VIEWPOINT

Human oocyte chromosome analyses need a standardized presentation of the results

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Introduction

Studies of DNA polymorphisms in human trisomic abortions and liveborn have revealed a chromosome-specific variation in the importance of meiosis I versus meiosis II errors. As a general rule, maternal meiosis I errors predominate among almost all trisomies (Hassold and Hunt 2001). It is evident that a direct analysis of female gametes would be useful in order to confirm these tendencies and elucidate causative mechanisms. This possibility actually exists because programmes of assisted reproduction provide oocytes that failed to become fertilized *in vitro*. Moreover, a few untreated gametes have been donated for research. During the past 20 years, more than 10,000 oocytes have been analysed cytogenetically by the conventional technique, which consists of cell fixation followed by homogeneous staining or, more rarely, by attempts to obtain banding of the chromosomes (Rosenbusch 2006a). Additional information has been obtained from molecular cytogenetic studies (Pellestor *et al*. 2005) that primarily employed fluorescence *in situ* hybridization (FISH).

The available oocytes are normally arrested at metaphase II (MII). Though their analysis will only allow conclusions on errors arising during the first meiotic division, the corresponding studies have substantially improved our understanding of the type and frequency of chromosomal abnormalities occurring during this stage of development. Of utmost importance was the demonstration of two aneuploidy-causing mechanisms in human oocytes, i.e. nondisjunction (ND) of bivalents and premature centromere division at meiosis I (predivision, PD). Briefly, ND will lead to the loss or gain of whole chromosomes whereas PD can cause the loss or gain of single chromatids (Angell 1991, 1997).

Unfortunately, this field of research also implies shortcomings and limitations that impair the interpretation of the accumulated data. The cytogenetic analysis of human oocytes is generally difficult owing to the particular morphology of the chromosomes which are characterized by compact, highly condensed arms (Rosenbusch 2006a). The success of any investigation primarily depends on the quality of chromosome spreading and it must be assumed that the results have been influenced by artefacts introduced by the fixation technique and/or by problems in interpreting the preparations (Rosenbusch 2004; Pellestor *et al*. 2005; Rosenbusch 2006a). Moreover, the conventional technique incorporating homogeneous staining only permits karyotyping according to the Denver classification, i.e. assignment of chromosomes into seven groups (A to G), whereas FISH is a priori restricted to the evaluation of some selected chromosomes of the haploid complement. Here, hybridization errors can occur and a significant bias might have been introduced by the fact that FISH has allowed evaluation of poor-quality chromosome spreads that often result from atretic or degenerated oocytes (Pellestor *et al*. 2005).

Another problem that becomes evident when screening the relevant literature is a lack of uniformity in description of the cytogenetic findings. Karyotypes have not always been specified, the distinction between ND and PD may be unclear or missing—even after the concept of PD (Angell 1991) had been introduced—and the nomenclature for PD events has been inconsistent. Needless to say, that assessing the rate of aneuploidy, identifying the affected chromosomes and defining the participating mechanisms is difficult under these circumstances.

However, at least the latter obstacle can be overcome. During the past years and based on own experience (Rosenbusch and Schneider 2000, 2006; Rosenbusch 2004) we have developed suggestions for a standardized presentation of human oocyte chromosome analyses that may serve as a possible guideline for future investigations. In view of emerging molecular cytogenetic techniques which allow identification of a single chromosomes of the haploid oocyte

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complement (Sandalinas *et al*. 2002; Gutiérrez-Mateo *et al*. 2005; Fragouli *et al*. 2006) and will hopefully stimulate new scientific interest, such standardization appears mandatory to avoid the difficulties we have faced with the interpretation of previous studies. The present commentary summarizes the basic features of our recommendations.

Suggested inclusion criteria and description of the data

Material

Because the aim is to detect aneuploidy in haploid MII oocytes that have extruded the first polar body (PB), the available gametes must be carefully evaluated. For instance, the occurrence of giant oocytes (Balakier *et al*. 2002; Rosenbusch *et al*. 2002) and immature MI oocytes lacking the first PB has to be denoted. Both types of gametes will be described as diploid and they should be excluded from the calculation of aneuploidy. Oocytes with two polar bodies contain sets of single chromatids (Pellestor *et al*. 2002) that may allow counting or karyotyping. In these cases, it should be indicated that chromatids have been analysed and that the findings also include information on the second meiotic division.

Fixation technique

The gradual fixation – air drying (GFAD) technique (Mikamo *et al*. 1994) should be applied preferentially. In contrast to the method developed by Tarkowski (1966), it offers the highest reliability for evaluating number and structure of chromosomes (Pellestor *et al*. 2005; Rosenbusch 2006a).

