Metaphase II human oocyte morphology: contributing factors and effects on fertilization potential and embryo developmental ability in ICSI cycles

Morphologic abnormalities in the oocyte are relevant for determining its developmental fate and could be related to controlled ovarian stimulation protocols and ovarian response. The contributing factors of oocyte dysmorphism incidence and its effects on fertilization potential and embryo development are the object of discussion in this study. (Fertil Steril® 2010;94:1115–7. ©2010 by American Society for Reproductive Medicine.)

A complex cascade of maturational events during the process of follicular development confers on the oocytes the capacity to undergo normal fertilization and subsequent embryonic development (1). Full physiologic maturation appears to require nuclear and cytoplasmic changes that need to be coordinately completed to ensure optimal cellular conditions (2). Nuclear maturity alone is not enough to determine the competency of an oocyte. Deficiency in cytoplasmic maturation could compromise all processes that prepare the oocyte for activation, adequate fertilization, and embryo development. In addition, a disturbance or asynchrony of these two processes has been shown to compromise oocyte quality, resulting in different oocyte dysmorphisms (3–8).

Usually, dysmorphisms are classified as intracytoplasmic and extracytoplasmic anomalies, including change in cytoplasmic granularity, presence of cytoplasmic inclusions such as vacuoles and smooth endoplasmic reticulum clusters, derivations from normal perivitelline space, and first polar body morphology (9, 10). A majority (60%–70%) of the oocytes retrieved from stimulated cycles exhibit one or more abnormal morphologic characteristics (11).

Morphologic variations of the oocyte may result from intrinsic factors, such as age (12) and genetic defects, or extrinsic factors, such as the controlled ovarian stimulation (COS) protocol and the ovarian response to COS (13, 14). Intracytoplasmic and extracytoplasmic anomalies developed during the maturation process may lead to fertilization failure (8, 15, 16), chromosome aneuploidy (3, 17), and developmental impairment of the embryo despite normal fertilization (4, 7, 8, 18, 19).

Analysis of the literature, however, shows that the effect of oocyte morphologic deviations after intracytoplasmic sperm injection (ICSI) remains controversial (11, 20, 21). Therefore, the aim of the present study was to evaluate whether COS protocols and ovarian response in ICSI cycles influence oocyte morphology and, therefore, its further development, such as fertilization and embryo quality.

A retrospective evaluation of oocyte morphology was undertaken in 3,148 metaphase II (MII) oocytes retrieved from 350 ICSI cycles performed from January 2007 to April 2008 at a private center for assisted reproduction. All cases of severe spermatogenic alteration, including frozen and surgically retrieved sperm, were excluded from the study. Written informed consent was obtained in which patients agreed to share the outcomes of their own cycles for research purposes. The study was approved by the local Institute Review Board.

We studied the influence on oocyte morphology of: 1) maternal age; 2) total dose of recombinant FSH administered for ovarian hyperstimulation; 3) number of aspirated follicles; 4) oocyte yield (no. of retrieved oocytes/no. of follicles); and 5) MII oocyte rate (no. of MII oocytes/total no. of retrieved oocytes). We also investigated the influence of oocyte dysmorphisms on: 6) normal fertilization (presence of two clearly pronuclei and polar bodies); and 7) embryo quality 68–72 hours after ICSI. High-quality embryos were defined as those showing 6–8 cells on the third day of development, <15% fragmentation, symmetric blastomeres, absence of multinucleation, and absence of zona pellucida dysmorphisms.

Controlled ovarian stimulation was achieved by long pituitary down-regulation using a GnRH agonist (Lupron Kit; Abbott, Societé Française des Laboratoires, Paris, France). This was followed by ovarian stimulation with recombinant FSH (Gonal-F; Serono, Geneva, Switzerland). Oocyte retrieval was performed 35 hours after administration of recombinant hCG (Ovidrel; Serono) through transvaginal ultrasonography.

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TABLE 1

General characteristics of stimulation ICSI cycles.

Characteristic	Data
Cycles (n)	350
Age (y)	34.2 ± 4.7
Total gonadotropin dose (IU)	2,480 ± 756
E_2 concentration on hCG day (pg/mL)	1,705 ± 1,420
Aspirated follicles (n)	16.9 ± 13.2
Retrieved oocytes (n)	11.8 ± 9.2
Retrieved oocytes/no. of follicles (%)	69
MII oocytes (n)	8.7 ± 6.9
MII oocytes/total no. of retrieved oocytes (%)	71.2
Normal fertilization rate (%)	68.7
Rate of high-quality embryos (%)	62.1

Note: Values expressed as mean ± SD unless indicated otherwise.
MII = metaphase II.

