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The effect of maternal age on chromosomal anomaly rate and spectrum in recurrent miscarriage

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STUDY QUESTION: Is there any effect of maternal age on chromosomal anomaly rate and spectrum in recurrent miscarriage?

SUMMARY ANSWER: There was no significant difference in the chromosome abnormality rate between sporadic and recurrent miscarriage but the chromosome abnormality rate increased significantly with maternal age.

WHAT IS KNOWN ALREADY: About 50–70% of non-recurrent miscarriages occur because of a chromosomal anomaly, but no agreement about the effect of either maternal age or the number of previous miscarriages on the chromosomal anomaly rate has been reached.

STUDY DESIGN, SIZE, DURATION: A retrospective cohort of 353 miscarriages successfully karyotyped in the same center between 2002 and 2011, grouped according to the number of miscarriages and maternal age.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Among the 353 women, 153 were below 35 years (73 with sporadic, 48 with two and 32 with recurrent miscarriage) and 200 were 35 years or more (81 with sporadic, 55 with two and 64 with recurrent miscarriage). The chromosomal anomaly rate and the anomaly spectrum were compared between sporadic and recurrent miscarriage, within the two maternal age groups, using the chi-square test and the Bonferroni correction for all the *P*-values. Risk of chromosomal anomaly was estimated for maternal age, number of miscarriages and previous live births by multivariate binary logistic regression analysis.

MAIN RESULTS AND THE ROLE OF CHANCE: Sporadic and recurrent miscarriage did not show significantly different chromosomal anomaly rates (68 versus 60%) and maternal age was the only statistically significant predictor of the chromosomal anomaly risk we identified. Some trends were observed in the chromosomal anomaly spectrum when sporadic was compared with recurrent miscarriage: recurrent miscarriage exhibited a decrease in viable trisomies (37 versus 11%) and an increase in non-viable trisomies (38 versus 57%) in women >35 years, together with an increase in unbalanced structural anomalies (4.9 versus 29%) in younger women.

LIMITATION, REASONS FOR CAUTION: The mixed origin of our study population, and the limited number of recurrent miscarriages, particularly in the younger group, limits statistical power to detect differences.

WIDER IMPLICATIONS OF THE FINDINGS: The most commonly observed chromosomal anomaly type in recurrent miscarriage depends on maternal age: non-viable autosomal trisomies in older women and unbalanced structural anomalies in younger women. When a chromosomal anomaly is identified as the cause of miscarriage, additional maternal evaluation may be avoided.

STUDY FUNDING/COMPETING INTERESTS: No competing interests declared.

Key words: chromosomal anomaly / cytogenetic analysis / recurrent miscarriage / pregnancy loss

Introduction

Recurrent miscarriage is generally defined as the loss of three or more pregnancies before 20 weeks of gestation (RCOG, 2003; ESHRE, 2006). This reproductive problem affects 1–5% of the reproductive age population (Roman, 1984; Salat-Baroux, 1988; Stirrat, 1990; Crosignani and Rubin, 1991) and is costly both for patients and for health care systems since the etiology often remains unknown.

Although it is well known that 50–70% of non-recurrent miscarriage occurs because of a chromosomal anomaly, mostly aneuploidy (Simpson, 1980; Sánchez et al., 1999), no agreement on the frequency of these anomalies in recurrent miscarriage has been reached. While some studies demonstrated a similar incidence (Coulam et al., 1996; Stern et al., 1996; Stephenson et al., 2002), others found fewer chromosomal anomalies in recurrent miscarriage (Ogasawara et al., 2000; Sullivan et al., 2004). Furthermore, the effect of maternal age in the chromosomal anomaly rate in recurrent miscarriage is also controversial: while some studies demonstrated a higher frequency of normal karyotypes in younger women, others did not support any relationship between aneuploidy and maternal age (Stephenson et al., 2002; Sullivan et al., 2004).

To resolve the controversies in the aforementioned evidence, we explored the relationship between the number of previous miscarriages, maternal age and the chromosomal anomaly rate and spectrum in a cohort of consecutive karyotyped miscarriages, using regression analysis to avoid confounding factors. To our knowledge, this is the first study in which a cohort of miscarriages (including sporadic and recurrent miscarriage) were karyotyped in the same center in order to explore the effects of maternal age and previous miscarriages on the chromosomal anomaly spectrum.