Nomenclature

Oocyte chromosome complements with prematurely separated chromatids should be described with a uniform nomenclature. Relevant suggestions exist (Rosenbusch and Schneider 2000) and have already been applied elsewhere (Pellestor *et al*. 2002). Though our proposals have been brought to the attention of the International Standing Committee on Human Cytogenetic Nomenclature, unfortunately it has been ignored in the recent edition (ISCN 2005). We have discussed earlier why the descriptions recommended by the ISCN do not seem appropriate (Rosenbusch and Schneider 2000).

Results

To provide a rapid and comprehensive summary of the results, we suggested their arrangement in four tables (Rosenbusch and Schneider 2006). Of course, this is only one possibility and the items shown in our tables will always have to be adjusted to the results of upcoming studies. Fusion of some tables may also be considered whereas any demonstration of correlations between aneuploidy and female age or other parameters may necessitate the inclusion of further illustrations. The following two points, however, are of utmost importance: (a) the obtained karyotypes should be specified and (b) there should be an unequivocal classification of aneuploid complements according to the underlying mechanism, i.e. a distinction of ND, PD and mixed or complex cases.

Some preparations consist of morphologically inferior chromosomes and/or chromatids that can be counted without permitting unambiguous karyotyping. It is suggested to describe these complements, but to exclude them from calculation of the rate of aneuploidy because of remaining uncertainties in the identification of the individual elements (chromosomes and chromatids).

Our detailed investigation of oocytes remaining unfertilized after intracytoplasmic sperm injection (ICSI) has demonstrated complements consisting of whole chromosomes, haploid or diploid counts of single chromatids, and mixtures of chromosomes and chromatids. These features may be ascribed to abortive activation of the oocyte, incorporating a partial or complete progression to anaphase II (Rosenbusch and Schneider 2006). Briefly, a set of 23 single chromatids should be associated with the presence of the second PB (see above) whereas 46 chromatids reflect abortive activation that was terminated before extrusion of the second PB. We have also pointed out that oocytes with a second PB and an abnormal number of single chromatids in the haploid range pose a problem concerning the origin of aneuploidy, because it is impossible to distinguish between meiosis I and meiosis II errors. Therefore, numerically abnormal sets of single chromatids in the haploid range cannot be used for calculating meiosis-I-derived aneuploidy.

The term 'balanced PD' refers to metaphases in which at least one chromosome has separated into its chromatids. The relevance of balanced PD has been at issue for a long time (Rosenbusch and Schneider 2000; Pellestor *et al*. 2003) because chromatid separation might as well represent an artefact or a sign of degeneration. Moreover, our recent analysis of unfertilized ICSI oocytes (Rosenbusch and Schneider 2006) suggests that balanced PD may sometimes be confounded with abortive activation, particularly if several chromosomes are affected. Therefore, balanced PD should be described but the definition of aneuploidy should be restricted to ND and unbalanced PD. If karyotypes of complements with balanced PD can be established, a limitation to three 'predivided' chromosomes has been proposed (Rosenbusch 2004).

Final comments

New molecular cytogenetic techniques such as spectral karyotyping (Sandalinas *et al*. 2002), centromere-specific multiplex fluorescence *in situ* hybridization (cenM-FISH) (Gutiérrez-Mateo *et al*. 2005) and single cell comparative genomic hybridization (CGH) (Fragouli *et al*. 2006) have recently been applied to human oocytes. It is to be expected that such studies comprising an evaluation of all 23 chromosomes will be extended and refined. This seems to make

sense because there are still interesting questions concerning, for instance, the distribution of ND and PD in aneuploid oocytes (Rosenbusch *et al*. 2001) and a striking discordance between direct oocyte chromosome analyses and preimplantation genetic diagnoses of the first PB (Rosenbusch 2006b). Briefly, PB analyses based on FISH allow drawing indirect conclusions on the chromosomal constitution of the oocyte (Kuliev *et al*. 2005). They indicate an approximately tenfold higher incidence of chromosome hyperhaploidy versus hypohaploidy and a preponderance of additional versus missing chromatids. These tendencies are not supported by conventional oocyte chromosome studies (Rosenbusch 2006b), suggesting the need for more data from both sources. Also, investigating the distribution of aneuploidy among individual chromosomes or the relationship between aneuploidy and various clinical or biological parameters would require precise and careful interpretation of further material.

To ensure comparisons between individual studies and to obtain valid and meaningful compilations of the results, the above-mentioned suggestions are considered obligatory. They may contribute to an increasing transparency in this field of research and to a more accurate estimate of aneuploidy in human oocytes. Of course, this proposal is open to criticism and the author welcomes any amendments and/or information on faults that have been overlooked.

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