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Combination of QF-PCR and aCGH is an efficient diagnostic strategy for the detection of chromosome aberrations in recurrent miscarriage

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Abstract

Background: Our aim was to conduct a comprehensive genetic evaluation using the combination of QF-PCR (quantitative fluorescence polymerase chain reaction) and aCGH (array comparative genomic hybridization) for the detection of the frequency and type of chromosome aberrations in recurrent miscarriage (RM) in the clinical setting.

Methods: This retrospective study was conducted on 73 first-trimester products of conception (POC) between September 2014 and February 2017. The POCs were collected from 73 women with at least one previous miscarriage and analyzed for chromosomal anomalies using QF-PCR and aCGH as part of the routine clinical evaluation.

Results: Chromosome aberrations were detected in 52/73 POCs (71.2%), of which 41 (56.2%) were identified by QF-PCR and an additional 11 (15.1%) by aCGH. Numerical aberrations constituted 92.3% of abnormalities, with trisomies as the most common subtype (72.9%). Causative structural aberrations were found in three samples (5.8%). The frequency of chromosome aberrations was not dependent on the number of previous miscarriages, whereas it significantly increased with advanced maternal age.

Conclusion: Our results confirm that chromosome aberrations are the most common cause of RM and that QF-PCR and aCGH combination should be included in the routine genetic analysis of POCs of couples with miscarriage.

KEYWORDS

aCGH, chromosome aberrations, QF-PCR, recurrent miscarriage

1 | BACKGROUND

Miscarriage, the spontaneous loss of pregnancy before the 22nd week of gestation or fetus weighing less than 500 g, is the most common complication of pregnancy (WHO, 1997). An estimated 70% of conceptions are miscarried, most of which are lost before the missed menstrual period (Macklon, Geraedts, & Fauser, 2002). Accordingly, approximately 10% of clinically recognized pregnancies result in a miscarriage.

Recurrent miscarriage (RM) is considered to be a distinct clinical entity defined as the occurrence of more than one miscarriage in a particular woman. However, the minimum

number of miscarriages (≥2 or ≥3), their order (consecutive or nonconsecutive), and partner specificity (with the same partner or not) required for the diagnosis of RM differ between guidelines recommended by professional societies (Jauniaux, Farquharson, Christiansen, & Exalto, 2006; Practice Committee of the American Society for Reproductive Medicine, 2012; Royal College of Obstetricians & Gynaecologists, 2011). Regardless of these discrepancies, the established causes include uterine anomalies and antiphospholipid syndrome in women, as well as balanced structural rearrangements in either partner. Collectively, these factors can be identified in 40% of couples at the most, leaving the majority without an identifiable cause (Stephenson, Awartani, & Robinson, 2002).

On the other hand, the increasing number of recent investigations suggests that embryonic chromosome aberrations are the most common cause of RM, similar to sporadic miscarriages (Hodes-Wertz et al., 2012; Sugiura-Ogasawara et al., 2012). Unfortunately, comprehensive genetic analysis of products of conception (POC) is still not a common practice despite the fact that it would reduce the number of diagnostic evaluations performed in reproductive couples with RM (Brezina & Kutteh, 2014) and also decrease the incidence of idiopathic RM (Foyouzi, Cedars, & Huddleston, 2012; Liu et al., 2015; Marquard, Westphal, Milki, & Lathi, 2010; Sugiura-Ogasawara et al., 2012).

Although the standard chromosome analysis of POCs has been performed using the G-banding method, the shortcomings of this cell-based analysis include high rate of cell culture failure, poor sample quality, low band resolution, and maternal cell or microbial contamination (Robberecht, Schuddinck, Fryns, & Vermeesch, 2009; Sahoo et al., 2017; Shah et al., 2017). Conversely, molecular cytogenetic methods, which do not require cell cultures, have a higher resolution and improve turnaround time. Recently, a clinical algorithm for efficient cytogenomic analysis of POCs and fetal tissues has been proposed, which includes quantitative fluorescent polymerase chain reaction (QF-PCR) followed by array comparative genomic hybridization (aCGH) on POCs with normal or uninformative results of QF-PCR (Morgen, Maire, & Kolomietz, 2012; Wou et al., 2016). This "no-culture or uncultured protocol" reduces failure rate, increases diagnostic yield, and is cost-effective in comparison to traditional karyotyping (Donaghue et al., 2017; Morgen et al., 2012; Wou et al., 2016). In previous studies, karyotyping of POCs of couples with RM was conducted using either G-banding, different molecular cytogenetic methods (e.g., fluorescence in situ hybridization, multiple ligation-dependent probe amplification), or aCGH but there are no previous reports in which the combination of QF-PCR and aCGH was systematically used in the clinical setting. Therefore, the aim of the present study was to investigate the frequency and type of chromosome aberrations in POCs of couples who previously had at least one miscarriage using the QF-PCR and aCGH strategy.

