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ARTICLE

Cytogenetic analyses of culture failures by comparative genomic hybridisation (CGH) – Re-evaluation of chromosome aberration rates in early spontaneous abortions

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Comparative genomic hybridisation (CGH) represents an alternative molecular-cytogenetic technique capable of detecting chromosomal imbalances by reverse fluorescence *in situ* hybridisation. As the technique uses genomic DNA for assessment it does not rely on metaphase chromosomes in the test material and thus circumvents technical problems associated with tissue culturing. In the present study, we applied CGH to identify chromosome anomalies in 60 spontaneous abortions of the first trimester, that had failed to grow in culture. In 57 out of 60 cases CGH analyses were successful. The overall aneuploidy rate detected was 72%. Trisomy was the predominant chromosome anomaly accounting for 68.0% of abnormal abortions, followed by triploidy (17.1%) and monosomy X (9.8%). An unbalanced structural rearrangement was found in one (2.4%) abortion. Most frequently involved in trisomies were chromosomes 16 (32.1%), 7 and 22 (10.7% each), 4, 13, 15, and 21 (7.2 % each). Three triploid cases and one complete mole were detected by microsatellite analysis as supplementary method. CGH data on culture failures were compared with data derived from 4693 successfully karyotyped first trimester spontaneous abortions, resulting in a chromosome aberration rate of 64.8%. The distribution of the different chromosome anomalies was similar with the exception of a higher rate of trisomies 7 and of XYY-triploidies in the culture failures. Based on our data we suggest that the genetic contribution to pregnancy loss is still underestimated. Investigating abortion tissues hitherto unassessed by conventional methods, we suggest that the contribution of chromosome aberrations to first trimester pregnancy loss is nearly 70%. *European Journal of Human Genetics* (2001) **9,** 539–547.

Keywords: Comparative genomic hybridisation (CGH); spontaneous abortions; culture failures; chromosome aberration rate

Introduction

Comparative genomic hybridisation (CGH) facilitates detection of DNA sequence gains and sequence losses throughout the genome in a singular double-colour FISH. It was first established to cytogenetically investigate tissues with multiple chromosomal rearrangements, ie tumour tissues.[1] More recently, the use of the technique has been extended to characterization of whole or partial aneuploidies in fetuses or neonatales.[2–5] The technique has also proved useful for retrospective identification of familial, even cryptic translocations, or identification of hitherto unclassifiable *de novo* chromosomal rearrangements.[6–8] CGH even allows investigation of archival formalin fixed and paraffin embedded

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placental or foetal tissues.[9,10] Latest results show the ability of CGH to detect aneuploidies in single cells eg in association with preimplantation diagnosis.[11] CGH circumvents problems arising from tissue culture and allows testing in samples unanalysable by conventional cytogenetic analysis. Suspecting adverse effects of hitherto unidentified chromosome imbalances on culture growth and thus a high aberration rate in culture failures, we applied CGH to archival frozen chorionic villi of early spontaneous abortions that had failed to grow in culture.

Material and methods

Tissue samples and controls

Foetal or chorionic tissues had been collected as part of a cytogenetic study on spontaneous abortions at the Department of Paedopathology Mainz (MZ=32 cases) and the Institute of Clinical Genetics Marburg (MR=28 cases). Samples were cleansed from maternal decidua, blood clots and mucus. Fifty to 200 mg CVS per case were stored deep frozen ($-80^{\circ}C$) to facilitate later DNA extraction. The remainder samples were used for conventional cytogenetic and histological examinations. If an embryo was present, it was examined and photographed. As for the abortion cases from Marburg, parental blood had been collected in addition and stored deep frozen. In the present investigation DNA was extracted from stored frozen samples in 60 cases of first trimester spontaneous abortions that had failed to grow in culture, culture failure having impeded cytogenetic analysis. DNA was used for retrospective chromosome analyses by CGH. DNA from parental blood samples was extracted for microsatellite analysis. A total of 6631 abortion specimens (MZ/MR 6198/433) of gestational weeks 6 to 12 having been tested by conventional cytogenetic analysis served as controls.

For statistical analysis Fishers' exact test was used. Statistical significance was accepted at the probability level of <0.05.

