Overview of Chromosome Abnormalities in First Trimester Miscarriages: A Series of 1,011 Consecutive Chorionic Villi Sample Karyotypes

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Keywords

Chorionic villi sampling · Chromosome abnormalities · First trimester miscarriages · Placental mosaicism

Abstract

In order to contribute to the knowledge of type and frequency of chromosome abnormalities in early pregnancy losses, we analyzed the cytogenetic results from a large series of first trimester miscarriages, using a diagnostic approach with a high success rate and no maternal contamination. A total of 1,119 consecutive chorionic villi samples were obtained before evacuation, and karyotypes were prepared after short-term culture (STC). In 603 samples, a long-term culture (LTC) was also performed. The overall and individual frequencies of the different types of chromosome abnormalities were established, including placental mosaicisms, and their relationship with maternal age and gestational weeks was assessed. An abnormal karyotype was detected in 70.3% of the samples. Single autosomal trisomy was the most frequent abnormality (64.6% of the abnormal cases), followed by triploidy (13.1%) and monosomy X (10.4%). Chromosome rearrangements were found in 5.2%, combined abnormalities in 8.9%, and placental mosaicism in 3.5% of the cases with STC and LTC performed. Individual trisomies behaved

differently with respect to maternal age and intrauterine survival. Due to the combination of STC and LTC, our study offers reliable information on the incidence and type of chromosome abnormalities and placental mosaicism in miscarriages and contributes to define the cytogenetic implication in their etiology.

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Around 10–15% of clinically recognized pregnancies result in miscarriage [Gardner et al., 2012], and about 50% of early pregnancy losses have chromosome abnormalities [Warburton, 2000]. Trisomies are the most frequently detected anomalies (61.2%), followed by triploidies (12.4%), monosomy X (10.5%), tetraploidies (9.2%), and structural chromosome anomalies (4.7%) [Eiben et al., 1990]. Thus, cytogenetic analysis of spontaneous miscarriages is essential to establish the etiology of fetal losses and to assess patients with risks of recurrence in future pregnancies. Generally, analyses are performed on fetal tissues collected after evacuation of the products of conception, and karyotyping usually has a low success rate. Conventional tissue culturing is laborious and impaired by bacterial contamination, culture failure, and/or overgrowth of maternal cells present in the sample [Bell et al., 1999]; the lat-

ter often leading to an overdiagnosis of normal female karyotypes [Lathi et al., 2014]. Analysis of chorionic villi (CV) obtained by sampling before evacuation minimizes external microbial contamination, and karyotyping after short-term culture (STC) guarantees the embryonic origin of the sample. In addition, it is a rapid method that allows the detection of a broad spectrum of chromosome abnormalities [Morales et al., 2008]. If a long-term culture (LTC) is added, placental mosaicism can be identified, but this is a completely unexplored field in miscarriages.

Recent molecular approaches such as chromosomal microarray-based analysis (CMA) use DNA isolated from CV or fetal tissue samples. Its success rate has been reported to be slightly higher than that expected for cytogenetic analysis, since it does not require cell culture. CMA has a much higher ability to detect subtle genomic changes than conventional cytogenetics, but it is unable to detect balanced structural rearrangements, some polyploidies [Robberecht et al., 2009; Dhillon et al., 2014], and presents difficulties to detect low-level mosaicisms. More recently, SNP-based CMA has increased the diagnostic power, adding the detection of uniparental disomy [Levy et al., 2014; Sahoo et al., 2017]. However, the involvement and relevance of small chromosome imbalances as well as uniparental disomy of individual chromosomes in early pregnancy losses still needs to be established, and variants of unknown significance can be detected [Dhillon et al., 2014], which could lead to anxiety in the parents and hinder the subsequent genetic counseling.

To contribute to the knowledge of type and frequency of chromosome abnormalities in early pregnancy losses, we analyzed the cytogenetic results in the largest Spanish series of first trimester spontaneous miscarriages (>1,000 samples), obtained through CV sampling before evacuation. We also compared our results with those of previously published large series obtained by culture of CV or fetal tissue samples. Furthermore, for the first time, we present data on placental mosaicism in miscarriages and an overview of the viability of different aneuploidies during the first trimester of pregnancy.

Methods

Since 1997, CV sampling has been offered to women with a diagnosis of miscarriage established at the ultrasound examination. Some of them had already been scheduled for CV sampling due to a high risk of fetal aneuploidy, and miscarriage was diagnosed in the scan prior to the procedure.

