Chromosomal analysis of unfertilized human oocytes*

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Summary. Unfertilized human oocytes were obtained from women in an in-vitro fertilization programme. The women had a mean age of 29.4 years (range 24–35 years). Chromosomal complements could be analysed in 50 oocytes. Q-banding of the chromosomes facilitated identification of individual chromosomes: 34 oocytes (68%) had the normal haploid chromosomal complement, 14 complements were hypohaploid (28%), 1 complement was hyperhaploid (2%) and 2 had structural abnormalities (4%). (One oocyte had numerical and structural abnormalities.) The 16 abnormal oocytes were obtained from 15 different women. A conservative estimate of aneuploidy in this sample is 4%; however, the frequency of aneuploidy may be higher if there is a predisposition to chromosome loss during oogenesis. This study provides information on the largest series of karyotyped unfertilized human oocytes published to date.

Introduction

Cytogenetic abnormalities are responsible for a substantial number of children born with birth defects and mental retardation. Chromosomal heteroploidy also causes reproductive loss, expressed as spontaneous abortion or fetal death. Estimates of the incidence of cytogenetic anomalies at human conception have varied from 5% (Hook, 1981) to 50% (Boué *et al.*, 1975). It has not been possible to be more precise in these figures because, until recently, human gametes were not available for study.

In 1978, Rudak *et al.* described a method which allows analysis of human sperm chromosomal complements after penetration of golden hamster oocytes. Using this technique, we have shown that normal human men have an average frequency of 8.9% chromosomal abnormalities in their spermatozoa (Martin, 1985).

It is believed that most meiotic chromosomal abnormalities occur in human females since various studies on the parental origin of the extra chromosome in Down syndrome have demonstrated that 70–80% are maternal in origin (Mattei *et al.*, 1979; Juberg & Mowrey, 1983). Studies on spontaneous abortions suggest an even greater maternal contribution for other trisomies (Hassold *et al.*, 1984). Human oocytes are difficult to obtain for analysis, but the advent of in-vitro fertilization programmes has provided 'spare' oocytes and embryos which are not needed clinically and may therefore be used for chromosomal analysis. There have only been a few reports on the chromosomal normality of human oocytes. Unfortunately these studies have been based on small numbers and have generally comprised only chromosome counts, not karyotyped oocytes. However, these observations are very valuable since they provide our sole source of direct information

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on cytogenetic abnormalities in human oocytes. In this report, we present data on chromosomal abnormalities in 50 karyotyped unfertilized human oocytes.

Materials and Methods

Criteria for selection of women for the in-vitro fertilization programme at the University of Calgary have been described previously (Taylor *et al.*, 1985). All of the women suffered from irreparable tubal occlusion. Ovarian stimulation was carried out using 50 mg clomiphene citrate starting on Day 2 or Day 4 of the menstrual cycle and continuing for 5 days (Day 1 being the first day of the menstrual flow). One vial of human menopausal gonadotrophin (hMG), containing 75 i.u. follicle-stimulating hormone (FSH) and 7 i.u. luteinizing hormone (LH), was given from the 2nd day of clomiphene citrate treatment for 4 days, followed by 1 or 2 vials of hMG until the mean diameter of the dominant follicle was 16 mm (by ultrasound determination). Blood samples were withdrawn 3 or 4 times daily for the detection of the LH surge. In the absence of an LH surge, 5000 i.u. human chorionic gonadotrophin (hCG) were administered when the mean diameter of the dominant follicle was ≥ 18 mm. Oocytes were recovered 32–36 h after the LH surge or hCG administration. The oocytes were washed free of follicular fluid and blood, and incubated in a modified Tyrode's medium (Whittingham, 1971), containing 10% heat-inactivated maternal serum. According to the guidelines of the University of Calgary/Foothills Hospital Ethics Committee, the 3 most mature oocytes were selected for in-vitro fertilization. The selection of mature oocytes was based on the expansion and appearance of the corona and the cumulus and the 'glassy' appearance of the cumulus matrix. These methods for ovarian stimulation and oocyte culture have been described in more detail elsewhere (Mahadevan *et al.*, 1983; Mahadevan & Baker, 1984; Mahadevan *et al.*, 1985).