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Oocyte morphology was assessed just before sperm injection (3–4 hours after retrieval) using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon, Tokyo, Japan) with a Hoffmann modulation contrast system under ×400 magnification. The following extracytoplasmic and intracytoplasmic morphologic abnormalities observed in the oocytes were recorded: 1) large perivitelline space size; 2) perivitelline space granularity; 3) fragmented first polar body; 4) increased cytoplasmic granularity; 5) smooth endoplasmic reticulum clusters; and 6) vacuoles in the ooplasm.

Linear regression models were performed to verify the relationship between patients' cycle characteristics and oocyte dysmorphism incidence. Results were described by the adjusted R^2 followed by the respective *P* value. The associations between morphologic oocyte features and the occurrence of normal fertilization and embryo quality were assessed using regression analysis. Data are presented as odds ratios (OR) (95% confidence interval [CI]) and *P* value. Results were considered to be significant at the 5% critical level ($P<.05$).

The general characteristics of the ICSI cycles are show in Table 1. From 350 ICSI cycles, 3,148 MII oocytes were retrieved. Of these oocytes, 1,934 (60.2%) showed at least one morphologic abnormality. Cytoplasmic and extracytoplasmic abnormalities were observed in 42.3% and 44.7% of the oocytes, respectively.

No correlation was found between age and the presence of oocyte dysmorphisms. On the other hand, we observed that the total dose of FSH positively influenced the presence of perivitelline space granularity ($R^2 = 0.125$; $P=.0181$). The number of aspirated follicles ($R^2 = 0.101$; $P=.0457$) and the number of retrieved oocytes ($R^2 = 0.125$; $P=.0179$) correlated positively with the presence of increased cytoplasmic granularity. Oocyte yield positively influenced the presence of granularity in the perivitelline space ($R^2 = 0.099$; $P=.0500$). No effect of any other cycle characteristics on morphologic features was observed.

Normal fertilization rate was significantly affected by the following oocyte abnormalities: increased oocyte cytoplasmic granularity (OR 1.22 (95% CI 1.03–1.45); $P=.011$), presence of vacuoles (OR 1.46 (95% CI 1.04–2.10); $P=.034$), and large perivitelline space (OR 1.25 (95% CI 1.02–1.51); $P=.004$). Overall, 62.1% of all embryos showed good quality 68–72 hours after ICSI. Embryo quality was significantly affected only by the presence of granularity in the perivitelline space (OR 1.33 (95% CI 1.06–1.69); $P=.024$). No effect of any other morphologic oocyte features on normal fertilization or embryo quality was observed.

Despite remarkable progress in both clinical and embryologic aspects of assisted reproductive technologies, the take-home baby rate per ICSI cycle started is still disappointingly low (22). The noninvasive identification of predictive markers for oocyte development potential, which can be assessed before fertilization using conventional microscopy, is a difficult task, because the mechanisms involved in the oocytes' morphologic abnormalities are multifactorial and complex. In addition, the use of different criteria for oocyte evaluation and polling oocyte features of different origins are indeed questionable and may be partly responsible for the discrepancies between different studies (11).

The present data showed that maternal age had no influence on the presence of oocyte abnormalities. Assuming that follicular atresia plays a role in determining the overall quality of follicles that reach the final stages of development, and considering that the mechanism of follicular selection becomes less stringent with increasing age, a reduced oocyte quality could be expected in older women (23). Therefore, the present results suggest that age-related decrease in oocyte quality could not be assessed through morphologic parameters. We noted that the total dose of administered FSH had an effect on extracytplasmic oocyte morphology. It is generally assumed that, in stimulated cycles, pharmacologic doses of gonadotropins create a supraphysiologic hormonal environment that induces the growth of a cohort of follicles, which, under natural conditions, would become atretic and regress (24). Oocyte extracytoplasmic abnormalities may be a physiologic maturation-related phenomenon affected by hyperstimulation protocols and less stringent follicular selection.

Furthermore, earlier studies described that a large perivitelline space could be related to oocyte overmaturity (25). In addition, the present data showed that excessive ovarian response, characterized by an increased number of aspirated follicles and retrieved oocytes, has a detrimental effect on oocyte quality, resulting in a higher incidence of intracytoplasmic defects. Deficient cytoplasmic maturity has been postulated to be reflected by cytoplasmic abnormalities (3). The oocytes retrieved from stimulated cycles may be derived from slower-developing follicles, and therefore the cytoplasm of these oocytes would be at different maturation stages upon the resumption of meiosis. The present data also showed that increased oocyte yield significantly affects the occurrence of debris in perivitelline space. Earlier studies indicate that in ~15% of meiotically mature human oocytes, an incomplete and premature exocytosis of cortical granules can occur (10) and that perivitelline space granularity may be a sign of gonadotropin overdose (6).