A total of 1,119 CV samples were received for cytogenetic diagnosis. Gestational ages ranged from 5 to 13 + 6 weeks according

Table 1. Classification of cases with abnormal karyotypes according to the main abnormality detected

Main abnormality	<i>n</i>	%
Single autosomal trisomy	459	64.6
Double/multiple trisomy	29	4.2
Sex chromosome trisomy	2	0.3
X monosomy ^a	74	10.4
Autosomal monosomy ^b	6	0.8
Triploidy ^c	93	13.1
Tetraploidy ^d	10	1.4
Balanced rearrangement ^e	6	0.8
Unbalanced rearrangement ^f	31	4.4
Chimera	1	0.1
Total	711	100.0

^a Including 4 cases with a combined trisomy. ^b Including 1 case with a combined trisomy and another with 2 small supernumerary marker chromosomes. ^c Including 9 hypertriploidies. ^d Including 1 hypertetraploidy. ^e Including 4 cases with a combined trisomy. ^f Including 2 cases with a combined monosomy X.

to ultrasound. In our Prenatal Diagnosis Unit, 1,002 CV samples were obtained by transcervical sampling before evacuation. Since 2006, we also received samples from other public centers around Barcelona (*n* = 117). All samples were subjected to the same procedures and evaluation criteria. Some of the early cases have been previously published [Morales et al., 2008; Muñoz et al., 2010; Grande et al., 2012].

All CV samples were collected in warmed RPMI 1640 medium (BioWhittaker, Cambrex, Belgium) and delivered to the laboratory within a few hours. They were inspected under the dissecting microscope to release villi from maternal decidua and blood clots. Villi were divided into fragments of 3–5 mg, which were processed independently by the semi-direct method after 24–48-h incubation (STC) to obtain G-banded metaphase chromosome preparations. Moreover, in 603 out of the last 905 samples, an LTC was also performed. G-banding was done using the Wright technique.

When an unexpected structural chromosome abnormality was found in a CV sample, parental karyotypes were performed according to standard protocols.

For the analysis of the results, in a first approach, pregnancies were analyzed altogether to establish the frequency of the different types of chromosome abnormalities. Afterwards, results for numerical chromosome abnormalities were reanalyzed, classifying pregnancies according to maternal age (<35 years and ≥35 years) and gestational weeks: group I (from 5 to 6 + 6 weeks), group II (from 7 to 8 + 6 weeks), group III (from 9 to 11 + 6 weeks), and group IV (from 12 to 13 + 6 weeks). Contingency tables were created and χ^2 test was used for statistics to analyze the relationship between maternal age or gestational weeks and chromosome abnormality. Significance was set at *p* < 0.05.

Table 2. Involvement of the different autosomes in the abnormal karyotypes

Chromosome	Single trisomy	Trisomy with balanced rearrangement	Trisomy with unbalanced rearrangement	Trisomy with monosomy	Polyploidy with extra chromosome	Double/multiple trisomy	Total extra chromosomes
2	8			1	1	4	14
3					1	4	5
4	12				1		13
5	1						1
6	7					1	8
7	13				1	2	16
8	8				1		9
9	12						12
10	13					1	14
11	3					2	5
12	6						6
13	30		2	1		6	39
14	15		5			4	24
15	65	1	2	2	1	4	75
16	86	2				7	95
17	2						2
18	24	1				5	30
20	13				3	7	23
21	56		1			9	66
22	85		2			5	92
Total	459	4	12	4	9	61 ^a	549

Chromosomes 1 and 19 were not involved. ^a These 61 chromosomes were found in 29 cases: 28 with 2 different extra chromosomes and 1 with 5 extra chromosomes.

Results

The STC was employed in the 1,119 samples received, and a complementary LTC in 603. Cytogenetic results could be obtained in 1,011 samples (in 411 of them exclusively from the STC, in 8 from the LTC, and in 592 from both cultures). Accordingly, the success rate was 90.3%.

Abnormal karyotypes were observed in 711 cases, giving an incidence of 70.3% for chromosome abnormalities. A normal male karyotype was observed in 156 cases, and a normal female karyotype in 144 cases, resulting in a sex ratio of 1.08 among normal cases. Table 1 summarizes the chromosome abnormalities detected in the present series.

Autosomal Trisomy

Autosomal trisomy was the most frequent chromosome abnormality diagnosed, found in 517 cases (including the cases with a polyploidy plus an extra chromosome). Among them, 459 were single autosomal trisomies: 110 involved chromosomes 13, 18, or 21; 236

involved chromosomes 15, 16, or 22 (“common” non-viable trisomies); and 113 the remaining chromosomes (“rare” non-viable trisomies), with the exception of chromosomes 1, 3, and 19. Mosaicism with a chromosomally normal cell line was observed in 14/459 cases. Table 2 shows the involvement of each autosome in single trisomies as well as in combined abnormalities. Among these combined or complex abnormalities, 29 cases showed double or multiple trisomy, 4 presented an additional monosomy, 4 a balanced structural rearrangement, 12 an unbalanced rearrangement and 9 a polyploidy.