2 | PATIENTS AND METHODS

2.1 | Editorial policies and ethical considerations

Informed consent for genetic testing was obtained from all women.

2.2 | Sample collection

Products of conception were obtained by dilatation and curettage from 73 consecutively admitted women with at least one previous miscarriage between September 2014 and February 2017. All POCs were referred to the Clinical institute of medical genetics, University medical centre Ljubljana, Slovenia. Twenty-nine women were admitted at second miscarriage, 32 at third miscarriage, and 12 at fourth to 10th miscarriage. All miscarriages occurred in the first trimester. None of the women had endocrine, metabolic, autoimmune or other systemic disorders, venous or arterial thrombosis, antiphospholipid syndrome or uterine anatomic abnormalities.

2.3 | Genetic testing

The protocol used for genetic analysis of POCs in our study is shown in Figure 1. This protocol is part of the routine genetic testing service provided at the Clinical institute of medical genetics, University medical centre Ljubljana, Slovenia and Institutional review board approval was therefore not obtained. The DNA was extracted from chorionic villi according to the manufacturer's protocol using Qiagen Mini kit (Qiagen). Quality and concentration parameters of the DNA

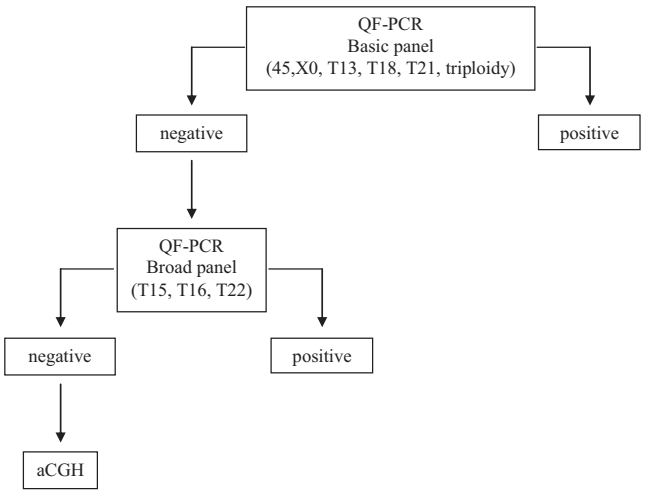

FIGURE 1 The protocol used for genetic analysis of products of conception

were measured with NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc.) and Qubit 2.0 fluorometer (Life Technologies Inc.).

2.3.1 | QF-PCR

The analysis of aneuploidy of chromosomes 13, 15, 16, 18, 21, 22, X, and Y was based on determining the number of copies of selected genetic markers on each chromosome using QF-PCR method. We used Aneufast Multiplex QF-PCR kit (molGENTIX SL) containing 21 genetic markers with a high degree of heterozygosity (five genetic markers for autosomes 13, 18, and 21, three markers for pseudoautosomal regions of chromosomes X and Y, one on the X chromosome-linked marker and markers on amelogenin, and SRY on chromosome Y). Devyser Extend M1 v2 kit by Devyser (Sweden) was used to analyze aneuploidy of chromosomes 15, 16, and 22, containing 15 genetic markers (five genetic markers for each autosome 15, 16, 22, and one additional marker on the chromosome 18) in one single QF-PCR reaction (Devyser Extend v2, Art.No.:8-A015.2, Devyser Extend M1 v2, Art. No.:8-A015.2-M1, For in vitro Diagnostic Use, Instructions for Use: Devyser Extend v2, CE-IVD, 7-A025-EN, version 2016-05-03).

Diagnosis of normal samples was acceptable if at least two markers on each chromosome had clear heterozygous pattern within the normal range. According to the manufacturer's instructions, markers indicated aneuploidy if at least two markers showed a normal triallelic profile or trisomic diallelic patterns and that other markers for a specific chromosome had an uninformative character (were homozygous).