The effect of morphologic abnormalities of oocytes on fertilization and embryo development is unfortunately unclear in the literature (11, 19). In the present study, we showed that both fertilization and quality of day 3 embryos are unfavorably influenced by specific oocyte dysmorphisms.

It has been reported that 13% of unfertilized oocytes after IVF present morphologic abnormalities (17). We observed that normal fertilization rate was significantly affected by oocyte abnormalities. How cytoplasmic inclusions such as deficiency in cytoplasm fluidity and ooplasm vacuolization affects fertilization remains

unclear (8, 15). This phenomenon could be related to cytoskeletal function and MII meiotic spindle structure (8, 10, 26). Cytoplasmic defects will, therefore, lead to detrimental effects to pronuclear formation, because the cytoskeleton cannot function properly and the MII spindle can be displaced from its polar position. In addition, while resumption of the first meiotic division takes place, synchronous cytoplasmic maturation is characterized by zonal ability to release calcium and cortical granules, mitochondrial changes, protein synthesis, and cytoskeletal change. Despite the presence of normal genetic material, ooplasmic factors play an important role in the fertilization process that could be compromised by cytoplasmic abnormalities (27).

There is a general tendency to disregard oocyte morphology in the decision process regarding the selection of an embryo to transfer. At the 4–8-cell developmental stage in human embryos, the embryonic genome is expressed by maternal encoded genomic products stored in the oocyte during the later stages of development of the ovary (28–30). The present data showed that oocytes with granularity in the perivitelline space have an increased risk of developmental incompetence. There is some evidence that suggests that some debris are the remnants of coronal cell processes (31). Earlier studies (32, 33) found extracellular matrix compromising granules and filaments in the perivitelline space, identical to the matrix observed between the cumulus and corona radiata cells. Stastna et al. (34) also described the presence of morphologically distinct secretory vesicles in the cortical cytoplasm of human preovulatory oocytes. A deviation of the normal morphology of human day 3 embryos could, therefore, be related to physiologic maturation-related oocyte mechanisms.

In conclusion, the cause for oocyte morphologic abnormalities is probably multifactorial. Ovarian stimulation and hormonal environment changes may result in the maturation of abnormal oocytes. Those oocyte dysmorphisms must be considered to be relevant to assess its developmental fate, and morphologic oocyte evaluation should be included in IVF scoring systems. Furthermore, the present results suggested that a lower oocyte cohort development may represent a more appropriate response to ovarian stimulation, allowing only the most competent follicles and oocytes to develop, increasing overall oocyte quality and maturity.

