Hum Genet (1992) 88:367–375

Human Genetics

Review article

Cytogenetics of human oocytes, zygotes, and embryos after in vitro fertilization

Maria Teresa Zenzes and Robert F. Casper

Division of Reproductive Science, Department of Obstetrics and Gynecology, University of Toronto, Toronto, Ontario, Canada

Received January 24, 1991 / Revised July 18, 1991

Summary. Chromosome errors, inherited or arising de novo during gametogenesis and transmitted at fertilization to the conceptus, may be a major cause of embryonic mortality. The in vitro fertilization and embryo transfer (IVF/ET) procedure provides extra material – oocytes, zygotes, and embryos – to investigate the contribution of chromosomal abnormality to implantation failure. This paper reviews the results of cytogenetic studies on such material. Estimates from a total of 1120 oocytes from 11 studies give an overall proportion of chromosomal abnormality of 35%. Single and multiple nullisomies and disomies are found, involving nonrandom chromosome gain or loss. Hypohaploid complements are more frequent than hyperhaploid complements. The higher rate of chromosome loss of hypohaploid karyotypes was found to be largely artifactual. The estimated overall frequency of aneuploidy is 13%. In embryos the level of chromosomal abnormality is 23%–40%. Errors of fertilization are responsible for a substantial number of triploid embryos, many of which develop into mosaics. Factors extrinsic to the conceptus, such as infertility, advanced maternal age, and ovarian hyperstimulation, may increase the level of chromosomal abnormality. More refined methods for accurately recognizing and selecting chromosomally normal embryos for transfer are needed to improve the success rate of this reproductive technology.

Introduction

For many couples trying to conceive, whether naturally or assisted by reproductive technology, the failure to achieve and maintain pregnancy remains a major problem. Abnormal conceptions may be lost either subclinically (due to embryonic mortality or failure to implant) or clinically (abortions, stillbirths), or may result in livebirths with congenital defects. Demographic data suggest that embryonal death is substantial. Fecundability, defined as the probability of producing a full-term infant per menstrual cycle during which intercourse occurred, is only 25% (Short 1979). Assessments of human chorionic gonadotropin (HCG) in the weeks after ovulation have revealed a transitory pregnancy among many women (Edmonds et al. 1982; Miller et al. 1980), suggesting a high rate of embryo mortality. The recent advent of reproductive technologies now permits us to assess and record the proportion of unrecognized losses. In in vitro fertilization and embryo transfer (IVF/ET), widely used as an established procedure for the treatment of certain types of infertility (Edwards et al. 1990), a significant proportion of embryos – 70% or more – fail to implant; at most 10% of transferred embryos will give rise to a full-term infant (Braude et al. 1986).

Cytogenetic surveys of clinical losses have often demonstrated a close association of abnormal conceptions with chromosome errors. Estimates of chromosomally abnormal abortuses range between 22% to 61% (weighted mean 41.63% ± 1.7%), with more than 90% of these abnormalities being lethal in utero (reviewed in Kline et al. 1989). Because the most severe abnormalities are likely to have a very limited survival, one can expect to find a higher rate and wider range of abnormalities shortly after conception. Chromosomal abnormalities, which may either be inherited or occur de novo during meiotic maturation of the gametes and also sometimes during fertilization or cleavage, can now be determined in IVF/ET material not used for transfer. This material includes unfertilized oocytes, uncleaved and multipronuclear zygotes, and the remaining (spare) untransferred embryos. As will be shown in this review, the availability of this material has hitherto been very limited.

Problems of technique

The procedure commonly used for chromosome preparation is based on the original method of Tarkowsky (1966) and needs technical skills. Handling of oocytes

Offprint requests to: M.T. Zenzes, Division of Reproductive Science, Toronto General Hospital, Room CCRW 3-815, 101 College St., Toronto, Ontario M5G 1L7, Canada

and embryos is always done under the stereomicroscope, using a mouth-controlled micropipette. Embryos require a short treatment with pronase to partially dissolve the zona pellucida. Hypotonic sodium citrate (0.5% to 1.0%) or KCl (0.075 *M*) at room temperature or 37°C are used. Some add bovine serum albumin to prevent cell lysis. After hypotonic treatment, each oocyte or embryo is transferred to a grease-free slide, and fixative (methanol: glacial acetic acid 3:1, vol/vol) is carefully added dropwise on top of the oocyte or embryo. The slide is then gently blown to evaporate the fixative and to enhance chromosome spreading.

Problems in obtaining chromosome preparations suitable for karyotyping greatly reduce this material. Following are the most important difficulties in technique:

Oocytes

Metaphase II chromosomes of oocytes are contracted, often with separated chromatids, and thus are difficult for banding. This limitation permits diagnosis of numerical abnormalities only. By far the main problem in technique is oocyte aging. Oocytes remaining unfertilized after insemination are prepared for chromosome analysis at least 40 h (or longer) after oocyte retrieval. Thus many have aged by the time of fixation. In addition, about 20% of the population of "unfertilized oocytes" available for research are actually fertilized (but are arrested in development and therefore are aged) and about 20% of oocytes are degenerated. Aged or degenerated oocytes are easily destroyed during fixation resulting in extensive chromosome loss. Fragmentation or clumping of chromosomes are commonly observed in the preparations of these oocytes. Also, to be analyzable, preparations must be selected for adequate chromosome spreading and little overlapping. Consequently these factors drastically reduce the yield of preparations suitable for karyotyping, resulting in a majority of studies having only low numbers of analyzed oocytes.

Embryos

Obtaining useful, easily interpreted chromosome data from the few cells available in early embryos presents several problems. The two-cell stage gives most success because the time for division to four cells can be anticipated by observing breakdown of the nuclear membranes, thus minimizing the time in colcemide. A majority of spare embryos, however, are in four-cell stage or later (e.g., four-six cells). Because it is difficult to judge the stage of the cell cycle when most nuclei are at mitosis, colcemide exposure is prolonged to around 18–20 h. This time increases the yield of mitoses but often produces contracted chromosomes unsuitable for banding. Another obvious difficulty is in obtaining preparations in which the chromosomes have sufficient but not excessive spreading.

