—Mini Review—

Intracytoplasmic Morphological Abnormalities in Human Oocytes

Junko Otsuki[1]

[1]*Nagai Clinic, 607-1 Kamihikona Misato, Saitama 341-0004, Japan*

Abstract: *In human oocytes, many types of abnormal phenotypes have been observed both within the cytoplasm and outside of the cytoplasm. The morphological evaluation of oocytes and its impact on embryo quality has been controversial. However, abnormal oocyte phenotypes can be directly influenced by the follicular environment, ovarian function and the effects of ovarian stimulation used in assisted reproductive technology. This review focuses on four critical intracytoplasmic anomalies; fluid-filled vacuoles, smooth endoplasmic reticulum clusters (sERC), refractile bodies / lipofuscin bodies and centrally located cytoplasmic granularity (CLCG). The fine structures, morphological and immunochemical characteristics and the possible mechanism giving rise to each phenotype are discussed.*

Key words: *Intracytoplasmic oocyte dysmorphism, Vacuole, Smooth endoplasmic reticulum cluster, Lipofuscin / Refractile body, Centrally located cytoplasmic granularity*

Introduction

Unlike in other mammalian species, several abnormal phenotypes may be observed within the cytoplasm of human oocytes after the follicular cells are denuded for intracytoplasmic sperm injection. In many cases of ART, multiple oocytes are retrieved following different types of ovarian stimulation protocols. Thus the ovarian stimulation sometimes induces follicle development of asymmetrical size, probably related to the irregular blood circulation in each follicle. Since there is a tendency for specific abnormal phenotype to recur in the same patients [1–4], it is likely that a correlation exists between the type of dysmorphism observed and ovarian function / environment produced.

This review focuses on four important intracytoplasmic dysmorphisms; fluid-filled vacuoles, smooth endoplasmic reticulum clusters (sERC), refractile bodies / lipofuscin bodies, centrally located cytoplasmic granularity (CLCG) in human oocytes. With regards to the influence of these phenotypes, it appears that there is an agreement that fertilization is not impaired by the oocyte morphology [2–10]. An exception is the excessive aberration, such as the large fluid-filled vacuole, large refractile / lipofuscin bodies and an excessively dark oocyte cytoplasm while any correlation between the morphology and embryo quality is controversial. This uncertainty arose partly because some of the studies did not focus on a single phenotype independently and partly due to the use of different embryo criteria to evaluate developmental competence. Since various cytoplasmic abnormalities may be derived from different differentiation pathways, we need to examine individually the influence on development, as well as the etiology, for each abnormal phenotype. For example, smooth endoplasmic reticulum stores Ca^{2+} that plays pivotal roles in oocyte maturation, fertilization and early embryonic development [11, 12]. Thus studies of Ca^{2+} signaling in sERC-positive oocytes may contribute to understanding the cause and the effect of sERC formation. Also it would be important to examine the unusual distribution and formation patterns of the sERC that may be involved in the abnormal regulation of Ca^{2+} signaling. In this review, the fine structure and characteristics of the dysmorphism are introduced and possible mechanisms involved in the establishment of each phenotype are discussed.

Fluid-filled Vacuoles

The fluid-filled vacuole is often observed in oocytes after denuding follicular cells for ICSI and may vary in size and number during embryo development to the

Received: February 28, 2009
Accepted: March 6, 2009
e-mail: otsuki.midori.junko@gmail.com

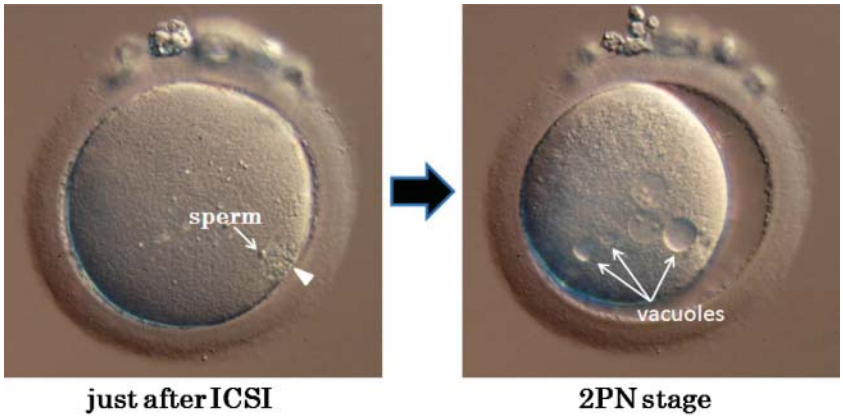

Fig. 1. A fluid filled vacuole caused by excessive injection of polyvinylpyrrolidone or culture medium with sperm during an ICSI procedure.