2.3.2 | Array CGH

DNA was processed according to Agilent protocol (Version 7.3 March 2014) using a commercially available male and female genomic DNA (Agilent Technologies, Human Reference DNA, Male and Female) or in-house DNA reference mix as a reference DNA. Agilent SurePrint G3 Unrestricted CGH ISCA v2, 8 × 60 K microarrays were used which provide a practical average resolution of 100 kb. Array images were acquired using Agilent laser scanner G2565CA, image files were quantified using Agilent Feature extraction software for Cytogenomics 3.0, and analyzed with Agilent Cytogenomics 3.0 software (Agilent Technologies).

2.3.3 | Classification of results

Called copy number variants (CNV) were aligned with known aberrations in publically available databases—ClinGen (http://dbsearch.clinicalgenome.org/search/), DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources https://decipher.sanger.ac.uk/), Database of Genomic Variants (DGV) (http://dgv.tcag.ca/dgv/app/home), as well as with the in-house database of detected variants and their clinical significance, ascertained by the trained analysts. All called CNVs were classified into three groups, benign, variants of unknown significance (VOUS), and pathogenic, according to ACMG Standards and Guidelines (Kearney, Thorland, Brown, Quintero-Rivera, & South, 2011).

Parental studies were performed in cases when unbalanced structural rearrangements were identified to confirm their origin.

2.4 | Statistical analysis

Statistical analyses were performed using Statistica for Windows, version 12 (StatSoft, Inc.). The distribution of numerical variables (maternal age) was tested using Kolmogorov–Smirnov test. Mann–Whitney *U* test was used for the comparison of the mean maternal ages in relation to karyotype results. Pearson's Chi-squared (χ^2) test was used to examine differences between the number of abnormal and normal karyotypes according to the number of previous miscarriages. $p < .05$ were considered statistically significant.

3 | RESULTS

The overall results of the QF-PCR and aCGH are summarized in Table 1. Chromosome aberrations were detected in 52 of the 73 POCs evaluated (71.2%), of which 41 (56.2%) were identified by QF-PCR and an additional 11 (15.1%) by aCGH. Numerical aberrations constituted the majority (92.3%) of abnormalities, with trisomies as the most common subtype (72.9%). Causative structural aberrations were found in three samples (5.8%). In addition, one VOUS was detected.

3.1 | Results of QF-PCR

In the 41 POCs with numerical aberrations detected by basic and broad panel QF-PCR (Table 2), the three most frequent aberrations were trisomy 22 (10/41; 24.4%), trisomy 16 (10/41; 24.4%), and triploidy (7/41; 17.1%). The common trisomies (13, 21) were present in five POCs (12.1%) and monosomy X in six POCs (14.6%). In addition, three POCs had trisomy 15 (7.3%). The only case of trisomy 18 was further analyzed and confirmed by aCGH as mosaicism due to uninformative results of QF-PCR.

3.2 | Results of aCGH

Array CGH was performed subsequently in 31 POCs with negative results of QF-PCR and one POC in whom trisomy

TABLE 1 Summary results of genetic analysis of 73 products of conception

	Results/*N* (%)		
	Positive	**Negative**	**Uninformative**
QF-PCR basic panel	18 (24.6)	54 (74.0)	1 (1.4)
QF-PCR broad panel	23 (31.5)	31 (42.5)	
aCGH[a]	11 (15.1)	20 (27.4)	
Total aberrations detected	52 (71.2)		
Numerical aberrations	48 (92.3)		
Trisomies	35 (72.9)		
Triploidy	7 (14.6)		
Monosomy X	6 (12.5)		
Unbalanced structural aberrations	3 (5.8)		
Variants of unknown significance	1 (1.9)		

Abbreviations: aCGH, array comparative genomic hybridization; POC, products of conception; QF-PCR, quantitative fluorescent polymerase chain reaction.
[a]One POC was excluded from analysis due to the insufficient amount of DNA sample.

18 was suspected (Table 3). However, one POC with negative results was excluded from analysis due to the insufficient amount of DNA sample. An additional seven aneuploidies were detected, including trisomy 2, 3, 9, 10, and 12, as well as two mosaic trisomies of chromosomes 10 and 18. Furthermore, aCGH also revealed three large unbalanced structural rearrangements, inherited from the balanced parental translocations. Finally, a de novo terminal deletion of chromosome 11q25 (size 2.44 ± 0.09 Mb) was detected in one male POC, which was classified as a VOUS after comparison with CNV databases.