Since spare embryos are those in excess of the three most rapidly and evenly dividing embryos, spares necessarily show variable quality. Many embryos show unequal blastomeres or moderate to severe fragmentation. Some fragmented embryos may divide in vitro as some normal-looking embryos do and their preparations show chromosomes. Preparations of such fragmented embryos, however, often show no chromosomes. Instead they have multinucleated cells with "nuclei" of different size. In addition, normal-looking embryos often show spontaneous cleavage arrest in vitro, thus markedly restricting the number of dividing embryos. Finally, a fraction of embryos are polyspermic and are also arrested in development. Fragmentation, cleavage arrest, and polyspermy together reduce the number of embryos with mitosis to about one-third the original number. From this third only those with adequate chromosome spreading can be used for analysis.

Oocytes

Two types of oocytes from IVF/ET programs have been used for chromosome analysis. These include fresh (donated) oocytes and oocytes that remain unfertilized after in vitro insemination. The former are difficult to obtain; the latter are used in the majority of studies. The frequency of chromosomal abnormalities in oocytes remaining unfertilized appears to be no higher than that in freshly recovered oocytes. The comparison, however, may be inaccurate because of the small number of fresh oocytes that have been analyzed. In two studies using freshly recovered, noninseminated oocytes (Martin et al. 1986; Wramsby et al. 1987a), the former group found that 34 of a total of 50 oocytes had a normal haploid complement and 16 (32%) had numerical abnormalities. Single, double, or triple autosomal nullisomies and one disomy were present. The latter group analyzed 17 oocytes and found 9 with a normal haploid set of chromosomes and 8 (47%) that were abnormal with extra or missing chromosomes, ranging in number from 19 to 25.

Among 1120 oocytes remaining unfertilized after insemination (determined by absence of pronuclei and cleavage within 24 h after insemination), the results of 11 studies (Martin et al. 1986; Plachot et al. 1986; Veiga et al. 1987; Wramsby and Fredga 1987; Bongso et al. 1988; Djalali et al. 1988; Pellestor and Selle 1988; Ma et al. 1989; Papadopoulos et al. 1989; Delhanty and Penketh 1990; Macas et al. 1990) show a wide range in the total frequencies of chromosomal abnormalities, varying between 23% and 65%, with a weighted mean of 35.0 ± 1.4% (our calculation). The proportion of total abnormalities differs very significantly among studies ($\chi^2 = 93.3$, $df = 10$, $P \ll 10^{-4}$). Data of these studies are summarized in Table 1.

The chromosomal abnormalities more commonly found are aneuploidies (hypo- and hyper-haploidy), followed by diploidies (with or without aneuploidy). Structural abnormalities are sometimes reported. These include deletions, gaps, and acentrics. Aneuploid karyotypes with single, double, triple, or quadruple nullisomies or disomies, some with structural alterations, have been reported. All chromosome groups are involved in aneuploidy. Using 109 aneuploid oocytes with single and multiple aneuploidies per karyotype, described in eight dif-

Table 1. Frequencies of normal, aneuploid, diploid and of total abnormal chromosome complements in oocytes remaining unfertilized from 11 different studies

Study	*n*	Normal haploid (%)	Hypo-haploid (%)	Hyper-haploid (%)	Total aneuploid (%)	Diploid (%)	Total abnormal[a] (%)	2 × % Hyper-haploid (%)
Martin et al. (1986)	51	66.6	22.9	2.0	4.0[b]	–[c]	33.4	4.0
Plachot et al. (1986)	53	52.8	5.6	5.6	11.2	3.8	47.2	11.2
Veiga et al. (1987)	117	76.9	2.6	7.7	15.4[b]	6.8	23.1	15.4
Wramsby and Fredga (1987)	52	34.6	51.9	5.8	57.7	3.8	65.4	11.6
Bongso et al. (1988)	251	76.6	13.0	8.0	21.0	2.0	23.4	16.0
Djalali et al. (1988)	90	65.5	20.0	2.2	22.2	8.8	34.5	4.4
Pellestor and Selle (1988)	201	76.1	12.4	7.5	19.9[d]	3.0	23.9	15.0
Ma et al. (1989)	65	52.3	15.4	10.8	26.2	12.3	47.7	21.6
Papadopoulos et al. (1989)	30	43.3	13.3	6.7	20.0	10.0	56.7	13.4
Delhanty and Penketh (1990)	155	45.8	43.2	2.6	10.0[b]	8.4	54.2	5.2
Macas et al. (1990)	55	65.4	10.9	14.5	29.1[d]	5.4	34.6	29.0

[a] Includes aneuploid, hyperdiploid, hypodiploid, polyploid, and structural alterations
[b] Estimated by study authors by doubling the hyperhaploid complements
[c] Diploid oocytes not mentioned by author
[d] Includes hyperdiploid complements

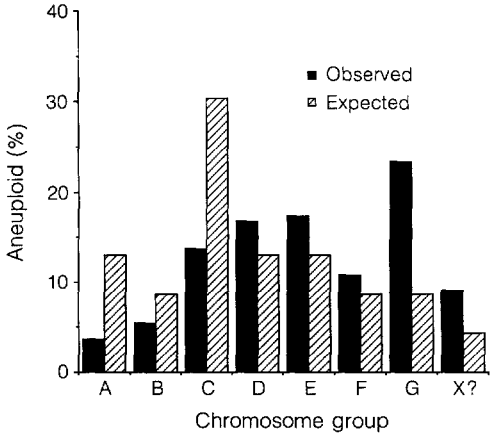

Fig. 1. Observed and expected frequencies of aneuploidy in the different chromosome groups. Data from 109 karyotypes of eight different studies

