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Review

Genetics of early miscarriage ☆

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ABSTRACT

A miscarriage is the most frequent complication of a pregnancy. Poor chromosome preparations, culture failure, or maternal cell contamination may hamper conventional karyotyping. Techniques such as chromosomal comparative genomic hybridization (chromosomal-CGH), array-comparative genomic hybridization (array-CGH), fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA) and quantitative fluorescent polymerase chain reaction (QF-PCR) enable us to trace submicroscopic abnormalities. We found the prevalence of chromosome abnormalities in women facing a single sporadic miscarriage to be 45% (95% CI: 38–52; 13 studies, 7012 samples). The prevalence of chromosome abnormalities in women experiencing a subsequent miscarriage after preceding recurrent miscarriage proved to be comparable: 39% (95% CI: 29–50; 6 studies 1359 samples). More chromosome abnormalities are detected by conventional karyotyping compared to FISH or MLPA only (chromosome region specific techniques), and the same amount of abnormalities compared to QF-PCR (chromosome region specific techniques) and chromosomal-CGH and array-CGH (whole genome techniques) *only*. Molecular techniques could play a role as an additional technique when culture failure or maternal contamination occurs: recent studies show that by using array-CGH, an additional 5% of submicroscopic chromosome variants can be detected. Because of the small sample size as well as the unknown clinical relevance of these molecular aberrations, more and larger studies should be performed of submicroscopic chromosome abnormalities among sporadic miscarriage samples. For recurrent miscarriage samples molecular technique studies are relatively new. It has often been suggested that miscarriages are due to chromosomal abnormalities in more than 50%, but the present review has determined that chromosomal and submicroscopic genetic abnormalities on average are prevalent in maximally half of the miscarriage samples. This article is part of a Special Issue entitled: Molecular Genetics of Human Reproductive Failure.

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1. Introduction

Miscarriage is the spontaneous loss of a clinically established intra-uterine pregnancy before the fetus has reached viability. It includes pregnancy losses until the maximum of 24 weeks of gestation [1]. It is the most frequent complication of a pregnancy. Between 10 and 15% of all clinically recognized pregnancies result in a spontaneous miscarriage. The overall prevalence of pregnancy losses, including biochemical pregnancies is generally assumed to be 4–5 times higher [1]. Around a quarter of all women experience at least one miscarriage during their lives [2,3].

Up to 5% of all couples will face recurrent miscarriage. The definition may vary but starts when at least two or more miscarriages have occurred [4,5]. The sequence of the miscarriages does not necessarily have to be consecutive [5]. Half of the first-trimester miscarriages are caused by fetal chromosome abnormalities diagnosed by conventional techniques [6].

Techniques such as Chromosomal Comparative Genomic Hybridization (CGH), array–Comparative Genomic Hybridization (array-CGH), Fluorescence in situ hybridization (FISH), Multiplex Ligation-dependent Probe Amplification (MLPA) and Quantitative Fluorescent Polymerase Chain reaction (QF-PCR) have overcome some disadvantages inherent to conventional cytogenetic techniques, including poor chromosome preparations, culture failure, or maternal cell contamination. These techniques have identified more abnormalities in early miscarriages [7–9]. Until now little is known about the contribution of the newer techniques to resolving the clinical problem.

Searching PubMed, Medline and EMBASE until October 2011 identified relevant studies. Search criteria used were related to the prevalence of cytogenetic abnormalities or submicroscopic abnormalities in relation to sporadic miscarriage and recurrent miscarriage. Estimates of 95% confidence intervals (CI) were determined for prevalence of chromosome abnormalities as well as for actual proportions of abnormalities as measured by the karyotyping, array-CGH, FISH, MLPA and/or QF-PCR. Pooled estimates were calculated based on

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weighted averages of the individual studies using random-effects meta-analyses in STATA 11.2.

In this review we present what is currently known about the prevalence and actual proportions of cytogenetic and submicroscopic abnormalities in sporadic as well as recurrent miscarriage. We will discuss the clinical findings that have come to light by using molecular techniques by paying attention to the capability of specific tests.

2. Current knowledge

Half of the sporadic early miscarriages (<12 weeks gestational age) are caused by fetal chromosome abnormalities, and nearly a third in second trimester miscarriages as was reported by a former review [6]. Cytogenetic studies have shown that most of these abnormalities are numerical chromosome abnormalities (86%), and a minority of the cases is caused by structural chromosome abnormalities (6%) and chromosome mosaicism (8%) [6].

It is hypothesized that the majority of chromosome abnormalities detected in spontaneous miscarriages occurs *de novo* and results from random errors produced during gametogenesis and embryonic development [10]. The risk of a fetal trisomy raises with increasing maternal age [11]. Since the average age of women bearing their first child has increased strongly over the last two decades in western countries, it is likely that fetal trisomy will become more frequent [12].

In women with recurrent miscarriage, chromosome abnormalities are prevalent as well, but the scale of the problem is less clear. One study showed that fetal aneuploidy contributes to recurrent miscarriage in a small proportion of the patients [13]. While others showed aneuploidy in larger number of patients [2,14–16].

The majority of miscarriages occur in chromosomally normal parents. Balanced chromosome abnormalities are present in about 2–5% of patients with recurrent miscarriage [4].

2.1. Conventional techniques

Conventional karyotyping is defined as the morphological characterization of the chromosomal complement of an individual including number, form and size of the chromosomes. It can detect abnormalities throughout the entire genome and is therefore used as the standard for detecting chromosome abnormalities in miscarriages samples (Table 1).