Materials and Methods

Population

The study population included miscarriages from three pregnancy groups. Idiopathic recurrent miscarriages from our Recurrent Miscarriage Unit were the first group. The second group included pregnancies at high risk of fetal aneuploidy already scheduled for chorionic villi sampling (CVS), due to a previous aneuploidy, advanced maternal age (38 years or more), increased nuchal translucency (NT) (above the 99th percentile) or a risk of fetal aneuploidy of >1/250 identified at the first trimester combined test, in which miscarriage was diagnosed in the scan prior to the procedure. In both these pregnancy groups, karyotyping was offered during the whole study period, from January 2002 to April 2011. Finally, the third group comprised low-risk women with a miscarriage, either diagnosed at a routine first trimester scan or after seeking consultation at our hospital. These women were offered CVS from January 2007 onwards. The inclusion criteria were miscarriage with a gestational sac observed by ultrasound and the woman's consent to undergo CVS before evacuation. Thus, pregnancies of unknown locations, biochemical pregnancy losses and ectopics were excluded from the study. The rationale behind obtaining chorionic villi before uterine evacuation was to improve our 37% cytogenetic success rate achieved in products of conception, and from January 2007, it was considered part of the standard clinical care. Miscarriages were further divided into three groups regarding the total number of the woman's miscarriages: one, two, three or more. Sporadic miscarriage was defined as a single miscarriage with no previous losses, and recurrent miscarriage when there was a history of three or more miscarriages.

At transvaginal ultrasound, the embryo crown-rump length (CRL) or, when no embryo was observed (pre-embryonic miscarriage), the gestational sac was measured. Embryonic demise was diagnosed on the basis of a minimum of 6-mm CRL with no cardiac activity in embryos and pre-embryonic miscarriage on the basis of a 16-mm gestational sac diameter with no embryonic pole (Morin et al., 2006). The gestational age was derived from the embryo CRL according to Robinson and Fleming (1975) when an embryo was observed by ultrasound, but it was considered to be 5 weeks when only the gestational sac was seen (Silver et al., 2011). Regarding the gestational period, miscarriages were considered to be: (i) pre-embryonic, when no embryo was observed within the gestational sac (5 weeks); (ii) embryonic, when the CRL was <32 mm (5–9 weeks) and (iii) fetal, when the CRL was 32 mm or more (10 weeks or more).

CVS and karyotyping

A vaginal speculum was inserted and the cervix was swabbed with povidone solution (Curadona 100 mg/ml, cutaneous solution, Lainco, S.A.). A round-tip curved steel forceps (1.9 mm in diameter and 25 cm in length), first introduced by Rodeck (Area Medica, Barcelona), were inserted transcervically under continuous ultrasound guidance. The number of insertions was not restricted to two, as it is the case in continuing pregnancies. The quality of the samples was evaluated by the clinician who performed the procedure, and they were transported in RPMI culture medium (BioWhittaker® RPMI 1640 medium with L-glutamine). At the laboratory, samples were inspected under the dissecting microscope to release villi from maternal material. The semi-direct method was used for cytogenetic analysis, and preparations of G-banded metaphase chromosomes by the Wright technique were obtained after 20–24 h incubation (Morales et al., 2008). Karyotype was analyzed with the use of the Cytovision (Applied Imaging, Sunderland, Tyne and Wear, UK) software. The number of cells analyzed ranged form 2 to 38.

Data analysis

The chromosomal anomaly rate and types were compared between groups defined by the number of miscarriages, specifically between sporadic (a single miscarriage) and recurrent miscarriage (three or more miscarriages), and also between pregnancy groups (from the recurrent miscarriage unit, high and low aneuploidy risk). Both analyses were performed splitting the whole series into two maternal age groups, using 35 years as a cut-off. Chromosomal anomalies were grouped into the following types: viable autosomal trisomies (trisomies 13, 18 and 21), non-viable autosomal trisomies (other than 13, 18 or 21), autosomal monosomies, sex trisomies, sex monosomies, polyploidies, unbalanced structural anomalies, balanced structural anomalies, double anomalies and mosaicisms. Mosaicism was defined as the finding of more than one cytogenetically distinct population of cells, and only those with a proportion above 10% were considered. The proportions of the different chromosomal anomalies types were compared in three groups of pregnancies defined by the number of losses. SPSS (Statistical Package for the Social Sciences) was used for database and data analysis. Comparison between groups was carried out using 95% confidence intervals (95% CI) for means and proportions. The chi-square test with the Bonferroni correction was used for specific comparisons between sporadic and recurrent miscarriage and between low- and high-risk pregnancy groups. Multivariate binary logistic regression analysis was performed to estimate risk of chromosomal anomaly (odds ratio, OR, with 95% CI for OR) including the following as co-variables: maternal age, number of losses and previous live births. OR for maternal age was expressed in intervals of 5 years.

