CASE REPORT

Maturation arrest of human oocytes as a cause of infertility

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Maturation arrest of human oocytes may occur at various stages of the cell cycle. A total failure of human oocytes to complete meiosis is rarely observed during assisted conception cycles. We describe here a case series of infertile couples for whom all oocytes repeatedly failed to mature during IVF/ICSI. Eight couples, all presenting with unexplained infertility, underwent controlled ovarian stimulation followed by oocyte retrieval and IVF/ICSI. The oocytes were stripped of cumulus cells prior to the ICSI procedure and their maturity status was defined. In each couple, oocyte maturation was repeatedly arrested at the germinal vesicle (GV) (n = 1), metaphase I (MI) (n = 4) and metaphase II (MII) (n = 3) stage. Oocyte maturation arrest may be the cause of infertility in some couples previously classified as having unexplained infertility. The recognition of oocyte maturation arrest as a specific medical condition may contribute to the characterization of the yet poorly defined entity currently known as ‘oocyte factor’. The cellular and genetic mechanisms causing oocyte maturation arrest should be the subject of further investigation.

Key words: infertility/IVF/meiosis/oocyte maturation arrest/recurrent maturation arrest

Introduction

In the human ovary, each fully-grown Graafian follicle contains a meiotically competent oocyte that has initiated the first stages of meiosis but is maintained at prophase I arrest by inhibitory factors produced by the follicle. Resumption of meiotic maturation in vivo requires hormonal stimulation and the pre-ovulatory gonadotrophin surge is thought to be the primary stimulus by which oocyte maturation is reinitiated. In-vitro, spontaneous, hormone-independent nuclear maturation is initiated when oocytes are removed from their follicular environment and cultured in simple buffered medium. Suppression of maturation occurs when different components of the follicle are added back to the culture system. Thus, intrafollicular oocytes are situated in the G2 phase of the cell cycle, poised to enter the M phase, but held in check by local follicular factors.

Women undergoing controlled ovarian stimulation prior to IVF are subjected to various treatment protocols aimed at inducing multiple follicular growth. Oocyte meiotic maturation is induced by HCG, acting as a surrogate LH surge. Normally, the resumption of meiosis and achievement of second metaphase occurs within 18 and 28–38 h respectively, following the LH surge (Seibel et al., 1982). Fortunately, with conventional protocols, by the time of retrieval the majority of oocytes have completed their maturation and are collected at the metaphase II (MII) phase. Although it is common for a few oocytes to remain immature despite ovarian stimulation and HCG administration (Bar-Ami et al., 1994), the complete failure of all oocytes to mature in vivo is extremely rare, and only a handful of such cases have been described in the literature (Rudak et al., 1990; Eichenlaub-Ritter et al., 1995; Hartshorne et al., 1999; Harrison et al., 2000; Barblett, 2000).

We describe here eight couples, all referred to our service for IVF–embryo transfer with the presumptive diagnosis of unexplained infertility. In all cases, complete oocyte maturation arrest at various stages of the cell cycle was observed following ovarian stimulation and oocyte retrieval.

Case reports

Eight couples with unexplained infertility are included in our report. All of them underwent a thorough infertility investigation without any abnormal findings identified. All female patients (aged 25–35 years) had regular ovulatory cycles and failed to conceive after at least six cycles of ovulation induction. Seven of the eight couples presented with primary infertility. All patients were in good general health, were non-smokers, and none of them reported a family history of infertility. None of the patients was exposed to any medications for other medical conditions during or just prior to IVF and embryo transfer, and a thorough evaluation of possible past exposure to environmental and occupational...
Oocyte maturation arrest

Table I. Clinical characteristics and ICSI outcome in the investigative cycles of patients with oocyte maturation arrest