2.1.1. Cytogenetic abnormalities and sporadic miscarriage

A review of the literature in 2000 reported that 49% of the sporadic miscarriage samples that had been karyotyped, showed chromosome abnormalities. The spectrum of these chromosome abnormalities included 86% numerical abnormalities, 6% structural abnormalities and 8% other chromosome abnormalities like mosaicism, or double and triple trisomies [6]. Table 2 provides a new overview of reported chromosome abnormalities since the 2000 review, found after karyotyping in a large sporadic miscarriage sample population [7–9,13,17–25]. The pooled prevalence in 7012 samples tested of a chromosome abnormality from 2000 to 2011 was 45% (95% CI: 38–52). This prevalence of chromosome abnormalities is comparable to the 49% found in the previous review (45% after 2000 versus 49% before 2000) [6]. The proportions of chromosome abnormalities across the different studies ranges from 23 to 61% and one study alone represents 5555 samples collected for karyotyping being 81% of all samples tested amongst studies, as shown by Table 2 [25]. The overall failure rate was 21% (95% CI: 13–30). To avoid overrepresentation of this large study, pooled estimates were calculated based on weighted averages of the individual studies using random-effects meta-analyses.

Subdivision into type of chromosome abnormalities remained comparable to the 2000 review: numerical abnormalities (91%) followed by structural abnormalities (6%) and others (6%), but the age related percentage of trisomies (63%) was increased compared to the 2000 review (52%). The last group included mosaicism, double, triple and quadruple trisomies, autosomal monosomy and one trisomy plus a balanced translocation.

2.1.2. Cytogenetic abnormalities and recurrent miscarriage

Seven studies investigated cytogenetic abnormalities in miscarriage samples of women with preceding recurrent miscarriage [2,13–17,26–28]. One study estimated the rate of chromosome abnormalities in recurrent miscarriage samples among women with three miscarriages only over the age of 35 [27]. Because of this age selection criterion we did not include this study in the summarized table. Six studies could be summarized, see Table 3, with an overall failure rate of 25% (95% CI: 9–41) [2,13–17,26–28]. Less than half of the total number of 1359 successful karyotyped miscarriage samples showed an abnormal karyotype (39%; 95% CI: 29–50). The spectrum of chromosome abnormalities included 90% numerical abnormalities, 3% structural abnormalities and 13% other chromosome abnormalities. It shows that remarkably high numbers of chromosome abnormalities occur in miscarriage samples of women with recurrent miscarriage. Two studies defined recurrent miscarriage as two or more miscarriages [13,14]. While two other studies used three or more miscarriages in their definition [3,15]. The last two studies did not mention the definition of recurrent miscarriages at all [2,26]. The pooled prevalence of chromosome abnormalities in miscarriage samples of women with recurrent miscarriage, although tested in a smaller amount of samples (39%, 95% CI: 29–50) proved to be comparable with the pooled prevalence of chromosome abnormalities in miscarriage samples of women facing a single sporadic miscarriage (45%, 95% CI: 38–52).

2.2. Submicroscopic genetic abnormalities and sporadic miscarriage

A part of the miscarriages may be due to submicroscopic chromosomal changes [28]. The introduction of array-CGH enabled us clinicians to search for submicroscopic 'miscarriage genes'. These submicroscopic chromosomal changes are also defined as DNA copy number variants (CNVs). The pathogenicity of a CNV can be made plausible by *de novo* origin, larger size and the presence of genes that have been associated with disease [29].

Seven studies investigated the use of array-CGH for detecting submicroscopic genetic abnormalities in sporadic miscarriage samples [7–9,20,30–32]. Table 4 provides an overview of the reported submicroscopic abnormalities among these studies, entailing 362 miscarriages in total [7–9,20,30–32]. These combined studies suggest that in 5% of all sporadic miscarriages CNVs are found which cannot be detected by conventional cytogenetic analysis. One study included both patients with sporadic and recurrent miscarriages [33]. Eight of the 25 included patients suffered from recurrent miscarriage. Four submicroscopic chromosome abnormalities were found by two different arrays (Xp22.31, 12q33.3, 5p15.33 and Xp22.31). Additionally, parents were examined to check the parental origin. The four submicroscopic chromosome abnormalities were not presented before in the databases of benign copy number changes [33].

However the clinical relevance of these CNVs remains unclear. Still, only a relatively low number of samples have been tested in this area of research so far. Only one study examined the parents additionally, to determine whether the changes were inherited or *de novo*, as such testing is of clinical value [32]. This small study identified six unique copy number variants (CNVs), formerly not reported, in 5 of the 17 embryos (19% of all cases). All six were <250 kb in size. On the basis of parental array-CGH analysis, only in one embryo a *de novo* origin of a CNV was determined (at 13q32.1). The number of cases reported is small and it is precisely for that reason that further studies are recommended to determine the size of distribution of *de novo* CNVs in this clinical group and whether these CNVs contribute to the cause of the miscarriage.

Table 1
Established advantages and disadvantages of techniques used for genetic testing of miscarriage samples.