Results

During the study period (from January 2002 to April 2011), 495 instances of CVS were performed in our Prenatal Diagnosis Unit after miscarriage. In 15% (76/495) of the pregnancies only maternal material was retrieved, and among the remaining 419 samples, successful cytogenetic analysis was achieved in 84% of the cases (353/419), more than 2-fold the previous 37% rate. Among the 66 cytogenetic failures, quantitative fluorescence-PCR provided information on selected chromosomes in 30 cases, but they were excluded from the study. The 353 miscarriages with a full karyotype formed our study population and included 96 pregnancies from our recurrent miscarriage unit, 123 at high risk of aneuploidy, mainly for advanced maternal age or previous history, and 134 at low risk. The mean maternal age was 34.9 years (range 22–45 years), and 57% (200/353) of women were 35 years or more. Forty-four percent (154/353) of women had a single miscarriage (sporadic miscarriage), 29% (103/353) had two and 27% (96/353) had 3 or more (recurrent miscarriage). The mean ultrasound gestational age at the moment of the miscarriage was 7.2 weeks (range 5–15 weeks). According the gestational period, this series included 23% (81/353) pre-embryonic (5 weeks), 62% (218/353) embryonic (5–9 weeks) and 15% (54/353) fetal (≥10 weeks) miscarriages. When comparing sporadic with recurrent miscarriage, a lower gestational age (7.9 versus 6.6 weeks; $P = <0.001$) and a higher frequency of pre-embryonic miscarriages (16 versus 31%; $P = 0.005$) were observed in the latter (Table I).

Normal male chromosomes (46,XY) were observed in 69 karyotypes, while a normal female (46,XX) was found in 54. The chromosomal anomaly rate was 65% (230/353) in the whole series, and it was not significantly different between sporadic (68%; 104/154) and recurrent miscarriage (60%;58/96) ($P = 0.25$) (Table I). The chromosomal anomaly rate increased with maternal age, being 54% (83/153) for women below 35 years and 74% (147/200) for 35 years or more ($P < 0.001$). Maternal age stratification by 5-year periods was as follows: 30% (<25 years), 52% (25–29 years), 58% (30–34 years), 66% (35–39 years) and 89% (>39 years). When comparing younger (<35 years) with older women (≥35 years), a similar remarkable increase (about 22% points) was observed. Thus, the chromosomal anomaly rate was 56 versus 78% in the sporadic miscarriage group ($P = 0.004$) and 44 versus 69% in the recurrent miscarriage group ($P = 0.018$) (Table I). In contrast, no significant differences were found when chromosomal anomaly rates were compared between sporadic and recurrent miscarriage within each of the two age groups: 56 versus 44% ($P = 0.241$) in the younger group and 78 versus 69% ($P = 0.22$) in the older group (Table I). Maternal age was the only significant predictor of chromosomal anomaly identified by the multiple logistic regression model (OR = 1.707, 95% CI = 1.363–2.127). The number of miscarriages and previous live births did not contribute significantly to the prediction model.

The most common chromosomal anomaly type in both maternal age groups was non-viable autosomal trisomies (31% in the younger and 49% in the older group), followed by polyploidies in the younger group (25%) and viable autosomal trisomies in the older group (22%). A trend toward a decreased rate (from 37 to 11%) of viable autosomal trisomies and to an increased rate (from 38 to 57%) of non-viable trisomies was observed in recurrent compared with sporadic miscarriage among women of 35 years or more (Table II). The pattern was different in the younger group where recurrent miscarriages showed a trend toward more unbalanced structural anomalies (4.9 versus 29%) (Table II). No balanced structural anomalies were found. Specific cytogenetic results, stratified by maternal age, are displayed in Table IV.

When the chromosomal anomaly rate was compared between the low-and high-risk pregnancy groups, in the older women a higher rate was observed (60 versus 81%; $P = 0.01$), apparently due to more viable autosomal trisomies. However, among the younger women, the chromosomal anomaly rate was similar between low-and high-risk pregnancy groups (57 versus 59%), in spite of more structural anomalies (1.8 versus 15%) and less non-viable trisomies (41 versus 0%) in the high-risk group (Table III).