Morphologically normal oocytes that were not selected for fertilization were transported from the in-vitro fertilization laboratory to the Genetics Research Laboratory in a container of medium in a prewarmed Thermos Flask containing water at 37°C. The oocytes were transferred to Ham's F10 medium (Flow Laboratories, Mississauga, Ontario, Canada) with 0.2% hyaluronidase (Type I-S, Sigma) at 37°C. Removal of cumulus cells was expedited by manipulation with a micropipette. The oocytes were rinsed twice in Ham's F10 medium and then transferred to a hypotonic solution (1% sodium citrate at room temperature) for 10 min. The oocytes were fixed on glass slides using Tarkowski's technique (Tarkowski, 1966). The chromosomes were stained with Giemsa and then banded using quinacrine dihydrochloride.

Results

Chromosomal complements could be analysed in 50 oocytes from 33 women: 19 women provided one oocyte, 11 women provided 2 oocytes and 3 women provided 3 oocytes. The age of the women ranged from 24 to 35 years, with a mean of 29.4 years. The oocytes were fixed 5–54 h after retrieval, with a mean of 20.5 h.

Thirty-four oocytes (68%) had the normal haploid chromosomal complement. Of these, 32 were clearly 23,X and two provided chromosome counts of 23. Fourteen complements were hypohaploid (28%), one complement was hyperhaploid (2%) and two complements had a structural abnormality (4%). One complement had both a numerical and structural abnormality. The frequency of hyperhaploid and hypohaploid complements differed significantly from the equal frequency expected from non-disjunction ($\chi^2 = 11.3$, $P < 0.001$). The 16 abnormal complements were obtained from 15 different women and were of the following complements:

Numerical abnormalities: hypohaploid	22,X, – 9
	22,X, – 22
	22,X, – E(17 or 18)

Numerical abnormalities: hyperhaploid
 Numerical and structural abnormalities:
 Structural abnormalities:

22,X,-B
 22,X,-C
 22,
 22,
 21,X,-B,-G
 21,-A,-D
 21,-C,-D
 21,
 20,X,-8,-9,-12,-22,+mar
 20,-C(?),-16,-19
 26,
 21,+A,-2C,-E,+frag
 23,X,ct del(3)

An example of a karyotype of a human oocyte is presented in Fig. 1.

Fig. 1. A chromosome karyotype of a 23,X human oocyte with solid stained (Giemsa) chromosomes shown above Q-banded chromosomes. $\times 3800$.

Discussion

This study provides information on 50 karyotyped unfertilized human oocytes. The second meiotic metaphase chromosomes of the human oocyte are much more difficult to karyotype than regular mitotic human chromosomes or human sperm chromosomes. In the following discussion, we have made comparisons between the frequency of chromosomal abnormalities in human spermatozoa and oocytes. It is important to recognize that our estimates in human spermatozoa are more reliable because of the morphology of human sperm chromosomes and our much larger data base.

The frequency of structural chromosomal abnormalities in the human oocytes was 4% (2/50). To our knowledge these complements (23,X,ct del[3];21,+A,-2C,-E,+frag) represent the first human metaphase II oocytes reported to have structural chromosomal anomalies. This frequency in oocytes is similar to the frequency observed in human sperm chromosomal complements (3.9%, 55/1426) (Martin, 1985). The frequency of aneuploid oocytes is difficult to estimate since the proportions of hyperhaploid and hypohaploid complements are not equal. Traditionally, some hypohaploid complements are considered to be artefactual and a conservative estimate of the frequency of aneuploidy is derived by doubling the hyperhaploid frequency. In our sample, this approach would yield an estimate of aneuploidy of 4%. Again, this frequency is similar to our observations in human spermatozoa (5%, 72/1426) (Martin, 1985).

Jagiello *et al.* (1975) removed oocytes from unstimulated human ovaries and cultured them *in vitro* to the metaphase II stage. Of 411 oocytes, 6 were found to be hyperhaploid. This provides an estimate of aneuploidy of 3%. However the chromosome complements were not karyotyped and the 6 oocytes were said to have 24 'bodies' since it was not clear whether the 'bodies' were chromosomes or chromatids. Zenzes *et al.* (1985) studied 3 unfertilized metaphase II oocytes in an in-vitro fertilization programme and found all 3 to be chromosomally normal. Wramsby & Liedholm (1984) analysed 8 metaphase II oocytes obtained from 9 women undergoing laparoscopy for infertility: 2 oocytes were hypohaploid, and both were obtained from one woman. Karyotyping was not possible in these oocytes and only chromosome counts were performed. It is likely that at least one of these hypohaploid oocytes was caused by technical loss of chromosomes since the number of chromosomes was only 16.

