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CASE REPORT *Embryology*

Cellular and genetic analysis of oocytes and embryos in a human case of spontaneous oocyte activation

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ABSTRACT: Unusual and consistent defects in infertility patients merit attention as these may indicate an underlying genetic abnormality, in turn necessitating tailored management strategies. We describe a case of repeated early pregnancy loss from *in vivo* conceptions, followed by cancelled embryo transfers after one IVF and one ICSI/PGD cycle. Following the unexpected presence of cleaved embryos at the fertilization check in the first IVF attempt, oocytes and embryos were subsequently analyzed in an ICSI/PGD case. Part of the oocyte cohort was fixed at retrieval for a cellular evaluation of microtubules, microfilaments and chromatin. The remaining oocytes were injected with sperm, and resultant embryos were biopsied for genetic analysis by fluorescence *in situ* hybridization (FISH), single-nucleotide polymorphism (SNP) microarray for 23 chromosome pairs, as well as with PCR for sex chromosomes. The presence of interphase microtubule networks and pronuclear structures indicated that oocytes were spontaneously activated by the time of retrieval. FISH revealed aneuploidy in all seven blastomeres analyzed, with all but two lacking Y chromosomes. Microarray SNP analysis showed an exclusively maternal origin of all blastomeres analyzed, which was further confirmed by PCR. From our multi-faceted analyses, we conclude that spontaneous activation, or parthenogenesis, was probably the pathology underlying our patient's recurrent inability to maintain a normal pregnancy. Such analyses may prove beneficial not only in diagnosing case-specific aberrations for other patients with similar or related failures, but also for furthering our general understanding of oocyte activation.

Key words: spontaneous activation / parthenogenesis / recurrent pregnancy loss / SNP microarray / microtubules

Introduction

Ovarian stimulation for IVF aims to result in the retrieval of mature metaphase II (MII) oocytes characterized by the presence of a meiotic spindle and a single polar body. Normal MII oocytes remain arrested at MII until fertilization, at which time the oocyte resumes meiosis. Meiotic arrest is maintained by a cytostatic factor activity, involving the c-Mos/mitogen-activated protein kinase (MAPK) pathway and EMI2, which is the early meiotic inhibitor 2 of the anaphase-promoting complex/cyclosome (APC) (reviewed by Madgwick and Jones, 2007; Vogt *et al.*, 2008; Wu and Kornbluth, 2008). Resumption of meiosis is normally triggered at fertilization with calcium oscillations and the activation of calmodulin-dependent kinase II and APC/C (reviewed by Jones, 2005; Perry and Verlhac, 2008). Between 2 and 13 h after sperm injection, meiosis II is completed, the second polar body is extruded and the oocyte and sperm chromosomes each become confined within a pronucleus (PN), resulting in a zygote with two enlarged and abutting pronuclei (Payne *et al.*, 1997). Thereafter, the diploid embryo undergoes a series of cleavage divisions in a relatively predictable and timely fashion (Edwards *et al.*, 1981). Human oogenesis and preimplantation embryogenesis encompass a highly complex set of molecular and cellular events, any disruption of which may underlie instances of developmental abnormalities. Enhanced understanding of such abnormalities will improve our ability to manage and treat a wider number of patients who have difficulty in conceiving following assisted reproduction treatment.

Here, we report a case with multiple pregnancy losses from *in vivo* conceptions. The patient subsequently underwent an IVF cycle followed by an ICSI/PGD cycle, both of which resulted in grossly abnormal embryos with no normal embryos available for transfer. In an effort to counsel, the patient and best manage this unusual case, we conducted both microscopic and genetic analyses aimed at diagnosing the cause of the patient's infertility and recurrent losses.

Methods and Results

The analyses reported in this case report were performed with the approval of the patient and her husband. Individual case reports such as this are exempt from the typical institutional review board approval at our institution.

Patient history

The patient was a G3P0SAB3 32-year-old woman when she first sought infertility treatment at our center. She presented with a reported total of three previous consecutive miscarriages, with losses at 5, 8 and 6 weeks, respectively. Of note, the last two pregnancies demonstrated fetal cardiac activity before the losses. No uterine evacuation was done for the first loss. The patient spontaneously passed the second pregnancy with a follow-up curettage yielding no villi. On the third loss, products of conception failed to grow in culture and so the cytogenetic analysis could not be performed. The first pregnancy was conceived spontaneously after 1 year of trying, while the second and third pregnancies resulted, respectively, from a second cycle of clomiphene therapy and a third cycle with Gonal-F/IUI (following three unsuccessful clomiphene/IUI cycles). Results from a clomid challenge test demonstrated that her Day 3 and Day 10 FSH levels were normal (5.9 and 6.8 mIU/ml, respectively). A recurrent loss evaluation revealed that the patient was a prothrombin gene mutation heterozygote, but was otherwise unremarkable. The infertility work-up was likewise unremarkable.