Sex Chromosome Trisomy

Only 2 sex chromosome trisomies were detected: one 47,XXX and one 47,XXY.

Monosomy

Monosomy was detected in 82 samples. Monosomy X accounted for 92.7% of the cases, thus representing 10.7% of the abnormal karyotypes. Sixty-nine cases showed a pure 45,X karyotype, 1 showed mosaicism (45,X/46,XX),

Table 3. Discrepancies between short-term- and long-term-culture results

Case	Short-term culture	Long-term culture
1	46,XY	47,XY,+16/46,XY
2	46,XY	46,XY,i(15)(q10)
3	46,XY	48,XY,+3,+16
4	46,XY	48,XY,+2,+15/46,XY ^a
5	46,XY	48,XY,+2,+15/46,XY ^a
6	46,XY	92,XXYY
7	46,XX	47,XX,+8
8	46,XX	47,XX,+14
9	46,XX	47,XX,+21
10	46,XX	46,XX,add(10)(p)
11	46,XX	46,XX,der(11)t(11;18)
12	46,XX	92,XXXX
13	47,XY,+6	46,XY
14	47,XY,+7	46,XY
15	45,X	46,X,+2
16	47,XX,+4	47,XX,+4/46,XX
17	47,XY,+7	47,XY,+7/47,XY,+18
18	47,XY,+16	47,XY,+16,inv(20)/47,XY,+16
19	48,XY,+2,+21	47,XY,+21/46,X,+21
20	48,XY,+15,+16	47,XY,+16
21	70,XXX,+3/69,XXX	69,XXX

^a Samples from a twin pregnancy and described as independent when received; but they must belong to the same twin.

and in 6 cases monosomy X was observed with another chromosome abnormality: autosomal trisomy (4 cases) or structural rearrangement (2 cases). The remaining monosomies involved chromosome 21 (4 cases), chromosome 8, and chromosome 13.

Triploidy

Triploidy was the second most frequent abnormality diagnosed ($n = 93$), accounting for 13.1% of the abnormal karyotypes. Eighty-four cases showed pure triploidy: 32 cases with 69,XXX, 51 cases with 69,XXY, and a single case with 69,YYY. Eight cases showed hypertriploidy with an extra autosome (Table 2), and the remaining case had an extra chromosome X.

Tetraploidy

Tetraploidy was found in 1.4% of the abnormal cases ($n = 10$). Five cases showed a 92,XXXX karyotype, 3 cases 92,XXYY, and 1 case had an extremely rare 92,XXXY karyotype. In the remaining case, an extra chromosome was present in addition to the tetraploidy (93,XXYY,+20) (Table 2). In 2 cases, the non-mosaic tetraploid cell line was only present in the LTC.

Chromosome Rearrangements

Six cases showed an apparently balanced chromosome rearrangement. Four of them were observed with an additional trisomy (Table 2). The balanced chromosome rearrangements diagnosed were 3 reciprocal translocations and 3 pericentric inversions. Four rearrangements were inherited (only one of the translocations was previously known), one was de novo and no information was available from the remaining case.

An unbalanced chromosome rearrangement was found in 4.4% of the abnormal cases ($n = 31$). Among them, there were 17 single unbalanced rearrangements (2 terminal deletions, 5 duplications, 6 unbalanced reciprocal translocations, and 4 isochromosomes), 12 unbalanced robertsonian translocations, and 2 cases with a combination of an unbalanced rearrangement and monosomy X. Three out of the 6 reciprocal translocations were previously unknown: 2 of them turned out to be familial and 1 de novo. Only 2 out of the 12 robertsonian translocations were previously known; among the unexpected ones, 8 were de novo, 1 inherited, and 1 with no information available.

Double/Multiple Chromosome Abnormalities

In 8.9% of the abnormal cases ($n = 63$), the chromosome abnormality was not a single alteration. Forty-two cases showed double or multiple numerical aberrations, including double trisomy, multiple trisomy, hypertriploidy, hypertetraploidy or trisomy plus monosomy. Twenty-one cases showed different combinations of numerical and structural abnormalities, including monosomy plus marker chromosomes, structural rearrangements with trisomy or monosomy, and a few cases of rare discrepancies between STC and LTC (included in Table 3).

Placental Mosaicism: Discrepancies between STC and LTC Results

In 603 cases where enough CV could be sampled, both STC and LTC were performed. However, in 8 of them no growth was obtained from the STC, and in 3 from the LTC; so a total of 592 samples gave results from both cultures. Among them, 21 (3.5%) showed discrepant karyotypes. In 12 cases, the STC result was normal and the chromosome abnormality was only present in the LTC, in 2 cases the abnormality was only present in the STC, and there were 7 cases with discrepant abnormal karyotypes between both cultures (Table 3).