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age (years)</th>
<th>Duration of infertility (years)</th>
<th>Day 3 FSH (IU/l)</th>
<th>Previous IVF attempts</th>
<th>Stimulation protocol</th>
<th>HCG day</th>
<th>Serum E$_2$ on HCG day (pg/ml)</th>
<th>No. of oocytes retrieved</th>
<th>Oocyte maturity</th>
<th>Normal fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>10</td>
<td>5.6</td>
<td>1</td>
<td>Long GnRHa</td>
<td>15</td>
<td>3475</td>
<td>5</td>
<td>5 GV</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>4</td>
<td>4.0</td>
<td>1</td>
<td>Long GnRHa</td>
<td>13</td>
<td>2746</td>
<td>11</td>
<td>11 MI</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>15</td>
<td>8.7</td>
<td>8</td>
<td>Long GnRHa</td>
<td>16</td>
<td>1893</td>
<td>7</td>
<td>7 MI</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>8</td>
<td>8.8</td>
<td>9</td>
<td>Long GnRHa</td>
<td>13</td>
<td>2430</td>
<td>21</td>
<td>17 MI</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>4.5</td>
<td>11</td>
<td>4</td>
<td>Long GnRHa</td>
<td>12</td>
<td>2160</td>
<td>16</td>
<td>16 MI</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>16</td>
<td>NA</td>
<td>9</td>
<td>Long GnRHa</td>
<td>9</td>
<td>NA</td>
<td>12</td>
<td>11 MI</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>2</td>
<td>3.5</td>
<td>3</td>
<td>Long GnRHa</td>
<td>11</td>
<td>1528</td>
<td>15</td>
<td>13 MI</td>
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<tr>
<td>8</td>
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<td>7</td>
<td>Long GnRHa</td>
<td>15</td>
<td>736</td>
<td>5</td>
<td>2 MI</td>
<td>0</td>
</tr>
</tbody>
</table>

NA = not available; GnRHa = gonadotrophin releasing hormone agonist; E$_2$ = estradiol.

toxicants and excessive X-ray irradiation yielded negative results. All patients exhibited a normal female (46,XX) karyotype. All patients had normal day 3 FSH levels (range 3.5–11 IU/l) and a normal response to both induction of ovulation and ovarian stimulation. Table I summarizes patients’ characteristics and cycle outcome.

All couples were treated according to our routine IVF treatment protocols, as previously described in detail elsewhere (Levran et al., 1998). Oocytes were retrieved ~36 h after HCG administration. Following retrieval, the oocytes were stripped of cumulus cells for the ICSI procedure and their maturity status was defined. The oocytes were further observed for 3 days after the insemination.

Since all patients underwent routine IVF procedures, an institutional review board (IRB) approval was not deemed necessary and therefore not obtained.

**Case 1**

A 31 year-old woman with a 10 year history of primary infertility was referred to our IVF clinic following failure of treatment with gonadotrophins for unexplained infertility. On her first IVF attempt, using the long GnRH agonist protocol, four of five oocytes that were retrieved remained at the germinal vesicle (GV) stage, even after 48 h of culture. The fifth oocyte was atretic. On the second attempt, we prolonged the HCG–oocyte retrieval interval to 38 h; again five oocytes were aspirated, all of them at the GV stage.

**Case 2**

A 29 year-old female with primary unexplained infertility of 4 years duration was referred to our IVF clinic following failure to conceive with gonadotrophin induction of ovulation. The couple had two attempts at IVF. Eight oocytes were retrieved in the first attempt and 11 in the second. All oocytes were at the metaphase I (MI) stage and failed to proceed in meiosis beyond this stage even after extended culture. An oocyte donation cycle, using the husband’s sperm, resulted in pregnancy which ended in miscarriage at 9 gestational weeks.

**Case 3**

A 33 year-old female had primary unexplained infertility of 15 years duration. Since 1989, the couple had undergone eight IVF attempts in several programmes in Europe and in Israel. In all attempts, four to nine oocytes were retrieved, all of them at the MI stage, without further progress in maturation after extended culture.

**Case 4**

A 29 year-old female presented with 8 years of primary unexplained infertility. The couple had been previously treated in two IVF centres. In nine attempts using the long GnRH agonist protocol, between 5–11 oocytes were retrieved, all of which did not proceed in maturation beyond the MI stage.