An arrow-head shows the place that polyvinylpyrrolidone or culture medium was excessively injected with sperm. The figure on the right shows vacuoles in the same egg at the pronuclear stage.

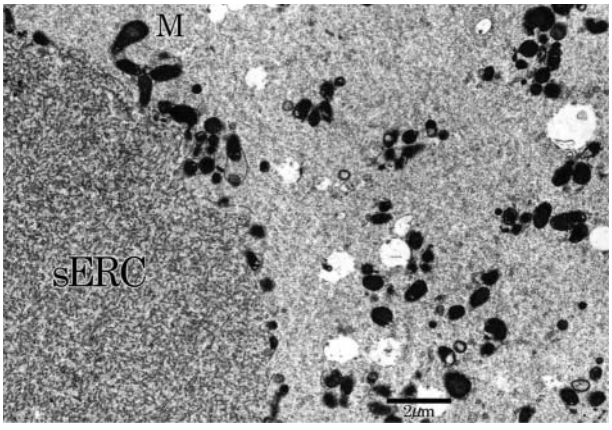

Fig. 2. Transmission electron micrographs of a smooth endoplasmic reticulum cluster.

A tubular type of large smooth endoplasmic reticulum cluster (sERC) was observed within the cytoplasm. M: mitochondria.

blastocyst stage. It has been reported that the fertilization rate was significantly reduced in oocytes in which the size of the vacuole is more than 14 μm or when multiple vacuoles are observed within the cytoplasm. On the other hand, small vacuoles that arise spontaneously in MII oocytes did not influence fertilization or development rates [9, 10]. It should be noted that vacuoles can also be produced by excessive injection (encapsulation) of polyvinylpyrrolidone or culture medium with sperm during ICSI procedures, thus generating a fluid-filled vacuole in the cytoplasm (Fig. 1).

Smooth Emdoplasmic Reticulum Clusters

A smooth emdoplasmic reticulum cluster (sERC) (Fig. 2) can be clearly distinguished morphologically from fluid-filled vacuoles (Fig. 3) using an inverted microscope. Oocytes that had possessed sERC and proceeded to ICSI followed by embryo transfer yielded low implantation rates and none produced pregnancies [2, 6]. Also, we reported that the pregnancy rate was low even with sERC-negative oocyte cohorts in cycles that produced sERC-positive oocytes. Furthermore, we observed higher estradiol concentrations on the day of hCG administration in cycles that yielded sERC-positive oocytes [3]. In addition, estradiol concentrations per aspirated oocyte, as well as the diameter of the largest follicle on the day of hCG administration, were significantly higher in cycles that produced sERC positive oocytes, compared with cycles that produced sERC negative oocytes [13]. These findings suggest the possibility of cytoplasmic deterioration and changes known to occur in aging human oocytes during extended culture. In the study, one baby born from a sERC-positive cycle was diagnosed with Beckwith-Wiedemann syndrome. However, the data has been misinterpreted in some papers, that claimed that the baby was derived from a sERC-positive oocyte. We did not transfer embryos derived from sERC-positive oocytes although the patient received an embryo obtained in a “sERC-positive cycle”. Ebner recently showed lower fertilization and blastocyst development rates in sERC positive oocytes compared with

Fig. 3. A smooth endoplasmic reticulum cluster in a mature oocyte.

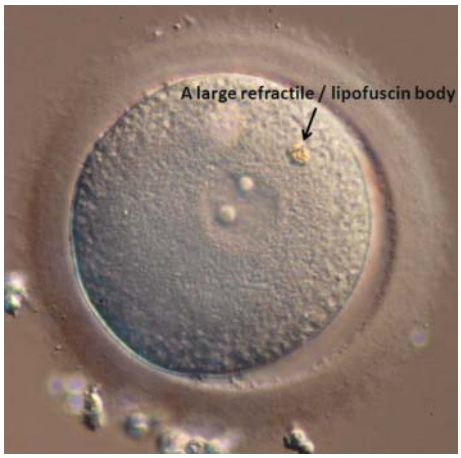

Fig. 4. A large refractile body / lipofuscin body in a pronuclear egg.