Chromosome Abnormalities and Maternal Age

Maternal age was available in 980 cases, and ranged from 17 to 48 years. The patients were divided into 2 age groups: advanced maternal age (AMA, ≥ 35 years; $n = 582$) and young maternal age (YMA, < 35 years; $n = 398$). The rate of chromosome abnormalities in the AMA group was significantly higher (76.8%) than in the YMA group (61.3%) ($p < 0.0001$). When we investigated differences regarding aneuploidy types between both groups (including only single aneuploid karyotypes), we found a significantly higher rate of trisomy 15, 21, and 22 in the AMA group compared to the YMA group: 8.93 versus 3.02% for trisomy 15 ($p = 0.0002$), 6.87 versus 3.77% for trisomy 21 ($p = 0.0381$), and 12.03 versus 4.02% for trisomy 22 ($p < 0.0001$). The group of rare non-viable trisomies also showed a significantly higher incidence in the older group of patients ($p = 0.0009$). On the contrary, triploidies were significantly higher in the YMA group than in the AMA group (14.07 vs. 5.33%, $p < 0.0001$). The rates for trisomy 13, 18, 16, monosomy X, and tetraploidy were similar between both groups (Table 4). There were 26 cases with double trisomy, representing 1.51% of the abnormal karyotypes in the YMA group and 3.26% in the AMA group (statistically not significant, $p = 0.1318$).

Chromosome Abnormalities and Weeks of Gestation

Gestational ages were established according to the ultrasound scan and were available in 976 cases. They were classified in the 4 groups described above: group I ($n = 263$), group II ($n = 323$), group III ($n = 288$), and group IV ($n = 102$). The percentage of chromosome abnormalities found in each group was 75.67, 77.09, 66.32, and 52.94%, respectively. The higher rate found in group I + II compared with group III + IV was statistically significant with $p < 0.0001$. When calculating rates for individual aneuploidies, trisomies related to unbalanced robertsonian translocations and aneuploidies combined with unrelated balanced reciprocal translocation were also included. There was a higher incidence of trisomy 13 in group IV compared to the rest of groups altogether (11.11 vs. 3.91%, $p = 0.0345$). Trisomy 21 was also significantly higher in group III + IV compared with group I + II (16.33 vs. 3.79%, $p < 0.0001$). This trend was also observed for trisomy 18, showing a rate of 7.76% in group III + IV and 1.34% in group I + II ($p < 0.0001$). On the contrary, the rates for trisomies 15, 16, and 22 were significantly increased in groups I and/or II compared to groups III and/or IV: 18.88% for trisomy 15 in group II versus 4.05% in group I + III + IV ($p < 0.0001$); 22.61% for trisomy 16 in group I versus 7.69% in group II + III + IV ($p < 0.0001$);

Table 4. Rates of numerical chromosome abnormalities in both maternal age groups

	Incidence, %	
	< 35 years	≥ 35 years
Trisomy 13	2.26 (9/398)	3.78 (22/582)
Trisomy 18	2.51 (10/398)	2.41 (14/582)
Trisomy 21	3.77 (15/398)	6.87 (40/582)
Trisomy 15	3.02 (12/398)	8.93 (52/582)
Trisomy 16	8.54 (34/398)	9.11 (53/582)
Trisomy 22	4.02 (16/398)	12.03 (70/582)
Trisomy 14	0.75 (3/398)	2.23 (13/582)
Other autosomal trisomies ^a	6.78 (27/398)	12.20 (71/582)
Triploidies	14.07 (56/398)	5.33 (31/582)
Tetraploidies	1.01 (4/398)	1.03 (6/582)
Monosomy X	8.54 (34/398)	5.67 (33/582)

Significantly different rates are in bold. ^a Only the most frequent individual trisomies are listed as separate categories, the remaining ones are grouped.

14.96% for trisomy 22 in group I + II versus 7.35% in group III + IV ($p = 0.0030$). Regarding monosomy X and triploidy, the rates in group I were statistically lower than those found in groups II + III + IV (1.01 vs. 13.56% for monosomy X and 4.52 vs. 15.99% for triploidy, $p < 0.0001$) (Table 5). For double trisomies, there was a statistically significant higher incidence in group I compared to the rest of groups altogether (7.54 vs. 1.82%, $p = 0.0005$).