IVF Cycle 1

During IVF Cycle 1, the patient was stimulated with a low dose luteal lupron down-regulation (LDLL) regimen using three ampules of Gonal F per day. She received 10 000 units of hCG on stimulation day 9 with an estradiol (E_2) concentration of 2155 pg/ml and five follicles $\geq$18 mm in maximal diameter. Thirty-six hours later, 19 oocytes were retrieved and inseminated with her husband's sperm, characterized by normal count and progressive motility. At the fertilization check (19 h after insemination), none of the 19 oocytes exhibited 2PN, although sperm binding to the zona pellucida was evident. With the exception of two oocytes without a polar body (staged as metaphase I, MI) and one oocyte with a polar body (staged as MII), all other oocytes were either degenerate ($n = 2$), had an abnormal number of PN ($n = 6$; five had 1PN and one had 3PN) or appeared as cleavage-stage embryos ($n = 8$; five were 2-cell embryos and three were 3-cell embryos).

Following the cancelled embryo transfer in this IVF cycle, the patient had a spontaneous chemical pregnancy, resulting in a fourth loss.

ICSI/PGD Cycle 2

Based on the complete absence of normal fertilization and the spectrum of abnormalities both in pronuclear number and in premature cleavage in IVF Cycle 1, we suspected that the oocytes had undergone parthenogenetic activation. Thus, for her second cycle, we recommended ICSI to increase the likelihood of normal fertilization. The couple agreed to allocation of all normal MII oocytes as well as half of the remaining oocytes to ICSI, followed by PGD for assessment of paternal contribution to the resulting embryos. The remaining oocytes were allocated to cytochemical analysis without any sperm exposure in order to assess the oocyte cytoarchitectural features. After stimulation with LDLL and hMG/FSH (Repronex/Gonal F: 1/2 ampules per day), 10 000 units of hCG were administered on stimulation day 10 with an E_2 of 3560 pg/ml and six follicles >18 mm in diameter. Nineteen oocytes were retrieved, and 3 h after retrieval oocytes were denuded of cumulus cells with 60 IU/ml of hyaluronidase. Upon assessment of the cumulus-free oocytes, all except two had a polar body. Of the 17 oocytes with a polar body, 3 appeared as normal MII oocytes with no PN and were injected, while 14 had a single PN. Of the 14 oocytes with a single PN, 7 were randomly selected for sperm injection, blastomeres were subsequently biopsied for single-nucleotide polymorphism (SNP) microarray or fluorescence *in situ* hybridization (FISH) analyses and the remaining seven oocytes were immediately fixed for microscopic evaluation of cytoarchitecture. To confirm the results from the initial assays, the SNP microarray was followed by PCR and the FISH analysis was followed by SNP microarray (Fig. 1).

At 14 h after ICSI, the 10 injected oocytes (the 3 MII and 7 with 1PN) were evaluated for evidence of fertilization. As shown in Fig. 2, none of the oocytes exhibited 2PN. Of the three injected MII oocytes, one had a single PN, and the other two did not exhibit any PN. Of the seven remaining

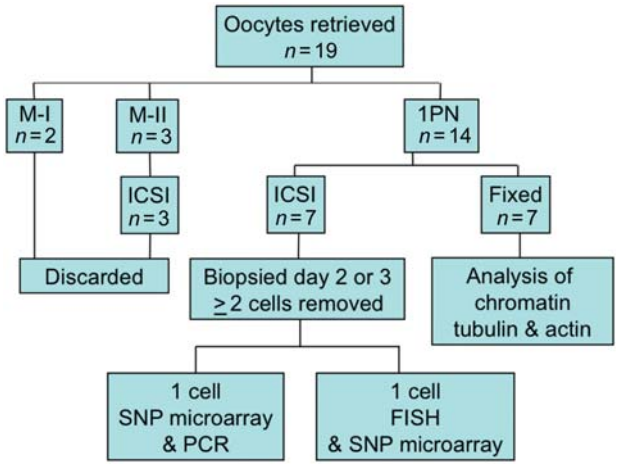

Figure 1 Allocation of retrieved oocytes in Cycle 2. The three injected MII oocytes were discarded and not biopsied due to an insufficient number of cells for two of them and the presence of a large fragment in the third one.