**Case 5**

The patient was referred to IVF in 1994 at the age of 23 because of primary unexplained infertility. The couple had four IVF attempts in which 13–19 oocytes were retrieved. The majority of oocytes were at the MI stage, while some did not proceed even beyond the GV stage. An oocyte donation cycle, using the husband’s sperm, resulted in a normal pregnancy and delivery of a healthy baby.

**Case 6**

A 35 year-old female had been married for 16 years without children. The patient failed to conceive after seven spontaneous pregnancies, five of which were blighted ova and two hydatidiform moles. The couple had nine IVF attempts in various centres in Israel. In all attempts, retrieved oocytes were at the MII stage. Following ICSI, in the majority of the oocytes more than two pronuclei were repeatedly observed (Figure 1), and in those having two pronuclei present, there was no extrusion of the second polar body. In one cycle using donor oocytes and husband’s sperm, normal fertilization and embryonic cleavage were observed, but pregnancy was not achieved.

**Case 7**

A 25 year-old female presented with 2 years of primary unexplained infertility. The couple had four IVF attempts in which 15–19 oocytes were recovered. Almost all of the oocytes (60/65) were at the MII stage. Following ICSI, none of the oocytes had completed the second meiotic division. They all remained at the MII stage; no signs of fertilization were observed.
observed. This patient’s husband’s sperm was used in a subsequent oocyte donor cycle resulting in normal fertilization and cleavage. A twin pregnancy was achieved that led to the birth of healthy twins.

Case 8
A 30 year-old female presented with 6 years of primary unexplained infertility. This patient had seven previous IVF attempts in which 2–5 oocytes were aspirated, most of them at MII stage. Embryo transfer was not carried out on this patient, because by means of ICSI, we either observed polynucleated fertilization or failure to extrude the second polar body. In the second cycle of oocyte donation, using the husband’s sperm, a normal singleton pregnancy was achieved, leading to the birth of a healthy baby.

Discussion
In the group of patients with the presumptive diagnosis of unexplained infertility undergoing IVF–embryo transfer one sporadically encounters cases in which the cause of infertility is attributed to a poorly defined ‘oocyte factor’. The series of patients reported here represent a previously unrecognized category of unexplained infertility with an ‘oocyte factor’, namely, oocyte maturation arrest.

All the patients in our series exhibited the same phenomena on at least two separate occasions. They all failed to conceive following numerous IVF attempts at several centres, and the same pattern of maturation arrest was repeatedly observed. Thus, oocyte maturation arrest appears to arise from an intrinsic oocyte abnormality, rather than due to a sporadic event of abnormal response to ovarian stimulation or poor culture conditions in a certain cycle or laboratory.

In patients undergoing IVF, it is common for a small percentage of oocytes to arrest at the GV or MI stage. In one study of human IVF, 8.6% of women treated with gonadotrophins yielded one or more oocytes that failed to resume meiosis after stimulation with HCG and subsequent 20 h in culture (Bar-Ami et al., 1994). When the percentage of incompetent oocytes was ≥25%, most of the IVF outcomes were markedly reduced. These women manifested lower than expected increases in estradiol (E2) after HCG administration. Overall, this study suggested a correlation between failure of meiotic competence, the developmental features of other follicles in terms of E2 secretion, fertilization and implantation potential of resultant embryos.

The incidence of complete oocyte maturation arrest as a cause of infertility is difficult to estimate. In view of the complex mechanisms associated with oocyte maturation, it is surprising that only very few cases have been reported until now. The series of patients in the present report was meticulously observed and collected over 5 years of intensive work at a busy centre. Thus, rather than stating that complete oocyte maturation arrest is a rare event, a valid conclusion on the prevalence of this phenomenon cannot be made.

Several sporadic cases of complete oocyte maturation arrest have been described in the literature. Rudak described one case of failure of oocyte maturation from the GV stage, and two cases with failure of polar body formation and cleavage (Rudak et al., 1990). Cases with maturation arrest at the GV stage have been described (Hartshorne et al., 1999; Barblett, 2000). Recently, two cases with repetitive oocyte maturation arrest at the MI stage, even after extended culture, were reported (Harrison et al., 2000). Finally, Eichenlaub-Ritter described a case with recurrent failure of polar body formation and premature chromosome condensation (Eichenlaub-Ritter et al., 1995).

