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Failure of oocyte maturation: Possible mechanisms for oocyte maturation arrest

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Abstract

For human IVF, the patient's ovaries are hormonally stimulated to ensure the collection of fully matured oocytes that are at the metaphase II stage. Only these oocytes can be successfully fertilized either when mixed with sperm or after ICSI. Nevertheless, in some cases immature or maturing oocytes are recovered from follicles. Surprisingly, sometimes these oocytes do not complete maturation when cultured *in vitro*, for unknown reasons. In this article we discuss some possible mechanisms that may be responsible for those atypical arrests.

Key words: maturation/meiotic arrest/oocytes

Introduction

Mammalian ovaries contain three basic populations of oocytes: (i) growing oocytes, which have not attained their full size and are unable to resume maturation when released from follicles and cultured *in vitro*; (ii) medium-sized oocytes that can resume maturation *in vitro*, although this process is not completed and oocytes are arrested in metaphase I (MI) stage; and (iii) fully grown oocytes that resume maturation in response to gonadotrophins or

when they are released from follicles and cultured *in vitro*. This process is completed after oocytes reach metaphase II (MII) stage. This is the only stage when oocytes, in most mammals, can be successfully fertilized (Eppig, 1993; Fulka *et al*., 1998). After oocytes begin to mature, their nuclei–germinal vesicles (GV) break down and chromosomes condense (germinal vesicle breakdown, GVBD). Chromosomes are then arranged in MI stage which is followed by anaphase I to telophase I (A–TI) transition and oocytes are arrested in MII, ready for fertilization (Trounson *et al*., 2001).

The process of maturation is under control of maturation promoting factor (MPF). More simply, in immature oocytes, MPF is present in an inactive phosphorylated form as a complex of Cdk 1 and cyclin B. This phosphorylation is controlled by Myt 1 kinase. The dephosphorylation of MPF is induced by Cdc25 phosphatase (probably by Cdc25B). The activity of MPF reaches its peak in MI and then decreases during the anaphase to telophase transition. Thereafter, high levels of MPF are again restored and oocytes are kept at this stage under the influence of a cytostatic factor (CSF) (Smith, 2001). MPF is fully degraded when oocytes are fertilized or parthenogenetically activated (Nebreda and Ferby, 2000; Tunquist and Maller, 2003). The process of maturation, however, is much more complex and not yet fully understood (Eichenlaub-Ritter and Peschke, 2002). In somatic cells, the transition from one stage to another one is perfectly controlled by so-called checkpoint controls. It is unclear whether equivalent control mechanisms also exist in mammalian oocytes (LeMaire-Adkins *et al*., 1997; Yin *et al*., 1998; Fulka *et al*., 2000). In general, we may suppose that the same, or similar, cell cycle control mechanisms regulate maturation of human oocytes (Yamashita *et al*., 2000).

Maturation arrests in human oocytes

Naturally, fully grown mammalian oocytes are arrested at two points of maturation. The first point of arrest is at GV stage when oocytes are awaiting the gonadotrophin signal or the release from an inhibitory follicular environment. The second point is at MII stage when oocytes are waiting for fertilization (Russell, 2001). In some recently published papers, certain atypical situations in human oocytes, which are not easy to explain, have been described (Bergere *et al*., 2001; Levran *et al*., 2002; Neal *et al*., 2002; Schmiady and Neizel, 2002). Thus, oocytes from some patients were collected at GV stage and did not resume meiosis when cultured *in vitro*. In some other cases, oocytes were collected in MI and were unable to complete meiosis up to MII. Moreover, in some patients, oocytes did not respond properly to fertilizing sperm. For our debate article we have selected some recently published results describing the analogical situations in oocytes from experimental animals, namely the mouse. We do believe that these data may, at least partially, shed some light on the meiotic arrest problem in assisted human reproduction.