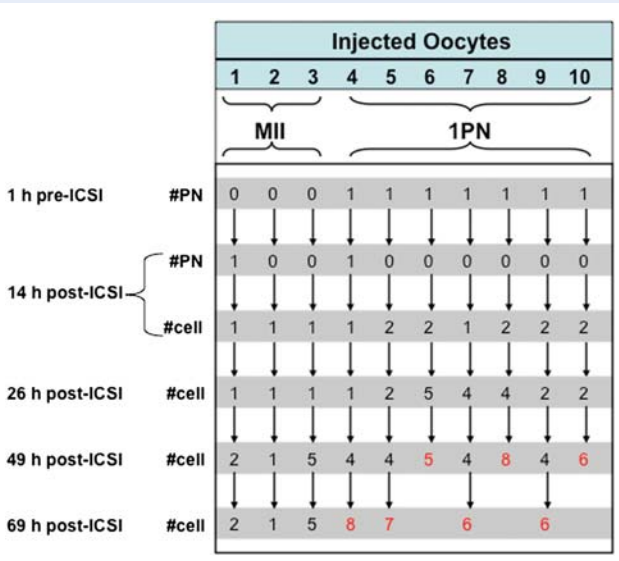

Figure 2 Developmental progression of injected oocytes in Cycle 2. Shown in red are embryos biopsied at 49 or 69 h post-ICSI.

1PN oocytes, five were at the 2-cell stage one retained a PN and the other no longer had a PN. Subsequent assessment at the early cleavage check (26 h after ICSI), and again at 49 and 69 h, revealed varying numbers of cells derived from each oocyte (Fig. 2). At 49 h, four embryos had ≥5 cells three of which were biopsied; one (Embryo #3) was not biopsied due to the presence of a large fragment. At 69 h, four additional embryos had ≥5 cells and were also biopsied.

Genetic analysis of embryos with PGD

For each of the seven biopsied embryos (three on Day 2 and four on Day 3), a minimum of two cells were removed, one of which was processed for FISH to determine the chromosomal ploidy for 10 chromosomes and the other for a 23-chromosome pair SNP microarray analysis to identify the complete genetic composition of the embryos. The DNA from the blastomere processed for SNP microarray was also used for a multiplex PCR for non-polymorphic short tandem repeat from the Y chromosome to confirm the sex of each embryo. FISH was conducted as previously described (Kearns and Pearson, 1994a). Ten DNA probes specific for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y were used during two rounds of FISH. For the 23-chromosome (22 autosomes and a sex chromosome pair) SNP microarrays, the DNA was extracted from a single cell from each embryo and from peripheral white blood cells from the wife and husband, as previously described (Kearns *et al.*, 2009). The DNA was first amplified using a modified multiple displacement amplification protocol followed by an additional round of whole genome amplification (Kearns *et al.*, 2009). Microarray analysis was then performed using the Illumina platform and the HumanHap 370 chip to obtain genotype data for ~370 000 SNPs. Bioinformatic analysis was conducted using deCODE Genetics Disease Miner Professional and Illumina's BeadStudio and KaryoStudio softwares. The sex of each embryo was confirmed by PCR on the amplified DNA product from each single embryonic cell. In short, our PCR analysis included male and female controls, a blank and DNA from a single cell from each embryo tested. Twenty microliters of a Master Mix (Promega, Madison, WI, USA), PCR primers and 0.2 μl of AmpliTaq Gold DNA polymerase (Promega) were added to each tube. The PCR was run for ~5.75 h. Upon completion, each sample was run through a 4% agarose gel and visualized.

After analysis, the cells processed for FISH were subsequently prepared for SNP microarray. In order to recover the fixed nucleus, slides were incubated in 1× phosphate-buffered saline for 20 min with gentle agitation. A modified diamond needle was then used to gently scrape the nucleus off the glass slide (Scalenghe *et al.*, 1981), which was subsequently placed into 5 μl of DNA stabilizing buffer. The nuclei underwent one round of multiple displacement DNA amplification for 16 h and one round of overnight whole genome amplification. Microarray analysis was performed as described above.