ferent studies (Martin et al. 1986; Plachot et al. 1986; Wramsby and Fredga 1987; Bongso et al. 1988; Djalali et al. 1988; Pellestor and Selle 1988; Ma et al. 1989; Macas et al. 1990), we estimated the frequency of aneuploidy for each chromosome group. We then compared these observed proportions with the corresponding expected proportions on the assumption that, at the time of meiosis, every chromosome has an equal probability of aneuploidy (therefore, the expected value is proportional to the number of chromosomes in each group). For each chromosome group the observed and expected proportions, which are illustrated in Fig. 1, are as follows (expected in parenthesis): A = 3.59% (13.04%); B = 5.39% (8.70%); C = 13.77% (30.43%); D = 16.77% (13.04%); E = 17.36% (13.04%); F = 10.78% (8.70%); G = 23.35% (8.70%); X (?) = 8.98% (4.35%). The goodness-of-fit chi-square shows a very significant deviation of the observed proportions from the expected (χ^2 = 83.23; *df* 7; $P \ll 0.001$). The ratios of observed proportion/expected proportion for each group are as follows: A = 0.28; B = 0.62; C = 0.45; D = 1.29; E = 1.33; F = 1.24; G = 2.68; X? = 2.07. These ratios fall into three nonoverlapping size classes: class I includes groups A, B, C (range 0.28–0.62); class II includes groups D, E, F (range 1.24–1.33), and class III includes group G (2.68) and the X? chromosome (2.07). Data on the X chromosome may not be accurate in this nonbanded chromosome material. The observed/expected ratios suggest that there is stronger selection against oocytes with aneuploidy of the large chromosomes than against oocytes with aneuploidy of the small chromosomes. The ratio for group G is higher than that of any other group and more than twice that of group F, which has the same expected frequency of aneuploidy as group G. This further suggests that selection operates not only in relation to chromosome size but also with regard to specific chromosomes.

As shown in Table 1, most studies report more oocytes with hypohaploid complements than with hyperhaploid. In addition to nondisjunction – the major source of aneuploidy – anaphase lag may produce chromosome loss during meiosis. There is recent evidence of anaphase lag from direct observations both in living and in fixed oocytes (Van Blerkom and Henry 1988; Zenzes et al. 1990a). The excess of hypohaploid oocytes thus may be the result of both processes, although technical error cannot be ruled out. As shown in Table 1, the frequency of aneuploidy ranges in the 11 studies between 4.0% and 57.7%. The overall (weighted) proportion is 25.5% ± 1.3% (our calculation). The proportions of aneuploid oocytes, however, differ very significantly among studies (χ^2 = 89.49, *df* = 10, $p \ll 10^{-4}$). Because it is not possible to distinguish hypohaploidy from chromosome loss due to technical errors, we also estimated the aneuploidy

rate in these studies by doubling the proportion of hyperhaploidy. This is shown in the last column of Table 1. The estimated weighted mean percentage of hyperhaploidy is 6.61% ± 0.74%, based on the 1120 oocytes from the 11 studies. Interestingly, these proportions do *not* vary significantly among studies ($\chi^2 = 17.45$; df 10; $P = 0.06$). This suggests that the above differences among studies were due mainly to differences in their rate of loss of chromosomes.

The estimated overall (weighted) corrected proportion of aneuploidy (2 × % hyperhaploidy) is 13.22 ± 1.48; this frequency of aneuploidy is half of that mentioned above. If this level of abnormality is a good approximation of the true value, it indicates that oocytes have a higher frequency of nondisjunction than do sperm. Analysis of sperm chromosomes from normal male donors visualized in the cytoplasm in zona-free hamster ova, have shown an estimated frequency of abnormality of about 8% with considerable variation among different studies (reviewed in Zenzes 1987). Using DNA polymorphisms as markers to determine parental origin of nondisjunction of the extra chromosome on children with trisomy 21, Antonarakis (1991) found 95% of the cases to be maternal and 5% to be paternal in origin, thus supporting our interpretation.

Diploid metaphase II oocytes usually result from lack of extrusion of the polar body. The rates of diploid oocytes, found in 10 of the above mentioned studies, range between 2% and 12.3%. The weighted proportion is 5.4% ± 0.7%. If fertilized, diploid oocytes have the potential to produce triploid embryos.

Errors during fertilization

The maturation stage of oocytes at the time of insemination may affect the efficiency of fertilization. The rate of fertilization is significantly lower when inseminations are done with immature than with fully mature oocytes (Zenzes et al. 1985; 1990a; Pieters et al. 1989). Sperm penetration into immature oocytes may not trigger activation of the oocyte, or may lead to incomplete development of the maternal and paternal nuclei, or to a marked asynchrony in their development (Zenzes et al. 1985, 1990a; Plachot et al. 1987a, b; Schmiady et al. 1987; Kessler et al. 1988). Asynchrony may affect normal postfertilization development, resulting in embryos with delayed cleavage, unequal blastomeres and cytoplasmic fragmentation. The viability of such morphologically abnormal embryos may be impaired (Plachot 1990). The observed asynchrony of postfertilization development has been related to asynchrony between meiotic and cytoplasmic maturation, which may occur in oocytes from stimulated cycles (Sundström and Nilsson 1984).

Some fertilized oocytes remain arrested in metaphase II and, in the cytological preparation, show prophasic sperm chromosomes in the oocyte cytoplasm. This phenomenon, called premature chromosome condensation (PCC), occurs in IVF at frequencies varying from 3% to 16% (Schmiady et al. 1986, 1987; Ma et al. 1989; Schmiady and Kentenich 1989; Papadopoulos et al. 1989; Pieters et al. 1989; Delhanty and Penketh 1990; Macas et al. 1990; Zenzes et al. 1990a). PCC may be related to a marked asynchrony in postfertilization development, as suggested by Schmiady and Kentenich (1989), and has been observed after insemination of both mature or immature oocytes.

Polyspermy is responsible for the largest proportion of errors created at the time of fertilization. Evidence suggests that the frequency of polyspermy is increased by immaturity or postmaturity of oocytes (Van der Ven et al. 1985; Angell et al. 1986b; Zenzes et al. 1985, 1990a). Multiple (three or more) sperm penetrations usually result in developmental arrest, probably because of limited availability of cytoplasmic factors in mammalian oocytes (Moltik 1989), which induce sperm chromatin decondensation and pronuclear formation. In the preparations of these arrested oocytes several decondensing sperm heads can be visualized in the oocyte cytoplasm. Polyspermic fertilization seems not to be definitely associated with the number of spermatozoa used in the insemination or with the hormonal protocol employed (Van der Ven et al. 1985; Webster et al. 1985), but may be an inherent inability of a particular oocyte to initiate and complete the zona reaction. Semen samples with subnormal seminal parameter values may produce polyspermy because of delayed fertilization, causing oocyte aging and, consequently, decreased efficiency of the zona reaction. Oocytes with delayed fertilization show pronuclear formation and cleavage 22 and 24 h, respectively, later than oocytes with normally timed fertilization (Plachot et al. 1988a). Such zygotes also have a significantly higher rate of chromosomal abnormalities including mosaicism.