Discussion

The 68% chromosomal anomaly rate we observed in sporadic miscarriages is near to the average rate of 63% reported in seven recently published series (Table V). In addition, we found no significant difference in the anomaly rate between sporadic and recurrent miscarriage (68 versus 60%), in agreement with some of the previously reported studies (Coulam *et al.*, 1996; Stern *et al.*, 1996; Stephenson *et al.*, 2002; Marquard *et al.* 2010), and in disagreement with those describing a decreased chromosomal anomaly rate in recurrent miscarriage (Ogasawara *et al.*, 2000; Sullivan *et al.*, 2004) (Table V). Interestingly, Ogasawara *et al.* (2000) described a rising miscarriage rate from 25 to 80% of pregnancies when the number of previous miscarriages increased from 2 to 7 or more, mostly because of an increasing frequency of miscarriages with a normal karyotype, whereas the frequency of chromosomally abnormal miscarriages remained stable.

We found that half of the miscarriages were due to a chromosomal anomaly in women younger than 35 years, increasing to 3/4 in those 35 years or more. In this study, we considered only the relative chromosomal anomaly rates in clinically diagnosed miscarriages, rather than real prevalences. Since the prevalence of miscarriage increases dramatically with maternal age from 9% at 20 years to 75% at 45 years or more (Nybo Andersen *et al.*, 2000), the increase in the true prevalence of chromosomal anomalies is more pronounced than that observed in relative rates. As expected, we found an increased chromosomal anomaly rate, by about 20% points, in women with 35 years or more compared with younger women, both in sporadic and recurrent miscarriage (Table I). Logistic regression demonstrated that maternal age is the only relevant parameter to define chromosomal anomaly risk, and the number of previous miscarriages was not found to contribute significantly in the predictive model. This fact may explain that differences in chromosomal anomaly rates between sporadic and recurrent miscarriage were found only in those reported series with lower mean maternal age (Table V).

When analyzing the chromosomal anomaly spectrum, a trend toward fewer viable autosomal trisomies was found in recurrent miscarriage in both maternal age groups. To which extent the trisomy 21 decline in older women (16 versus 1.6%, data not shown) may be due to first trimester prenatal diagnosis and elective termination before fetal demise occurs is not established, but it is unlikely, since only 15% of miscarriages occurred beyond the 10th week. Alternatively,

Table I Maternal, pregnancy and sampling characteristics according to the number of miscarriages and stratified by maternal age.

	One miscarriage (sporadic)	Two miscarriages	Three or more miscarriages (recurrent)	Total	*P*-value
Maternal age (years) (mean and 95% CI)	34.5 (33.6–35.4)	34.7 (33.7–35.6)	35.8 (34.9–36.7)	34.9 (34.4–35.4)	0.107
<35 years	29.6 (28.8–30.4)	30.4 (29.5–31.3)	31.3 (30.1–32.4)	30.2 (29.7–30.8)	0.063
≥35 years	38.9 (38.3–39.5)	38.4 (37.7–39.1)	38.1 (37.5–38.7)	38.5 (38.2–38.9)	0.176
Gestational age (weeks) (mean and 95% CI)	7.9 (7.5–8.2)	6.8 (6.5–7.2)	6.6 (6.2–6.9)	7.2 (6.9–7.4)	<0.001
<35 years	7.9 (7.4–8.5)	6.9 (6.3–7.4)	6.2 (5.8–6.6)	7.2 (6.9–7.6)	<0.001
≥35 years	7.8 (7.3–8.4)	6.8 (6.3–7.3)	6.8 (6.3–7.2)	7.2 (6.9–7.5)	0.004
Pre-embryonic rate (% and 95% CI)	16% (10–22)	25% (17–34)	31% (22–41)	23% (19–27)	
<35 years	25/154, 14% (6–22)	26/103, 27% (15–40)	30/96, 31% (15–47)	81/353, 22% (15–28)	0.005
≥35 years	10/73, 19% (10–27)	13/48, 24% (12–35)	10/32, 31% (20–43)	33/153, 24% (18–30)	0.035
Cytogenetic success rate (% and 95% CI)	15/81, 80% (75–86)	13/55, 87% (80–93)	20/64, 89% (83–95)	48/200, 84% (81–88)	0.075
<35 years	154/192, 82% (74–90)	103/119, 83% (73–93)	96/108, 82% (70–94)	353/419, 82% (77–88)	0.05
≥35 years	73/89, 79% (71–87)	48/58, 90% (83–98)	32/39, 93% (87–99)	153/186, 86% (81–90)	0.9
Chromosomal anomaly rate (% and 95% CI)	81/103, 68% (60–75)	55/61, 66% (57–75)	64/69, 60% (51–70)	200/233, 65% (60–70)	0.01
<35 years	104/154, 56% (45–68)	68/103, 58% (44–72)	58/96, 44% (27–61)	230/353, 54% (46–62)	0.2
≥35 years	41/73, 78% (69–87)	28/48, 73% (61–85)	14/32, 69% (57–80)	83/153,74% (67–80)	0.2
	63/81	40/55	44/64	147/200	0.2

