship between smooth endoplasmic reticulum clusters and genomic imprinting defects. It will be of interest to examine the unusual distribution pattern of the smooth endoplasmic reticulum cluster formation that may be involved in the abnormal regulation of Ca$^{2+}$ signalling. More studies are required to understand the important functions of the smooth endoplasmic reticulum in oocyte maturation and Ca$^{2+}$ signalling for embryonic development.

Effect of ovarian stimulation on oocyte quality and morphological appearance

Earlier studies suggested that high concentrations of LH during follicular phase of stimulation could have a negative impact on oocyte quality, pregnancy rate and the incidence of miscarriage (Howles et al., 1986; Homburg et al., 1988; Regan et al., 1990). Several studies examined the effect of polycystic ovarian syndrome on the maturation and quality of the oocyte but the results were controversial (Franks et al., 2002). However, most of the studies published so far examining the effect of clinical parameters on oocyte quality have been dependant upon oocyte maturity rather than morphology. Few articles examined the effect of different stimulation protocols with different types of gonadotrophins on the morphology of the oocyte (Imthurn et al., 1996; Yu Ng et al., 2001; Rashidi et al., 2005).

Imthurn et al. (1996) examined the effect of highly purified follicle stimulating hormone (HP-FSH) on nuclear maturity and morphological appearance of the oocyte in couples undergoing ICSI and compared the results with human menopausal gonadotrophin (HMG) stimulation. Patient characteristics did not differ between the two groups. A significantly higher proportion of oocytes in the FSH-HP group were nuclearily mature (metaphase II) than in the HMG group. The percentage of oocytes with cytoplasmic abnormalities was similar between the groups. However, when a subgroup analysis of these cytoplasmic abnormalities was performed, it was evident that significantly fewer oocytes with dark cytoplasm were encountered in the FSH-HP group, whereas the group of oocytes showing course-grained granulations was similar in both groups. Similar fertilization, cleavage and pregnancy rates were obtained for both groups. More recently Ng studied the effect of HMG versus FSH stimulation on oocyte maturity as well as on extracytoplasmic and cytoplasmic morphology of the oocyte (Ng et al., 2001). The percentage of mature oocytes (metaphase II) was similar between the groups. The incidence of oocytes with extracytoplasmic abnormalities such as zonal abnormality or first polar body abnormality, as well as oocytes with cytoplasmic abnormalities was also similar. Rashidi et al. (2005) 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.

There appear to be no reliable and in-depth observations regarding the effect of different stimulation protocols, different gonadotrophin preparations and dosage, duration of ovarian stimulation and oestradiol concentrations on the maturity and competence of oocytes. Data from women with PCOS suggest that oocyte and embryo quality and implantation may be
impaired in these patients treated with assisted reproduction (Urman et al., 2004). This may be due to impaired gamete quality resulting from the high oestrogenic milieu or intrinsic problems. However, not all results are in agreement and clinical outcome of PCOS patients appears to be equivalent to other infertility factors.

Conclusions

Based on the published data for IVF procedures cumulus–corona morphology, grading may be useful for oocyte selection for insemination, but not used for ICSI.

The results in the literature are controversial regarding first polar body evaluation for determining the fate of the embryo. It may serve as an additional tool for oocyte selection for insemination or cryopreservation at the pronuclear stage.

Although results regarding the effect of only extracytoplasmic morphological deviations are still controversial, few studies were able to demonstrate their detrimental effect on the implantation potential of the embryo. Therefore, these types of oocyte dysmorphisms should perhaps not be considered as abnormalities, but only a phenotypic deviation resulting from the heterogeneity of the oocytes retrieved. They can be useful as a selection criterion in countries that ban the utilization of other embryo selection tools after the fertilization process.

Based on the published data, it is very clear that severe cytoplasmic deviations of the oocyte (such as organelle clustering, centrally severe granularity, excessive vacuolization) do impair the developmental and implantation potential of the embryo. Therefore, it maybe 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.

The above notwithstanding, it should be noted, however, that there are several studies in the literature with discordant results and oocyte morphology today should be regarded as one of the many markers for predicting embryo quality and implantation potential. Decisions regarding insemination and transfer of derived embryos based on oocyte morphology may not be accurate.

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