CGH analysis

Stored CVS/foetal tissue samples were disaggregated under liquid nitrogen. Genomic DNA was isolated by the salting-out method.[12] Reference DNA was extracted from peripheral blood of a karyotypically normal male. CGH metaphase preparations, DNA labelling, hybridisation and detection were performed as described previously.[13] To detect ratio changes in the test DNA equal amounts (300–500 ng each) of FITC labelled sample- and rhodamine labelled control-DNA were precipitated in the presence of a 30 μg Cot-1-DNA fraction (GIBCO–BRL).

Slides were analysed with a Zeiss epifluorescence microscope with selective filtres equipped with a charged-coupled device camera. Three colour images with green (FITC), red (rhodamine) and blue (DAPI for counterstaining of the chromosomes) were obtained from 10–15 metaphases in each hybridisation. The image representing DAPI was inverted and used for semiautomatic karyotyping. Green (test-DNA) and red (reference DNA) fluorescence ratios were determined for each chromosome. After background substraction, fluorescence intensities were measured along the length of each homologue chromosome. For each chromosome, fluorescent ratio profiles (FR) were calculated as quotients of green and red fluorescence intensities and normalised to 1.0 representing the balanced state of the chromosomal copy number. An upper threshold of >1.20 was used to define gain of chromosomal material, while a lower threshold of <0.80 was used to indicate loss of chromosomal material.[14] For processing the captured images and for analysis function of automated generation of chromosome fluorescence intensity profiles, the ISIS digital image analysis system (MetaSystems, Altlussheim, Germany) and the Cytovision CGH program (Applied Imaging Co-operations) were used.

FISH and microsatellite analysis

All samples shown to be chromosomally balanced in CGH underwent interphase FISH or microsatellite analysis to determine ploidy. Known labelling artifacts associated with GC rich regions of 1p32→pter, 16p, 19, and 22 can lead to a deviation from a 1.0 FR value and thus to false positive interpretations.[13,15] Whenever these chromosomes were involved, we applied supplementary methods. Interphase FISH was performed on CVS touch preparations. Chromosome specific centromeric probes were implemented according to standard protocols for their hybridisation and detection. Nuclei were counterstained with DAPI and the number of hybridisation signals per cell were counted in at least 50 nuclei. For microsatellite analysis chromosome specific polymorphic markers were used. All primers were obtained from Research Genetics Inc. (Huntsville, AL, USA) using the Map Pair set 6.0. Individual markers were chosen primarily based on their heterozygosities, most being 0.8 and higher. Details of the primers used may be obtained on request from the authors. For the studies, DNA was amplified using standard conditions and a 'high touch down protocol' with annealing temperatures of $63^{\circ}C$[16]. PCR products were separated by 8% polyacrylamide gel-electrophoresis (PAGE) and visualised by silver staining. Determinations were based on at least two informative markers. Triploidy was excluded/confirmed by analysis of DNA polymorphisms of different autosomes.

Results

Sixty placental samples of first trimester spontaneous abortions were analysed for unbalanced chromosome aberrations by CGH (Table 1). In 57 out of 60 (95%) cases CGH analyses were successful. In three cases (5%) the FR-profiles were not interpretable due to poor, non-homogeneous hybridisation signals. CGH did not reveal any chromosome abnormalities in 20 of the 57 successfully analysed cases. The

copy number representation of sex chromosomes required an appropriate choice of both reference DNA and reference metaphase spreads. Therefore, CGH analyses were performed by co-hybridisation of abortion DNA with 'male' control DNA to normal 'male' metaphase spreads. In 13 of the 20 normal cases where abortion DNA (XX) and control DNA (XY) were discordant with respect to sex, the entire X chromosome was over-represented in the CGH profile, and the Y chromosome showed a relative loss, consistent with Y material derived only from the control DNA. The remaining chromosomes showed CGH profiles with no significant deviation from a value of 1.0. Ploidy changes in the seemingly 20 normal cases were investigated by microsatellite analyses in 16 cases and by interphase FISH in four cases. Three cases (MZ-1566, MZ-2557, MR-172) which had produced a balanced 46,XX status on CGH displayed polyploidy on microsatellite analysis with a 69,XXX karyotype. In one case (MR-112), microsatellite analysis demonstrated paternally inherited alleles only, thus verifying a complete mole which had been suspected previously by histology. Altogether, CGH identified 37 of the 41 chromosomally aberrant cases (90%). In four cases chromosome aberration was detected by supplementary methods.