3.3 | Chromosome aberrations in relation to the number of previous miscarriages and maternal age

There were no statistically significant differences in the total number of abnormal and normal karyotypes according to the number of previous miscarriages, that is, between two and three miscarriages ($X^2 = 0.56$, $p = .456$), two, three, and four or more miscarriages ($X^2 = 2.40$, $p = .301$), nor two and three or more miscarriages ($X^2 = 0.06$, $p = .811$; Table 2).

The mean maternal ages according to different karyotype results is shown in Table 4. The mean age was higher in mothers with abnormal karyotype results than those with normal results, although the *p*-value was of borderline statistical

TABLE 2 Frequency and types of numerical chromosome aberrations detected by QF-PCR

	Order of miscarriage/*N* (%)			
Karyotype	**Second**	**Third**	**Fourth to 10th**	**Total**
Monosomy X	2 (13.3)	1 (5.0)	3 (42.9)	6 (14.6)
Triploidy	2 (13.3)	5 (25.0)		7 (17.1)
Trisomies				
Trisomy 13	1 (6.7)			1 (2.4)
Trisomy 18	1 (6.7)[a]			
Trisomy 21	1 (6.7)	2 (10.0)	1 (14.2)	4 (9.8)
Trisomy 15	1 (6.7)	2 (10.0)		3 (7.3)
Trisomy 16	3 (20.0)	3 (15.0)	3 (42.9)	10 (24.4)
Trisomy 22	4 (26.6)	6 (30.0)		10 (24.4)
Total abnormal	14 (48.3)	20 (62.5)	7 (58.3)	**41 (56.1)**
Total normal	14 (48.3)	12 (37.5)	5 (41.7)	**31 (42.5)**
Total uninformative	1 (3.4)			**1 (1.4)**
Statistical significance (total abnormal vs. normal)	$X^2 = 0.56$, $p = .456$[b] $X^2 = 2.40$, $p = .301$[c] $X^2 = 0.06$, $p = .811$[d]			

Abbreviations: aCGH, array comparative genomic hybridization; QF-PCR, quantitative fluorescent polymerase chain reaction.
[a]Uninformative result, which was further analyzed and confirmed by aCGH.
[b]Two versus three miscarriages.
[c]Two versus three versus four or more miscarriages.
[d]Two versus three or more miscarriages.
Bold values indicate statistical significance (total abnormal vs. normal).

TABLE 3 Frequency and types of chromosome aberrations detected by aCGH

Order of miscarriage	Number of POCs tested[a]	Result	Total abnormalities/ *N* (%)
Second	15	Numerical aberrations: arr[hg19] (2)×3 arr[hg19] (3)×3 arr[hg19] (12)×3 arr[hg19] (18)×2−3 Unbalanced structural rearrangements: arr[hg19] 11q13.1q25(64,238,508–134,868,407)×3,17p13.3p13.1(76,263–3,696,032)×1 arr[hg19] 3p26.3p25.3(93,949–10,002,512)×1,7q32.1q36.3(128,187,082–159,124,131)×3	6 (40)
Third	12 (−1)[b]	Numerical aberrations: arr[hg19] (9)×3 arr[hg19] (10)×3 arr[hg19] (10)×2–3 Unbalanced structural rearrangements: arr[hg19] 2q22.3q37.3(146,419,130–243,068,396)×3,13q31.3q34(92,126,915–115,092,648)×1 mat VOUS: 11q25 deletion de novo	5 (45.4)
Fifth	1	Normal	0
Sixth	1	Normal	
Seventh	1	Normal	
Eight	1	Normal	
Tenth	1	Normal	
Total	31		11 (35.5)

Abbreviations: aCGH, array comparative genomic hybridization; POC, products of conception; QF-PCR, quantitative fluorescent polymerase chain reaction; VOUS, variants of unknown significance.

[a]All POCs had normal QF-PCR results except for one POC in the category of two miscarriages in whom uninformative results were obtained.

[b]One POC was excluded from analysis due to the insufficient amount of DNA sample.

significance ($p = .049$). Furthermore, the average maternal age was significantly higher in the group POCs with trisomies in comparison to both the POCs of normal karyotype ($p = .007$) and to all other karyotypes ($p < .001$).

TABLE 4 Chromosome aberrations in relation to maternal age

	Mean age[a]	*SD*	*p* value[d,e]	*p* value[d,f]
Total[b]	34.00	4.75		
Normal[c]	32.20	4.32		
Abnormal[b]	35.00	4.81	.049	
Monosomy X[c]	32.50	4.32	.738	.541
Triploidy	32.71	4.27	.638	.588
Trisomy	35.49	4.82	.007	<.001

[a]72 women.