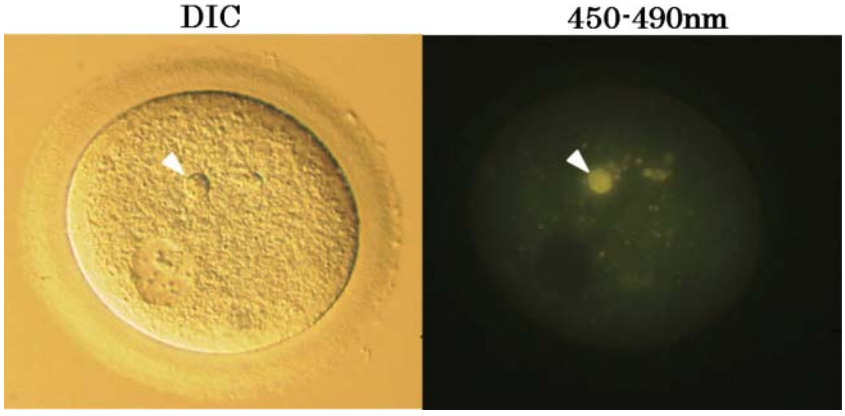

Fig. 5. Autofluorescent refractile bodies in human oocytes.
An arrowhead shows a large refractile body (~10 μm), in a GV stage human oocyte, that exhibited autofluorescence when excited at 450–490 nm.

unaffected sibling oocytes [14]. Patients who had one or more gametes showing sERC had significantly higher spontaneous abortion rates. Furthermore, in sERC positive cycles, significantly higher obstetric problems as well as neonatal deaths were observed and birth-weight was significantly lower in patients who had cycles with at least one or more sERC-positive oocytes [14].

Refractile Bodies / Lipofuscin Bodies

Refractile bodies (Fig. 4) are one of the main morphological abnormalities that can be observed in the cytoplasm of human oocytes. They were found to have autofluorescence (Fig. 5) which was consistent with the typical autofluorescence of lipofuscin [4]. Viewed by transmitted electron microscopy, the refractile bodies contained the conventional morphology of lipofuscin inclusions (Fig. 6) and consisted of a mixture of lipids and dense granule material [4]. Large refractile bodies (> 5 μm) were positively stained by the Schmorl reaction and were considered to contain lipofuscin [4]. Oocytes containing these larger lipofuscin inclusions were found to have a significantly reduced fertilization by IVF while these differences were not seen in similar oocytes treated by ICSI. Also, blastocyst development was found to be significantly lower when the size of the refractile bodies was larger than 5 μm [4].

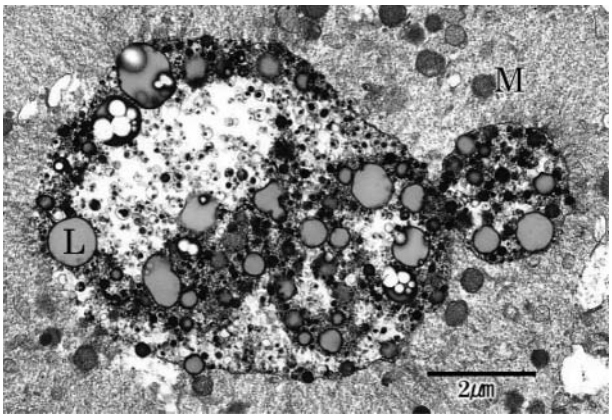

Fig. 6. Transmission electron micrographs of a large refractile body.

A large refractile body (~8 μm in diameter) contained fine amorphous electron-dense substance and lipids. The inclusions were partly encircled by a membrane (not completely surrounding the inclusion). M: mitochondria, L: lipid.

Centrally Located Cytoplasmic Granularity (CLCG)

In contrast to sERC, CLCG is considered to be associated with cytoplasmic immaturity in oocytes [1]. Low implantation and reduced ongoing pregnancy rates have been reported for embryos arising from oocytes with CLCG [1]. However, the quality of cleavage stage embryos and overall pregnancy rates were not significantly different after transfer of embryos derived from oocytes with or without CLCG. Also in the same study a very high aneuploidy rate (52.2%) was reported in embryos derived from oocytes with CLGC. Meriano [2] showed CLCG that appeared in repetitive cycles was a negative predictor of pregnancy and implantation rates, even though the cleavage stage embryo quality was not affected. Our recent unpublished pictures show that numerous small refractile / lipofuscin bodies were localized within the CLCG (Fig. 7) suggesting that these cytoplasmic abnormalities may have a common origin.