All of the seven biopsied embryos were scored as abnormal upon genetic analysis by FISH, with a variety of aneuploidies noted (Table I). For the white cells from the wife and husband, the microarray detection and genotype call rates were >90%. For the embryos, the microarray detection rate and genotype call rate were >80%. The karyotypes obtained by microarray on the 23 chromosome pairs also revealed various aneuploidies in each blastomere investigated (Table I). Although performed on two different blastomeres, the FISH and SNP microarray data largely confirmed each other with respect to the aneuploidies of the 10 autosomes probed by both methodologies. When excluding Embryo #7 (with a binucleated blastomere used for FISH), the only difference for the autosomes was in Embryo #10 for which normal diploidies for chromosomes 16 and 18 were detected by microarray in contrast to trisomies by FISH. Interestingly, again when excluding binucleated Embryo #7, the results for the sex chromosomes differed between FISH and microarray in three out of the seven embryos (#4, #5 and #10; Table I); also of note were the uncertain interpretations for the Y chromosome FISH testing for both Embryos #4 and #10. A P-P-C Heritability Frequency comparing embryonic with parental DNA genotypes showed only the presence of maternal-derived DNA within the cell of each embryo analyzed initially by FISH, as well as in the other cell processed initially for SNP microarray. For over 22 000 SNPs, there was 100% concordance for exclusively a maternal contribution in all seven blastomeres, with 0% concordance for paternal contribution (Supplementary data, Table S1). To confirm the sex of the blastomeres, we also performed PCR on the DNA from the blastomeres originally processed for SNP microarray. This further analysis confirmed the absence of Y chromosomes in all seven blastomeres analyzed (Supplementary data, Fig. S1).

Table I Chromosome ploidies of blastomeres during ICSI/PGD Cycle 2 by FISH and SNP microarray.

Embryo	FISH karyotype (evaluated 13,14,15,16, 17,19,21,22, X,Y)	SNP microarray
#4	−13, −14, −15, −16, −17, −19, −21, −22, XY	23, X, (−1 through 22)
#5	+13, +14, +15, +16, +17, +19, +21, +22, XX	68, XX, (+1 through 22)
#6	+15, X	46, X, −4, +10, +15
#7	+13, +14, +15, +16, +17, +19, +21, +22, XXXX	50, XX, +13, +14, +15, +22
#8	+15, XX	47, XX, +15
#9	+16, XX	48, XX, +4, +16
#10	+13, +14, +15, +16, +17, +19, +21, +22, XXYY	51, XX, +1, −7, +13, +14, +17, +21, +22

The data shown are the abnormal ploidies obtained in each embryo from FISH in one blastomere (for the 10 chromosomes probed) or from SNP microarray in a second blastomere (for the 24 chromosomes). For SNP microarray, the first number is the total number of chromosomes. A − sign preceding a chromosome number denotes a monosomy and a + sign a trisomy (with the exception of the cell from Embryo #7 analyzed by FISH; this was a binucleated cell with 4–6 of each chromosome counted per nucleus). Note that while Y chromosomes were assigned to the karyotypes of Embryos #4 and #10 by FISH, there were uncertainties as to their presence and the data interpretation.

Cellular analysis of oocytes with immunocytochemistry

Each of the seven non-injected oocytes that were allocated to fixation for cytoarchitecture analysis (Fig. 1) appeared to have a single PN upon cumulus cell removal and gross morphological evaluation. The oocytes were fixed 4 h after retrieval and processed for the simultaneous immunocytochemical analysis of microtubules, microfilaments and chromatin as previously described (Combelles *et al.*, 2002).

Chromatin analysis showed a single PN in 6/7 (86%) oocytes (Fig. 3A) while one oocyte contained two regions of uncondensed chromatin (Fig. 3B); either this second smaller PN was not detected upon morphological evaluation due to its small size or, alternatively, it formed between the initial examination and fixation. In those oocytes with a single PN, the PN was consistently positioned eccentrically within the oocyte (Fig. 3A, a). Staining for actin microfilaments confirmed the presence of normal-sized polar bodies in all seven oocytes. While four oocytes (57%) had more than one polar body structure, only one of these structures in each