Four of the 41 chromosomally abnormal cases revealed imbalances of the sex chromosomes. In two cases CGH analysis showed two copies of the X chromosome in the test DNA and a balanced state of Yp indicating a XXY-status. In two cases gain of a Y chromosome became evident indicating a XYY gonosome constellation. These cases were highly suspective of triploidy. In all cases triploidy was confirmed by the appearance of a third allele or by an increased dosage of a unique parental allele raising the total number of triploids to seven. Partial hydatidiform mole in six of the seven triploid cases suggested paternal origin of the additional haploid chromosome set. This could be confirmed by microsatellite analysis in three of the cases in which parental DNA was available.

In four of the 41 abnormal cases the average green/red FR profile indicated a single X chromosome and absence of a Y chromosome in the test genome. The 45,X karyotype could be confirmed in two out of four cases by microsatellite analysis revealing loss of the paternal X chromosome. Autosomal trisomies, comprising chromosomes 2, 4, 7, 8, 10, 13, 15, 16, 18, 19, 21 and 22, were evidenced by CGH in 28 of the 41 chromosomally abnormal cases (Table 1, Figure 1). In three cases (MZ-1682, MR-162, MZ-4677) the average FR-ratio of chromosomes 2, 19, and 22, respectively, was <1.2 along the entire chromosome. Microsatellite analysis, however, revealed two alleles of normal and one allele of reduced intensity. Based on these data these karyotypes could be defined as mosaic karyotypes. CGH analysis detected one segmental aneusomy (MZ-1520). Weak staining of the distal region of the short arm of chromosome 5 indicated underrepresentation of this chromosomal region in the test genome. The parents were excluded as translocation carriers. Thus, CGH allowed the identification of a *de novo* del(5p) defining the breakpoint at 5p14 (Figure 1). In total, aneuploidy was detected in 40 cases (70.2%), respectively in 41 cases (72.0 %) if the abortion with paternal disomy of all chromosomes and complete hydatidiform mole of the placenta was included (Table 2). Trisomy was the predominant chromosome anomaly accounting for 68.3% of all abnormal abortions, followed by triploidy (17.1%), and monosomy X (9.8%). An unbalanced structural rearrangement was found in one (2.4%) abortion. Chromosomes 16 (32.1%), 7 and 22 (10.7% each), 4, 13, 15, and 21 (7.2% each) were most frequently involved in trisomies. Three mosaic karyotypes were found corresponding to 7.5% of the chromosomally abnormal specimens (Table 2).

CGH results of culture failures were compared to consecutive series of unselected first trimester spontaneous abortions. A total of 6198 cases were analysed in Mainz (MZ) and 433 cases in Marburg (MR). The two studies were discordant regarding the culture failure rate (12.5% in MR *vs* 30.4% in MZ) and the sex ratio. In the MR-study the male : female sex ratio of 110 spontaneous abortions with normal karyotype was 1.0. The MZ-study showed a sex ratio of 0.6 with a predominance of chromosomally normal female abortions (553 : 990). However, the frequency and type of chromosome anomalies detected in the two studies were similar. Pooled data are summarised in Table 3. Abnormal karyotypes were found in 64.8% of the cases and included autosomal trisomies in 53.9%, monosomy X in 11.2%, triploidy in 15.6%, tetraploidy in 11.3%, and unbalanced structural rearrangements in 9.0% of the cases. Among the triploid abortions the gonosomal constitution of XXY prevailed (n=252), followed by XXX (n=168) and XYY (n=14).

Mean gestational week at foetal loss and maternal age did not differ between the culture failures with normal/abnormal karyotypes (9.5/10.0 weeks and 29.9/32.4 years) and the successfully karyotyped cases (9.8/9.9 weeks and 30.3/31.4 years).

