CLINICAL ASSISTED REPRODUCTION

Chromosome 21 Detection in Human Oocyte Fluorescence In Situ Hybridization: Possible Effect of Maternal Age

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Purpose: The purpose of this study was to evaluate, among 100 uncleaved oocytes, the incidence of numerical and structural chromosome 21 and X abnormalities and to analyze the influence of various factors, such as in vitro (IVF) indications, follicle stimulation protocols, and women's age.

Methods: We investigated 150 uncleaved oocytes from 128 patients after an IVF attempt. After cytogenetic analysis (Giemsa) 100 oocytes (66%) were selected for fluorescence in situ hybridization (FISH). Fluorescent probes for human chromosomes X and 21 were used simultaneously according to standard procedures for their hybridization and detection.

Results and Conclusions: We analyzed by the FISH protocol 100 metaphase II oocytes with 22 to 25 chromosomes. Our results demonstrate a high rate of disomy for chromosome 21 in human oocytes. Among them, eight were disomic (8%) and three were nullosomic (3%) for chromosome 21. Only one disomy of chromosome X was noted. The various indications of IVF and the different folliculogenesis stimulating protocols did not seem to influence the results but suggested a correlation between the maternal age and the aneuploidy rate of chromosome 21.

KEY WORDS: chromosome 21 abnormalities; oocytes in vitro fertilization programs; maternal fluorescence in situ hybridization.

INTRODUCTION

The incidence of chromosomal aneuploidy in human oocytes is higher than for other animal species. This phenomenon is the major etiological factor in the high rate of pregnancy wastage in humans. Since the introduction of human in vitro fertilization (IVF), several cytogenetic studies of oocytes have provided information concerning chromosome aneuploidies (1–3). These studies demonstrated the high incidence of aneuploidy in uncleaved oocytes and the importance of chromosomes abnormalities in reproductive failure and explained the high rate of pregnancy loss in IVF programs.

However, oocyte meiotic chromosomes are very difficult to label using conventional banding methods. Therefore, fluorescence in situ hybridization (FISH) is a useful technique for chromosomal identification (4,5). Chromosome painting allows the detection of chromosome aneuploidy in metaphase II oocytes and the corresponding first polar body. Specific fluorescent hybridization is analyzed in both metaphase oocytes and polar bodies and shows the nondisjunctions.

The purpose of this study was to evaluate the incidence of numerical or structural chromosome 21 and X abnormalities in oocytes stopped at the second meiotic metaphase. Chromosomes 21 and X were chosen because of the high frequency of these pathologies at birth (6–8).

In this study we analyzed the influence of several factors, such as IVF indications, follicle stimulation protocols, and women's age. Actually, the influence of advanced maternal age on fetal aneuploidy at birth is well documented but contradictory studies exist concerning the rate of aneuploidy in oocytes (reviewed in Ref. 9).

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MATERIALS AND METHODS

IVF attempts were performed on oocytes obtained from 135 patients aged between 22 and 44 years. Indications for IVF were tubal sterility (32% of the cases), male infertility (63% of the cases) and idiopathic infertility (5% of the cases). Stimulation of follicular development was induced by downregulation of the pituitary gland with a luteinizing hormone-releasing hormone agonist for a short period in 65% of the cases and for a long period in 27% of the cases followed by stimulation of the ovaries using daily injections of human menopausal gonadotropin (hMG). In a few cases (8%), hMG alone was used.

The ovarian response was monitored using estradiol blood levels and ultrasonography beginning on day 6. Ovulation was induced with 10,000 UI of human chorionic gonadotropin (hCG) when the largest follicle was over 20 mm and the mean E₂ level 300 pmol per large follicle (>18 mm). The oocytes were collected approximately 36 hr after hCG injection by celioscopy or vaginally using ultrasound guidance. Oocytes were inseminated.

Uncleaved oocytes, 40 hr after insemination, were fixed according to Tarkowski's air-drying method (10) and submitted to cytogenetic analysis. Interpretable oocytes were processed for FISH. The slides were treated with RNAase and pepsin, post-fixed with paraformaldehyde, washed, and dehydrated in ethanol. Slides and probes were denatured separately in 70% formamide/2× SSC, pH 7, at 72°C for 3 and 5 min, respectively. Fluorescent probes for human chromosomes X and 21 (Biosys Cambio) were used simultaneously, according to standard procedures for their hybridization and detection. Briefly the two probes were applied on slides. Hybridization was completed overnight at 37°C. A fluorescein-avidin antibody system that gives a yellow signal was used for detection of probes (Figs. 1 and 2). Metaphases were examined with a fluorescence microscope (Zeiss).

RESULTS

Among the 150 fixed oocytes, cytogenetic analysis showed that 50 of them were not suitable for FISH. Three oocytes were arrested in diakinesis or metaphase I (2%), 4 were diploid metaphase II (2.7%), 24 were not analyzable because of an aberrant number of chromosomes (16%), and 19 (12.6%) did not present any chromosome or showed degenerated chromosomes or

chromosomes whose in situ hybridization was not possible because of a too-poor quality.

We analyzed by the FISH protocol only metaphase II oocytes with 22 to 25 chromosomes. Cytogenetic examination showed that 100 (66.6%) oocytes could be processed for FISH according to these criteria. Among these 100 available oocytes, 12% showed aneuploidy for chromosome 21: 9 were hyperhaploid (1 trisomy and 8 disomies) and 3 were hypohaploid, exhibiting only the hybridization signal specific for chromosome X. Only one oocyte showed disomy for chromosome X (Table I). The supernumerary chromosomes 21 and X always showed two chromatids (Figs. 1–3).