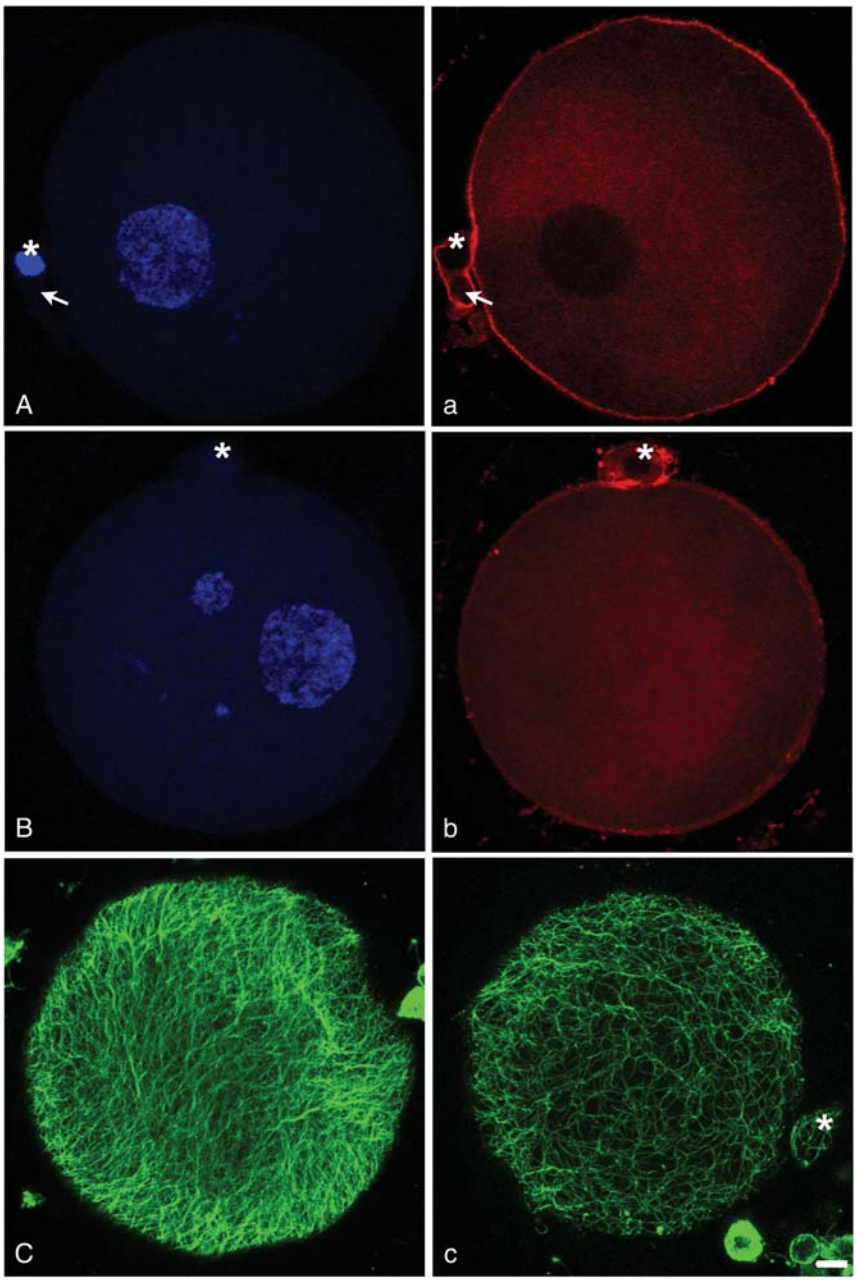

Figure 3 Chromatin and cytoskeletal organization of oocytes 4 h post-retrieval. Confocal reconstructions (10 optical slices) are shown for DNA (**A** and **B**), microfilaments (**a** and **b**) and microtubules (**C**, **c**). Most oocytes (6/7) possessed a single PN (A) with only one oocyte with two pronuclei (B). Four out of seven oocytes had a fragmented single polar body with the polar body confirmed to contain DNA and microtubules (A, a, asterisk) in contrast to the anuclear fragment (A, a, arrow). The other three oocytes exhibited a single polar body with DNA and microtubules (B, b, c, asterisk). In all oocytes, microtubules were organized as a network, most dense in the cortical regions (C, with parallel bundles) when compared with the rest of the ooplasm (c, with a mesh-like pattern). Scale bar: 10 μm.

oocyte contained chromatin; the remaining 'polar body' structures appeared as cellular fragments with microtubules and microfilaments but lacking chromatin (Fig. 3A, a). For the other three oocytes (3/7; 43%), the single polar body exhibited chromatin, microtubules and microfilaments (Fig. 3B, b). All seven (100%) fixed oocytes lacked a spindle and condensed chromosomes; instead, they displayed a very dense cortical array of microtubules with predominantly parallel bundles in the cortical region (Fig. 3C), with the remainder of the ooplasm containing a less dense mesh-like network of microtubules (Fig. 3c).