Discussion

To our knowledge, the present study represents the largest Spanish series of cytogenetic analyses of first trimester spontaneous miscarriages. The strength of our dataset is the reliability of the results due to the homogeneous cytogenetic procedures carried out in a single laboratory, the high success rate of the strategy used, and the minimization of misdiagnosis due to maternal cell contamination. Moreover, for the first time, we provide information about placental mosaicism in miscarriages and an overview of the viability of different aneuploidies during the first trimester of pregnancy.

Success Rate, Abnormality Rate, and Overall Frequencies

We obtained a 90.3% rate of successful chromosome analysis, which is higher than the rates reported in some of the previous large studies that performed cytogenetic

Table 5. Distribution of chromosome abnormalities according to gestational age

	Incidence, <i>n</i> (%) ^a					
	group I (5–6 + 6 wg)	group II (7–8 + 6 wg)	group III (9–11 + 6 wg)	group IV (12–13 + 6 wg)	unknown	total
Normal	64 (24.33)	74 (22.91)	97 (33.68)	48 (47.06)	17 (48.57)	300 (29.67)
Abnormal	199 (75.67)	249 (77.09)	191 (66.32)	54 (52.94)	18 (51.43)	711 (70.33)
Trisomy 13 ^b	9 (4.52)	7 (2.81)	9 (4.71)	6 (11.11)	1 (5.56)	32 (4.50)
Trisomy 18 ^b	2 (1.01)	4 (1.61)	14 (7.33)	5 (9.26)	0 (0.0)	25 (3.52)
Trisomy 21 ^b	4 (2.01)	13 (5.22)	30 (15.71)	10 (18.52)	0 (0.0)	57 (8.02)
Trisomy 15 ^b	11 (5.53)	47 (18.88)	7 (3.66)	0 (0)	2 (11.11)	67 (9.42)
Trisomy 16 ^b	45 (22.61)	7 (2.81)	26 (13.61)	5 (9.26)	3 (16.67)	86 (12.10)
Trisomy 22 ^b	28 (17.07)	39 (15.66)	15 (7.85)	3 (5.56)	1 (5.56)	86 (12.10)
Trisomy 14 ^b	5 (2.51)	10 (4.02)	3 (1.57)	1 (1.85)	0 (0.0)	19 (2.67)
Other trisomies ^b	50 (25.13)	21 (8.43)	21 (10.99)	5 (9.26)	1 (5.56)	98 (13.78)
Monosomy X ^b	2 (1.01)	40 (16.06)	22 (11.52)	5 (9.26)	1 (5.56)	70 (9.85)
Triploidy ^b	9 (4.52)	45 (18.07)	24 (12.57)	10 (18.52)	4 (22.22)	92 (12.94)
Tetraploidy ^b	4 (2.01)	2 (0.80)	3 (1.57)	1 (1.85)	0 (0.0)	10 (1.41)
Others	30 (15.08)	15 (5.62)	17 (8.90)	3 (5.56)	5 (27.78)	70 (9.70)
Total	263	323	288	102	35	1,011 (100)

wg, weeks of gestation. ^a The percentage for each type of abnormality was calculated in relation to the total number of abnormal karyotypes in the respective group of gestational age. ^b This category comprises single chromosome abnormalities, including trisomies related to unbalanced robertsonian translocations, and aneuploidies combined with unrelated balanced rearrangements.

Table 6. Summary of the data from the present study and other large series previously published focusing on cytogenetic analysis of spontaneous miscarriages

Study	Samples, <i>n</i>	Gestational weeks range	Tissue	Type of culture	Success rate, %	Abnormality rate, %	Sex ratio ^a
Eiben et al., 1990	983	5–25	CV	CV STC	76	50.1	0.71
Menasha et al., 2005	2,180	NR	CV or FT	CV LTC or solid tissue culture	88	57	0.5
Shearer et al., 2011 ^b	3,418	NR	CV	CV LTC	90	55	1.0
Jenderny, 2014	534	7–34	CV	CV LTC	73	61	0.94
Wang et al., 2014	5,457	NR	CV or FT	CV LTC or solid tissue culture	75	45.7	NR
Present study	1,119	5–13	CV	CV STC (+ LTC in 54%)	90.3	70.3	1.08

NR, not reported; CV, chorionic villi; FT, fetal tissue; STC, short-term culture; LTC, long-term culture. ^a Sex ratio in normal samples. ^b Partial data, elective abortion samples included.

analyses on miscarriages (Table 6) [Eiben et al., 1990; Menasha et al., 2005; Shearer et al., 2011; Jenderny, 2014; Wang et al., 2014]. Our success rate is similar to the 92.4% rate obtained in the recent study of Sahoo et al. [2017] using mostly SNP-array analysis in fresh tissue samples, but lower than the 99.9% rate reported by Levy et al. [2014], although in this case 22% of the samples were not valid due to maternal cell contamination. The reason for our high success rate is that CV samples were obtained before evacuation and were processed within a few hours, thus minimizing microbiological contamination and allowing a high success in STC karyotype achievement.