Discussion

According to the literature, chromosome abnormalities account for more than 50% of early foetal losses, thus constituting the single most frequent cause of pregnancy loss in several surveys.[17] Meanwhile nearly 20 000 spontaneous abortions have been karyotyped and the general distribution of anomalies is well established (Figure 2). It is remarkable, however, that the highest overall abnormality rates in early spontaneous abortions were found in series with the high culture success rates.[18–20] This suggests, that culture failures are most likely to be chromosomally abnormal and that the incidence of aneuploidy is still clearly underestimated since potential abnormalities in culture failures have not yet been evaluated. To fill this gap we applied the CGH technique to detect chromosome

Table 1 CGH results and interpretation for 57 first trimester spontaneous abortions which failed to grow in culture

ID No	*CGH*	*Verification of CGH*	*Proposed karyotype (origin of aberration)*	*Maternal age*	*Gestational age*
MZ-383	rev ish XY	m	46,XY	29	9+1
MZ-2011	rev ish XY	f	46,XY	30	9
MR-40	rev ish XY	m	46,XY	28	10
MR-198	rev ish XY	m	46,XY	28	8
MR-259	rev ish XY	m	46,XY	33	9
MR-531	rev ish XY	f	46,XY	33	10+2
MR-599	rev ish XY	m	46,XY	33	1. trim
MZ-531	rev ish XX	m	46,XX	31	10+4
MZ-637	rev ish XX	f	46,XX	29	7
MZ-1593	rev ish XX	f	46,XX	21	10+2
MZ-2826	rev ish XX	m	46,XX	33	8
MZ-4077	rev ish XX	f	46,XX	38	8–9
MR-59	rev ish XX	m	46,XX	28	7–8
MR-72	rev ish XX	m	46,XX	33	1.trim
MR-92	rev ish XX	m	46,XX	24	8
MR-118	rev ish XX	m	46,XX	28	11
Complete mole					
MR-112	rev ish XX	m	46,XX	30	8–9
Triploidy					
MZ-1566	rev ish XX	m/f	69,XXX	37	9
MZ-2557	rev ish XX	m/f	69,XXX	39	1.trim
MR-172	rev ish XX	m	69,XXX (pat)	42	11+4
MZ-526	rev ish XX and Y	m	69,XXY	34	12+6
MZ-1438	rev ish XX and Y	m/f	69,XXY	31	6
MR-81	rev ish enh (Y)	m	69,XYY (pat)	35	9
MR-216	rev ish enh (Y)	m	69,XYY (pat)	24	9
Trisomy					
MZ-1682	rev ish XY, enh(2)	m	46,XY/47,XY,+2	30	7
MZ-2816	rev ish XY, enh(4)		47,XY,+4	28	9
MR-380	rev ish XX enh(4)	m	47,XX,+4	33	9
MZ-570	rev ish XX, enh(7)	f	47,XX,+7	33	1.trim
MR-120	rev ish XX, enh(7)	m	47,XX,+7 (mat)	33	8
MR-137	rev ish XY, enh(7)	m	47,XY,+7 (pat)	38	11
MR-138	rev ish XX, enh(8)	m	47,XX,+8	30	11
MZ-4618	rev ish XX, enh(10)		47,XX,+10	38	9–10
MZ-2070	rev ish XX, enh(13)		47,XX,+13	39	1. trim
MR-67	rev ish XY, enh(13)	m	47,XY,+13 (mat)	35	10
MZ-4348	rev ish XY, enh(15)		47,XY,+15	37	1. trim
MR-60	rev ish XY, enh(15)		47,XY,+15	33	7
MZ-1832	rev ish XX, enh(16)	f	47,XX,+16	31	10
MZ-2025	rev ish XY, enh(16)	m/f	47,XY,+16	31	10
MZ-2992	rev ish XX, enh(16)		47,XX,+16	29	11
MZ-3586	rev ish XY, enh(16)	m	47,XY,+16	34	11
MZ-4440	rev ish XX, enh(16)		47,XX,+16	30	5
MR-106	rev ish XY, enh(16)	m	47,XY,+16 (mat)	28	9
MR-159	rev ish XY, enh(16)	m	47,XY,+16 (mat)	30	11
MR-258	rev ish XY, enh(16)		47,XY,+16	23	9
MR-291	rev ish XX, enh(16)	m	47,XX,+16(mat)	19	14
MZ-4616	rev ish XX, enh(18)		47,XX,+18	32	10
MR-162	rev ish XY, enh(19)	m	46,XY/47,XY,+19	21	10
MZ-2600	rev ish XX, enh(21)	m	47,XX,+21	39	11
MR-95	rev ish XX, enh(21)	m	47,XX,+21	35	12
MZ-3943	rev ish XY, enh(22)		47,XY,+22	35	10
MZ-4677	rev ish XY, enh(22)	m	46,XY/47,XY,+22	33	8/9
MR-41	rev ish XY, enh(22)	m	47,XY,+22 (mat)	38	10
Monosomy X					
MZ-3572	rev ish X		45,X	29	12
MR-45	rev ish X	m	45,X (pat)	38	10