Triploidy is the most frequent chromosomal error responsible for developmental failure during cleavage and before or after implantation. Tripronuclear zygotes are determined by the presence of three equal-sized pronuclei in the cytoplasm. Triploidy usually results from penetration of two spermatozoa (diandry), or by fertilization of a diploid oocyte (digyny) produced in low proportions, as mentioned above, or, rarely, from fertilization by a diploid spermatozoon. Some tripronuclear zygotes cleave to two-cell embryos and have a triploid or near-triploid chromosome complement before first cleavage (Rudak et al. 1984; Zenzes et al. 1985; Michelmann et al. 1986; Kola et al. 1987; Macas et al. 1988; Pieters et al. 1988). Since deviations from triploidy may occur during the subsequent cleavage divisions, Rudak et al. (1984) analyzed pronuclear chromosomes of tripronuclear zygotes to assess the chromosome constitution of the gametes. These authors could ascertain the parental origin of several complements with aneuploidies. About one-third of tripronuclear zygotes develop into diploid/triploid mosaic embryos (Van Blerkom et al. 1984; Michelmann et al. 1986; Plachot et al. 1987b; Macas et al. 1988; Mettler and Michelmann 1988; Papadopoulos et al. 1988; Plachot 1989), as a mechanism to revert to the diploid stage. The implantation and survival capacity of 2n/3n mosaic embryos probably will depend on increased proportions of normal diploid cell lines. A majority of tripronuclear zygotes, however, do not develop into tri-

ploid embryos but rather cleave into three cells via a tripolar spindle. This causes disorganized movement of chromosomes resulting in mosaic embryos (Plachot et al. 1987b; Angell et al. 1986b; Angell 1989; Plachot 1989). The chromosome complements found in different cells from 20 triponuclear zygotes ranged in two studies from 16 to 57 (Kola et al. 1987; Macas et al. 1988). Some zygotes have been observed to cleave into two cells plus an extrusion and are karyotypically diploid. Their embryos show normal cleavage, being morphologically indistinguishable from embryos produced by normal fertilization (Van Blerkom and Henry 1988; Angell et al. 1986b).

Embryos

A high frequency of embryo loss seems to be unique to humans; it has not been observed in other mammals (Hanly 1961; Santaló et al. 1986). In the IVF/ET procedure, despite the successful rate (90%) of fertilizations that reach pronuclear stage and undergo cleavage (Trounson et al. 1982; Edwards et al. 1983, 1984; Testart et al. 1983; Lopata 1983), the implantation rate remains very low. Furthermore, about 26% of the embryos that do implant and reach the stage of clinical pregnancy subsequently abort (Plachot 1989; Roessler et al. 1989). This fact highlights the need for chromosome analyses of IVF/ET embryos.

Because of the usual unavailability of IVF/ET embryos, only a few analyses of chromosome complements have been done in them. These include "research" embryos (oocytes donated by women seeking sterilization and fertilized with donor sperm) analyzed in three studies (Angell et al. 1986a; Wramsby et al. 1987b; Papadopoulos et al. 1988) and "spare" untransferred embryos (Angell et al. 1983, 1986a; Rudak et al. 1985; Zenzes et al. 1985; Plachot et al. 1987a, b; Veiga et al. 1987; Watt et al. 1987; Papadopoulos et al. 1988; Wimmers and Van der Merve 1988; Angell 1989; Pieters et al. 1989). The types of chromosome abnormalities found include aneuploids (single and multiple monosomies and trisomies), haploids, mosaics (diploid/haploid; diploid/triploid; haploid/triploid), and structural alterations. Only one study (Angell et al. 1986a) showed comparative data between spare and research embryos. The ratios (abnormal/normal) found in this study between the two groups were 4/15 for research and 2/3 for spare embryos. The 12 research (only) embryos analyzed by Wramsby et al. (1987b) were normal diploid. Papadopoulos et al. (1988) had chromosome data pooled from both types of embryos.

The total frequencies of chromosomal abnormality in research and spare embryos together (excluding multipronuclear embryos) range between 23% and 40% in four studies that analyzed a reasonable number of embryos (Plachot et al. 1987b; $n = 30$, 23%; Papadopoulos et al. 1988: $n = 35$, 40%; Wimmers and Van der Merve 1988: $n = 50$, 36% Angell 1989: $n = 31$, 23%). Mosaics with normal and aneuploid cell lines appear to be the most common (Plachot et al. 1987a, b; Papadopoulos et al. 1989). Haploids occur at a frequency of about 2% (Plachot et al. 1988a).

Table 2. Summary of specific types of chromosome aneuploidies in spare and research embryos

Study	Karyotype
Angell et al. (1983)	22,X,−15
Angell et al. (1983)	47,XY+D
Rudak et al. (1985)	44,XY,−1,−G
Zenzes et al. (1985)	24,XX
Plachot et al. (1987a)	Trisomy 18[a]
Plachot et al. (1987a)	46,XY;51,XXY,+C,+C,+F,+F[b]
Plachot et al. (1987b)	43,XY,−C,−C,−F
Plachot et al. (1987b)	45,XY,−C
Plachot et al. (1987b)	45,XY,−D
Plachot et al. (1987b)	21,X,−D,−G
Plachot et al. (1987b)	24,X,+G
Watt et al. (1987)	47,XY,+1
Angell (1989)	47,XX+G
Angell (1989)	47,XY+G
Angell (1989)	45,XY−15
Angell (1989)	45,XY−G
Veiga et al. (1987)	20,X,−20
Papadopoulos et al. (1988)	47,XY,+E,+F,−2;46;46[b]
Papadopoulos et al. (1988)	48,XX,+C,−2;91[b]
Papadopoulos et al. (1988)	47,XX,+D;46,XX;41;36[b]
Zenzes et al. (1990b)	92,XXYY,−C,+G
Zenzes et al. (1990b)	47,XXY
Zenzes et al. (1990b)	45,XX,−A
Zenzes et al. (1990b)	43,XX,−C,−E,−G