In our series, the frequency of abnormal karyotypes was 70.3%, which is also higher than the abnormality rates reported in the previously published studies (Table 6). The reasons for these differences probably lie in the fact that gestational ages in our series are lower than in the other studies, and our diagnostic approach, with combination of STC and LTC, reduces the risk of misdiagnosis due to maternal cell contamination to the minimum. The only possibility of misdiagnosis would be in the very unlikely case of a normal female karyotype in STC and a chromosome abnormality confined to mesenchyme with complete overgrowth of maternal cells in LTC.

Table 7. Relative frequency of the different types of chromosome abnormalities in our series and those previously published

Study	Single trisomy	Monosomy X	Triploidy ^a	Tetraploidy ^b	Multiple aneuploidy ^c	Structural rearrangement
Eiben et al., 1990	60.3	10.5	12.4	4.5	1.8	4.8
Menasha et al., 2005	66	9	10.6	1.6 ^d	6.1	4.2
Shearer et al., 2011	58.4	14.2	12.1	3	2.8	7.3
Jenderny, 2014	47	7.6	13.1	3.8	4.6	4.2
Wang et al., 2014	66	13	14	4	NR	2
Present study	64.9	10.7	13.1	1.4	4.5	5.2

All values are given in percentages. NR, not reported. ^a Including near-triploidy. ^b Including near-tetraploidy, excluding mos 92/46 (probably produced in vitro). ^c Including double or multiple trisomy and trisomy plus monosomy. ^d Near-tetraploidy not included.

The relative frequencies of the different groups of cytogenetic anomalies in our series are comparable with those reported in the previously published large studies (Table 7).

Sex Ratio in Normal Karyotypes

An interesting finding was the male:female ratio of 1.08 among normal karyotypes, showing an excess of males in chromosomally normal miscarriages. This figure is in contrast to that observed in the other studies, which show sex ratios equal to or less than 1.0 (Table 6). The excess of female karyotypes can be attributed to maternal cell contamination in the studies based on LTC of CV. However, our data are in contrast to the findings of Eiben et al. [1990]. The differences between both studies include gestational ages, the success rate, and the abnormality rate, both much higher in our study. According to Eiben et al. [1990] the excess of normal female karyotypes increases with gestational age. Earlier miscarriage studies, such as the present one, would account for an increase of normal male karyotypes. However, our sex ratio in karyotypically normal miscarriages is significantly similar to the present Spanish male:female ratio at birth, 1.07 (http://www.indexmundi.com/spain/sex_ratio.html). Our data suggest an initial excess of male conceptuses and argue against a male-specific developmental disadvantage.

Chromosome Abnormalities, Maternal Age, and Gestational Age

Single autosomal trisomies represent the largest class of chromosome abnormalities in spontaneous miscarriages. Trisomy 16 is the most frequent one (18.7% of the single autosomal trisomies), followed by trisomy 22

(18.5%), trisomy 15 (14.2%), and trisomy 21 (12.2%). Trisomy 13 (6.5%) and trisomy 18 (5.2%) are the following categories in frequency.

In our series, monosomy X is the third-most single chromosome abnormality, after trisomy 16 and trisomy 22. All cases except one were non-mosaic. This finding supports the idea that lethality in 45,X cases may be explained by the absence of “rescue” cell lines (i.e., 46,XX) in critical tissues for a correct development (i.e., placenta) [Hook and Warburton, 2014].

A significant correlation was found between AMA and numerical chromosome abnormalities. As expected, the overall abnormality rate was significantly higher in older women. However, analyzing in detail each type of aneuploidy, we found that this relation was only sustained for trisomies 15, 21, and 22. Among the most frequent single trisomies, those of chromosomes 13, 16, and 18 did not correlate significantly with maternal age. The prevalence of monosomy X is slightly higher in young women, but it does not achieve statistical significance. Triploidy was significantly more prevalent in young women, while tetraploidy showed no difference.

Focusing on gestational age, trisomies involving chromosomes 13, 18, and 21 were more prevalent in advanced weeks of gestation, while the ones involving chromosomes 15, 16, and 22 were increased in earlier pregnancies. The same latter results were found for the group of rare non-viable trisomies, with a 24.64% detection rate in group I and a 12.35% rate in group II + III + IV ($p < 0.0001$). These results were expected, since trisomies that can survive until birth (13, 18, 21) are expected to allow longer fetal development, compared with those that, although able to achieve pregnancy, are considered delete-

rious. Our results provide an overview of the viability of different aneuploidies during the first trimester of pregnancy, not reported in previous studies.