Discussion

In this contribution, we describe a patient with recurrent pregnancy losses from spontaneous pregnancies, from clomid/IUI and from

gonal F/IUI cycles; the patient then had two failed assisted reproduction treatment cycles due transfer cancellation for abnormal embryos. The collective immunocytochemical and genetic findings we report in this paper suggest an underlying oocyte-specific deficit resulting in parthenogenesis due to spontaneous activation.

During each of the two assisted reproduction treatment cycles, obvious abnormalities were apparent at the fertilization check. There was a complete absence of zygotes with 2PN following either standard IVF insemination or ICSI, consistent with complete fertilization failure. However, cell division had already occurred by 19 h post-insemination, with 8 of the 15, and 5 out of 10 mature non-degenerating unfertilized oocytes already exhibiting two or three cells in Cycles 1 and 2, respectively. While the normal timing of the first cleavage is highly variable in human embryos, it typically ranges between 22 and 34 h after insemination (Balakier *et al.*, 1993). The high incidence of fast cleavers and the association between accelerated cleavage and chromosome abnormalities (Munne *et al.*, 1995; Magli *et al.*, 2007) could be consistent with aneuploidy as the underlying reason for the patient's prior multiple pregnancy losses. However, our genetic analysis of biopsied cells, together with the cellular evaluation of oocytes we report here, support the conclusion that her failure to achieve a viable pregnancy was more probably due to spontaneous parthenogenetic activation, wherein some development proceeds without the paternal contribution (Mittwoch, 1978). Given the strict requirement for both maternally and paternally imprinted genes for normal development to proceed, mammalian parthenotes cannot develop to term (reviewed by Rougier and Werb, 2001).

Following controlled ovarian stimulation and oocyte retrieval, meiotically mature, MII oocytes normally contain a meiotic spindle with condensed chromosomes with no microtubules present in the cytoplasm. However, the immunocytochemical analysis of our patient's oocytes instead revealed that, at the time of retrieval, all oocytes exhibited cytoarchitectural characteristics similar to those present in a fertilized oocyte, i.e. clear signs of interphase, with pronuclear chromatin and cytoplasmic microtubules. These findings lead us to conclude that spontaneous activation probably explained the present observations. This conclusion is supported by the following additional lines of evidence: (i) the observed microtubule arrays (dense in the cortex and mesh-like in the rest of ooplasm) resemble those forming after completion of meiosis II at fertilization in humans (Van Blerkom *et al.*, 1995); and (ii) the distribution and density of microtubule arrays observed were similar to those present in parthenotes after either artificial activation in oocytes from numerous species (human: Winston *et al.*, 1991; Simerly *et al.*, 1995; non-human primate: Wu *et al.*, 1996; pig: Kim *et al.*, 1997; bovine: Morito *et al.*, 2005) or following spontaneous activation (hamster: Hewitson *et al.*, 1997). Moreover, we conclude that this spontaneous activation was programmed, rather than resulting from oocyte aging (Eichenlaub-Ritter *et al.*, 1988; Pickering *et al.*, 1988), as the oocytes were fixed within 4 h of retrieval.

Spontaneous activation may result from defects in the cell cycle regulators that control meiotic progression in mammalian oocytes, with the c-Mos/MAPK pathway and EMI2 playing central roles in normal MII arrest (reviewed by Madgwick and Jones, 2007; Wu and Kornbluth, 2008). Of relevance is the cell cycle phenotype of *c-mos* knockout mice whose oocytes fail to arrest at MII and undergo parthenogenetic activation (Colledge *et al.*, 1994; Hashimoto *et al.*, 1994; Hirao and Eppig, 1997). Animal studies further highlight the specialized and conserved roles of MAPK and EMI2 in establishing and maintaining MII arrest, respectively (reviewed by Perry and Verlhac, 2008; Suzuki *et al.*, 2010). While our analysis cannot discern between failure to maintain MII arrest versus spontaneous exit from or stimulation of MII exit, we can postulate that the patient's oocytes possess a molecular defect(s) that fails to prevent the meiotic to mitotic conversion after meiosis I. It remains to be determined whether this is similar to that in the LT/Sv mouse strain which also has oocytes that exhibit an MI arrest and/or parthenogenetically activate (Stevens and Varnum, 1974; Eppig *et al.*, 1996). As perhaps is the case in our patient's oocytes, the LT/Sv defect is complex and polygenic in nature (Maciejewska *et al.*, 2009).