GV stage arrest

As mentioned above, those oocytes not attaining their full size are unable to

undergo GVBD and remain arrested at this stage or eventually they may mature only to MI. In pig and cattle, the ability to initiate maturation is related to the follicle size from which the oocyte is collected (Motlik and Fulka, 1986). It cannot be supposed that for human IVF the oocytes are isolated from such small follicles. However, it remains a question if, in some rare cases, the follicular growth is not accompanied with relevant oocyte growth. Thus the follicle would attain the appropriate size at the time of aspiration whilst the oocyte is still slightly developmentally behind. It has been shown that chromatin configurations differ in immature human oocytes collected from large antral oocytes. It is assumed that only those oocytes in which the nucleolus is surrounded with a ring of condensed chromatin mature better and are more developmentally competent after fertilization (Wickramasinghe and Albertini, 1993; Combelles *et al*., 2002; Miyara *et al*., 2003a). It has been proposed that the artificial GV stage arrest may permit the oocyte to reach a more advanced developmental stage and thus to increase the competence of oocytes to mature and develop better after fertilization. In humans, the prolonged GV stage arrest, when oocytes are incubated in medium with phosphodiesterase type 3 inhibitor, does not enhance their maturation competence (Nogueira *et al*., 2003).

Levran *et al*. (2002), however, reported that the inability of oocytes to mature was observed repeatedly, thus we may rather suppose some rare heritable molecular defects that are responsible for the inability of these oocytes to initiate the activation of MPF. It is impossible to define these defects precisely but a recent paper by Lincoln *et al*. (2002) showed that this possibility may theoretically exist. These authors generated $Cdc25B^{-/-}$ mice and found that oocytes from these females were ovulated at GV stage and when further cultured *in vitro* were unable to undergo GVBD and remained GV stage-arrested. The wildtype Cdc25B mRNA microinjection into these oocytes triggers the resumption of meiosis. The possible treatment for the patient described in Levran's paper would be, theoretically, the transfer of GV from a patient's oocytes into a donor's enucleated oocytes (Fulka *et al*., 2002; Palermo *et al*., 2002), with their subsequent maturation and IVF (ICSI).

Metaphase I arrest

In the second group of patients, oocytes were collected in MI stage and, when cultured *in vitro*, they were unable to reach MII. Here, at least three possible explanations could be offered. First, when collected, oocytes still did not attain the full competence to mature. Second, this arrest may result from the absence of meiotic recombination, which, under normal conditions, occurs in pachytene stage. For example, as demonstrated in the mouse, the targeted disruption of the DNA mismatch repair genes *Mlh1* or *Mlh3*, which results in the absence of MLH 1 (MLH 3) proteins, sharply reduces the meiotic recombination and maturing oocytes are arrested in MI-like stage (Woods *et al*., 1999; Lipkin *et al*., 2002). A similar defect can be observed in the absence of Spo 11p (Lichten, 2001), and also the mouse meiotic mutation *mei1* disrupts chromosome synapsis and oocytes are arrested in MI stage (Libby *et al*., 2002

). This clearly indicates the necessity of meiotic recombination for the final phases of oocyte maturation. The third possibility may be in an inability of oocytes to produce the key cell cycle regulating factors. Spruck *et al*. (2003) demonstrated in the mouse that the lack of Cks 2 results in the production of oocytes unable to pass the first meiotic metaphase. Hampl and Eppig (1995) reported that oocytes from LT/Sv mice do not progress beyond MI. They showed that in these oocytes the natural degradation of $p34^{cdc2}$ and cyclin B, which is typical for the normal transition from MI to AI, did not occur. That protein kinase C (PKC) may be among the participating factors regulating this transition has been shown by Viveiros *et al*. (2001). Certainly some other factors, namely those which are responsible for a correct spindle function, play a crucial role in the transition either from MI or MII (Kubiak *et al*., 1993; Brunet *et al*., 1999; Petronczki *et al*., 2003). Microfilaments also play an important role because they can influence the migration of spindle to the oocyte periphery. If inhibited, for example when oocytes are incubated in medium with high concentrations of cytochalasin D or in the absence of *Formin-2* gene, oocytes are arrested in MI. Interestingly, these oocytes can be fertilized but embryos generated from them are polyploid (Soewarto *et al*., 1995; Leader *et al*., 2002).