	Advantages	Disadvantages
Whole genome techniques		
Karyotyping	• Gold standard • Abnormalities detected throughout the entire genome • High specificity	• Limited resolution (>3–5 Mb) • Microdeletion/duplication syndromes not detectable • Origin of small supernumerary marker chromosomes not detectable • Subtle rearrangements of subtelomeric regions undetected • Chance of culture failure or maternal contamination • Time intensive (10–21 days) • Laborious procedure • High costs
Chromosomal comparative genomic hybridization (CGH)	• Resolution is limited to metaphase chromosomes (5–10 Mb pairs). • Abnormalities detected throughout the entire genome • Detection of deletions, duplications, or amplifications • Archival formalin fixed and paraffin embedded placenta or fetal tissues can be investigated • Quick results (5 days)	• Ploidy status not detectable • Balanced rearrangements not detectable • High costs (most expensive procedure) • Laborious procedure • Chance of false positives test results including lack of useable DNA products
Array-comparative genomic hybridization (array-CGH)	• Resolution ranging from oligonucleotides (25–80 bp) to bacterial artificial chromosomes (BACs) (80–200 kb) • Abnormalities detected throughout the entire genome, • Detection of deletions, duplications, or amplifications (including submicroscopic imbalances) • Archival formalin fixed and paraffin embedded placenta or fetal tissues can be investigated • Quick results (5 days)	• Ploidy status not detectable • Balanced rearrangements not detectable • High costs (most expensive procedure) • Chance of false positives test results including lack of useable DNA products
Chromosome specific techniques		
Fluorescence in situ hybridization (FISH)	• Polyploidy, monosomies and trisomies are detected • Performed directly on interphase cells and eliminates the requirement for cell culture • Quick results (1–2 days)	• Abnormalities distinct from the genomic segments for which probes have been designed cannot be detected. • Structural chromosome abnormalities not detectable • Chance of false positives test results including lack of useable DNA products
Multiplex ligation-dependent probe amplification (MLPA)	• Targets a large number of small DNA sequences (50–70 nt) • Quick results (1–3 days) • Low costs	• Mosaicism, polyploidy and balanced chromosome rearrangements not detectable • Maternal contamination not detectable • Chance of false positives test results including lack of useable DNA products
Quantitative fluorescence polymerase chain reaction (QF-PCR)	• Quick results (3–4 days) • Detection of maternal contamination	• Provides only information on the limited number of chromosomes the test is designed for, usually chromosome 13, 18, 21 and sex chromosomes • Chance of false positives test results including lack of useable DNA products

2.3. Submicroscopic genetic abnormalities and recurrent miscarriage

Idiopathic recurrent miscarriage presents a large problem for patient and clinician. Despite extensive evaluation, in many cases no underlying explanation for the recurrence of miscarriages can be found [3]. So there is a possibility that submicroscopic chromosomal changes, not detectable by conventional cytogenetic analysis, can account for some part of these miscarriages. Theoretically a parental CNV could contribute to the pregnancy loss if a gene is, or genes are, disrupted involving early development of the embryo, but this seems to be an unlikely cause of recurrent miscarriage.

One recent small study investigated 27 miscarriages samples and 22 partners from 20 couples with idiopathic recurrent miscarriage [29]. In 13 miscarriages samples of 8 couples with recurrent miscarriage, 11 unique CNVs could be identified. All CNVs identified have not previously be described, proved to be inherited and are therefore not likely to be of clinical value. Furthermore, this study has a small sample size [29].

2.4. Accuracy of the whole genome techniques

Conventional karyotyping is the standard for detecting chromosome abnormalities in miscarriages samples. It is one of the whole genome techniques. However, it may be hampered by maternal contamination, culture failure or overgrowth and poor quality of the chromosomal preparations. No fetal karyotype result is available in about 20% of the samples tested [25]. Another disadvantage is the limited resolution (<3–5 Mb). For a full overview of advantages and disadvantages of all techniques used for detecting chromosome abnormalities, see Table 1. Chromosomal

Table 2
Cytogenetic findings among reported series of spontaneous miscarriage samples by conventional karyotyping (percentages in parentheses).

Study	Total number of samples studied	Failures[a]	Successful karyotypes		Trisomy	Polyploidy	Monosomy X	Structural chromosome abnormalities	Others[b]
			Normal	Abnormal					
	n	n	n (%)	n (%)	n (% of abn)	n (% of abn)	n (% of abn)	n (% of abn)	n (% of abn)
Lomax 2000	301	48 (16)	98 (39)	155 (61)	111 (72)[c]	25 (16)	12 (8)	7 (5)	0
Tabet 2001	21	0	10 (48)	11 (52)	6 (55)	1 (9)	1 (9)	3 (27)	0
Jobanputra 2002	57	5 (9)	22 (42)	30 (58)	17 (57)	6 (20)	2 (7)	0	5 (17)
Schaeffer 2004	41	0	25 (61)	16 (39)	13 (81)	1 (6)	1 (6)	1 (6)	0
Sullivan 2004	150	17 (11)	77 (58)	56 (42)	53 (63)	12 (21)	5 (9)	3 (5)	0
Hu 2006	38	7 (18)	15 (48)	16 (52)	12 (75)	2 (13)	2 (13)	0	0
Bruno 2006	78	11 (14)	38 (57)	29 (43)	17 (59)	3 (10)	2 (7)	7 (24)	0
Diego-alvarez 2007	221	119 (54)	62 (61)	40 (39)	24 (60)	5 (13)	6 (15)	1 (3)	4 (10)
Menten 2009	100	28 (28)	55 (76)	16 (23)	9 (53)	3 (18)	2 (12)	2 (12)	0
Robberecht 2009	103	26 (25)	55 (71)	22 (29)	10 (45)	5 (23)	6 (27)	1(5)	0
Zhang 2009	115	23 (20)	37 (40)	55 (60)	36 (65)	8 (15)	5 (9)	2 (4)	4 (7)
Doria 2009	232	59 (25)	107 (62)	66 (38)[d]	36 (55)	13 (20)	6 (9)	5 (8)	15 (23)
Shearer 2011	5555	2194 (39)[e]	1627 (48)	1734 (52)[f]	1074 (62)	278 (16)	260 (15)	135 (8)	89 (5)
Total	7012	2537	2228	2246	1418	362	310	167	117
Prevalence		21%	55%	45%	63%	17%	11%	6%	6%
(95% CI)[g]		(13–30)	(48–61)	(38–52)	(59–68)	(15–19)	(8–14)	(4–8)	(2–11)