Continued

Table 1 *(Continued)*

ID No	CGH	Verification of CGH	Proposed karyotype (origin of aberration)	Maternal age	Gestational age
MZ-1768	rev ish X		45,X	29	9/10
MR-62	rev ish X	m	45,X (pat)	29	11
Structural anomalies					
MZ-1520	rev ish XX, dim(5)(p14pter)		46,XX,del(5)(p14)	38	10

m=microsatellite analysis, f=FISH, mat=maternal, pat=paternal. The CGH results are described according to ISCN (1995). Enhanced (enh) and diminished (dim) regions are those where the fluorescence ratio is increased or decreased relative to the euploid (balanced) state.

aberration rates in 60 cases of early spontaneous abortions which had failed to grow in culture.

As an alternative cytogenetic technique CGH circumvents technical problems associated with tissue culturing. In combination with microsatellite analysis and interphase FISH, CGH can identify whole chromosome aneuploidies and all major unbalanced structural chromosome aberrations that are detected by conventional cytogenetic analyses. In our series, CGH shows a lower failure rate (5%) than conventional cytogenetic analyses. A recent study on spontaneous abortions using CGH in combination with flow cytometry revealed a similar failure rate providing more accurate cytogenetic results by avoiding maternal cell contamination and tissue culture artifacts.[21]

Application of CGH to abortion material in our study detected chromosome abnormalities in 72% of culture failures. This pointed to an adverse effect of chromosome imbalances on culture growth and thus to a higher chromosome aberration rate in culture failures. Our rate of culture failures was 30.4% (MZ) and 12.5% (MR) in first trimester abortions. In the literature, data of overall failure rates range from 10 to 60%.[22,23] Our control group comprised 4693 successfully karyotyped first trimester spontaneous abortions showing an aberration rate of 64.8%, exceeding the average value of most previous studies. This result is even more striking when the cases from Marburg with an aberration rate of 71% are regarded separately. As the MR-study shows higher success rates in tissue culturing and a low rate of maternal contaminations, as concluded from a sex ratio of 1.0 in the chromosomally normal cases, more 'critical cases', that may or may not grow in culture are included in the control group. The rate of incorrect karyotype assessment by maternal cell contamination has been suggested to be as high as 29–58% in conventional analysis.[24] Similarities in distribution of anomalies could, therefore, be due to the fact that both collectives studied overlap and that successfully karyotyped 'critical cases' tend to be chromosomally abnormal.

Autosomal trisomies were most common in both groups, yet of a higher abnormality rate (68.3%) in chromosomally abnormal CGH cases compared to a rate of 53.9% in our non-CGH cases and approximately 55% in pooled literature data. Complete trisomy for each chromosome, including chromosome 1, has meanwhile been identified in early spontaneous abortions with trisomies 16, 21 and 22 being the most frequent.[25,26] The distribution of individual trisomies in our CGH and non-CGH cases, however, are comparable with the exception of trisomy 7. The high incidence of trisomy 7 (10.2%) in our CGH series is most striking. Together with trisomy 22, trisomy 7 is the second most common trisomy compared to a 3.2% rate of our non-CGH control group and a 3.4% rate of other studies.[26] However, intergroup differences in the frequency of trisomy 7 were of borderline significance (P=0.08). Recent molecular studies of autosomal trisomies 16, 18, and 21 have shown that non-disjunction originated predominantly from maternal meiosis I and that factors such as abnormal recombinations play an important role in its genesis.[27–29] Zaragoza *et al*[30] confirmed this for trisomies 15 and 22 but revealed a different etiology for chromosome 7. In more than half of the cases trisomy 7 originated from mitotic non-disjunction. Furthermore, chromosome 7 has been shown to be imprinted. Pre- and postnatal growth failure associated with maternal uniparental disomy 7 has been reported indicating the presence of several imprinted growth genes or growth receptors such as peg1/mest, GRB10.[31] However, trisomy 7 is a relatively common pseudomosaic finding in prenatal cytogenetic analysis suggesting that at least in the mosaic condition proliferative disadvantage may be compensated by euploid cells.[32,33]