[b]Median.

[c]Mean.

[d]Mann–Whitney *U* test.

[e]Mean age in relation to age of normal karyotypes.

[f]Mean age in relation to age of all other karyotypes.

4 | DISCUSSION

Our results confirm that chromosome aberrations are the most common cause of miscarriage and that QF-PCR and aCGH testing strategy is efficient in the genetic analysis of POCs. Both of these findings have the potential to advance the diagnostic management of couples with miscarriage.

We detected an overall 69.9% of causative numerical and structural chromosome aberrations in our study sample, which is among the highest reported frequencies in RM. The frequency of chromosome aberrations in previous studies conducted exclusively on POCs of couples with RM varies depending on the method used for their detection. For example, the frequencies range from 24.6% to 64% using G-banding (Carp et al., 2001; Choi, Lee, Park, Jeong, & Moon, 2014; Grande et al., 2012; Hassold, 1980; Liu et al., 2015; Ogasawara, Aoki, Okada, & Suzumori, 2000; Stephenson et al., 2002; Stern, Dorfmann, Gutiérrez-Najar, Cerrillo, & Coulam, 1996; Sugiura-Ogasawara et

al., 2012; Sullivan, Silver, LaCoursiere, Porter, & Branch, 2004; Zhang et al., 2014), 66.6% using aCGH (Ozawa et al., 2016), and 55.9 to 70.7% using single-nucleotide polymorphism array (Robberecht et al., 2012; Maslow et al., 2015; Wang et al., 2017). In addition, preimplantation genetic diagnosis performed using fluorescence in situ hybridization or aCGH revealed a high incidence of embryonic chromosome aberrations, ranging from 41% to 70.7% (Hodes-Wertz et al., 2012; Pellicer et al., 1999; Rodrigo et al., 2014; Rubio et al., 2003; Rubio et al., 2009; Vidal et al., 1998). The percentage of numerical and unbalanced structural aberrations obtained in our study (92.3% vs. 5.8%) is also in accordance with those of previous studies. Moreover, the classification of numerical aberrations into subcategories further confirmed that trisomies are the most common abnormalities in POCs of couples with RM, followed by triploidy and monosomy X. The most prevalent trisomies are the nonviable trisomy 16 and 22 (Carp et al., 2001; Carp et al., 2006; Choi et al., 2014; Hassold, 1980; Robberecht et al., 2012; Stephenson et al., 2002). Our results are also consistent with previous findings that the frequency of chromosome aberrations significantly increases with advanced maternal age, which is especially notable for trisomies (Grande et al., 2012; Marquard et al., 2010; Sugiura-Ogasawara et al., 2012).

On the other hand, unbalanced structural rearrangements comprise less than 10% of all chromosome aberrations in POCs of couples with RM (Carp et al., 2006; Choi et al., 2014; Liu et al., 2015; Ozawa et al., 2016; Robberecht et al., 2012; Stephenson et al., 2002; Sugiura-Ogasawara et al., 2012; Sullivan et al., 2004; Wang et al., 2017). We detected large rearrangements in three POCs resulting in partial trisomies of chromosomes 11q13, 7q32, and 2q22, as well as partial monosomies of chromosomes 17p13, 3p26, and 13q31, respectively. Considering that all chromosomal gains and losses were larger than 30 and 3 Mb, respectively, they can be classified as pathological variants that led to miscarriage. We subsequently performed selective karyotyping of parents and confirmed that all three rearrangements were inherited from one of the parents. The incidence of balanced structural aberrations in reproductive couples who have had at least two miscarriages is between 2% and 6% ++(Barber et al., 2010; De la Fuente-Cortes et al., 2009; El-Dahtory, 2011; Franssen et al., 2005; Fryns & Van Buggenhout 1998; Gonçalves et al., 2014; Jaslow, Carney, & Kutteh, 2010; Karim et al., 2017; Kochhar, & Ghosh, 2013; Sheth et al., 2013; van den Boogaard et al., 2011). Although translocations are heterogeneous in couples with RM, certain chromosome segments appear to be affected more often than others. Accordingly, the segments affected in our study sample were previously described either in partners with RM who were carriers of balanced translocations or POCs who inherited an unbalanced rearrangement (Fan et al., 2016; Ghazaey et al., 2015; Goddijn et al., 2004; Iyer, Vyas, Ranjan, & Saranath, 2009; Kochhar & Ghosh, 2013; Shimokawa et al., 2006). Among them, the 7q32 breakpoint is one of the most frequently reported in the literature.