[a] In the diploid cell of a 2n/3n mosaic embryo
[b] Mosaic embryos

The specific types of chromosome groups with aneuploidies found in spare and research embryos are summarized in Table 2. These include single monosomies for chromosome 15, and for groups A, C, D, and G, found in seven embryos (Plachot et al. 1987b; Angell 1989; Papadopoulos et al. 1988; Zenzes et al. 1990b); multiple nullisomies involving chromosome 1 and groups C and F or groups C, E, and G, found in two embryos (Plachot et al. 1987b; Zenzes et al. 1990b); double trisomies involving groups C and F or E and F, found in two embryos (Plachot et al. 1987a; Papadopoulos et al. 1988); single trisomies 18, D, and G, found in three embryos (Plachot et al. 1987a, b; Angell 1989); a single trisomy 1, and a monosomy of group A, found in two embryos (Watt et al. 1987; Zenzes et al. 1990b); an XXY sex chromosome complement found in two embryos (Plachot et al. 1987b; Zenzes et al. 1990b); a disomy X found in a haploid embryo (Zenzes et al. 1985); monosomies 15 and 20, found in two other haploid embryos (Angell et al. 1983; Veiga et al. 1987); monosomies together with trisomies, found in two mosaic embryos (Papadopoulos et al. 1988) and in a tetraploid embryo (Zenzes et al. 1990b). The latter arose presumably through failure of cytokinesis at first cleavage. The overall results suggest that autosomal monosomy (single and multiple) is a condition that may not always be lethal in early cleavage stages of development. It may be lethal after the onset of embryonic gene

expression, which is known to occur after third cleavage (Tesarik et al. 1986).

An important observation was that embryos that were chromosomally normal could not be distinguished morphologically from those with abnormal chromosome complements (Angell et al. 1986a; Plachot et al. 1987a; Zenzes et al. 1990b). The latter embryos cleaved evenly and their rate of cleavage fell within the same range as those with normal chromosomes. This may be because at the time of transfer, when spare embryos are prepared for chromosome analysis (between two- and eight-cell stage), gene expression of the embryo itself is not yet manifested; selective elimination will probably occur sometime after the onset of embryonal gene activation. Therefore, chromosome data from untransferred embryos may give us an estimate of the probability that transferred embryos will fail to implant. Embryos unlikely to reach implantation would probably be those with haploidy, monosomies, deletions, or chromosome fragmentation, while those with trisomies or triploidies would probably abort at a stage soon after implantation.

Clinical parameters

In vitro fertilization and embryo transfer is a therapeutic procedure for infertility in which success depends on the interaction of various clinical and biological parameters. Four variables in the procedure may increase the risk of chromosomal abnormality. These include: (1) infertility: tubal factor, unexplained, endometriosis, male factor; (2) advanced maternal age: average age from several studies 33 years, range 27 to 43 years; (3) ovarian hyperstimulation regimes: multiple follicular development is usually induced with either clomiphene citrate (CC) and human menopausal gonadotropin (HMG) or with human follicle stimulating hormone (FSH); ovulation is induced with human chorionic gonadotropin; and (4) in vitro manipulation of gametic cells. Some of these variables have been addressed in a few studies as shown below.

Maternal age

Because a significant increase in nondisjunction has been established with advanced maternal age, both in animal studies (Gosden 1983; Maudlin and Fraser 1978) and in human abortuses (Hassold et al. 1980; Hook 1981; Hassold and Chiu 1985), it is clear that women older than 35 years, seeking IVF/ET, constitute a high-risk group. There are data showing increased frequency of aneuploidy with increased maternal age in oocytes of IVF/ET patients, but some studies are contradictory. Thus, while two studies (Djalali et al. 1988; Pellestor and Sele 1988) found no increase in the aneuploidy rate in oocytes from women older than 35 years, a tendency to increased nondisjunction among older women was reported by others (Martin et al. 1986; Plachot et al. 1988b; Wramsby and Fredga 1987; Ma et al. 1989; Bongso et al. 1990; Delhanty and Penketh 1990; Macas et al. 1990). Two studies (Bongso et al. 1988; Macas et al. 1990) reported an elevated mean maternal age of 36.7 and 36.2 years, respectively, in patients with increased aneuploidy.

Data on embryos to address this important question are very limited. A study (Angell 1989) indicated that the rate of nondisjunction in IVF/ET embryos appears to be higher than in clinically recognized pregnancies. The increase in nondisjunction in this small series of embryos ($n = 6$) occurred mainly on specific types of chromosomes (acrocentrics of groups D and G), abnormalities of which are known to be associated with increased maternal age (Hassold et al. 1980b).

In a recent study Segal and Caspar (1990) found that women older than 35 have, on average, a lower rate of implantation than younger women (28–35 years), even though both groups showed similar responses to hormonal stimulation of follicular development and produced a similar number of mature, fertilized oocytes. The older group, however, produced a significantly lower number of cleaved embryos for transfer. A reason for the difficulty in achieving pregnancy may be the increased frequency of chromosomal abnormalities in the oocytes or embryos of older women. Oocytes donated by young, fertile women to women 40–44 years old produced rates of implantation and ongoing pregnancies similar to matched controls (Sauer et al. 1990). This suggests that the endometrium of older women retains ability to respond to gonadal steroids thus permitting embryo implantation.

Other clinical parameters

Only one multicenter study (Plachot et al. 1988b) analyzed an appreciable number of oocytes and zygotes and correlated several clinical parameters with the rate of chromosomal abnormality. The parameters included:

1. Infertility (275 oocytes, 1409 zygotes). The tubal factor group included patients with previous ectopic pregnancies and salpingitis; the unexplained infertility group included patients with at least 30 months of infertility; the endometriosis group included endometriosis alone or associated with tubal factors. The male infertility group included patients with seminal abnormalities (sperm number, motility and morphology) when their wives were normal.