The frequency of triploidy in the growth failure CGH series (17.1%) was slightly above that of our control group (15.6%). Warburton *et al*[34] distinguished between triploid abortions due to dispermy and digyny, and calculated relations of 1XXX : 2XXY : 1XYY for the former and 1XXX : 1XXY for the latter condition. In practice XYY triploids have rarely been observed, accounting for about 3% of all triploids and were associated with early embryonic demise.[34,35] In our own material we found a relation of 1.5XXX : 1XXY : 1XYY (n=7) in the CGH series and of 1XXX : 1.5XXY : 0.08XYY (n=459) in the cytogenetically analysed abortion material. The observed differences in

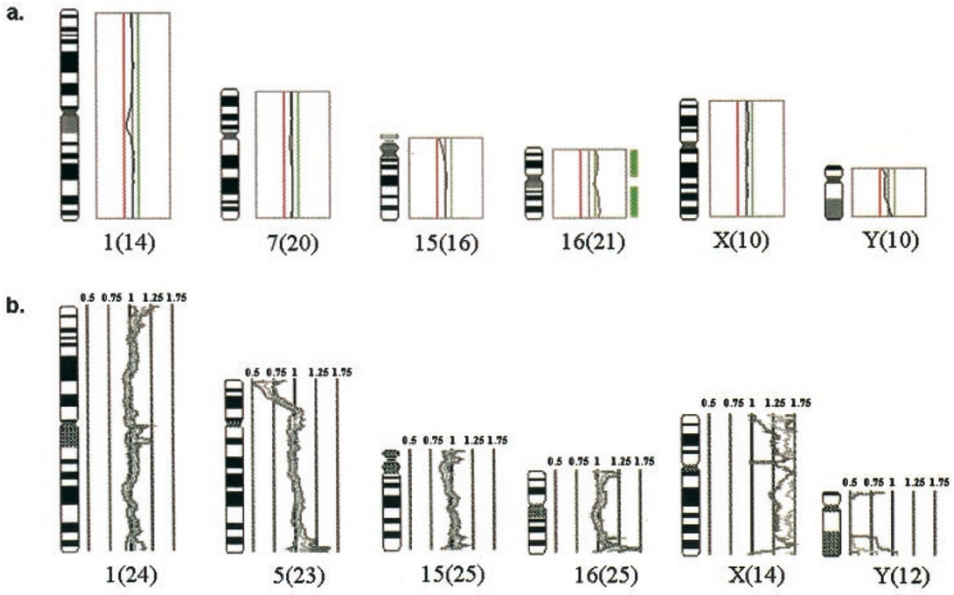

Figure 1 (a) missed abortion in 11th g.w. (MR-159); partial CGH profile showing male karyotype with gain of chromosome 16. Chromosomes 1, 7, 15 are added for comparison. (b) missed abortion in 10th g.w. (MZ-1520); partial CGH profile showing female karyotype with loss of chromosome 5p14→pter (chromosome 1, 15, 16 shown for normal profiles).

Table 2 Characteristics of spontaneous abortions which failed to grow in culture

CHG data of abortion with culture failure	*Cases*		*Mean maternal age (years)*	*Mean gestational age (weeks)*	*Sex ratio m : f*
	n	*%*			
Total number	60				
Successfully analysed	57	95			
Normal	16	28.0	29.9±3.9	9.5±1.4	0.8
Aberrant*1	41	72.0	32.4±5.0	10.0±1.7	0.9
Autosomal trisomy*2	28	68.3	32.0±5.0	9.9±1.6	1.3
Monosomy X	4	9.8	31.3±3.9	10.8±0.8	–
Triploidy	7	17.1	34.6±5.4	9.7±2.0	1.3
Tetraploidy	–	–	–	–	–
Structural	1	2.4	38	10	–
Other	1	2.4	30	9	–
Mosaics ChA%*2	3	7.3	28.0±5.0	8.7±1.2	3.01

*1 including one complete mole; *2 including three mosaic karyotypes. Mosaic karyotypes have been included in the major aberration group. ChA=Chromosomal abnormality.

