Undocumented embryos: do not trash them, FISH them

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Pronuclear formation is routinely assessed 16-20 h after oocyte insemination in in-vitro fertilization (IVF). Occasionally, the pronuclei disappear before this time, rendering them as 'undocumented'. Since the number of pronuclei detected is used to distinguish normal from abnormal embryos in the context of ploidy, the diploidy of undocumented embryos is questionable, and therefore they are routinely discarded. The introduction of fluorescent in-situ hybridization (FISH) technology allows the assessment of ploidy status in undocumented embryos that continue to cleave to form blastomeres. In this study, we used FISH to analyse the chromosomal status of 23 undocumented embryos obtained from 10 patients. Biopsied blastomeres were fixed and probed for five chromosomes (X, Y, 13, 18, 21). Diploidy was confirmed in 13 (57%) embryos while the remaining 10 embryos displayed various chromosomal anomalies. Six of the diploid embryos were transferred subsequently to the patients. One ongoing pregnancy was achieved following transfer of an undocumented, analysed embryo, which was already cleaved when assessed 20 h after insemination. We suggest that accelerated dismantling of the pronuclear membrane and subsequent cleavage do not necessarily indicate abnormal chromosomal content and may result in normal pregnancy. In a patient with a small number of embryos, FISH may be used to ascertain diploidy of undocumented embryos, thereby increasing the number of available embryos for transfer.

Key words: embryo FISH/ ploidy/preimplantation diagnosis/ pronuclei/undocumented embryo

Introduction

The morphological correlate of normal fertilization of the human oocyte is the appearance of two pronuclei which can be detected as early as 5 h post-insemination (Trounson and Osborn, 1993). The pronuclear membranes break down 20-22 h post-insemination, resulting in their disappearance, and allowing subsequent cleavage. The time frame of pronuclei appearance and disappearance is of utmost importance in an in-vitro fertilization (IVF) procedure, since their number is taken to reflect the ploidy of the zygote: two pronuclei suggest diploidy, while three or more pronuclei indicate polyploidy. Such polyploid embryos are discarded in an effort to minimize the risk of abnormal pregnancy. Unipronuclear zygotes have been the subject of intense investigation, in which fluorescent in-situ hybridization (FISH) technology has been used (Staessen et al., 1993; Palermo et al., 1995; Sultan et al., 1995).

Routine IVF protocols dictate pronuclei assessment 12-20 h after insemination (Trounson and Osborn, 1993); however, occasionally the pronuclei disappear before they can be assessed. Since the chromosomal composition of these embryos is questionable, most IVF programmes will define them as 'undocumented embryos' and choose not to transfer them.

The exact frequency of early pronuclei disappearance is not known, but can be estimated based on data regarding timing of first cleavage. Cummins et al. (1986) have shown that first cleavage division occurs 33.6 h after insemination. Osborn (1991) presented data on accelerated first cleavage. Specifically, 1.1, 3.5 and 16% of zygotes undergo first cleavage division at 20-22, 22-24 and 24-26 h after insemination respectively. In a series of 1364 inseminated oocytes, Trounson and Osborn (1993) have identified accelerated first cleavage in eight embryos (0.6%). We have evaluated 2000 inseminated oocytes in our programme. In 20 fertilized oocytes (1%) the pronuclei disappeared before their first assessment, 16-20 h post-insemination (unpublished data).

A single undocumented embryo in a batch of multiple, well-documented embryos constitutes a minor challenge, since discarding this embryo will hardly influence the chances of achieving pregnancy in a given treatment cycle. In contrast, a situation in which a patient has only a small number of embryos, of which one (or more) is undocumented, presents a clinical dilemma. The development of FISH technology, and its implementation in blastomere pre-implantation diagnosis, offers a potential solution to these situations. FISH has been used for preimplantation diagnosis and in the chromosomal assessment of abnormally developing IVF embryos (Griffin et al., 1994; Munne et al., 1994a,b, 1995; Harper et al., 1994, 1995a,b). The aim of the present communication is to report our experience with FISH analysis of undocumented embryos. Our data demonstrate that undocumented embryos, or in the broader sense, embryos displaying accelerated cleavage, are not necessarily abnormal.

Materials and methods

 Patients and embryos

Ten IVF patients were enrolled in this study. Ovarian stimulation with daily injections of human menopausal gonadotrophin was
Undocumented embryos are not necessarily abnormal

Table I. Summary of IVF treatment cycles

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Age (years)</th>
<th>Oocytes retrieved</th>
<th>IVF/ICSI*</th>
<th>Assessment time $^b$ (h)</th>
<th>2 PN zygotes</th>
<th>Undocumented embryos</th>
<th>No. of cells$^c$</th>
<th>Embryo grade</th>
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</table>

$^a$The number of metaphase II oocytes as determined in stripped oocytes prepared for ICSI is given in parentheses
$^b$Hours post-insemination.
$^c$Only two embryos could be analysed.
$^d$At the time FISH was performed.

initiated after pituitary down regulation in six patients [mid-luteal phase decapcep6$^a$ CR 3 75 mg (o-Trp-luteinizing hormone-releasing hormone (LHRR), Ferring, Malmo, Sweden), or on cycle day 3 in four patients. Patients were monitored by daily serum oestradiol and progesterone measurements, and serial transvaginal sonography

Ovulation was triggered by human chorionic gonadotrophin (HCG) (10 000 IU, Chongon, Teva, Petach Tikva, Israel) when the leading follicle was >16 mm in diameter. Oocyte retrieval was performed 36 h later under vaginal ultrasound guidance. All 10 patients had undocumented embryo(s) in at least one IVF treatment cycle. An undocumented embryo was defined as an embryo in which no pronuclei could be identified 16–20 h after insemination. Six patients had regular IVF, while intracytoplasmic sperm injection (ICSI) was performed in five treatment cycles due to severe male infertility.