[a] Failures included: No fetal tissue found and failure of technique.
[b] Others included: mosaicism, double, triple and quadruple trisomies, autosomal monosomy and one trisomy plus a balanced translocation.
[c] Described as aneuploidy.
[d] 66 cases with an abnormal karyotype; 75 chromosome abnormalities are found.
[e] Failures included 1366 unidentified tissue samples.
[f] Subtypes of chromosome abnormalities could only be calculated in 1836 abnormal miscarriage samples including 102 unidentified tissue samples.
[g] Pooled estimates of the proportions with 95% CI were calculated using a random-effects meta-analyses in STATA 11.2.

Comparative Genomic Hybridization (CGH), array-Comparative genomic hybridization (array-CGH), fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA) and quantitative fluorescent polymerase chain reaction (QF-PCR) are molecular techniques that are often said to deal with (a part of) the limitations of conventional karyotyping. These molecular techniques can be subdivided in whole genome techniques (chromosomal-CGH and array-CGH) and chromosome specific techniques (FISH, MLPA and QF-PCR). However, a good comparison of these techniques has not been done so far.

2.4.1. Chromosomal comparative genomic hybridization (CGH)

Chromosomal-CGH is a technique that allows for the detection of losses and gains in DNA copy number across the entire genome. Resolution is limited to metaphase chromosomes (5–10 Mb pairs). Four studies evaluated the accuracy of chromosomal-CGH compared to conventional karyotyping. In total 369 sporadic miscarriage samples were investigated both by karyotyping as chromosomal-CGH (Table 5) [17,18,21,34]. It is important to realize that the studies differ in resolution of the tests used, sample sizes and diverge with regard to proportion of chromosome abnormalities detected by chromosomal-CGH (range of chromosome abnormalities 33–67%). Compared to chromosomal-CGH (52% (95% CI: 36–68), karyotyping detected a comparable amount of chromosome abnormalities (60% (95% CI: 49–72) in sporadic miscarriage samples. Also chromosomal-CGH missed a comparable amount of the abnormalities (6% (95% CI: 0–12)) compared to karyotyping which missed 2% (95% CI: 1–4) of the abnormalities.

Only one study compared chromosomal-CGH with karyotyping in miscarriage samples from women with recurrent miscarriage [35]. In this relatively small study chromosomal-CGH detected more abnormalities than conventional karyotyping (13 versus 5 abnormalities). Chromosomal-CGH and conventional karyotyping were performed on 12 samples, karyotyping detected five chromosome abnormalities while chromosomal-CGH detected six abnormalities. The other seven abnormalities were detected among 15 samples in which karyotyping failed. These abnormalities were unbalanced translocations. At first sight this seems logical, however the number of samples tested is simply too small to draw firm conclusions.

The use of chromosomal-CGH alone in clinical cytogenetics has severe limitations. Ploidy status or balanced chromosome abnormalities

Table 3
Cytogenetic findings among reported series of recurrent miscarriage samples by conventional karyotyping (percentages in parentheses).

Study	Total number of samples studied	Failures[a]	Successful karyotypes		Trisomy	Polyploidy	Monosomy X	Structural chromosome abnormalities	Others[b]
			Normal	Abnormal					
	n	n (%)	n (%)	n (%)	n (% of abn)	n (% of abn)	n (% of abn)	n (% of abn)	n (% of abn)
Stern 1996	94	0	40 (43)	54 (57)	45 (83)[c]	9 (17)	Not documented	Not documented	Not documented
Ogasawara 2000	458	224 (49)	114 (49)	120 (51)	63 (52)	18 (15)	5 (4)	0	34 (28)
Carp 2001	167	42 (25)	89 (71)	36 (29)	24 (67)	5 (14)	5 (14)	2 (6)	0
Stephenson 2002	472	58 (12)[d]	225 (54)	195 (46)	122 (63)	37 (19)	18 (9)	8 (4)	10 (5)
Sullivan 2004	135	13 (10)	91 (75)	31 (25)	21 (68)	6 (19)	3 (10)	1 (3)	0
Halder 2006	33	18 (55)	12 (80)	3 (20)	1 (33)	1 (33)	0	0	1 (33)
Total	1359	355	571	439	276	76	31	11	45
Prevalence		25%	61%	39%	65%	17%	8%	3%	13%
(95% CI)[e]		(9–41)	(50–71)	(29–50)	(54–76)	(14–21)	(4–12)	(0–6)	(0–26)

[a] Failures included: No fetal tissue found an failure of technique.
[b] Others included: mosaicism, double, triple and quadruple trisomies and autosomal monosomy.
[c] Described as aneuploidy.
[d] In 58 samples cytogenetic analysis was unsuccessful, however there were six sets of diamnotic twins therefore there were 420 samples with a successful karyotyping.
[e] Pooled estimates of the proportions with 95% CI were calculated using a random-effects meta-analyses in STATA 11.2.

Table 4
Additional submicroscopic findings in miscarriage samples detected by array-CGH and not detected with conventional karyotyping among reported series of women with sporadic miscarriage (percentages in parentheses).