numbers of XYY-triploids was statistically significant (P=0.02). We, therefore, suggest in accordance with the early observations of Boué *et al*[17] that the small number of 69,XYY constitution in the reported surveys can be explained by the fact that this karyotype leads to very early developmental arrest and to insufficient growth in tissue culture. The excess of females observed in spontaneous abortions may be due to karyotyping of maternal cells giving rise to an underestimation of the true incidence of aneuploidy in spontaneous abortions.[24,36,37] Interestingly, CGH evidenced an altered male : female sex ratio of 1 : 1.3 (0.77). In CGH, it is methodically unlikely for this altered sex ratio to be due to maternal cell contamination. We, therefore, postulate that the predominance of the females in culture failures represents a biological phenomenon.

Despite our promising results, however, CGH does have some limitations in the evaluation of abortion specimens. Ploidy changes found in approximately 30% of abortion specimens are not detected as shown for all 69,XXX cases in our series. One further troubling aspect of CGH for the detection of copy number anomalies is that this assay is insensitive in regions of high variability which is relatively

Table 3 Control group comprising 4693 successfully karyotyped first trimester spontaneous abortions (pooled data from Mainz and Marburg)

First trimester abortion specimens	*Cases* *n*	*%*	*Mean maternal age (years)*	*Mean gestational age (weeks)*	*Sex ratio m : f*
Total number	6631 (43)	–			
Successfully analyzed	4693 (379)	70.8 (87.5)			
Normal	1653 (110)	35.2 (29.0)	30.3±4.5	9.8±1.6	0.6 (1.0)
Aberrant[a]	3040 (269)	64.8 (71.0)	31.4±4.8	9.9±1.4	0.9[b] (0.9)
Autosomal trisomy	1638 (158)	53.9 (58.8)	32.8±4.6	9.8±1.3	0.9 (1.1)
Monosomy X	355 (41)	11.7 (15.3)	29.8±4.0	10.6±1.3	–
Triploidy	459 (25)	15.6 (9.3)	29.6±3.9	9.7±1.5	1.6 (1.3)
Tetraploidy	344 (13)	11.3 (4.8)	29.9±4.3	9.6±1.4	0.7 (1.2)
Structural	275 (31)	9.0 (11.5)	30.8±4.6	9.5±1.4	0.7[c] (1.8)
Mosaic %ChA	457 (26)	15.0 (11.6)	30.7±4.5	9.6±1.4	0.6[c] (1.0)

[a]Complete hydatiform moles with 46,XX-karyotype included. [b]Complete hydatiform moles with 46,XX-karyotype and cases with monosomy X excluded. [c]Cases with monosomy X excluded. Number in brackets mark separate data from Marburg showing higher culture success and chromosomal aberration rates and lower maternal contamination indicated by a sex ratio of 1.0. Mosaic karyotypes have been included in the major aberration group since differing numbers of cells analysed made a more accurate estimation of mosaicism difficult.