95% CIs for means and proportions are quoted between periods.

this decrease in viable trisomies may be related to the higher frequency of pre-embryonic miscarriages observed in recurrent miscarriage, in agreement with the association between pre-embryonic miscarriages and non-viable autosomal trisomies, previously reported by our group (Muñoz *et al.*, 2010). Three series have previously assessed the trisomy rates in sporadic and recurrent miscarriage with discrepant results: in one, fewer trisomies were observed in recurrent miscarriage (Ogasawara *et al.*, 2000), while in the others, no differences were found (Stephenson *et al.*, 2002; Sullivan *et al.*, 2004). However, if we would aggregate viable and non-viable trisomies into a single autosomal trisomies group for the whole series, the trend toward a different rate between sporadic and recurrent miscarriage (62 versus 57%, data not shown) would vanish. Thus, our 57% autosomal trisomy rate in recurrent miscarriage is near to 53–68% rates reported by other groups (Ogasawara *et al.*, 2000; Sullivan *et al.*, 2004).

In women below 35 years, chromosomal unbalanced structural anomalies are the most common anomaly in recurrent miscarriage. We assume that the prevalence of unbalanced structural anomalies is maintained with maternal age, while the observed decreased proportion may be due to a rising prevalence of aneuploid recurrent miscarriages. Unbalanced structural anomalies are, in fact, the type of anomalies expected in recurrent miscarriage. However, in a European study, only 5% of the couples with recurrent miscarriage were found to be carriers of a balanced structural anomaly (de Braekeleer and Dao, 1990), and cost effectiveness analysis disrecommended routine parental karyotyping (Barber *et al.*, 2010; Van den Boogaard *et al.*, 2011).

The most common chromosomal anomaly types observed in recurrent miscarriage appear to be dependant on maternal age: non-viable autosomal trisomies in older women and unbalanced structural anomalies in younger women. When a maternal etiology for recurrent miscarriage is identified, treatment should be provided, such as hysteroscopy and resection in uterine septum, progesterone or ovulation induction for luteal phase defects, low-dose aspirin and heparin for antiphospholipid syndrome, heparin in trombophilias and diet and exercise in maternal obesity. When chromosomal anomalies are identified as the cause of the miscarriage, additional maternal work-up may be avoided, resulting in a significant reduction in economic costs (Wolf and Horger, 1995), and a better prognosis for subsequent pregnancies has been reported (Carp *et al.*, 2001).

We have to point out that the main weakness of our study is the mixed origin of our study population, including three different pregnancy groups: recurrent miscarriages, low- and high-aneuploidy-risk pregnancies. Other limitations are the diagnosis of pre-embryonic pregnancies being hampered by embryo reabsortion, and the limited number of recurrent miscarriage, accounting for 27% of the studied population that may restrict the significance of differences. In younger women, recurrent miscarriage accounts for 21% of the cases, and this may explain our disagreement with the lower chromosomal anomaly rate in recurrent miscarriage reported by Stephenson *et al.* (2002). If recurrent miscarriage had been defined as a history of

Table II Distribution of chromosomal anomaly types according to the number of miscarriages, and maternal age groups.