Metaphase II arrest and abnormal situations after fertilization

With respect to Levran *et al*. (2002), cases 6, 7 and 8 are very difficult to explain. In case 6, oocytes were collected in MII stage and after ICSI two pronuclei were detected in them but no extrusion of the second polar body. This may indicate some MII spindle abnormalities as there is a very high probability that the separation of individual chromatids occurred as a consequence of oocyte activation. Typically, if this does not occur, the oocyte is not properly activated (Fulka *et al*., 1994). This may be the explanation for case 7, where oocytes remained in MII stage after ICSI, but the absence of some essential cell cycle molecules which are involved in the regulation of a proper response after activation cannot be ruled out. The failure of oocyte activation prevents the formation of both pronuclei. Thus, MII is preserved and the sperm head chromatin does not undergo the decondensation. Instead, the individualization of paternal chromatin and the formation of the sperm head chromosome group can be detected (Flaherty *et al*., 1995; Schmiady *et al*., 1996; Rosenbusch, 2003). This phenomenon is named ‘sperm premature chromosome condensation (PCC)’. The detailed analysis of human MII oocytes after IVF failure indicates that the morphology of spindles was rather abnormal in almost all cells and this results in the failure of normal fertilization (Miyara *et al*., 2003b). Case 8 has MII oocytes that either did not extrude the second polar bodies or contained multiple pronuclei. The absence of second polar bodies may be theoretically explained as a consequence of ageing. In aged oocytes the metaphase spindle is not located on the periphery and moves to the oocyte centre. When these oocytes are parthenogenetically activated, they either cleave to 2-cell-like stage (immediate cleavage) or contain two pronuclei. The polynucleated fertilization with second polar bodies may be indicative of certain spindle defects. That situation resembles the observations made by Simerly *et al*. (2003) when they tried to produce primate clones. The absence of two essential spindle proteins (NuMA and HSET), which were removed when the cytoplast was prepared, resulted, after nucleus transfer, in abnormal embryonic divisions.

It is possible that levels of the above or similar proteins were reduced and that this resulted in an abnormal separation of maternal chromosomes in MII. Even if we accept that the first meiotic division differs from the second one, the consequences of non-extrusion of polar bodies are basically the same and result in chromosomally abnormal oocytes (Soewarto *et al*., 1995). Moreover, all the above cases are very difficult to explain. For example, if we accept that some spindle defects may be responsible, it is then not easy to understand how oocytes reached the MII. This indicates the absence of cell cycle checkpoint controls, but we rather suggest that there is a high probability that in some patients, oocytes are deficient in some key cell cycle regulating molecules (Schmiady and Neitzel, 2002).

Conclusion

Maturation arrest in human oocytes as a cause of infertility has been discussed in some recently published papers. With the exception of oocytes from LT/Sv mice (Hampl and Eppig, 1995), this phenomenon has not been described in oocytes from any other mammalian species. We have listed some examples that are known from animal experiments and that result in a meiotic arrest at different stages of oocyte maturation. It has not been our intention to present a complete review and account of all papers covering this problem. Moreover, the arrest may be detected not only when oocytes mature but it may occur even after they are successfully fertilized (Balczon *et al*., 2002; Wu *et al*., 2003). We intend that our paper should open a further discussion on this topic and will demonstrate to clinicians that this problem is well known and complex. Neither was it our intention to indicate in detail the possible approaches which may solve at least some of the complications described (i.e. cytoplasmic transfer, germinal vesicle replacement, metaphase spindle transfer). When these approaches are eventually used, new issues like the 'heteroplasmy' and 'epigenetic modifications' must be considered (De Rycke *et al*., 2002; Hawes *et al*., 2002; St John, 2002).