Although none of our patients as well as neither of the above studies reported a family history of infertility, there is some evidence in mammals for genetic factors in meiotic arrest. Oocytes of strain LT mice, and related strains such as LTXBO, exhibit a high incidence of arrest in the progression of meiosis at metaphase I (MI) and in spontaneous parthenogenetic activation (Eppig et al., 2000).

Meiotic maturation of human oocytes consists of a cell cycle punctuated by a series of start/stop control points. Specifically, primary oocytes generated prenatally remain arrested in meiotic prophase until ovulation when, as a result of gonadotrophin stimulation, the cell cycle is reinitiated, proceeds through maturation, and is halted at metaphase of meiosis II. The capacity to resume and complete meiotic maturation is probably acquired during oogenesis. This capability is known as meiotic competence acquisition (Fulka et al., 1998). Follicular somatic cells within the cumulus–oocyte complex suppress the expression of meiotic competence in oocytes in vivo, and also mediate the stimulation of meiotic maturation and its extent.

Thus, failure to resume meiosis in vivo may arise at one of the following three levels: (i) absent or incomplete LH effect; (ii) derangements in the signalling mechanism from the surrounding cumulus cells; and (iii) intrinsic oocyte factors.

In-vivo, abnormal or insufficient LH effect may interfere with progression of meiosis by one of the following mechan-
Oocyte maturation arrest

isoms: incorrect timing of the HCG injection, lack of LH activity (HCG batch problem, i.e. inactive isofom), disturbed hormone delivery or dysfunctional LH receptors. All of the above theories seem unlikely in view of the repetitive nature of this phenomenon, as well as normal ovarian steroidogenesis (E2 and progesterone output) before and after HCG administration (data not shown) and the ease of oocyte aspiration, all of which represent normal LH effects.

In-vivo derangements in the signalling mechanism from the surrounding cumulus cells may account for meiotic maturation arrest noted in some of our cases. It has been recently demonstrated in the mouse that follicles lacking the gap junction protein connexin43 failed to develop a multilaminar granulosa cell layer, and exhibited retarded oocyte growth (Ackert et al., 2001). In all cycles in our series, apparently mature cumulus cells with normal appearance surrounded the oocytes. In only one case with oocyte maturation arrest at the GV stage (case number 1), the corona cells appeared immature. We have unsuccessfully attempted to induce germinal vesicle breakdown (GVBD) in these oocytes in vitro using extended culture and incubation with sperm, a technique recently described by our team (Farhi et al., 1997).

Like us, others have also failed to induce completion of meiosis in cases with oocyte maturation arrest at the GV stage. In the case reported by Rudak et al. extended incubation in vitro for several days failed to induce oocyte maturation beyond the GV stage (Rudak et al., 1990). Hartshorne et al. have noted variable degrees of cumulus expansion following oocyte retrieval in a case with meiotic arrest at the GV stage (Hartshorne et al., 1999). They were able to induce expansion of cumuli after exposure to FSH and HCG in vitro, suggesting that the cumulus cells were capable of responding to gonadotrophins but their response was not translated to the oocyte or that the oocyte was unable to respond to the signals from the cumulus.

In all our cases, no advance in meiotic stage was observed following removal of the corona cells, which is normally performed on the day of oocyte retrieval or 24 h later in ICSI and IVF respectively. Thus, although the possibility of abnormal signalling between the corona cells and the oocytes cannot be completely excluded, it seems more likely that the reasons for meiotic arrest at the GV stage lie within the oocyte.

In primates and in rodents, it has been demonstrated that oocytes may become competent to resume meiosis only after they have acquired a certain size and that further progress in the meiotic process up to the second metaphase is also dependent on oocyte growth and rimming (Fulka et al., 1998). Growing oocytes are yet unable to respond to maturation signals both in vivo and in vitro and remain arrested in the diplotene stage or, if more advanced, progress only to MI. Only fully-grown oocytes respond to gonadotrophic signals and mature to MII oocytes in pre-ovulatory follicles or in culture.