Study	Total number of samples studied n	Submicroscopic abnormalities n (%)	Type of submicroscopic abnormalities	Study description
Schaeffer 2004	41	3/41 (7)	Duplication of the 10q telomere region; deletion at 9p21; duplication of the 15q telomere region	41 samples were analyzed by both array-CGH and conventional karyotyping
Benkhalifa 2005	26	2/26 (8)	Duplication of 1p-terminal; deletion at 22q13[a]	26 samples were analyzed by array-CGH that failed to grow in culture
Shimokawa 2006	20	1/20 (5)	Deletion at 3p26.2–p26.3 corresponding to clone RP11-30m15	20 samples with a normal karyotype were analyzed by array-CGH
Menten 2009	100	3/100 (3)	Duplication of (13)(q32.1qter) combined with deletion at (20)(pterp12.1); deletion at (7)(q36qter); deletion at (X)(q28qter)	100 samples were analyzed by both array-CGH in combination with FCM and conventional karyotyping
Robberecht 2009	103	1/91 (1)	Deletion of five clones (787.5 kb) at the steroid sulfatase locus	103 samples were analyzed by both array-CGH and conventional karyotyping
Zhang 2009	58	5/58 (9)	Deletion at 9p21.1; duplication of 2p12; duplication of 9q22.33; duplication of 19p11.2; duplication of 18p11.31	58 samples with normal karyotypes and non-poliploidy after culture failure were analyzed by array-CGH
Rajcan-Separovic 2010	14	5/14[b] (36)	Duplication of 10p15.3; duplication of 17p13.1; deletion at 1q25.3; duplication of Xq28; duplication of 13q32.1; deletion at 7q14.3	14 euploid miscarriage samples were analyzed by array-CGH
Total Prevalence (95% CI)[c]	362	20 Proportion 5% (1–8)		

CGH: comparative genomic hybridization.
FCM: flow cytometry.
[a] In one patient a monosomy 21 and a small amplification of 2 BACs at the X-chromosome were detected; the monosomy 21 being the main cause of miscarriage.
[b] 6 chromosome abnormalities in 5 embryos.
[c] Pooled estimates of the proportions with 95% CI were calculated using a random-effects meta-analyses in STATA 11.2.

remain undetected (see Table 1). Polyploidy can easily be detected by performing FISH or flow cytometry (FCM) additionally. One study combined chromosomal-CGH and FCM in a total of 253 spontaneous miscarriages samples [17]. The combination of the two techniques revealed 12/253 additional chromosome abnormalities compared to conventional karyotyping alone, because of culture failure. These results suggest that chromosomal-CGH supplemented with FCM can overcome the limitations of chromosomal-CGH alone and karyotyping.

Karyotyping may suffer from culture failure. One study performed chromosomal-CGH on 57 samples after culture failure by using karyotyping [36]. More than half of the miscarriage samples (65%, 37/57) showed chromosomal abnormalities by using chromosomal-CGH. Chromosomal-CGH can be used as an additional technique when karyotyping cannot be performed.

2.4.2. Array-comparative genomic hybridization (array-CGH)

Array-CGH screens the whole genome for submicroscopic chromosomal changes. So, more abnormalities can be detected by array-CGH compared to chromosomal CGH. Four studies evaluated the accuracy of array-CGH compared to conventional karyotyping on, in total, 264 sporadic miscarriage samples (Table 6) [8,9,20,37]. The studies differ in resolution of the tests used, sample sizes and diverge with regard to proportion of chromosome abnormalities detected by array-CGH (range of chromosome abnormalities 27–41%). Whereas array-CGH was used for detecting chromosomal aberrations, less abnormalities remained undetected compared to conventional karyotyping (array-CGH missed 2% (95% CI: 0–5) compared to 10% (95% CI: 6–14) by karyotyping).This could be explained by the fact that karyotyping has a higher failure rate (18% (95% CI: 7–30)) compared to array-CGH (5% (95% CI: 0–10)). Also, array-CGH detected submicroscopic abnormalities which remained undetectable for karyotyping (20 submicroscopic abnormalities of the 362 samples tested, 5%).

Overall, conventional karyotyping detected an equal number of chromosome abnormalities (30% (95% CI: 23–37) when compared to array-CGH (31% (95% CI: 14–38) in sporadic miscarriage samples. Evidence on the value of array-CGH in recurrent miscarriage is limited. Array-CGH can be used as an additional technique when conventional karyotyping fails. Table 7 shows that after culture failure array-CGH detects additional abnormalities. These abnormalities accounted for respectively 17 and 58% of the miscarriages samples in which karyotyping was unsuccessful, and were mostly trisomies and monosomies [30,33]. If only karyotyping was used these test results would not be possible.

As mentioned above (Section 2.4.1 Chromosomal comparative genomic hybridization), the use of array- CGH alone in clinical cytogenetics has severe limitations. To overcome these limitations one study combined array-CGH and FCM [8]. Compared to conventional karyotyping alone, the combination of array-CGH and FCM showed 10/100 additional chromosome abnormalities. To overcome the limitations of array-CGH alone, this technique can be supplemented with FCM. This combination also can overcome the limitations of conventional karyotyping.

2.5. Accuracy of the chromosome region specific techniques

2.5.1. Fluorescence in situ hybridization (FISH)

FISH is a mapping technique, which uses fluorescently labeled DNA probes to detect gains or losses of specific segments of DNA. It can be used as an additional technique for accurate cytogenetic evaluation of spontaneous miscarriages materials or to confirm the cytogenetic results, because FISH uses probes with specific genomic segments. Table 8 shows that FISH is a reliable technique to detect abnormalities where karyotyping failed [25,38–41]. However, the probe kits used, differed per study as shown in Table 8. One study included the largest number of miscarriage samples (n = 5555) and performed FISH successfully in 727/762 (95%) samples after culture failure [25]. So, in 95% a result could be obtained. We excluded unidentified tissue samples because of the reported high risk of maternal contamination.