Another indication for a cell-cycle defect is the observed pace of embryonic cell division, which was accelerated significantly in both the IVF and ICSI cycle. Oocyte meiosis and embryonic cleavage divisions are both under maternal control, and failure to maintain arrest at MII in the oocyte and the accelerated cleavage in the embryo may thus arise from a common, maternally stored defect that regulates cell division. Interestingly, LT/Sv mice share such defects in regulation of both meiosis and early mitotic cleavage (Maciejewska *et al.*, 2009).

Interestingly, deletion of the *c-mos* gene in mice results in increased polar body size and delayed degradation together with further division of the first polar body (Choi *et al.*, 1996). The c-Mos/MAPK pathway thus appears to play a role in polar body formation and degradation, at least in the mouse. However, in the present case report, the evaluation of microfilaments revealed normal-sized polar bodies and the fragmentation of polar bodies in only about half of the oocytes. The exact significance and underlying causes of polar body fragmentation are unknown, although it is possible that the observed polar body fragmentation is related to the cell cycle defect in the oocyte. Based on polar body numbers and characteristics, it is presumed that second polar body extrusion did not occur, although only a ploidy analysis of retrieved oocytes could prove so with certainty.

It is important to note that our analyses could not discern the timing of oocyte spontaneous activation in relation to the hormonal stimulation used for treatment. Although we can only speculate, LH signaling could prematurely initiate calcium transients that together with a presumed cell cycle defect may perhaps result in spontaneous activation. An evaluation of immature oocytes retrieved prior to any LH surge may augment a basic understanding of the oocyte defect. In addition, if pre-LH oocytes were not found to be activated, *in vitro* maturation could be considered as a treatment option. However, further testing would be required first to ensure the safety of using such *in vitro* matured oocytes with potential abnormalities in embryonic divisions.

In addition to the knowledge gained from the immunocytochemical analysis, the genetic evaluation of our patient's blastomeres also proved informative. Indeed, the SNP microarray and PCR data helped determine the genetic contribution by each parent to the biopsied embryos, with all analyzed cells determined to be of strictly maternal origin. Both FISH and SNP microarray indicated a wide range of chromosomal abnormalities, with instances of monosomies, disomies and trisomies for autosomes and/or the sex chromosomes. The defects in the patient's oocytes thus clearly included errors in chromosomal separation, as well as susceptibility to spontaneous

activation. In human single pronucleate embryos, previous studies revealed an increase in irregular chromosome segregations (Cohen et al., 1995; Staessen and Van Steirteghem, 1997; Feenan and Herbert, 2006). One study showed a range of ploidies in embryos from 1PN zygotes after IVF or ICSI, with 24% haploidy, 49% diploidy, 3% polyploidy and 24% mosaic (Staessen and Van Steirteghem, 1997). In that study, a sperm contribution was demonstrated in 70–75% of 1PN embryos, thereby differing strikingly from the embryos in our patient. Nonetheless, it is relevant to note that spontaneously activated oocytes vary in their ploidy and can arise from very different pathways (Mittwoch, 1978; Rougier and Werb, 2001). For instance, haploidy may arise from development of a normally reduced oocyte; diploidy may occur from various routes, including pre- or post-meiotic endoreduplication, suppression of first or second polar body extrusion, or complete suppression of meiosis; lastly, polyploidy may result from suppression of first and second polar body extrusion or suppression of the first cleavage division in a diploid oocyte. The range of chromosomal ploidies we observed in this patient is thus consistent with the range of possible chromosomal constitutions of parthenotes.

Together, the SNP microarray and PCR data demonstrated the absence of paternal genotypes for all of the 23 chromosome pairs tested. However, a sperm was injected in each of the oocytes since the patient was seeking treatment and we were trying to obtain embryos for transfer. It is thus interesting that sperm chromatin must not have been able to incorporate successfully with the female PN, and with subsequent possible degradation of the sperm chromatin there was lack of any evidence for paternal genome in every blastomere DNA analyzed. This was evidenced in our study by both SNP microarray and PCR. There is no prior knowledge of the ability of parthenogenetically activated oocytes to be fertilized, and this case would also indicate an inability to do so. This may not be surprising given the abnormal cell cycle state of the oocyte (namely 1PN) at the time of sperm injection.