	<35 Years				≥35 Years			
	One miscarriage (sporadic)	Two miscarriages	Three or more miscarriages (recurrent)	Total	One miscarriage (sporadic)	Two miscarriages	Three or more miscarriages (recurrent)	Total
Normal chromosomes	32, 44% (33–55)	20, 42% (28–56)	18, 56% (39–73)	70, 46% (38–54)	18, 22% (13–31)	15, 27% (16–39)	20, 31% (20–43)	53, 27% (20–33)
Abnormal chromosomes	41, 56% (45–68)	28, 58% (44–72)	14, 44% (27–61)	83, 54% (46–62)	63, 78% (69–87)	40, 73% (61–85)	44, 69% (57–80)	147, 74% (67–80)
Viable autosomal trisomies (13,18,21)	3, 7.3% (0.07–15)	4, 14% (1.3–27)	0, 0% (0–0)	7, 8.4% (2.5–14)	23, 37% (25–48)	5, 13% (2.3–23)	5, 11% (2–21)	33, 22% (16–29)
Non-viable trisomies	14, 34% (20–49)	9, 32% (15–49)	3, 21% (0.01–43)	26, 31% (21–41)	24, 38% (26–50)	23, 58% (42–73)	25, 57% (42–72)	72, 49% (41–57)
Autosomal monosomies	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	1, 1.6% (0.15–4.7)	0, 0% (0–0)	0, 0% (0–0)	1, 0.7% (0.06–0.2)
Sex trisomies	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	1, 1.6% (0.15–4.7)	0, 0% (0–0)	0, 0% (0–0)	1, 0.7% (0.06–2)
Sex monosomies	9, 22% (9.3–35)	2, 7.1% (0.24–17)	1, 7.1% (0.63–21)	12, 15% (6.9–22)	3, 4.8% (0.05–10)	5, 13% (2.3–23)	3, 6.8% (0.06–14)	11, 7.5% (3.2–12)
Polyploidies	9, 22% (9.3–35)	9, 32% (15–49)	3, 21% (0.01–43)	21, 25% (16–35)	4 6.3% (0.3–12)	4, 10% (0.7–19)	6, 14% (3.5–24)	14, 9.5% (4.8–14)
Unbalanced structural anomalies	2, 4.9% (0.17–12)	1, 3.6% (0.33–10)	4, 29% (4.9–52)	7, 8.4% (2.5–14)	4, 6.3% (0.3–12)	0, 0% (0–0)	1, 2.3% (0.21–6.7)	5, 3.4% (0.5–6.3)
Double anomalies	3, 7.3% (0.07–15)	3, 10.7% (0.07–22)	1, 7.1% (0.63–21)	7, 8.4% (2.5–14)	2, 3.2% (0.12–7.5)	3, 7.5% (0.07–16)	3, 6.8% (0.06–14)	8, 5.4% (1.8–9.1)
Mosaicisms	1, 2.4% (0.23–7.2)	0, 0% (0–0)	2, 14% (0.4–33)	3, 3.6% (0.04–7.6)	1, 1.6% (0.15–4.7)	0, 0% (0–0)	1, 2.3% (0.21–6.7)	2, 1.4% (0.05–3.2)
Total	73, 100, 00%	48, 100, 00%	32, 100, 00%	153, 100, 00%	81, 100, 00%	55, 100, 00%	64, 100, 00%	200, 100, 00%

95% CIs were calculated for all the proportions. After Bonferroni correction, no significant differences were found between sporadic and recurrent miscarriage.

Table III Distribution of chromosomal anomaly types according to the three pregnancy groups and the two maternal age groups.

	<35 Years				≥35 Years			
	Recurrent miscarriage	Low aneuploidy risk	High aneuploidy risk	Total	Recurrent miscarriage	Low aneuploidy risk	High aneuploidy risk	Total
Normal chromosomes	18, 56% (39–73)	43, 43% (34–53)	9, 41% (20–62)	70, 46% (38–54)	20, 31% (20–43)	14, 40% (24–56)	19, 19% (11–26)	53, 27% (20–33)
Abnormal chromosomes	14, 44% (27–61)	56, 57% (47–66)	13, 59% (39–80)	83, 54% (46–62)	44, 69% (57–80)	21, 60% (44–76)	82, 81% (74–89)	147, 73% (67–80)
Viable autosomal trisomies (13,18,21)	0, 0% (0–0)	4, 7.1% (0.4–14)	3, 23% (0.2–46)	7, 8.4% (2.5–14)	5, 11% (2–21)	3, 14% (0.07–29)	25, 31% (21–41)	33, 22% (16–29)
Non-viable trisomies	3, 21% (0.01–43)	23, 41% (28–54)	0, 0% (0–0)	26, 31% (21–41)	25, 57% (42–72)	12, 57% (36–78)	35, 43% (32–53)	72, 49% (41–57)
Autosomal monosomies	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	1, 1.2% (0.1–3.6)	1, 0.7% (0.06–2)
Sex trisomies	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	0, 0% (0–0)	1, 1.2% (0.1–3.6)	1, 0.7% (0.06–2)
Sex monosomies	1, 7.1% (0.6–21)	8, 14% (5.1–24)	3, 23% (0.2–46)	12, 15% (6.9–22)	3, 6.8% (0.06–14)	3, 14% (0.07–29)	5, 6.1% (0.9–11.3)	11, 7.5% (3.2–12)
Polyploidies	3, 21% (0.01–43)	15, 27% (15–38)	3, 23% (0.2–46)	21, 25% (16–35)	6, 14% (3.5–24)	2, 9.5% (0.3–22)	6, 7.3% (1.7–13)	14, 9.5% (4.8–14)
Structural anomalies	4, 29% (4.9–52)	1, 1.8% (0.1–5.3)	2, 15% (0.4–35)	7, 8.4% (2.5–14)	1, 2.3% (0.2–6.7)	0, 0% (0–0)	4, 4.9% (0.2–9.5)	5, 3.4% (0.05–6.3)
Double anomalies	1, 7.1% (0.6–21)	4, 7.1% (0.4–14)	2, 15% (0.4–35)	7, 8.4% (2.5–14)	3, 6.8% (0.06–14)	1, 4.8% (0.4–14)	4, 4.9% (0.2–9.5)	8, 5.4% (1.8–9)
Mosaicisms	2, 14% (0.4–33)	1, 1.8% (0.1–5.3)	0, 0% (0–0)	3, 3.6% (0.04–7.6)	1, 2.3% (0.2–6.7)	0, 0% (0–0)	1, 1.2% (0.01–3.6)	2, 1.4% (0.05–3)
Total	32, 21%	99, 65%	22, 14%	153, 100%	64, 32%	35, 18%	101, 51%	200, 100 , 00%