Another study used FISH with Y chromosome probe (DYZ3: p11.1–q11.1 region) in 45 samples with an 46,XX karyotype to detect maternal contamination [42]. In two cases the Y-probe proved positive thus making maternal contamination plausible. Maternal contamination may result in false negative outcomes.

Only one study reporting on 57 sporadic miscarriage samples used FISH as additional technique to conventional karyotyping [19]. In this study, FISH identified chromosome abnormalities in four out of five

Table 5
Studies reporting on a comparison of CGH versus conventional karyotyping in sporadic miscarriage samples (percentages in parentheses).

Study	Total number of samples studied	Failures using CGH[a]	Failures using karyotyping[a]	CGH chromosome abnormalities	Karyotyping chromosome abnormalities	Abnormalities missed by CGH/ confirmed by karyotyping[b]	Abnormalities missed by karyotyping/ confirmed by CGH[c]
	n	n (%)	n (%)	n (%)	n (%)	n	n
Lomax 2000[d]	301	48 (16)	48 (16)	161/253 (64)	155/253 (61)	0	6
Bell 2001[e]	9	0	0	6/9 (67)	9/9 (100)	3	0
Tabet 2001[f]	21	0	2 (8)	7/21 (33)	11/21 (52)	3	0
Hu 2006	38	0	7 (18)	17/38 (45)	16/31 (45)	0	1
Total	369	48	57	191	191	6	7
Proportion (95% CI)[g]		12% (1–22)	16% (12–19)	52% (36–68)	60% (49–72)	6% (0–12)	2% (1–4)

CGH: comparative genomic hybridization.
FCM: flow cytometry.
[a]Failures included: No fetal tissue found an failure of technique.
[b]Abnormalities missed by CGH: aneuploidy, polyploidy and structural chromosome abnormalities.
[c]Abnormalities missed by karyotyping: aneuploidy, polyploidy and structural chromosome abnormalities.
[d]CGH + FCM analysis.
[e]CGH performed in 9 samples with confirmed chromosome abnormalities by karyotyping.
[f]Samples included first, second and third trimester miscarriages.
[g]Pooled estimates of the proportions with 95% CI were calculated using a random-effects meta-analyses in STATA 11.2.

cases with culture failure. Also two cases with normal karyotype proved to be false negative and showed a chromosome abnormality using FISH. This can be explained by maternal contamination. Otherwise, FISH missed four abnormalities because the probe was not designed to detect the specific trisomy.

2.5.2. Multiplex ligation-dependent probe amplification (MLPA)

MLPA is a multiplex PCR-based technique detecting abnormal copy numbers of up to 50 different genomic DNA sequences, using only small amounts of DNA (20–200 ng) [39]. Only few studies report on MLPA as a technique to detect chromosome abnormalities in sporadic or recurrent miscarriages samples. Only three studies compared MLPA versus karyotyping in sporadic miscarriages samples; Table 9 [10,22,37]. However, these studies used different MLPA kits, so the data cannot be pooled. All chromosome abnormalities missed by karyotyping were due to culture failure. The drawback of using MLPA is that polyploidy cannot be detected. One MLPA study successful investigated 74 samples [22]. Of the 74 samples, MLPA missed two triploid chromosome abnormalities. One study compared MLPA versus karyotyping in recurrent miscarriages samples [43]. MLPA technique missed more frequently a diagnosis (36/284 (13%)) compared to conventional karyotyping (8/284 (3%)). Otherwise, conventional karyotyping failed in 50/284 (18%) of the cases, due to culture failure (41 samples) and putrefaction of the tissue samples (9 samples). While MLPA showed no result in 4/284 (1%) of the samples, due to poor tissue quality (3 samples) and a low mass of DNA (1 sample). So, in case of culture failure, MLPA may be an alternative, but polyploidy remains undetected. If MLPA with subtelomeric probes was used when karyotyping provided no results, 37% of the cases showed chromosome abnormalities [44].

2.5.3. Quantitative fluorescent polymerase chain reaction (QF-PCR)

QF-PCR is a method in which DNA polymorphic markers on chromosomes are used to determine the presence of different alleles. The assay based on the use of informative polymorphic small tandem repeat (STR) markers (microsatellites) is used for prenatal and postnatal diagnosis of aneuploidies of chromosomes 13, 18, 21, X and Y. In 2005, this technique was first used to evaluate the value of short tandem repeats (microsatellites) of numerical chromosomal anomalies in 160 sporadic miscarriage samples with markers on nine different chromosomes (chromosomes 2, 7, 13, 15, 16, 18, 21, 22 and X) compared to karyotyping. Discrepancies between the two techniques occurred in eight out of 89 cases (9%). QF-PCR identified five chromosomally male miscarriage samples in which karyotyping showed a female karyotype, caused by maternal overgrowth. In two of these samples a trisomy 13 was identified by QF-PCR. In three cases an aneuploidy (the type of aneuploidy was not mentioned) was diagnosed by karyotyping, while QF-PCR showed a normal female pattern [23]. Another study used QF-PCR to detect chromosome abnormalities in 61 sporadic miscarriages samples and 48 controls. They used

Table 6
Studies reporting on a comparison of array-CGH versus conventional karyotyping in sporadic miscarriage samples (percentages in parentheses).