Of interest, Y chromosomes were detected in one blastomere from each of the two embryos (Embryos #4 and 10) by FISH. While chromosomal mosaicism was suspected initially (Munne et al., 1994), no paternal genome was found in a subsequent 23-chromosome pair SNP microarray analysis of these two cells. Such discordance between the FISH and microarray data could be explained by signal cross-hybridization between the X and Y chromosomes; this is a previously reported limitation of FISH using probes against the pseudoautosomal regions of sex chromosomes (Kearns and Pearson, 1994b). Furthermore, it is relevant that at the time of interpretation of the FISH data, there were noted uncertainties as to the presence of a Y chromosome in both Embryos #4 and #10 by the analyst on the score sheet. Such uncertainty lends credence to the possibility of cross-hybridization between the X and Y chromosomes. Lastly, SNP microarray and PCR data in a second cell confirmed the sole presence of a maternal genome in embryos thus presumed to have developed abnormally and parthenogenetically (Supplementary data, Table S1 and Supplementary data, Fig. S1).

There are only rare instances of spontaneous human parthenogenesis *in vivo*, all of which have been reported in the ovary and have been suggested to underlie ovarian teratocarcinomas (Krafka, 1939; Shettles, 1958; Linder et al., 1975; Surani, 1995; Makabe and Van Blerkom, 2006). Several studies have reported the incidence of single pronucleate oocytes, deemed parthenogenetic, among inseminated oocytes (Plachot et al., 1989; Staessen et al., 1993; Van Blerkom et al., 1994; Feenan and Herbert, 2006). *In vitro*, the spontaneous activation of human oocytes has been documented after *in vitro* maturation (Combelles et al., 2002; Combelles et al., 2005); however, none of these spontaneously activated oocytes were cultured for assessment of their developmental potential. Parthenogenetic development has also been induced artificially in human oocytes; parthenotes could constitute surrogates to fertilized embryos, and thus they can serve as study material or a source of stem cells (Paffoni et al., 2008). Of relevance, stem cell lines derived from human parthenogenetic embryos exhibit abnormalities in centriole numbers and the expression of cell cycle checkpoints (Brevini et al., 2009). With optimized protocols, artificially induced parthenotes can develop to the blastocyst stage in humans (Rogers et al., 2004; Paffoni et al., 2007). Although parthenogenetic development can proceed post-implantation in the mouse (Kaufman et al., 1977) and implantation of parthenotes can occur in primates (Marshall et al., 1998), there is only a single report of a viable parthenogenetic–normal chimera in humans (Strain et al., 1995). Regardless, non-manipulated parthenotes cannot develop to term in mammals and, as previously proposed, some early human pregnancy losses may be attributable to undetected oocyte activation with a likely poor development of extra-embryonic tissues (Edwards, 1986; Surani, 1995). Our patient's infertility and recurrent pregnancy losses could thus be explained by likely universal parthenogenesis of her pre-ovulatory oocytes. To our knowledge, such a condition has never been documented previously, although previous reports suggest the predisposition of a subset of oocytes in certain patients to digyny (Pergament et al., 2000; Check et al., 2009).

With respect to patient counseling, much can be gained from a diagnostic cycle such as the ICSI/PGD Cycle 2 reported here, in which some of the oocyte cohort are injected and the rest are fixed shortly after retrieval. For either set of oocytes, a follow-up diagnostic approach (employing a cellular and genetic analysis of both non-injected and injected oocytes) proved most informative. We propose that the inability of this patient's oocytes to remain in normal meiotic arrest prior to fertilization was probably due to a genetic defect resulting in spontaneous activation. A multi-faceted approach such as described in this report may help us to diagnose and hopefully someday manage as yet unrecognized cases of parthenogenesis in patients with unexplained infertility and recurrent pregnancy losses. The patient in this case report was counseled to proceed to egg donation, but the couple eventually decided not to pursue such treatments.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

C.M.H.C. performed the cellular analysis of oocytes and took the lead in the data analyses and drafting the manuscript. W.G.K. performed the genetic analyses of blastomeres. J.H.F. oversaw the clinical management and monitoring of the patient couple throughout the course of their IVF treatment, and participated in editing the manuscript. C.R. was responsible for the study design, overseeing the completion of the study, editing and finalizing the manuscript.

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