Structural Rearrangements

Structural rearrangements were found in 5.2% of the abnormal cases, 6 balanced and 31 unbalanced. This rate is similar to that reported in previous studies (Table 7). Only 6 cases had previously known familial rearrangements. The remaining 31 cases were unexpected findings (83.8% of the rearrangements). Accordingly, in our series, 3.1% of the samples showed an unexpected chromosome rearrangement. Parental karyotyping disclosed 3 reciprocal translocations, 1 robertsonian translocation, and 2 pericentric inversions to be inherited. In 2 cases parental karyotypes were not available. Focusing on balanced abnormalities, only 2 cases (both inherited) could not be related to the fetal demise. The remaining 4 had an additional numerical abnormality.

Acrocentric chromosomes were the only ones involved in structural rearrangements plus trisomy, in the form of unbalanced robertsonian translocations. Noteworthy, at least 66% of them were de novo (1 case was unknown); this fact adds evidence of the high frequency of this kind of chromosome mutations [Bandyopadhyay et al., 2002].

Chromosome rearrangements have especially great impact for couples and their future offspring, and their knowledge allows extending the genetic counseling to other relatives at risk for unbalanced offspring.

Placental Mosaicisms

Discrepant karyotypes between STC and LTC were found in 3.5% of the samples with both cultures performed. Since fetal tissues were not available for study, we might consider that the LTC karyotype most probably reflects the fetal karyotype. In this theoretical scenario, 57% of the discrepancies would represent type V placental mosaicism [Hahnemann and Vejerslev, 1997], 10% would correspond to type I mosaicism, and the remaining 33% to type VI. Accordingly, 90% of the discrepant cases would represent true fetal mosaicism, which would be responsible for the fetal death. The aneuploidy found in the trophoblast of the confined placental mosaicism cases might compromise the placental function and contribute to the demise of a chromosomally normal fetus.

A structural rearrangement was found in 4 cases, and in all of them the anomaly was only present in the LTC. In three of them partial or complete aneusomy was involved (cases 2, 10, and 11), which can be related to the

fetal demise. The rearrangement inv(20) (case 18), was only present in 1 cell line, and is probably a culture artifact arising after an unusually long culture.

Double abnormalities were more frequent in LTC than in STC (33.3 vs. 14%). In 3 cases, the double abnormality was only present in the LTC (cases 3, 4, and 5); in 3 other cases, the second abnormality appeared in the LTC in addition to the first STC abnormality (cases 15, 17, and 18); and in a single case, a third abnormality, not present in the STC, was detected (case 19). Three cases which showed a double numerical abnormality in the STC (cases 19, 20, and 21) lost one of the extra chromosomes in the LTC.

Overall, in the majority of cases (16/21), the LTC karyotype added a new abnormality not present in the STC. This finding is consistent with the results of the large CV study published by Malvestiti et al. [2015], in which mosaicism with an abnormal karyotype in LTC was more frequent than when the abnormality was present in the STC. The higher complexity of the LTC karyotypes might reflect artifacts caused by the prolonged culture time, or contribute to the fetal demise due to the accumulation of chromosome abnormalities in the mesenchyme, representative of the fetal tissues.

Conclusion

We present a large single-center data set on chromosome abnormalities detected in first trimester miscarriages, obtained through CV sampling and conventional karyotyping. Our approach had a high success rate with absence of maternal cell contamination. The combination of STC and LTC revealed an abnormality rate of 70%, which is the highest detection rate, probably due to the exclusive selection of first trimester miscarriages. Moreover, this is the first work reporting data on placental mosaicism in miscarriage samples. Our results on the incidence and types of chromosome abnormalities are similar to those reported in the previous large studies and contribute to define the cytogenetic implication in the etiology of miscarriages.

CMA techniques offer advantages and disadvantages over karyotyping, but their absolute detection rate of causative chromosome abnormalities in the study of miscarriages still needs to be established. Therefore, the complete cytogenetic analysis of the placental tissues is still the most reliable diagnostic approach to reveal the causes of miscarriage. Moreover, due to the diagnosis of both numerical and structural abnormalities (both balanced and unbalanced) by karyotyping, information about the

recurrence risk of miscarriage can be obtained. The disclosure of the cause of the fetal demise decreases significantly parental anxiety and allows providing accurate genetic counseling to families.