2. Maternal age (296 oocytes, 1393 zygotes). Mean age was 33.4 years; range 27–42 years. It included two groups of women, ≤ 35 years or > 35 years.

3. Stimulation treatments (290 oocytes, 1258 zygotes): these included CC/HMG; HMG alone; pure FSH and LHRH (luteinizing hormone release hormone) analogues/HMG.

4. Doses of gonadotropin (293 oocytes, 1012 zygotes). From 6 to 46 ampules of HMG (one ampule of HMG contained 75 IU of FSH and 75 IU of LH).

The results for oocytes showed no correlation between type of infertility, stimulation treatments, and doses of gonadotropin administered and chromosome abnormalities. A slight ($P < 0.05$) increase in aneuploidy rate was

found among patients > 35 years. Ideally, these results in oocytes retrieved from stimulated cycles should be compared with those from normal cycles but these are unavailable.

Conclusions

The rather high estimate of the frequency of aneuploidy in oocytes remaining unfertilized after insemination suggests that chromosomally abnormal oocytes may be selected for low fertilizability. Chromosome data from oocytes donated by fertile, young women should test this assumption. The significant deviations of the observed frequencies of aneuploidy from the expected frequencies indicates that aneuploidy of specific chromosomes (e.g., the large chromosomes) is usually lethal to the oocyte.

Efficient handling and manipulation of the gametic cells in the IVF/ET procedure can reduce the risk of errors during fertilization. Some defects can be detected and, consequently, avoided. For example, if inseminations are done with immature oocytes or if the semen samples show subnormal values, the outcome may be a low fertilization rate, or abnormal or delayed fertilization. Replacement into the mother of embryos that developed from multipronuclear zygotes may result in embryonal loss. In view of the potential pathological consequences of polyspermy, some IVF teams advocate examination of the pronuclei so that multipronuclear zygotes are not replaced. Other anomalies of fertilization, including asynchrony of pronuclear development and PCC, have been related to asynchrony in nuclear and cytoplasmic maturation of oocytes from stimulated cycles. Therefore, the stimulation protocols in IVF/ET programs must be constantly reevaluated.

Because numerical chromosome abnormalities are a common cause of early spontaneous abortion, they may account for much of the failure of transferred IVF embryos to implant. Since the estimated overall frequency of chromosomal aneuploidy is 13% in oocytes (f_o) and 8% in sperm (f_s), and abnormalities may be transmitted at conception by the oocyte only, the sperm only, or both gametes, the expected probability of aneuploidy at fertilization is $f_o\ (1-f_s)+f_s(1-f_o)+f_of_s$ or 20%. Other numerical abnormalities such as diploidy, triploidy, tetraploidy, and mosaicism will increase this frequency.

Observations in early preimplantation embryos of karyotypes with monosomy or trisomy indicate that severe chromosome abnormalities of oocyte or sperm do not prevent fertilization and zygotic formation. Different degrees of embryo viability are probably associated with specific chromosomal abnormalities. Data on abortuses are informative here. The types of chromosomal abnormalities most commonly found in abortuses include autosomal trisomies (51.9% ± 1.5%), followed in frequency by monosomy X (18.5% ± 1.5%) and triploidy (16.5% ± 1.5%); unweighted means calculated from five large-scale studies; in Kline et al. (1989). In contrast to abortion material where autosomal monosomies are very rarely found, the few analyses on preimplantation (IVF/ET) embryos have found single and multiple autosomal monosomies. Such embryos will probably not survive long after the onset of embryonic gene activation. Thus embryos with severely depleted complements such as haploidy, single and multiple monosomies, or with fragmented chromosomes most likely contribute to the high rate of subclinical loss afflicting IVF/ET couples.

The high (33 years) mean maternal age of women in IVF/ET makes them a risk group. The lower pregnancy rate found among older women, and their longer periods of infertility, may well be related to the increased frequency of chromosomal abnormality in their oocytes. A greater tendency to loss or gain of the small satellited chromosomes (group G) is probably associated with advanced maternal age. Aneuploidies of this specific group of acrocentric chromosomes are known to be associated with increased maternal age in large cytogenetic surveys of abortuses. Patients over 35 years seeking IVF should be advised on the possible increased risks of implantation failure or abortion.

Finally, a few attempts to correlate other parameters in the IVF procedure with an increased level of chromosomal abnormality, such as infertility and superovulation regimes, have so far failed. The negative results may be due to the limitations in the technique, which generates low numbers of chromosome data. Because of these limitations we still cannot compare the levels of chromosomal abnormality after in vitro fertilization with the in vivo situation. We can, however, predict that the reproductive success in this selected population of IVF/ET couples largely depends on our ability to recognize and select chromosomally normal embryos for transfer.

Acknowledgements. We thank Professor T. E. Reed for important suggestions in the manuscript and an anonymous referee for helpful comments.

References

Angell RR (1989) Chromosome abnormalities in human preimplantation embryos. Prog Clin Biol Res 294:181–187

Angell RR, Aitken RJ, Look PFA van, Lumsden MA, Templeton AA (1983) Chromosome abnormalities in human embryos after in vitro fertilization. Nature 303:336–338

Angell R, Templeton AA, Aitken RJ (1986a) Chromosome studies in human in vitro fertilization. Hum Genet 72:333–339

Angell RR, Templeton AA, Messinis IE (1986b) Consequences of polyspermy in man. Cytogenet Cell Genet 42:1–7

Antonarakis S et al (1991) Parental origin of the extra chromosome in trisomy 21 as indicated by analysis of DNA polymorphisms. N Engl J Med 324:872–876

Bongso A, Chye NS, Ratnam S, Sathanantyan H, Wong PC (1988) Chromosome anomalies in human oocytes failing to fertilize after insemination in vitro. Hum Reprod 3:645–649

Braude RR, Bolton VN, Johnson MH (1986) The use of human pre-embryos for infertility research. In: Bock G, O'Connor M (eds) Human embryo research – yes or no? (Ciba Foundation) Tavistock, London New York, pp 63–82

Delhanty JDA, Penketh RJA (1990) Cytogenetic analysis of unfertilized oocytes retrieved after treatment with LHRH analogue, buserelin. Hum Reprod 5:699–702