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References

Balczon, R., Simerly, C., Takahashi, D. and Schatten, G. (2002) Arrest of cell cycle progression during first interphase in murine zygotes microinjected with anti-PCM-1 antibodies. *Cell Mot. Cytoskel.*, **52**, 183–192.[CrossRef][Medline]

Bergere, M., Lombroso, R., Gombault, M., Wainer, R. and Selva, J. (2001)

▪ References

An idiopathic infertility with oocytes metaphase I maturation block. *Hum. Reprod.*, **16**, 2136–2138.[Abstract/Free Full Text]

Brunet, S., Santa Maria, A., Guillaud, P., Dujardin, D., Kubiak, J.Z. and Maro, B. (1999) Kinetochore fibers are not involved in the formation of the first meiotic spindle in mouse oocytes, but control the exit from the first meiotic M phase. *J. Cell Biol.*, **146**, 1–11.[Free Full Text]

Combelles, C.M.H., Cekleniak, N.A., Racowsky, C. and Albertini, D.F. (2002) Assessment of nuclear and cytoplasmic maturation in in-vitro matured human oocytes. *Hum. Reprod.*, **17**, 1006–1016.[Abstract/Free Full Text]

DeRycke, M., Liebaers, I. and Van Steirteghem, A. (2002) Epigenetic risks related to assisted reproductive technologies. *Hum. Reprod.*, **17**, 2487–2494.[Abstract/Free Full Text]

Eichenlaub-Ritter, U. and Peschke, M. (2002) Expression in in-vivo and in-vitro growing and maturing oocytes: focus on regulation of expression at translational level. *Hum. Reprod. Update*, **8**, 21–41.[Medline]

Eppig, J.J. (1993) Regulation of mammalian oocyte maturation. In Adashi, E.Y. and Leung, P.C.K. (eds), *The Ovary*. Raven Press, New York, pp. 185–208.

Flaherty, S.P., Payne, D., Swann, N.J. and Mathews, C.D. (1995) Aetiology of failed and abnormal fertilization after intracytoplasmic sperm injection. *Hum. Reprod.*, **10**, 2623–2629.[Abstract]

Fulka, J., Jr, Moor, R.M. and Fulka, J. (1994) Sister chromatid separation and the metaphase–anaphase transition in mouse oocytes. *Dev. Biol.*, **165**, 410–417.[CrossRef][Medline]

Fulka, J., Jr, First, N.L. and Moor, R.M. (1998) Nuclear and cytoplasmic determinants involved in the regulation of mammalian oocyte maturation. *Mol. Hum. Reprod.*, **58**, 1177–1187.

Fulka, J., Jr, Tesarik, J., Loi, P. and Moor, R.M. (2000) Manipulating the human embryo: cell cycle checkpoint controls. *Cloning*, **2**, 1–7.[CrossRef]

Fulka, J., Jr, Loi, P., Fulka, H., Kren, R., Dean, W., Mrazek, M. and Reik, W. (2002) Nucleus replacement in mammalian oocytes. *Cloning Stem Cells*, **3**, 181–187.[CrossRef]

Hampl, A. and Eppig, J.J. (1995) Analysis of the mechanism(s) of metaphase I arrest in maturing mouse oocytes. *Development*, **121**, 925–933.[Abstract/Free Full Text]

Hawes, S.M., Sapienza, C. and Latham, K.E. (2002) Ooplasmic donation in humans. *Hum. Reprod.*, **17**, 850–852.[Abstract/Free Full Text]

Kubiak, J.Z., Weber, M., de Pennart, H., Winston, N.J. and Maro, B. (1993) The metaphase II arrest in mouse oocytes is controlled through microtubule-dependent destruction of cyclin B in the presence of CSF. *EMBO J.*, **12**, 3773–3778.[Abstract]

Leader, B., Lim, H., Carabatsos, M.J., Harrington, A., Ecsedy, J., Pellman, D., Maas, R. and Leder, P. (2002) Formin-2, polyploidy, hypofertility and positioning of the meiotic spindle in mouse oocytes. *Nature Cell Biol.*, **4**, 921–928.[CrossRef][Medline]

LeMaire-Adkins, R., Radke, K. and Hunt, P.A. (1997) Lack of checkpoint control at the metaphase/anaphase transition: a mechanism of meiotic nondisjunction in mammalian females. *J. Cell Biol.*, **139**, 1611–1619.[Abstract/Free Full Text]

Levran, D., Farhi, J., Nahun, H., Glerezman, M. and Weissman, A. (2002) Maturation arrest of human oocytes as a cause of infertility. *Hum. Reprod.*, **17**, 1604–1609.[Abstract/Free Full Text]