In the case reported by Hartshorne et al. the nuclei of GV-arrested oocytes were examined by fluorescence microscopy following chromatin staining (Hartshorne et al., 1999). The appearance of the chromatin in all oocytes was consistent with initiation of meiotic progression from prophase I towards MI, characteristic of arrest at entry to M-phase of the cell cycle. This is the normal stage of meiotic arrest in small oocytes. The diameter of the oocytes, however, was found to be within the normal range required for resumption and completion of maturation and fertilization in vitro (Durinzi et al., 1995). Similarly, in all patients and in all cycles described here, the oocytes appeared to be normal in size as expected for mature oocytes, although specific measurements were not made. Therefore, it seems unlikely that disturbed oocyte growth was the cause of meiotic incompetence.

Recently, it has been postulated that the acquisition of full developmental competence may extend beyond the growth phase and involves instead prematuration changes in the fully-grown oocyte (Fulka et al., 1998). Thus, given that an ill LH effect, disturbed cumulus–oocyte interaction and abnormal oocyte growth are unlikely, the possibility of an intrinsic oocyte defect remains the most viable option as the cause of oocyte maturation arrest.

The potential of an oocyte to progress through meiosis is acquired only after certain structural and biochemical changes that have occurred in the various compartments of the nucleus and the cytoplasm (Fulka et al., 1998). Some of the structural changes that occur in the cytoplasm include modifications to the Golgi complex, accumulation of ribosomes and an increase in the number and a change in the morphology of mitochondria (Knobil and Neill, 1988). At this stage, one can also notice prominent nuclear changes expressed by a transition from a diffuse, reticulated configuration to a dense uniform body. These modifications reflect a period of intensive RNA synthesis, which gradually ceases as the oocytes grow.

At the biochemical level, the maturation-promoting factor (MPF) is an M-phase specific kinase resident in oocytes that must be activated for meiotic resumption. MPF is a conserved dimeric complex consisting of a catalytic subunit, the p34cdc2 kinase, and a regulatory subunit, known as cyclin (Nurse, 1990). Cyclin is degraded abruptly following each cell cycle and reaccumulates during the next cycle, hence the terminology cyclin. When cyclin degradation is inhibited, MPF remains active and oocytes may reach MII.

These factors are expressed during oogenesis and the formation of functional MPF appears to be one step in the regulation of meiotic arrest and resumption. Once MPF is activated, this kinase phosphorylates an array of protein targets that lead to significant remodelling of the nuclear and cytoplasmic compartments during oocyte maturation. The physiological control mechanisms intrinsic to the oocyte and involving MPF expression and activation must be modulated precisely during follicle growth and ovulation to coordinate these events with meiotic maturation.

In four of our cases meiotic arrest at MI was observed. Progression from MI to anaphase requires proteolysis of cyclin molecules (Kobayashi et al., 1991). Further advancement in meiosis to MII again requires high levels of active MPF production. A c-mos protooncogene product, Mos, acts as a stabilizer on MPF and arrests cells cell progression at MII. Resumption of meiosis after fertilization is associated with loss of kinase activity that could be regulated by intracellular Ca2+ oscillation and loss of MPF activity. This process involves degradation and inactivation of c-mos immediately after sperm
penetration that causes an increase in intracellular Ca$^{2+}$. MPF activity declines and the metaphase arrested chromosomes divide resulting in extrusion of the second polar body, leaving behind a haploid set of chromosomes (Sagata, 1996). In addition, studies in mice have shown that in the absence of a normal meiotic spindle homologue chromosome separation does not occur and the oocytes are arrested in MI (Soewarto et al., 1995). Although some degree of meiotic maturation from MI can be overcome in mouse oocytes by exposure to calcium ionophores (Eppig et al., 1994), this goal cannot be achieved without a functional spindle (Soewarto et al., 1995). It can thus be speculated that either deranged regulation of MPF activity or abnormal spindle formation are responsible for the meiotic arrest at MI.