Study	Total number of samples studied	Failures using array-CGH[a]	Failures using karyotyping[a]	array-CGH chromosome abnormalities	Karyotyping chromosome abnormalities	Abnormalities missed by array-CGH/confirmed by karyotyping[b]	Abnormalities missed by karyotyping/confirmed by array-CGH[c]
	n	n (%)	n (%)	n (%)	n (%)	n	n
Schaeffer 2004	41	0	0	17/41 (41)	16/41 (39)	0	4
Menten 2009[d,e]	100	2 (2)	28 (28)	26/98 (27)	17/72 (24)	1	10
Robberecht 2009	103	12 (12)	26 (25)	26/91 (29)	22/77 (29)	4	10
Desphande 2010	20	0	0	8/20 (40)	8/20 (40)	0	0
Total	264	14	54	77	63	5	24
Proportion (95% CI)[f]		5% (0–10)	18% (7–30)	31% (14–38)	30% (23–37)	2% (0–5)	10% (6–14)

CGH: comparative genomic hybridization.
[a] Failures included: No fetal tissue found an failure of technique.
[b] Abnormalities missed by array-CGH: aneuploidy, structural chromosome abnormalities.
[c] Abnormalities missed by karyotyping: aneuploidy, polyploidy, mosaicism and submicroscopic abnormalities.
[d] CGH + FCM analysis.
[e] Samples included first, second and third trimester miscarriages.
[f] Pooled estimates of the proportions with 95% CI were calculated using a random-effects meta-analyses in STATA 11.2.

Table 7
Studies reporting on chromosome testing by using array-CGH alone or after failed conventional karyotyping in sporadic miscarriage samples (percentages in parentheses).

Study	Total number of samples studied	Array-CGH chromosome abnormalities	Explanation
	n	n (%)	
Benkhalifa 2005	26	15/26 (58)	Array-CGH performed in 26 samples after culture failure[a]
Warren 2009[b]	30	5/30 (17)	Karyotyping not performed in 26 samples, and normal in 4 samples[c]

CGH: comparative genomic hybridization.
[a] The detected abnormalities were triploidy, trisomy/monosomy 1, 8, 13, 14, 16, 18, 21, 22 and X, one loss on 22q13, one gain 1p terminal and one amplification on the X chromosome was detected.
[b] Samples: fetal loss between 10 and 20 weeks of gestation.
[c] The detected abnormalities were trisomy 21, monosomy X, triploidy and a triploidy with an trisomy 13.

microsatellite markers of eight most commonly found chromosome abnormalities (chromosomes 13, 14, 15, 16, 18, 21, 22 and sex chromosomes). Twenty-two (22/61; 36%) samples were diagnosed with a numerical abnormality. Only two cases showed another outcome than the result of karyotyping; both karyotyping as well as QF-PCR technique missed one abnormality [45]. QF-PCR missed a 46XY/46XX karyotype confirmed by karyotyping. Another sample karyotyping showed 46 XX, while QF-PCR showed irregular peaks.

One study used PCR-based microsatellite genotyping for the identification of trisomy or polyploidy in those cases of culture failure or maternal cell contamination [7]. PCR-based genotyping identified 2/23 (9%) cases with triploidy.

Since the number of chromosomes tested for in this technique is limited, the question whether QF-PCR is a reliable technique on its own, leads to discussion. A great disadvantage is that this technique works with markers for specific chromosomes and does not screen the whole genome. So the success rate largely depends on the chromosome markers used. Few studies reported about the use of QF-PCR, so more research is required to determine whether this technique can be used on its own as a diagnostic tool to detect chromosome abnormalities in miscarriage samples. An advantage above karyotyping is that it is fast and relatively cheap. Furthermore, compared to MLPA, it can detect polyploidies.

3. Discussion

Testing miscarriage samples for the purpose of determining the genetic contribution causing the loss is of undisputable value. A miscarriage can be regarded as a rescue mechanism, to prevent an implanted but abnormal pregnancy from further growth [46]. Although it has been frequently mentioned that the prevalence of chromosome abnormalities in miscarriage samples is higher than 50% and the finding of submicroscopic chromosome abnormalities contributes to an even higher percentage of chromosome abnormalities, this could not be confirmed after carefully reviewing existing literature [7–9]. The prevalence of chromosome abnormalities whether tested by the gold standard, chromosome region specific techniques and whole genome techniques in sporadic and recurrent miscarriage samples on average never exceeded half of the samples tested. A chromosomal cause for the loss therefore only exists in maximum 50% of all miscarriages.

Opinions differ as to the usefulness of karyotyping of miscarriage samples for routine clinical practice. There is no clear relevance for clinical decision-making, but a genetic test result may provide information for the woman or couple in question [47]. Guidelines on the topic of recurrent miscarriage vary with regard to their advices. The European Society of Obstetrics and Gynaecology (ESHRE) advises genetic evaluation of miscarriage samples only within the setting of scientific studies [48]. The Royal College of Obstetricians and Gynaecologists (RCOG) recommends fetal karyotyping for chromosome abnormalities whereas in contrast the Dutch Society of Obstetrics and Gynaecology (NVOG) recommends no fetal cytogenetic analysis [49,50].

This review provides an overview of what is currently known about (submicroscopic) genetic abnormalities in sporadic and recurrent miscarriage samples by the use of the whole genome techniques (karyotyping, chromosomal-CGH and array-CGH) and chromosome region specific techniques (FISH, MLPA and QF-PCR). Cytogenetic abnormalities in miscarriages are re-evaluated by providing an overview of recent literature since 2000. The prevalence of chromosome abnormalities in women facing a single sporadic miscarriage proved to be 45% (95% CI: 38–52) in 7012 samples collected. The prevalence of chromosome abnormalities in women undergoing a subsequent miscarriage after preceding RM was 39% (95% CI: 29–50) in 1359 samples collected. The overall pooled prevalence of submicroscopic abnormalities additionally found next to conventional karyotyping in sporadic miscarriage samples was 5%. However, the clinical relevance of submicroscopic abnormalities, whether it is a causal factor, is not clear in most reported cases.