Libby, B.J., De La Fuente, R., O'Brien, M.J., Wigglesworth, K., Cobb, J., Inselman, A., Eaker, S., Handel, M.A., Eppig, J.J. and Schimenti, J.C. (2002) The mouse meiotic mutation *mei1* disrupts chromosome synapsis with sexually dimorphic consequences for meiotic progression. *Dev. Biol.*, **242**, 174–187.[CrossRef][Medline]

Lichten, M. (2001) Meiotic recombination: breaking the genome to save it. *Curr. Biol.*, **11**, R253–R256.[CrossRef][Medline]

Lincoln, A.J., Wickramasinghe, D., Stein, P., Schultz, R.M., Palko, M.E., DeMiguel, M.P., Tessarollo, l. and Donovan, P.J. (2002) Cdc25b phosphatase is required for resumption of meiosis during oocyte maturation. *Nature Genet.*, **30**, 446–449.[Medline]

Lipkin, S.M., Moens, P.B., Wang, V., Lenzi, M., Shanmugarajan, D., Gilgeous, A., Thomas, J., Cheng, J., Touchman, J.W., Green, E.D., Schwartzberg, P., Collins, F.S. and Cohen, P.E. (2002) Meiotic arrest and aneuploidy in MLH3-deficient mice. *Nature Genet.*, **31**, 385–390.[CrossRef][Medline]

Miyara, F., Migne, C., Dumont-Hassan, M., Le Meur, A., Cohen-Bacrie, P., Aubriot, F.X., Glissant, A., Nathan, C., Douard, S., Stanovici, A. and Debey, P. (2003a) Chromatin configuration and transcriptional control in human and mouse oocytes. *Mol. Reprod. Dev.*, **64**, 458–470.[CrossRef][Medline]

Miyara, F., Aubriot, F.X., Glissant, A., Nathan, C., Douard, S., Stanovici, A., Herve, F., Dumont-Hassan, M., LeMeur, A., Cohen-Bacrie, P. and Debey, P. (2003b) Multiparameter analysis of human oocytes at metaphase II stage after IVF failure in non-male infertility. *Hum. Reprod.*, **18**, 1494–1503.[Abstract/Free Full Text]

Motlik, J. and Fulka, J. (1986) Factors affecting meiotic competence in pig oocytes. *Theriogenology*, **25**, 87–96.[CrossRef]

Neal, M.S., Cowan, L., Louis, J.P., Hughes, E., King, W.A. and Basrur, P.K. (2002) Cytogenetic evaluation of human oocytes that failed to complete meiotic maturation in vitro. *Fertil. Steril.*, **77**, 844–845.[CrossRef][Medline]

Nebreda, A.R. and Ferby, I. (2000) Regulation of the meiotic cell cycle in oocytes. *Curr. Opin. Cell Biol.*, **12**, 666–675.[CrossRef][Medline]

Nogueira, D., Albano, C., Adriaenssens, T., Cortvrindt, R., Bourgain, C., Devroey, P. and Smitz, J. (2003) Human oocytes reversibly arrested in prophase I by phosphodiesterase type 3 inhibitor *in vitro*. *Biol. Reprod.*, DOI:10.1095/biolreprod.103.015982.

Palermo, G.D., Takeuchi, T. and Rosenwaks, Z. (2002) Technical approaches to correction of oocyte aneuploidy. *Hum. Reprod.*, **17**, 2165–2173.[Abstract/Free Full Text]

Petronczki, M., Siomos, M.F. and Nasmyth, K. (2003) Un ménage a quatre: the molecular biology of chromosome segregation in meiosis. *Cell*, **112**, 423–440.[Medline]

Rosenbusch, B.E. (2003) Frequency and patterns of premature sperm chromosome condensation in oocytes failing to fertilize after intracytoplasmic sperm injection. *J. Assist. Reprod. Genet.*, **5**, 253–259.

Russell, J.B. (2001) In vitro oocyte maturation. In Wolf, D.P. and Zelinski-Wooten, M. (eds), *Assisted Fertilization and Nuclear Transfer in Mammals*. Humana Press, Totowa, pp. 67–79.