Michelmann & Mettler (1985) have reported on 33 unfertilized human oocytes. They found 1 out of 33 (3%) unfertilized oocytes to be hyperhaploid. The results from this study provide a similar estimate of aneuploidy as the conservative estimate from our study.

Many cytogenetic studies on mammalian oocytes have demonstrated more hypohaploid than hyperhaploid complements (Rohrborn, 1972; Martin *et al.*, 1976; Hansman & Probeck, 1979). The process of meiotic non-disjunction should produce an equal number of $n + 1$ and $n - 1$ gametes. Therefore the excess of hypohaploid complements is generally ascribed to technical loss of chromosomes during oocyte fixation. However, it is possible that chromosomes are lost during oogenesis through other mechanisms, such as anaphase lag. In a study comparing 1000 human sperm pronuclear chromosomal complements and 1000 hamster oocyte pronuclear complements, we determined that the male pronuclei had an equal frequency of hyperhaploid and hypohaploid complements whereas the female pronuclei had a significant excess of hypohaploid complements (Martin, 1984). Since chromosome fixation of both male and female pronuclei occurred under the same conditions within the same fertilized oocyte, it is difficult to ascribe the excess of female hypohaploid complements to technical artefact. This does suggest that the process of oogenesis might be more susceptible to chromosome loss than spermatogenesis. It could be argued that since the species are different, the excess of hypohaploid complements is characteristic of the hamster and not female mammals in general. However, this excess of hypohaploid oocytes has been observed in many other female mammals (Rohrborn, 1972; Martin *et al.*, 1976; Hansman & Probeck, 1979), and was also observed in the human oocytes in this study. Therefore the frequency of numerical abnormalities in human oocytes may be as high as 30%. However since 7 out of 14 hypohaploid complements were missing 2 or 3 chromosomes, it is likely that some of these complements were

caused by technical loss of chromosomes. Thus the frequency of aneuploidy in human oocytes probably lies somewhere between these two estimates (4% and 30%).

As well as these studies on unfertilized human oocytes, there have been a small number of studies of human fertilized oocytes and embryos from in-vitro fertilization programmes. Angell *et al.* (1983) studied three 8-cell human embryos and found that two were aneuploid. Michelmann & Mettler (1985) studied 15 fertilized oocytes that failed to cleave and found that one maternal pronucleus had a hyperhaploid complement; of 11 polyploid embryos, none was aneuploid. Rudak *et al.* (1985) studied multipronuclear oocytes which yielded 42 pronuclei in which chromosomes could be counted and 27 pronuclei which could be karyotyped. The estimate of aneuploid gametes was 16.7% from the pronuclei with chromosome counts and 22.2% from the karyotyped pronuclei. The frequency of hyperhaploid and hypohaploid complements was approximately equal. It was possible to attribute parental origin to only one pronucleus, therefore the aneuploid frequency was a combination of maternal and paternal meiotic errors.

These preliminary studies on unfertilized and fertilized human oocytes suggest that the frequency of aneuploid oocytes lies between 4 and 30%. Most studies on unfertilized oocytes support the lower estimate. This estimate would indicate a level of aneuploidy very similar to that observed in human spermatozoa. Reports on fertilized oocytes suggest the higher estimate of aneuploidy. Our study indicates that the higher estimate would only be accurate if various processes in oogenesis result in more chromosome loss than gain. In all material obtained from in-vitro fertilization studies, there is a possibility that the frequency of chromosomal abnormalities is inflated since the mean maternal age is elevated and oocytes that are not reimplanted (and are therefore available for study) may be morphologically inferior and more likely to have chromosomal abnormalities.

The study of unfertilized human oocytes allows unambiguous assessment of the maternal contribution to aneuploidy. Data from multipronuclear oocytes would be more valuable if attempts were made to identify the maternal and paternal origin of the pronuclei. If this were possible, more data could be collected to study the intriguing possibility that chromosome loss occurs more frequently in oogenesis than spermatogenesis. Despite the technical difficulties and ethical dilemmas involved in this area of research, the information gained from these studies will be of great benefit in understanding the aetiology of chromosomal abnormalities in humans.

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