At present, more chromosome abnormalities are found by using the gold standard (conventional karyotyping) than by the use of FISH or MLPA. However, data about these techniques are limited and the detection rate of FISH or MLPA strongly depends on the number and type of chromosomes tested as karyotyping provides a whole

Table 8
Studies reporting on outcomes of FISH in sporadic miscarriage samples (percentages in parentheses).

Study	Chromosome probes used	Total number of samples studied	Samples with chromosome abnormalities detected by FISH	Explanation
		n	n (%)	
Lebedev 2004	1, 5, 13–16, 18, 19, 21, 22, X, Y	60[a]	32/60 (53)	FISH after culture failure
Lescoat 2005	13, 16, 18, 21, X, Y	202	83/196 (42)	FISH after culture failure
Vorsanova 2005[b]	1, 9, 13, 14, 15, 16, 18, 21, 22, X, Y	148	89/148 (60)	Only FISH applied
Jobanputra 2011	13, 15, 16, 18, 21, 22, X, Y	324	108/324 (33)	FISH in 171 samples with a normal karyotype and in 153 samples after culture failure or contamination
Shearer 2011	13, 16, 18, 21, 22, X, Y	762	181/727 (25)	FISH after culture failure, unidentified tissue excluded

FISH: fluorescence in situ hybridization.
[a] 146 samples were collected, FISH analysis was performed in 60 samples.
[b] Failures are not reported.

Table 9
Studies reporting on a comparison of MLPA versus conventional karyotyping in sporadic miscarriage samples (percentages in parentheses).

Study	MLPA kit	Total number of samples studied	Samples with chromosome abnormalities detected by MLPA	Samples with chromosome abnormalities detected by karyotyping	Samples with abnormalities missed by MLPA/detected by karyotyping	Samples with abnormalities missed by karyotyping/ detected by MLPA
		n	n (%)	n (%)	n	n
Bruno 2006	Subtelomeric probe kits	78	28/74 (38)	29/67 (43)	2	1
Desphande 2010	Subtelomeric probe kits	20	4/20 (20)	8/20 (40)	2	0
Carvalho 2010[a,b]	Aneuploidy (chromosomes 13, 18, 21, X and Y)	489	38/489 (8)	98/328 (30)	60	13

MLPA: Multiplex ligation-dependent probe amplification.
[a] 38 out of the 489 samples were intra-uterine fetal deaths, the other samples were miscarriage samples.
[b] In 161 samples only MLPA results were available.

genome analysis. A great advantage of using these techniques is that no cell culture is needed, as 20% of the cell cultures fails [25]. QF-PCR is also a chromosome region specific technique but performed as good as conventional karyotyping, although evidence was limited and its result depends on the chromosome markers or probes used.

When using whole genome techniques, chromosomal-CGH detected an equal amount of chromosome abnormalities (52% (95% CI: 36–68)) and missed a comparable amount of chromosome abnormalities (6% (95% CI: 0–12)) compared to karyotyping (60% (95% 49–72)) and (2% (95% CI: 1–4)). Array-CGH detected an equal number of chromosome abnormalities (31% (95% CI: 14–38)) compared to karyotyping (30% (95% CI: 23–37)), but missed less chromosome abnormalities (2% (95% CI: 0–5)) compared to karyotyping (10% (95% CI: 6–14)). This can be explained by the fact that karyotyping had a higher failure rate (18% (95% CI: 7–30)) compared to array-CGH (5% (95% CI: 0–10)).

The molecular techniques offer the advantage of quick results and higher resolutions. The expectations of the molecular techniques, in particular the array-CGH, MLPA and FISH techniques are high, especially by using array-CGH, because it screens the whole genome. Surprisingly, no more chromosome abnormalities were detected by chromosomal-CGH, array-CGH and FISH, and costs are higher compared with the gold standard. More importantly, certain abnormalities like polyploidy or balanced chromosome abnormalities remain undetectable by chromosomal-CGH, array-CGH and MLPA. QF-PCR can detect polyploidy, but the detection rate of chromosome abnormalities is strongly correlated with the markers used.

Molecular techniques like array-CGH, FISH and MLPA may have certain advantages apart from routine cytogenetic analysis of miscarriage samples for investigation of chromosomal abnormalities. FISH and MLPA can detect only pre-selected submicroscopic abnormalities while array-CGH can detect chromosome abnormalities in the whole genome. These techniques enable us to detect submicroscopic chromosome abnormalities which cannot be found by traditional techniques. More, larger, studies should be performed to determine the clinical relevance of the submicroscopic chromosome abnormalities.

In case of culture failure or maternal contamination, molecular techniques may contribute to detect additional chromosome abnormalities in these miscarriages samples in addition to standard karyotyping. The value of knowledge on prevalence of cytogenetic abnormalities in miscarriage samples is undisputed, but the relevance of the molecular techniques for daily clinical practice is still a point of discussion.

4. Conclusions and recommendations

Chromosomal-CGH, array-CGH, FISH, MLPA and QF-PCR can possibly play a complementary role to karyotyping, especially in case of culture failure. By using these techniques independently, instead of conventional karyotyping, unfortunately they show no added clinical value. It is precisely for that reason that knowledge of submicroscopic abnormalities and the molecular techniques needs to be improved.

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