95% CIs were calculated for all the proportions. After Bonferroni correction, no significant differences were found between pregnancies at low and high risk for aneuploidy.

Table IV Cytogenetic results stratified by maternal age groups.

Cytogenetic result	<35 years *n* (%)	≥35 years *n* (%)	Total
Normal karyotype			
46XY	42 (27.5)	27 (13.5)	69 (19.5%)
46XX	28 (18.3)	26 (13.0)	54 (15.3%)
Viable autosomal trisomies			
+21	3 (2.0)	18 (9.0)	21 (5.9%)
+18	2 (1.3)	3 (1.5)	5 (1.4%)
+13	2 (1.3)	12 (6.0)	14 (4.0%)
Non-viable autosomal trisomies			
+2	1 (0.7)	1 (0.5)	2 (0.6%)
+4	1 (0.7)	3 (1.5)	4 (1.1%)
+5	1 (0.7)	—	1 (0.3%)
+7	1 (0.7)	6 (3.0)	7 (2.0%)
+8	1 (0.7)	1 (0.5)	2 (0.6%)
+9	—	2 (1.0)	2 (0.6%)
+10	—	1 (0.5)	1 (0.3%)
+12	—	2 (1.0)	2 (0.6%)
+14	2 (1.3)	—	2 (0.6%)
+15	5 (3.3)	11 (5.5)	16 (4.5%)
+16	7 (4.6)	21 (10.5)	28 (7.9%)
+20	1 (0.7)	2 (1.0)	3 (0.8%)
+22	6 (3.9)	22 (11.0)	28 (7.9%)
Autosomal monosomies			
−21	—	1 (0.5)	1 (0.3%)
Sex trisomies			
47XXX	—	1 (0.5)	1 (0.3%)
Sex monosomies			
45X	12 (7.8)	11 (5.5)	23 (6.5%)
Polyploidies			
69XXX	9 (5.9)	6 (3.0)	15 (4.2%)
69XXY	12 (7.8)	6 (3.0)	18 (5.1%)
92XXXX	—	2 (1.0)	2 (0.6%)
Unbalanced structural anomalies			
45X,-21,+der(14);t(14;21)(q13;q22.1)pat	1 (0.7)	—	1 (0.3%)
46XY,der(13;14)(q10;q10),+14mat	1 (0.7)	—	1 (0.3%)
46XX,der(14;21)(q10;q10),+21mat	1 (0.7)	—	1 (0.3%)
46XX,der(22;22)(q10;q10),+22	—	1 (0.5)	1 (0.3%)
46XY,der(4)t(4;11)(q35;q23)pat	1 (0.7)	—	1 (0.3%)
46XY(der14;15)(q10;q10),+15	—	1 (0.5)	1 (0.3%)
46XX,der(8)t(6;8)(p25;p23)pat	—	1 (0.5)	1 (0.3%)
46XX,i(8)(q10)	—	1 (0.5)	1 (0.3%)
46XX,add(10)(p?15)	1 (0.7)	—	1 (0.3%)
46XY,add(1)(p?)	1 (0.7)	—	1 (0.3%)
47XY,+i(20)(p10)	—	1(0.5)	1 (0.3%)
47XY,+der(5)t(3;5)(p23;q33)pat	1 (0.7)	—	1 (0.3%)
Double anomalies			
46X,+15	1 (0.7)	—	1 (0.3%)
48XY,+3,+16	1 (0.7)	—	1 (0.3%)