Discussion

On the basis of the reported studies, the origin of four dysmorphic phenotypes that are detected in human oocytes is different. Furthermore, embryos derived from oocytes containing such dysmorphisms have lower successful pregnancy and birth rates although correlation between the cytoplasmic morphology and embryo quality has been controversial. The problem of the exact effects of the morphological abnormalities could be due to different embryo criteria that were used to evaluate developmental competence in each study. More importantly, some investigators have grouped these phenotypes together as cytoplasmic inclusions even though the origin of the different structures and their influence on implantation should be considered separately.

Fluid-filled vacuoles in oocytes have been considered to arise spontaneously as membrane-bound cytoplasmic inclusion filled with fluid that is virtually identical to perivitelline fluid [15]. Also vacuoles have been assumed to arise spontaneously [15].

In contrast cytoplasmic vacuolation has been

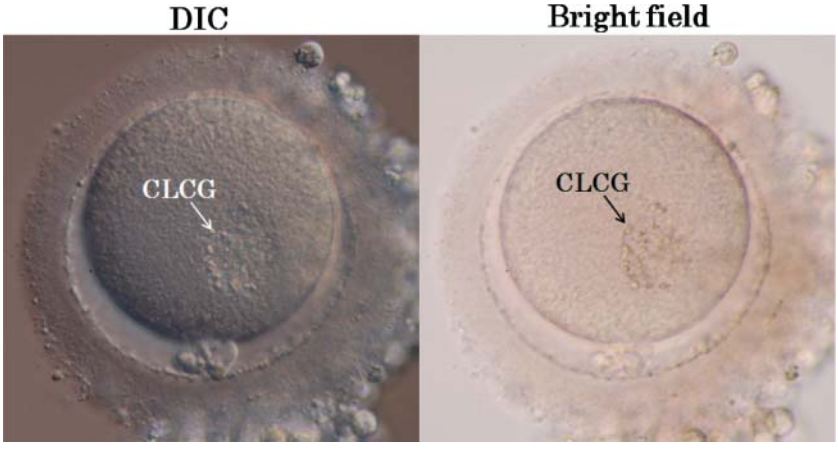

Fig. 7. Centrally located cytoplasmic granularity (CLCG).

Numerous small refractile / lipofuscin bodies were localized within the CLCG.

considered to be associated with cellular degeneration [16] and oocyte atresia [17]. It seems that spontaneous vacuolation should be distinguished from necrotic / apoptotic vacuolation. Interestingly it has been reported that larger and multiple vacuolations reduced fertilization and embryo development rates. This may be explained by the possibility that more extensive vacuolization interferes with segregation of chromosomes and cytokinesis following the fertilization process. Alternatively, such extensive abnormalities may represent the occurrence of necrosis / apoptosis in oocytes.

sERC is most likely to be associated with aberrant events of unknown origin and the consequence of the sERC in oocytes remain to be explored. Thus the transfer of embryos derived from oocytes that contain sERC should be avoided.

The presence of large refractile / lipofuscin bodies in oocytes was found to impair fertilization by IVF but not by ICSI [4, 18]. Some of the early stages of IVF could be disrupted by oxidative stress or cytoplasmic deterioration (for example, induction of a premature cortical granule reaction and/or damage of the plasma membrane which may disturb sperm-egg fusion). ICSI bypasses some of the critical early stages of fertilization. This could account for some of the differences between embryos that arise from IVF and ICSI and the effects of cytoplasmic disturbances related to lipofuscin formation and associated abnormalities. The much higher fertilization rates obtained in the current studies, compared to those report by Veeck, may be related to improvements in culture media and sperm preparation over the last 10 years.

It is very interesting that refractile / lipofusin bodies accumulated within CLCG, suggesting that there may be a common origin of the two different phenotypes. The accumulation of lipofuscin may occur during the growth phase of the oocytes when dominant follicles are being recruited into the preovulatory pathway. Also, the occurrence of both the larger lipofuscin bodies and CLCG may be partly related to the physiological conditions of the developing ovarian follicles, such as peri-follicular blood circulation and follicular fluid composition. Oocytes affected by the presence of the larger refractile / lipofuscin bodies, were found to have a significantly reduced fertilization [4, 18] and blastocyst development [4] rates.

All factors considered, it seems that embryos derived from these four dysmorphic phenotypes influence pregnancy outcome. Although there is a possibility for improving pregnancy rates with the further refinement of culture media that may reduce the occurrence of the defects, the influence on the birth rate and on the health of the babies derived from each phonotypes should be further considered. However, it is impossible to evaluate the consequence of oocyte morphology on the implantation potential of affected embryos when multiple embryos are transferred. However, following the recent regulations that promote single embryo transfer, the influence of each dysmorphic phonotype on pregnancy rate and outcome can be expected to be clarified in the near future. At this stage, it may be advisable not to transfer embryos derived from obviously dysmorphic oocytes when non-affected sibling embryos are available.