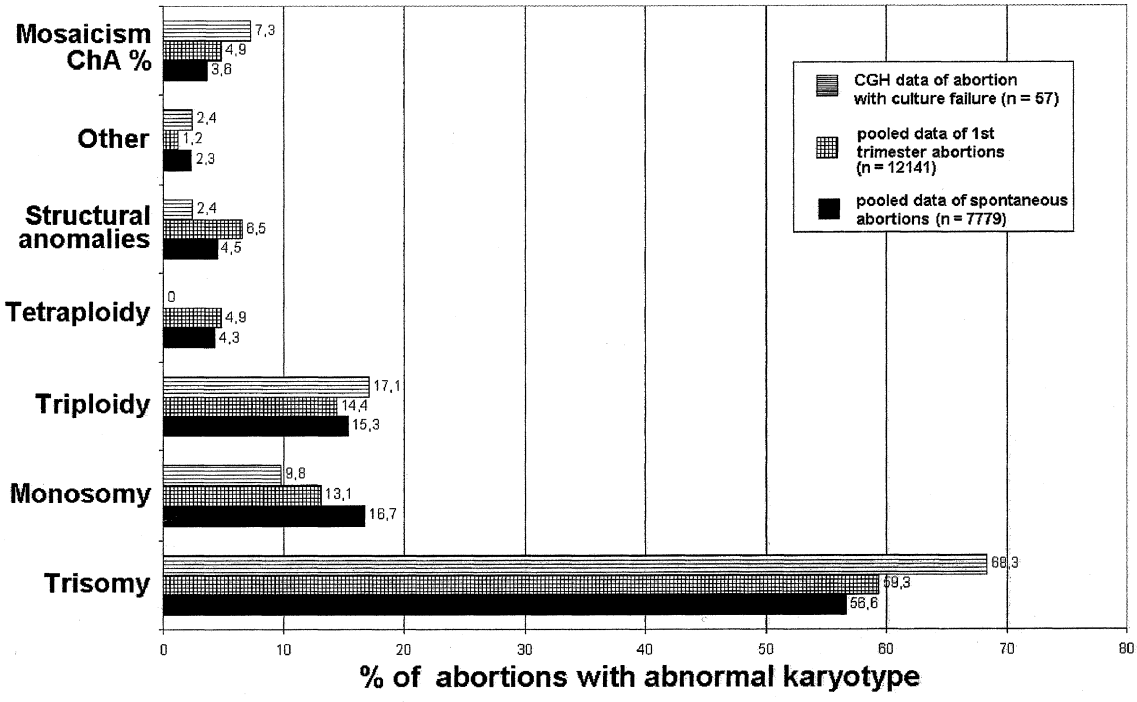

Figure 2 Distribution of chromosome anomalies: comparison of CGH data on abortions after culture failure with pooled data of cytogenetically analysed first trimester spontaneous abortions[19,20,23,42–45, this study] and with pooled data of spontaneous abortions including abortions beyond 14 g.w.[18,22,34,36,46–56]

important for the chromosome regions 1p32→pter, 16p, 19 and 22.[15] Trisomy 16 and 22, however, are frequently found in abortions accounting for about 40–50% of the trisomic specimens.[26] Therefore, additional methods such as microsatellite analysis or interphase FISH are required, and as shown in our series can bridge this gap. Theoretically, detection of deletions by CGH is in the range of 2–5 Mb.[38] To date, however, only deletions in the 10–15 Mb range have been successfully demonstrated by CGH.[5,7,39] The 5p deletion comprising the region 5pter→p14 identified by

CGH in our series was well above the lower detection limit (Figure 1).

At the time CGH is predominantly restricted to research applications, but recently, this method was successfully applied in clinical cytogenetics to detect and characterise whole or segmental aneuploidies on different kinds of tissues such as blood, chorionic villi, and amniocytes.[6–8,40,41] Since CGH requires such highly specialised techniques as DNA extraction, labelling using nick-translations kits and hybridisation, setting up CGH for clinical use demands time and effort. An average cytogenetic laboratory needs about 6 months to establish the technique.[15] This can be confirmed by our laboratory. The main disadvantage of CGH for a cytogenetic service laboratory, however, may be the cost of the required image analysis system. Once established, we estimate, that the cost of CGH is within a reasonable range compared with other cytogenetical methods using *in situ* hybridisation.

Nevertheless, CGH extends the diagnostic spectrum considerably facilitating diagnosis of chromosome aberrations in hitherto unresolved abortion cases. On the basis of the present data it seems reasonable to assume that the genetic contribution to pregnancy losses is still underestimated. Pooled data of 22 studies using conventional cytogenetic methods result in an average frequency of chromosome abnormalities of 52% (Figure 2). Restricting estimations to first trimester abortion cases only, the frequency rate is higher at 57%. Estimations thus far, however, do not take into account cases of culture failures amounting to 10–60% of conventional analyses. If we transfer our results of a 72% chromosome aberration rate among cases with culture failure onto the total number of culture failure in our cytogenetic study we reach an aberration rate of 67% among first trimester spontaneous abortions. Therefore, in view of our investigation into this hitherto unexplored segment the total percentage of aberrant early foetal losses has to be estimated well beyond 65%. By elimination of maternal cell contamination the aberration rate would be further enhanced. This rate of chromosomal aneuploidy in early spontaneous abortions is higher than, in general, currently accepted. Since the outcome of subsequent pregnancies varies on the presence/absence of chromosome disturbances in a previous abortion correction towards a higher aberration rate gives a more positive outlook in counselling patients with a past history of early foetal losses.

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