Continued

Table IV *Continued*

Cytogenetic result	<35 years *n* (%)	≥35 years *n* (%)	Total
48XX,+11,+13	—	1 (0.5)	1 (0.3%)
48XX,+13,+20	1 (0.7)	—	1 (0.3%)
48XY,+13,+20	—	2 (1.0)	2 (0.6%)
48XY,+14,+22	—	1 (0.5)	1 (0.3%)
48XY,+16,+22	—	1 (0.5)	1 (0.3%)
48XX,+18,+21	1 (0.7)	—	1 (0.3%)
48XX,+20,+21	—	1 (0.5)	1 (0.3%)
70XXY,+2	1 (0.7)	—	1 (0.3%)
70XXX,+7	—	1 (0.5)	1 (0.3%)
70XXX,+15	—	1 (0.5)	1 (0.3%)
70XXX,+20	1 (0.7)	—	1 (0.3%)
47,XX,t(8;11)(p2?;q1?),+15	1 (0.7)	—	1 (0.3%)
Mosaicisms			
mos45X/46XX	—	1 (0.5)	1 (0.3%)
mos51XX+3+7+14+15+20/46XX	1 (0.7)	—	1 (0.3%)
mos47XX+10/46XX	1 (0.7)	—	1 (0.3%)
mos47XY+22/46XX	—	1 (0.5)	1 (0.3%)
mos48XX+?C+18/49XX+3+?C+18	1 (0.7)	—	1 (0.3%)
Total	153 (100)	200 (100)	353 (100%)

? means unknown breakage point.

Table V Reported chromosomal anomaly rates in sporadic and recurrent miscarriage.

	n	Mean maternal age	Cytogenetic success rate (%)	Chromosomal anomaly rate in sporadic miscarriage (%)	Chromosomal anomaly rate in recurrent miscarriage (%)	Chromosomal anomaly rate stratified by maternal age
Stern *et al.* (1996)	224	—	—	57	57	No
Ogasawara *et al.* (2000)	234	31	51	72	51	No
Carp *et al.* (2001)	125	32	75	—	29	No
Stephenson *et al.* (2002)	420	34	88	48	46	Yes
Sullivan *et al.* (2004)	255	31	88	42	25	Yes
Marquard *et al.* (2010)	137	39	—	70	78	No
Present study	376	35	84	68	60	Yes
Total	1771		77	63	47	

two or more losses, instead of three or more, the recurrent miscarriage group would have increased up to 56%, while the trend toward a decrease in viable autosomal trisomies in the older group would have become significant (37 versus 12%, $P = 0.02$), and a trend toward sex monosomies decrease (from 22 to 7.1%) would have appeared in the younger group (data not shown). On the other hand, the lack of differences in the chromosomal anomaly rate between high- and low-aneuploidy risk groups in younger women may be explained by the poor predictive value of past history, given that less than one-third of these miscarriages occurred after 11 weeks, the gestational age at which NT is typically assessed. In addition, the use of the semi-direct method in chorionic villi processing is prone to detect anomalies confined to the placenta, given that about 1–2% of placentas can have a chromosomal constitution different from that of the embryo. In living embryos, the typical discrepancy is based on a trisomic placenta and a normal embryo, and we wonder whether in fetal demise the opposite may be more common. We are aware that our CVS offer to

women with low-risk miscarriages is controversial, but we learned from high-risk women that karyotyping and the subsequent counseling provided was considered valuable information for their reproductive choices (Muñoz *et al*., 2010).

On the other hand, a strength of the semi-direct method applied to chorionic villi retrieved before evacuation is that it minimizes maternal cell contamination and the underdiagnosis of chromosomal anomalies. Recently, maternal cell contamination has been described to affect up to 31% of the normal female karyotypes obtained by culture of the products of conception (Jobanputra *et al*., 2011). In our study, we observed more male than female karyotypes. We consider that one of the main strengths of our study is that methods for both sample retrieving and cytogenetic analysis were uniform for all the study population, in contrast to some series including cases and controls studied in different laboratories. In addition, our study is the first to compare the chromosomal anomaly spectrum between sporadic and recurrent miscarriage within a single cohort. Although the distribution of chromosomal anomaly types was not significantly different between sporadic and recurrent miscarriage after the Bonferroni correction, the observed trends toward increased unbalanced structural anomalies in younger women and decreased viable autosomal trisomies in the older group must be taken into account, given that other reported series did not perform such adjustments. These results may become significant in larger studies.

Authors' roles

M.G. and A.B. carried out the study design, the analysis and interpretation of data and the draft of the manuscript. A.B., R.G.-P., V.B. and M.M. contributed to the design and acquisition of samples. A.So. and A.Sa. carried out the analysis of the samples and revised the manuscript. A.B., M.C. and J.B. revised the article for important intellectual content.

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Conflict of interest

None declared.

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