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References

- Bandyopadhyay R, Heller A, Knox-DuBois C, McCaskill C, Berend SA, et al: Parental origin and timing of de novo Robertsonian translocation formation. *Am J Hum Genet* 71:1456–1462 (2002).
- Bell KA, Van Deerlin PG, Haddad BR, Feinberg RF: Cytogenetic diagnosis of “normal 46,XX” karyotypes in spontaneous abortions frequently may be misleading. *Fertil Steril* 71:334–341 (1999).
- Dhillon RK, Hillman SC, Morris RK, McMullan D, Williams D, et al: Additional information from chromosomal microarray analysis (CMA) over conventional karyotyping when diagnosing chromosomal abnormalities in miscarriage: a systematic review and meta-analysis. *BJOG* 121:11–21 (2014).
- Eiben B, Bartels I, Bähr-Porsch S, Borgmann S, Gatz G, et al: Cytogenetic analysis of 750 spontaneous abortions with the direct-preparation method of chorionic villi and its implications for studying genetic causes of pregnancy wastage. *Am J Hum Genet* 47:656–663 (1990).
- Gardner RJM, Sutherland GR, Shaffer LG: *Chromosome Abnormalities and Genetic Counseling*, ed 4 (Oxford University Press, Oxford 2012).
- Grande M, Borrell A, Garcia-Posada R, Borobio V, Muñoz M, et al: The effect of maternal age on chromosomal anomaly rate and spectrum in recurrent miscarriage. *Hum Reprod* 27:3109–3117 (2012).
- Hahnemann JM, Vejerslev LO: European collaborative research on mosaicism in CVS (EU-CROMIC)–fetal and extrafetal cell lineages in 192 gestations with CVS mosaicism involving single autosomal trisomy. *Am J Med Genet* 70:179–187 (1997).
- Hook EB, Warburton D: Turner syndrome revisited: review of new data supports the hypothesis that all viable 45,X cases are cryptic mosaics with a rescue cell line, implying an origin by mitotic loss. *Hum Genet* 133:417–424 (2014).
- Jenderny J: Chromosome aberrations in a large series of spontaneous miscarriages in the German population and review of the literature. *Mol Cytogenet* 7:38 (2014).
- Lathi RB, Gustin SLF, Keller J, Maisenbacher MK, Sigurjonsson S, et al: Reliability of 46,XX results on miscarriage specimens: a review of 1,222 first-trimester miscarriage specimens. *Fertil Steril* 101:178–182 (2014).
- Levy B, Sigurjonsson S, Pettersen B, Maisenbacher MK, Hall MP, et al: Genomic imbalance in products of conception: single-nucleotide polymorphism chromosomal microarray analysis. *Obstet Gynecol* 124:202–209 (2014).
- Malvestiti F, Agrati C, Grimi B, Pompilii E, Izzi C, et al: Interpreting mosaicism in chorionic villi: results of a monocentric series of 1001 mosaics in chorionic villi with follow-up amniocentesis. *Prenat Diagn* 35:1117–1127 (2015).
- Menasha J, Levy B, Hirschhorn K, Kardon NB: Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. *Genet Med* 7:251–263 (2005).
- Morales C, Sánchez A, Bruguera J, Margarit E, Borrell A, et al: Cytogenetic study of spontaneous abortions using semi-direct analysis of chorionic villi samples detects the broadest spectrum of chromosome abnormalities. *Am J Med Genet A* 146A:66–70 (2008).
- Muñoz M, Arigita M, Bennasar M, Soler A, Sanchez A, Borrell A: Chromosomal anomaly spectrum in early pregnancy loss in relation to presence or absence of an embryonic pole. *Fertil Steril* 94:2564–2568 (2010).
- Robberecht C, Schuddincx V, Fryns JP, Vermeesch JR: Diagnosis of miscarriages by molecular karyotyping: benefits and pitfalls. *Genet Med* 11:646–654 (2009).
- Sahoo T, Dzidic N, Strecker MN, Commander S, Travis MK, et al: Comprehensive genetic analysis of pregnancy loss by chromosomal microarrays: outcomes, benefits, and challenges. *Genet Med* 19:83–89 (2017).
- Shearer BM, Thorland EC, Carlson AW, Jalal SM, Ketterling RP: Reflex fluorescent in situ hybridization testing for unsuccessful product of conception cultures: a retrospective analysis of 5555 samples attempted by conventional cytogenetics and fluorescent in situ hybridization. *Genet Med* 13:545–552 (2011).
- Wang BT, Chong TP, Boyar FZ, Kopita KA, Ross LP, et al: Abnormalities in spontaneous abortions detected by G-banding and chromosomal microarray analysis (CMA) at a national reference laboratory. *Mol Cytogenet* 7:33 (2014).
- Warburton D: Cytogenetics of reproductive wastage: from conception to birth, in Mark HFL (ed): *Medical Cytogenetics*, pp 213–246 (Marcel Dekker, New York 2000).

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.