REFERENCES

1. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev 1996;17:121–55.
2. Eppig JJ. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. Reprod Fertil Dev 1996;8:485–9.
3. Kahraman S, Yakin K, Donmez E, Samli H, Bahce M, Cengiz G, et al. Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. Hum Reprod 2000;15:2390–3.
4. Loutradis D, Drakakis P, Kallianidis K, Milingos S, Dendrinos S, Michalas S. Oocyte morphology correlates with embryo quality and pregnancy rate after intracytoplasmic sperm injection. Fertil Steril 1999;72:240–4.
5. Eichenlaub-Ritter U, Schmiady H, Kentenich H, Soewarto D. Recurrent failure in polar body formation and premature chromosome condensation in oocytes from a human patients: indicators of asynchrony in nuclear and cytoplasmic maturation. Hum Reprod 1995;10:2343–9.
6. Hassan-Ali H, Hisham-Saleh A, El-Gezeiry D, Baghdady I, Ismaeil I, Mandelbaum J. Perivitelline space granularity: a sign of human menopausal gonadotrophin overdose in intracytoplasmic sperm injection. Hum Reprod 1998;13:3425–30.
7. Otsuki J, Okada A, Morimoto K, Nagai Y, Kubo H. The relationship between pregnancy outcome and smooth endoplasmic reticulum clusters in MII human oocytes. Hum Reprod 2004;19:1591–7.
8. Ebner T, Sommergruber M, Moser M, Shebl O, Schreier-Lechner E, Tews G. Basal level of antimullerian hormone is associated with oocyte quality in stimulated cycles. Hum Reprod 2006;21:2022–6.
9. Veeck LL. Oocyte assessment and biological performance. Ann N Y Acad Sci 1988;541:259–74.
10. Van Blerkom J. Occurrence and developmental consequences of aberrant cellular organization in meiotically mature human oocytes after exogenous ovarian hyperstimulation. J Electron Microsc Tech 1990;16:324–46.
11. Balaban B, Urman B. Effect of oocyte morphology on embryo development and implantation. Reprod Biomed Online 2006;12:608–15.
12. de Bruin JP, Dorland M, Spek ER, Posthuma G, van Haaften M, Looman CW, et al. Age-related changes in the ultrastructure of the resting follicle pool in human ovaries. Biol Reprod 2004;70:419–24.
13. Rashidi BH, Sarvi F, Tehrani ES, Zayeri F, Movahedin M, Khanafshar N. The effect of hMG and recombinant human FSH on oocyte quality: a randomized single-blind clinical trial. Eur J Obstet Gynecol Reprod Biol 2005;120:190–4.
14. Ng EH, Lau EY, Yeung WS, Ho PC. hMG is as good as recombinant human FSH in terms of oocyte and embryo quality: a prospective randomized trial. Hum Reprod 2001;16:319–25.
15. Dozortsev D, Rybouchkin A, De Sutter P, Qian C, Dhont M. Human oocyte activation following intracytoplasmic injection: the role of the sperm cell. Hum Reprod 1995;10:403–7.
16. Rienzi L, Ubaldi FM, Iacobelli M, Minasi MG, Romano S, Ferrero S, et al. Significance of metaphase II human oocyte morphology on ICSI outcome. Fertil Steril 2008;90:1692–700.
17. Van Blerkom J, Henry G. Oocyte dysmorphism and aneuploidy in meiotically mature human oocytes after ovarian stimulation. Hum Reprod 1992;7:379–90.
18. Xia P. Intracytoplasmic sperm injection: correlation of oocyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality. Hum Reprod 1997;12:1750–5.
19. Ebner T, Moser M, Tews G. Is oocyte morphology prognostic of embryo developmental potential after ICSI? Reprod Biomed Online 2006;12:507–12.
20. Yakin K, Balaban B, Isiklar A, Urman B. Oocyte dysmorphism is not associated with aneuploidy in the developing embryo. Fertil Steril 2007;88:811–6.
21. Balaban B, Urman B, Sertac A, Alatas C, Aksoy S, Mercan R. Oocyte morphology does not affect fertilization rate, embryo quality and implantation rate after intracytoplasmic sperm injection. Hum Reprod 1998;13:3431–3.
22. Patrizio P, Bianchi V, Lalioti MD, Gerasimova T, Sakkas D. High rate of biological loss in assisted reproduction: it is in the seed, not in the soil. Reprod Biomed Online 2007;14:92–5.
23. te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update 2002;8:141–54.
24. Fortune JE. Selection and maintenance of the dominant follicle: an introduction. Biol Reprod 2001;65:637.
25. Mikkelsen AL, Lindenberg S. Morphology of in-vitro matured oocytes: impact on fertility potential and embryo quality. Hum Reprod 2001;16:1714–8.
26. Ebner T, Yaman C, Moser M, Sommergruber M, Jesacher K, Tews G. A prospective study on oocyte survival rate after ICSI: influence of injection technique and morphological features. J Assist Reprod Genet 2001;18:623–8.
27. Collas P, Poccia D. Remodeling the sperm nucleus into a male pronucleus at fertilization. Theriogenology 1998;49:67–81.
28. Tesarik J, Kopecny V, Plachot M, Mandelbaum J. Early morphological signs of embryonic genome expression in human preimplantation development as revealed by quantitative electron microscopy. Dev Biol 1988;128:15–20.
29. Tesarik J, Kopecny V, Plachot M, Mandelbaum J. Activation of nucleolar and extranucleolar RNA synthesis and changes in the ribosomal content of human embryos developing in vitro. J Reprod Fertil 1986;78:463–70.
30. Cohen J, Scott R, Alikani M, Schimmel T, Munne S, Levron J, et al. Ooplasmic transfer in mature human oocytes. Mol Hum Reprod 1998;4:269–80.
31. Sathananthan AH. Ultrastructure of the human egg. Hum Cell 1997;10:21–38.
32. Dandekar P, Aggeler J, Talbot P. Structure, distribution and composition of the extracellular matrix of human oocytes and cumulus masses. Hum Reprod 1992;7:391–8.
33. Dandekar P, Talbot P. Perivitelline space of mammalian oocytes: extracellular matrix of unfertilized oocytes and formation of a cortical granule envelope following fertilization. Mol Reprod Dev 1992;31:135–43.
34. Stastna J, Dvorak M, Pilka L. Electron microscopic and cytochemical study of the cortical cytoplasm in the preovulatory human oocytes. Z Mikrosk Anat Forsch 1983;97:675–87.