One case of recurrent failure in polar body formation and premature chromosome condensation has been previously described (Eichenlaub-Ritter et al., 1995). The patient, who had undergone four failed IVF attempts, showed neither a polar body or pronuclei when examined for fertilization. The inseminated oocytes were spread for karyotype analysis. One set of MII haploid chromosomes and a remarkably condensed structure, representing the sperm chromatin at a state of premature chromatin condensation, possibly due to a block in oocyte maturation, were observed. The authors postulated that a rapid maturation to MII before retrieval and prolonged arrest at this stage before fertilization, accompanied by degeneration of the first polar body, were responsible for the condition.

The whole process of oocyte maturation is regulated by cell cycle checkpoints. Cell cycle checkpoints are mechanisms that control the order and timing of cell cycle transition and ensure that key events such as DNA replication and chromosome separation are completed correctly (Elledge, 1996). It has been postulated that oocytes and embryos, as opposed to somatic cells, have a limited ability to detect abnormalities in cell cycle progression. One example of this phenomenon is the inability of GV stage mouse oocytes to detect replicating DNA. Mouse oocytes undergo GVBD even in the presence of unreplicated chromatin (Fulka et al., 1995) and after premature fertilization continue meiosis despite the presence of condensed sperm DNA (Pyrzynska et al., 1996).

Information on cell cycle checkpoint mechanisms in human oocytes is rather limited and incomplete. An updated detailed review of this topic can be found elsewhere (Fulka et al., 1995). Nevertheless, based on the current status of knowledge, our hypothesis is that molecular events associated with cell cycle checkpoints may be responsible for the variety of maturation arrest patterns observed in our series of patients. The clinical manifestation common to these derangements is unexplained infertility.

Patient number 6 represents an unusual case of severe derangements in the meiotic and fertilization processes. Her past obstetrical history reveals two molar pregnancies and five pregnancies with blighted ova, which may all represent severe abnormalities in meiosis or cytokinesis. Edwards et al. closely observed fertilization, syngamy and embryonic development in a patient with a history of four previous complete hydatidiform moles (Edwards et al., 1992). Of 14 oocytes, three embryos had developed normally or near normally, and the others displayed immediate cleavage or, similar to our case, had one or three pronuclei. The fact that normal fertilization and in-vitro embryo development were observed in our couple with the use of donor oocytes is suggestive of a primary oocyte defect.

In cases number 7 and 8, normal fertilization had never been observed, as they had either arrested in MII (all oocytes from patient number 7 and part of the oocytes in case number 8) or exhibited polynucleated fertilization (case number 8). It cannot be excluded that the arrest was caused by some incompatibility between the partners and not by a primary failure of the oocytes to mature. This hypothesis could have been tested with the use of donor sperm. However, the achievement of normal pregnancies and newborns in both cases by the use of donor oocytes suggests that the underlying abnormality lies within the oocyte.

Like others, we cannot suggest a therapeutic approach that would help overcome the blocks in oocyte maturation and sustain successful IVF in our patients. Extending the HCG to retrieval interval, increasing the HCG dose, and extended in-vitro culture all failed in our hands to overcome the meiotic arrest. Additional therapeutic approaches could include in-vitro maturation (IVM) of immature oocytes retrieved in either stimulated or non-stimulated cycles, extended culture in media enriched with yet undetermined factors necessary for oocyte maturation, and intracytoplasmic injection of donor cytoplasm or maturation promoting factors. All of these interventions should be currently regarded as speculative, and the breakthrough in management of these difficult cases has to await better understanding and knowledge of the processes governing oocyte maturation.

Currently, oocyte donation seems to be the most viable option. In five of our patients (cases number 2, 5, 6, 7 and 8), oocyte donation was employed and resulted in live births of healthy babies in three. For many patients, however, emotional and religious considerations make this option unacceptable. More information on the physiology and pathophysiology of oocyte maturation is needed before the exact nature of the defects interfering with meiotic competence can be determined and effective therapy can be suggested.

References


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