Djalali M, Rosenbusch B, Wolf M, Steriz K (1988) Cytogenetics of unfertilized human oocytes. J Reprod Fertil 84:647–652

Edmonds DK, Lindsy KS, Miller JF, Williamson E, Wood PJ (1982) Early embryonic mortality in women. Fertil Steril 38:447–453

Edwards RG (1986) Causes of early embryonic loss in human pregnancy. Hum Reprod 1:185–198
Edwards RG, Steptoe PC, Purdy JM (1980) Establishing full-term human pregnancies using cleaving embryos grown in vitro. Br J Obstet Gynaecol 87:737–756
Edwards RG, Fishel SB, Purdy JM (1983) In vitro fertilization of human eggs: analysis of follicular growth, ovulation and fertilization. In: Beier HM, Lindner HD (eds) Fertilization of the human egg in vitro. Springer, Berlin Heidelberg New York, pp 169–188
Edwards RG, Fishel SB, Cohen J, Fehilly CB, Burdy JM, Slater JM, Steptoe PC, Webster JM (1984) Factors influencing the success of in vitro fertilization for alleviating human infertility. J In Vitro Fert Embryo Transfer 1:3–7
Golbus MS, Bahrman R, Wiltse S, Hall BD (1976) Tetraploidy in a liveborn infant. J Med Genet 13:329–336
Gosden RG (1983) Chromosomal anomalies in preimplantation embryos in relation to maternal age. J Reprod Fertil 25:351–354
Hanly S (1961) Prenatal mortality in farm animals. J Reprod Fertil 1:182–194
Hassold T, Chiu D (1985) Maternal age specific rates of numerical chromosome abnormalities with special reference to trisomy. Hum Genet 70:11–17
Hassold T, Jacobs PA, Kline J, Stein Z, Warburton D (1980) Effect of maternal age on autosomal trisomies. Ann Hum Genet 44:29–36
Hook EB (1981) Rates of chromosome abnormalities at different matrnal ages. Obstet Gynecol 58:282–284
Jacobs PA, Hassold TJ (1980) The origin of chromosome abnormalities in spontaneous abortion. In: Porter IH, Hook EB (eds) Human embryonic and fetal death. Academic Press, New York, pp 289–298
Kessler HH, Winter R, Dohr G, Urdl W, Pusch HH (1988) The importance of an early diagnosis of fertilization disorders. Hum Reprod 3:767–772
Kline J, Stein Z, Susser M (1989) Conception to birth: epidemiology of prenatal development. Oxford University Press, Oxford New York, pp 81–101
Kola I, Trounson A, Dawson G, Rogers P (1987) Tripronuclear human oocytes: altered cleavage patterns and subsequent karyotypic analysis of embryos. Biol Reprod 37:395–401
Lopata A (1983) Concepts in human in vitro fertilization and embryo transfer. Fertil Steril 40:289–301
Ma S, Kalousek DK, Zouves C, Yuen BH, Gomel V, Moon YS (1989) Chromosome analysis of human oocytes failing to fertilize in vitro. Fertil Steril 51:992–997
Macas E, Subschanek E, Griselj V, Puharic I, Simmic V (1988) Chromosomal preparations of human triploid zygotes and embryos fertilized in vitro. Eur J Obstet Gynecol Reprod Biol 29:299–304
Macas E, Floersheim Y, Hotz E, Imthurn B, Keller PJ, Walt H (1990) Abnormal chromosomal arrangements in human oocytes. Hum Reprod 5:703–707
Martin RH, Mahadevan MM, Taylor PJ, Hildebrandt K, Long-Simpson L, Peterson D, Yamamoto J, Fleetham J (1986) Chromosomal analysis of unfertilized human oocytes. J Reprod Fertil 78:673–678
Maudlin I, Fraser RL (1978) Maternal age and the incidence of aneuploidy on first cleavage mouse embryos. J Reprod Fertil 54:423–426
Mettler L, Michelmann HW (1988) Chromosome abnormalities in early human embryo development after in vitro fertilization and embryo transfer. In: Lizuka R, Semm K (eds) Human reproduction: current status future prospects. Elsevier, Amsterdam, pp 503–507
Michelmann HW, Bonhoff A, Mettler L (1986) Chromosome analysis in polyploid human embryos. Hum Reprod 1:243–246
Miller J, Williamson E, Glue J, Gordon YB, Grudzanskas JG, Sykes A (1980) Foetal loss after implantation. Lancet II:554–556
Moltik J (1989) Cytoplasmic aspects of oocyte growth and maturation in mammals. J Reprod Fertil Suppl 38:592–597
Papadopoulos G, Templeton AA, Fisk N, Randall J (1988) The frequency of chromosomal anomalies in human preimplantation embryos after in-vitro fertilization. Hum Reprod 4:91–98
Papadopoulos G, Randall J, Templeton AA (1989) The frequency of chromosome anomalies in human fertilized oocytes and uncleaved zygotes after insemination in vitro. Hum Reprod 4:568–573
Pellestor F, Selle B (1988) Assessment of aneuploidy in the human female using cytogenetics of IVF failures. Am J Hum Genet 42:274–279
Pieters MHEC, Geraedts JPM, Dumoulin JCM, Evers JLH, Bras M, Kornips FHAC, Menheere PPCA (1989) Cytogenetic analyses of in vitro fertilization (IVF) failures. Hum Genet 81:367–370
Plachot M (1989) Chromosome analysis of spontaneous abortions after IVF: a European survey. Hum Reprod 4:425–429
Plachot M (1990) New approaches to test viability of human preimplantation embryos. Hum Reprod 5 [Suppl]:365
Plachot M, Junca AM, Mandelbaum J, Grouchy J de, Salat-Baroux J, Cohen J (1986) Chromosome investigations in early life: human oocytes recovered in an IVF program. Hum Reprod 1:547–551
Plachot M, Junca AM, Mandelbaum J, Grouchy J de, Salat-Bardoux J, Cohen J (1987a) Chromosome investigations in early life. II. Human preimplantation embryos. Hum Reprod 2:29–35
Plachot M, Grouchy J de, Junca AM, Mandelbaum J, Turleau C, Couillin P, Cohen J, Salat-Bardoux J (1987b) From oocyte to embryo: a model deduced from in vitro fertilization for natural selection against chromosome abnormalities. Ann Genet 30:22–32
Plachot M, Grouchy J de, Junca AM, Mandelbaum J, Salat-Baroux J, Cohen J (1988a) Chromosome analysis of human oocytes and embryos: does delayed fertilization increase chromosome imbalance. Hum Reprod 3:125–127
Plachot M, Veiga A, Montagut J, Grouchy J de, Calderon G, Lepetre S, Junca AM, Santalo J, Carles E, Mandelbaum J, Barri P, Degoy J, Cohen J, Egozcue J, Sabatier JC, Salat-Baroux J (1988b) Are clinical and biological parameters correlated with chromosomal disorders in early life: a multicenter study. Hum Reprod 5:627–635
Roessler M, Wise L, Katayama KP (1989) Karyotype analysis of blighted ova in pregnancies achieved by in vitro fertilization. Fertil Steril 51:1065–1066
Rudak E, Jehoshua D, Mashiac S, Nebel L, Goldman B (1984) Chromosome analysis of multipronuclear human oocytes fertilized in vitro. Fertil Steril 41:538–545
Rudak E, Jehoshua D, Mashiac S, Nebel L, Goldman B (1985) Chromosome analysis of human oocytes and embryos fertilized in vitro. Ann NY Acad Sci 422:476–486
Santaló J, Estop AM, Egozcue J (1986) The chromosome complement of first-cleavage mouse embryos after in vitro fertilization. J In Vitro Fert Embryo Transfer 3:99–105
Sauer MV, Paulson RJ, Lobo RA (1990) A preliminary report on oocyte donation extending reproductive potential to women over 40. N Engl J Med 323:1157–1160
Schmiady H, Kentenich H (1989) Premature chromosome condensation after in vitro fertilization. Hum Reprod 4:689–695
Schmiady H, Sperling H, Kentenich H, Stauber M (1986) Prematurely condensed human sperm chromosomes after in vitro fertilization (IVF). Hum Genet 74:441–443
Schmiady H, Kentenich H, Stauber M (1987) Chromosome studies of human in vitro fertilization (IVF) failures. In: Obe G, Basler A (eds) Cytogenetics: basic and applied aspects. Springer, Berlin Heidelberg New York, pp 185–197
Segal S, Casper RF (1990) The response to ovarian stimulation in older women during in vitro fertilization. Hum Reprod 5:255–257