Results
The clinical data describing the IVF cycles that resulted in undocumented embryos are given in Table I. In three patients (nos 1, 5 and 7) the number of undocumented embryos was higher than the number of two pronuclei embryos

Table I. Summary of IVF treatment cycles

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<thead>
<tr>
<th>Patient no</th>
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FISH analysis of biopsied undocumented embryos with sub-
documented by amniocentesis. This specific embryo was found
Patient
Table II. Summary of blastomere biopsies from undocumented embryos

<table>
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</tr>
</tbody>
</table>

*aDetection of three or four signals of 21/13 is interpreted as normal (Wester and Gray, 1992).
*bAbsence of subsequent cleavage precluded transfer of this embryo.
*cThe patients chose not to use these embryos after FISH analysis; therefore, they were left for observation, and subsequently frozen if they progressed to the blastocyst stage.
*dFurther analysis with 21/13 was not attempted in embryos with abnormal signals for X, Y, or 18 chromosomes.
*eThe signal for 21/13 was not detected because of a technical problem.
*fThis embryo has an ongoing pregnancy.
*gCleaved at 22-25 h post-insemination.
*hCleaved at 18-20 h post-insemination.

one ongoing pregnancy. Of special interest was patient no. 7, who had all her four embryos analysed. FISH revealed 4PN mosaicism in her only embryo that was 'documented'. In contrast, two of her three undocumented embryos proved to be normal and were transferred (in April, 1996), resulting in an ongoing pregnancy. A normal female fetus (46 XX) was documented by amniocentesis. This specific embryo was found to have cleaved when first assessed 20 h after insemination.

Illustrations of normal and abnormal blastomeres are given in Figure 1.

Discussion
To the best of our knowledge, this is the first report describing FISH analysis of biopsied undocumented embryos with subsequent transfer of normal embryos. Our preliminary experi-

dence indicates that early dismantling of the pronuclei membranes and cleavage do not necessarily reflect abnormal embryo development, and probably should be regarded as an intriguing biological variation. Our data also document the possibility of achieving normal pregnancy from accelerated-cleaved embryos. Increasingly, as the mean age of our IVF patients increases, we face patients with only a few oocytes and embryos. In these cases, all efforts should be made to maximize the number of transferred embryos, including chromosomal analysis of undocumented embryos.

The literature on this entity is quite limited, probably due to the lack of tools to analyse these embryos. Harper et al. (1994) have used FISH to document fertilization in embryos that underwent accelerated cleavage. While fertilization was ascertained in all five embryos assessed, a wide range of X chromosome signals were observed. The authors speculate that early oocyte activation may stem from penetration by multiple spermatozoa. Since 13 out of 23 embryos (57%) in our series were found to be diploid, our data document the possibility that accelerated disappearance of the pronuclear membrane following either regular IVF or ICSI, does not necessarily reflect aneuploidy. Furthermore, the mean age of the study patients (32.8) is comparable to the mean age of our general patient population, suggesting that age is not a significant contributory factor in the context of the occurrence of undocumented embryos.

Of interest is the patient who had repeatedly produced embryos that showed accelerated cleavage, during both IVF and ICSI cycles. It is tempting to speculate that this phenomenon was imprinted in her oocytes, which upon fertilization entered an accelerated sequence of development. It will be of interest to see if her embryos can produce a viable pregnancy.

Also worthy of note is the patient who had four embryos, three of which were undocumented. FISH showed that the seemingly 'normal' embryo (which upon initial assessment had two pronuclei) was, in fact, tetraploid mosaic. On the other hand, two undocumented embryos were found to be normal. The pregnancy achieved by one of these two embryos (currently ongoing) establishes the ability of undocumented embryos to implant.

While FISH is an efficient and accurate method (Delhanty et al., 1993), based on a previous report, covering 555 blastomeres (Munne et al., 1995), it should be emphasized that successful FISH analysis can be expected in ~90% of the blastomeres assessed. In addition, Munne et al. (1995) raise the possibility of normal diploid embryos in combination with one to three polyploid cells. Hence, in using this technique for assessment of undocumented embryos, patients should be counselled with regard to these potential limitations. The above notwithstanding, the extensive literature on the use of FISH in preimplantation diagnosis (Munne et al., 1994b; Harper et al., 1995a), has driven the technology to a stage of selected clinical use.

Our preliminary experience is still premature to draw conclusions concerning the percentage of undocumented embryos which are genetically normal, and can result in normal pregnancy. However, our data strongly suggest that accelerated dismantling of the pronuclear membrane may constitute a
Undocumented embryos are not necessarily abnormal

Figure 1. (A) A normal blastomere (X,Y,18,18) before applying the 21/13 probe. The blue and red signals denote the Y and X chromosomes respectively. The two green signals denote the pair of chromosomes 18. (B) A normal blastomere (X,X,18,18,21/13X4) after applying the 21/13 probe. The two green signals representing the two chromosomes 18 are seen, together with six red signals representing the two X and four 21/13 chromosomes. (C) An abnormal blastomere (X,X,18,18,18) before applying the 21/13 probe. The two red signals represent the X chromosomes, while three green signals reflect the three chromosomes 18.

biological variation that, although rare, does not necessarily reflect abnormal chromosomal composition

References


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