Schmiady, H. and Neitzel, H. (2002) Arrest of human oocytes during meiosis I in two sisters of consanquineous parents: first evidence for an autosomal recessive trait in human infertility. *Hum. Reprod.*, **17**, 2556–2559.[Abstract/Free Full Text]

Schmiady, H., Tandler-Schneider, A. and Kentenich, H. (1996) Premature chromosome condensation of the sperm nucleus after intracytoplasmic sperm injection. *Hum. Reprod.*, **11**, 2239–2245.[Abstract]

Simerly, C., Dominko, T., Navara, C., Payne, C., Capuano, S., Gosman, G., Chong, K.Y., Takahashi, D., Chace, C., Compton, D., Hewitson, L. and Schatten, G. (2003) Molecular correlates of primate nuclear transfer failures. *Science*, **300**, 297.

Smith, G.D. (2001) Control of oocyte nuclear and cytoplasmic maturation. In Wolf, D.P. and Zelinski-Wooten, M. (eds), *Assisted Fertilization and Nuclear Transfer in Mammals*. Humana Press, Totowa, pp. 53–65.

Soewarto, D., Schmiady, H. and Eichenlaub-Ritter, U. (1995) Consequences of non-extrusion of the first polar body and control of the sequential segregation of homologues and chromatids in mammalian oocytes. *Hum. Reprod.*, **10**, 2350–2360.[Abstract]

Spruck, C.H., de Miguel, M.P., Smith, A.P.L., Ryan, A., Stein, P., Schultz, R.M., Lincoln, A.J., Donovan, P.J. and Reed, S.I. (2003) Requirement of Cks2 for the first metaphase/anaphase transition of mammalian meiosis. *Science*, **300**, 647–650.[Abstract/Free Full Text]

StJohn, J.C. (2002) Ooplasm donations in humans. *Hum. Reprod.*, **17**, 1954–1958.[Abstract/Free Full Text]

Trounson, A., Anderiesz, C. and Jones, G. (2001) Maturation of human oocytes in vitro and their developmental competence. *Reproduction*, **121**, 51–75.[Medline]

Tunquist, B.J. and Maller, J.L. (2003) Under arrest: cytostatic factor (CSF)-mediated metaphase arrest in vertebrate eggs. *Genes Dev.* **17**, 683–710.[Free Full Text]

Viveiros, M.M., Hirao, Y. and Eppig, J.J. (2001) Evidence that protein kinase C (PKC) participates in the meiosis I to meiosis II transition in mouse oocytes. *Dev. Biol.*, **235**, 330–342.[CrossRef][Medline]

Wickramasinghe, D. and Albertini, D.F. (1993) Cell cycle control during mammalian oogenesis. *Curr. Top. Dev. Biol.*, **28**, 125–153.[Medline]

Woods, L.M., Hodges, C.A., Baart, E., Baker, S.M., Liskay, M. and Hunt, P.A. (1999) Chromosomal influence on meiotic spindle assembly: abnormal meiosis I in female *Mlh1* mutant mice. *J. Cell Biol.*, **145**, 1395–1406.[Abstract/Free Full Text]

Wu, X., Viveiros, M.M., Eppig, J.J., Bai, Y., Fitzpatrick, S.L. and Matzuk, M.M. (2003) *Zygote* arrest 1 (*Zar1*) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. *Nature Genet.*, **33**, 187–191.[CrossRef][Medline]

Yamashita, M., Mita, K., Yoshida, N. and Kondo, T. (2000) Molecular mechanisms of the initiation of oocyte maturation: general and species-specific aspects. In Meijer, L., Jezequel, A. and Ducommun, B. (eds) , *Progress in Cell Cycle Research*. Kluwer Academic/Plenum Publishers, New York, pp. 115–129.

Yin, H., Cukurcam, S., Betzendahl, I., Adler, I.D. and Eichenlaub-Ritter, U. (1998) Trichlorfon exposure, spindle aberrations and nondisjunction in mammalian oocytes. *Chromosoma*, **107**, 514–522.[CrossRef][Medline]

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