Short RV (1979) When a conceptus fails to become a pregnancy. (Ciba foundation symposium no 64) Excerpta Medica, Amsterdam, pp 377–394

Sundström P, Nilsson O (1984) Sequential changes of cytoplasmic features duting maturation of the human oocyte. Prog Clin Biol Res 294:299–310

Tarkowsky AK (1966) An air drying method for chromosome preparation from mouse eggs. Cytogenetics 5:394–400

Tesarik J, Kopecny V, Plachot M, Mandelbaum J, Da Lage C, Flechon JE (1986) Nucleogenesis in the human embryo developing in vitro: ultrastructural and autoradiographic analysis. Dev Biol 115:193–203

Testart J, Lasalle B, Frydman R, Belaisch JC (1983) A study of factors affecting the success of human fertilization in vitro. I. Influence of semen quality and oocyte maturity on fertilization and cleavage. Biol Reprod 28:425–431

Trounson AO, Mohr LR, Wood C, Leeton J (1982) Effect of delayed insemination on in-vitro fertilization, culture and transfer of human embryos. J Reprod Fertil 64:285–294

Van Blerkom J, Henry G (1988) Cytogenetic analysis of living human oocytes: cellular basis of developmental consequences of perturbation in chromosomal organization and complement. Hum Reprod 3:777–790

Van Blerkom J, Henry G, Porreco R (1984) Preimplantation human embryonic development from polypronuclear eggs after in vitro fertilization. Fertil Steril 41:686–696

Van der Ven H, Al-Hasani S, Diedrich K, Hamerich U, Lehmann F, Krebs D (1985) Polyspermy in in vitro fertilization of human oocytes: frequency and possible causes. Ann NY Acad Sci 422: 88–95

Veiga A, Calderón G, Santaló J, Barri P, Egozcue J (1987) Chromosome studies in oocytes and zygotes from an IVF programme. Hum Reprod 5:425–430

Watt JL, Templeton AA, Messinis I, Bell L, Cunningham P, Duncan R (1987) Trisomy 1 in an eight cell human embryo. J Med Genet 24:60–64

Webster BW, Wentz AC, Osteen KG, Rogers J, Vaughn WK (1985) Hormonal stimulation correlates with polyspermy. Ann NY Acad Sci 422:332–336

Wimmers MSE, Van der Merve JV (1988) Chromosome studies on early human embryos fertilized in vitro. Hum Reprod 3: 894–900

Wramsby H, Fredga K (1987) Chromosome analysis of human oocytes failing to cleave after insemination in vitro. Hum Reprod 2:137–142

Wramsby H, Fredga K, Liedholm P (1987a) Chromosome analysis of human oocytes recovered from preovulatory follicles in stimulated cycles. N Engl J Med 316:121–124

Wramsby H, Fredga K, Liedholm P (1987b) Ploidy in human cleavage stage embryos after fertilization in vitro. Hum Reprod 3:233–236

Zenzes MT (1987) Chromosome analyses using human spermatozoa. In: Obe G, Basler A (eds) Cytogenetics: basic and applied aspects. Springer, Berlin Heidelberg New York, pp 198–224

Zenzes MT, Belkien L, Bordt J, Kan I, Schneider HPG, Nieschlag E (1985) Cytologic investigation of human in vitro fertilization failures. Fertil Steril 43:883–891

Zenzes MT, De Geyter C, Bordt J, Schneider HPG, Nieschlag E (1990a) Abnormalities of sperm chromosome condensation in the cytoplasm of immature human oocytes. Hum Reprod 5: 842–846

Zenzes MT, Wang P, Casper RF (1990b) Chromosome analyses in human preimplantation embryos. J In Vitro Fert Embryo Transper 7:45