The frequency of chromosome X and 21 abnormalities was evaluated according to maternal age in four groups of patients. Results revealed that aneuploidy rates for chromosome 21 increased significantly, from 8.1% in the 25- to 30-year-old women to 11.9, 17.6, and 25%, respectively, in the 31- to 35-, 36- to 40-, and >40-year-old women. For chromosome X, there was no significant increase in aneuploidy in the groups of older women (Table II).

Our data did not demonstrate any influence of the folliculogenesis stimulating protocol: two oocytes (11.1%) were aneuploid for chromosome 21 with the long stimulating protocol, and nine with the short protocol (13.8%). In every type of infertility, the following rates of aneuploidy were observed: 10.5% in tubal infertility, 11.8% in male infertility, and 18.2% in idiopathic infertility (Table III).

DISCUSSION

Accurate identification of precise chromosomes involved in nondisjunction in uncleaved oocytes has not always been possible by standard cytogenetic methods, because of the often poor quality of metaphases observed after fixation. Chromosome painting represents an efficient method to identify accurately one or several chromosomes and to determine the incidence of aneuploidy in uninseminated oocytes. However, as reported in the literature, realization of FISH experiments on these cells is subject to many technical difficulties (2,11).

The hybridization efficiency (100%) on these cells indicates that our method is reliable for the detection of chromosomes 21 and X. Our results confirm the frequent implication of chromosome 21 in nondisjunction during female meiosis. Indeed, a high rate of disomy for this chromosome was found in our sample.

Fig. 1. Example of FISH analysis on unfertilized oocytes. Haploid metaphase hybridized with chromosome 21 (double arrows) and chromosome X (one long arrow).

Chromosome painting is also useful to detect structural abnormalities on metaphases. None was found in our samples.

Previous cytogenetic studies described a significant excess of nondisjunction in the acrocentric D and G groups: the overall frequency of aneuploidies in mature oocytes was 22.8% (12–14), with 7.6% (2) for the acrocentric G group alone. The fact that 77% of trisomies 21 are of a maternal origin is also in agreement with our data. It is generally assumed that first meiotic division errors arise through nondisjunction of whole chromosomes. Angell (15) described a mechanism of trisomy formation by precocious division of univalents at the first meiotic anaphase in human oocytes forming single chromatids at the second meiotic metaphase. In our observations, the supernumerary chromosomes 21 and X always showed two chromatids.

Nevertheless, to confirm these results, detection of chromosome aneuploidy in metaphase II and the corresponding first polar body will have to be performed in a larger study (16).

Using probes specific for chromosomes X and 21, we did not demonstrate an increased rate of chromosomal disorders in two folliculogenesis-stimulating pro-

ocols. This is in agreement with a similar study by Gras *et al.* (17) in which the incidence of chromosomal aneuploidy in stimulated and unstimulated uniseminated human oocytes was tested. In a review, Plachot (9) concluded that aneuploidy rates are not influenced by the type of hormonal stimulation in humans.

Several authors found an increased incidence of abnormalities in oocytes from women with tubal or unexplained infertility compared to women whose husbands were infertile as the sole cause of the couple's infertility (2,13). As shown in Table III, the incidence of abnormalities for chromosome 21 was lower in unfertilized oocytes from the idiopathic infertility group than in those from women with tubal sterility or whose husbands were infertile, but in the different groups, our results are not significantly different. However, these data need to be confirmed in a larger number of oocytes from different clinical groups of patients.

A correlation between the aneuploidy rate for chromosome 21 and the maternal age has been reported by Angell *et al.* (18), in opposition to the conclusions of Benkhalifa *et al.* (19). Angell *et al.* (18) showed that chiasmatic anomalies were more frequent in older

Figs. 2 and 3. Two metaphases with chromosome 21 disomy (double arrows) and with chromosome X monosomy (one long arrow).

Table I. Frequency of Chromosome 21 and X Aneuploidy

	21 FISH results on 100 oocytes	X FISH results on 100 oocytes
Nullisomy	3	0
Disomy	8	1
Trisomy	1	0

Table II. Frequency of Chromosome 21 and X Aneuploidy and Maternal Age

Maternal age	Number of oocytes	Anomalies of	
		Chromosome 21	Chromosome X
25–30	37	3 (8.1%)	0
31–35	42	5 (11.9%)	1 (2.4%)
36–40	17	3 (17.6%)	0
>40	4	1 (25%)	0

Table III. Chromosomal Abnormalities and IVF Indications

Indication	Number of oocytes	Anomalies of	
		Chromosome 21	Chromosome X
Tubal	38	4 (10.5%)	1 (2.63%)
Male	51	6 (11.8%)	0
Idiopathic	11	2 (18.2%)	0
Number of oocytes	100	12	1

oocytes and correlated with the nondisjunction rate. The origin of these nondisjunctions is unclear and there are contradictory studies (9,19).

Indeed, Hawley *et al.* (20) reported that 60–70% of nondisjunctions of chromosomes X and 21 derived from the production of bivalents. Several hypotheses have established a correlation between nondisjunctions and a disturbance in spindle formation ability being related to cytoplasmic maturation (21). Animal experiments showed that maternal age increased the incidence of chromosome aneuploidies (22), but this needs confirmation in larger series of human oocytes.

In conclusion, chromosome painting is an efficient method to identify chromosomes involved in aneuploidy. Our study confirms the high incidence of chromosome 21 abnormalities occurring as a consequence of meiotic nondisjunction in human oocytes and probably related to maternal age.

Larger studies are needed to confirm this probable effect of maternal age on chromosome 21 abnormality incidence in human oocytes.

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