Acknowledgement

The author would like to thank Dr. Alexander Lopata for editing this paper.

References

1) Kahraman, S., Yakin, K., Donmez, E., Samli, H., Bahce, M., Cengiz, G., Sertyel, S., Samli, M. and Imirzalioğlu, N. (2000): Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. Hum. Reprod., 15, 2390–2393.
2) Meriano, J.S., Alexis, J., Visram-Zaver, S., Cruz, M. and Casper, R.F. (2001): Tracking of oocyte dysmorphisms for ICSI patients may prove relevant to the outcome in subsequent patient cycles. Hum. Reprod., 16, 2118–2123.
3) Otsuki, J., Okada, A., Morimoto, K., Nagai, Y. and Kubo, H. (2004): The relationship between pregnancy outcome and smooth endoplasmic reticulum clusters in MII human oocytes. Hum. Reprod., 19, 1591–1597.
4) Otsuki, J., Nagai, Y. and Chiba, K. (2007): Lipofuscin bodies in human oocytes as an indicator of oocyte quality. J. Assist. Reprod. Genet., 24, 263–270.
5) De Sutter, P., Dozortsev, D., Qian, C. and Dhont, M. (1996): Oocyte morphology does not correlate with fertilization rate and embryo quality after intracytoplasmic sperm injection. Hum. Reprod., 11, 595–597.
6) Serhal, P.F., Ranieri, D.M., Kinis, A., Marchant, S., Davies, M. and Khadum, I.M. (1997): Oocyte morphology predicts outcome of intracytoplasmic sperm injection. Hum. Reprod., 12, 1267–1270.
7) Xia, P. (1997): Intracytoplasmic sperm injection: correlation of oocyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality. Hum. Reprod., 12, 1750–1755.
8) Balaban, B., Urman, B., Sertac, A., Alatas, C., Aksoy, S. and Mercan, R. (1998): Oocyte morphology does not affect fertilization rate, embryo quality and implantation rate after intracytoplasmic sperm injection. Hum. Reprod., 13, 3431–

3433.
9) Ebner, T., Moser, M., Sommergruber, M., Gaiswinkler, U., Shebl, O., Jesacher, K. and Tews, G. (2005): Occurrence and developmental consequences of vacuoles throughout preimplantation development. Fertil. Steril., 83, 1635–1640.
10) Ebner, T., Moser, M. and Tews, G. (2006): Is oocyte morphology prognostic of embryo developmental potential after ICSI? Reprod. Biomed. Online, 12, 507–512.
11) Homa, S.T., Carroll, J. and Swann, K. (1993): The role of calcium in mammalian oocyte maturation and egg activation. Hum. Reprod., 8, 1274–1281.
12) Tesarik, J. (1997): Use of immature germ cells for the treatment of male infertility. Baillieres Clin. Obstet. Gynaecol., 11, 763–772.
13) Otsuki, J., Okada, A., Momma, Y., Takahashi, K., Nagai, Y. and Kubo, H. (2004): The smooth endoplasmic reticulum clusters in oocytes associated with higher estradiol and progesterone and larger follicles. In: Abstract Book for the 19th ESHRE Annual Meeting, p. 147.
14) Ebner, T., Moser, M., Shebl, O., Sommerguber, M. and Tews, G. (2008): Prognosis of oocytes showing aggregation of smooth endoplasmic reticulum. Reprod. Biomed. Online, 16, 113–118.
15) Van Blerkom, J. (1990): Occurrence and developmental consequences of aberrant cellular organization in meiotically mature human oocytes after exogenous ovarian hyperstimulation. J. Electron. Microsc. Tech., 16, 324–346.
16) Zamboni, L., Thompson, R.S. and Smith, D.M. (1972): Fine morphology of human oocyte maturation in vitro. Biol Reprod., 7, 425–457.
17) Nayudu, P.L., Lopata, A., Jones, G.M., Gook, D.A., Bourne, H.M., Sheather, S.J., Brown, T.C. and Johnston, W.I. (1989): An analysis of human oocytes and follicles from stimulated cycles: oocyte morphology and associated follicular fluid characteristics. Hum. Reprod., 4, 558–567.
18) Veeck, L.L. (1991): Atlas of the human oocyte and early conceptus, pp. 121–166, Williams & Wilkins, Baltimore.