In addition to its ability to detect large unbalanced structural rearrangements, aCGH has the potential to identify submicroscopic imbalances (Dhillon et al., 2014). However, despite the fact that various small-size de novo and inherited CNVs have been discovered in POCs of couples with RM, their contribution to developmental failure is mostly unknown (Rajcan-Separovic et al., 2010; Wang et al., 2017). It is estimated that the incidence of such VOUS in miscarriage is 2% (Dhillon et al., 2014). In our study, a single de novo terminal deletion of chromosome 11q25 was detected (size 2.44 ± 0.09 Mb). The deleted region harbors nine OMIM genes, which have not been associated with human diseases when present in a single copy. However, larger 11q terminal deletions lead to the 11q terminal deletion disorder or Jacobsen syndrome (OMIM #147791), the characteristics of which do not include spontaneous abortions. We identified one description of a similar deletion in the ClinGen database, which was classified as pathogenic (nssv575983) in a patient with a coordination disorder. In addition, one similar deletion (ID259180) was found in the DECIPHER database in a patient with mental retardation, which was inherited from a healthy parent. Finally, we found no description of similar deletions in the DGV.

Despite the fact that the standpoint on whether RM should be defined as two or three or more miscarriages is still not unanimous, the results of our study indicate that the frequency of chromosome aberrations is not statistically different in relation to the number of previous miscarriages, corroborating the findings of several previous studies (Goldstein, Svirsky, Reches, & Yaron, 2017; Stern et al., 1996). Also, the frequency of chromosome aberrations in RM appears to be similar to those observed in sporadic miscarriages (Goldstein et al., 2017; Grande et al., 2012; Stern et al., 1996). Nevertheless, regardless of the number of previous miscarriages, chromosome aberrations are the major factor leading to undesired pregnancy loss. Additionally, whether women with a previous aneuploid miscarriage are at an increased risk of future chromosome aberrations is still a matter of debate. The conflicting results might be the consequence of the fact that conclusions are based on the evaluation of a small number of patients conducted in a small number of studies. Recurrent aneuploidy has been reported to occur in 10%–76.2% of cases and does not appear to be related to a single chromosome (Bianco, Caughey, Shaffer, Davis, & Norton, 2006; Hassold, 1980; Hassold, Jacobs, Pettay, & Rao, 1988; Sugiura-Ogasawara et al., 2012; Sullivan et al., 2004).

The results of our comprehensive genetic analyses of POCs of couples with RM could have significant clinical implications considering that we confirmed that the QF-PCR and aCGH is an efficient testing strategy in the clinical practice. Due to the high frequency of chromosome aberrations, we suggest that a standard genetic analysis of POCs is performed in all miscarriages. Consequently, such analysis would enable proper genetic counseling and, no less important, reduce the number of unnecessary pharmaceuticals that are usually administered to women with RM regardless of therapeutic indications. The application of this testing strategy will alter the ratio of couples with explained and truly unexplained RM, which will inevitably improve the clinical approach to couples with RM. Even though parental cytogenetic studies are included in the routine diagnostic evaluation of RM, cost analysis studies indicate that obtaining a karyotype of POCs of couples with RM as a first step in diagnostic evaluation is less costly than an extensive parental work-up (Bernardi, Plunkett, & Stephenson, 2012; Foyouzi et al., 2012; Petracchi, Paez, & Igarzabal, 2017).

Future studies should focus on the discovery of the origin of aneuploidies and long-term follow-up of couples to explore the true frequency of recurrent aneuploidy. In addition, next-generation sequencing could be conducted on POCs with negative results after both QF-PCR and aCGH to determine the contribution of the smallest DNA variants to miscarriage.

5 | CONCLUSIONS

Chromosome aberrations were detected in 71.2% of POCs of couples with RM using QF-PCR and aCGH testing strategy. Numerical aberrations comprised more than 92.3% of all abnormalities. The frequency of chromosome aberrations was not dependent on the number of previous miscarriages, whereas it significantly increased with advanced maternal age. We suggest that the genetic analysis of POCs using QF-PCR and aCGH is introduced in the routine clinical evaluation of couples with miscarriage.

CONFLICTS OF INTEREST

The authors report no conflict of interest.

CAPSULE

The QF-PCR and aCGH testing strategy reveals a high frequency of chromosome aberrations in POCs of couples with recurrent miscarriage.

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