RBMO

REVIEW

Cytogenetic testing of pregnancy loss tissue: a meta-analysis

BIOGRAPHY

Myrthe Smits works as a PhD candidate and fertility doctor at the Center for Reproductive Medicine in the Amsterdam University Medical Center. As clinician and researcher, her focus is mainly on pregnancy loss and ovarian ageing.

Myrthe A.J. Smits[1], Merel van Maarle[2], Geert Hamer[1], Sebastiaan Mastenbroek[1], Mariëtte Goddijn[1], Madelon van Wely[1,*]

KEY MESSAGE

Chromosomal abnormalities are identified in almost half of pregnancy losses. The percentage of detected abnormalities is comparable in women that have suffered from sporadic or recurrent pregnancy loss. Routine testing of pregnancy loss tissue for chromosomal abnormalities has no clinical benefit.

ABSTRACT

Many clinics offer routine genetic testing of pregnancy loss tissue. This review presents a comprehensive literature search and meta-analysis on chromosomal abnormality rates of pregnancy loss tissue from women with a single or recurrent pregnancy loss. A total of 55 studies published since 2000 were included, analysed on the prevalence of test failure rates, abnormality detection rates and percentages of trisomy, monosomy X, structural abnormalities and other clinically (ir)relevant abnormalities detected by conventional karyotyping, array-comparative genomic hybridization (aCGH), single nucleotide polymorphism (SNP) array, fluorescence in-situ hybridization (FISH) and multiplex ligation-dependent probe amplification (MLPA). The detected prevalence of chromosomal abnormalities was 48% (95% confidence interval [CI] 39–57) using aCGH, 38% (95% CI 28–49) with FISH, 25% (95% CI 12–42) using MLPA, 60% (95% CI 58–63) using SNP array and 47% (95% CI 43–51) with conventional karyotyping. The percentage of detected abnormalities did not differ between women that suffered sporadic (46%; 95% CI 39–53) or recurrent (46%; 95% CI 39–52) pregnancy loss. In view of the high prevalence of chromosomal abnormalities in pregnancy loss tissue, and the low chance of recurrence of the same chromosomal aberration, it was concluded that detection of specific chromosomal abnormalities in pregnancy loss tissue has no clinical benefit. Therefore, routine testing of pregnancy loss tissue for chromosomal abnormalities is not recommended.

[1] Amsterdam UMC, University of Amsterdam, Center for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute Amsterdam, the Netherlands
[2] Department of Clinical Genetics, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, Amsterdam AZ 1105, the Netherlands

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*Corresponding author E-mail address: m.vanwely@amsterdamumc.nl (M. van Wely). https://doi.org/10.1016/j.rbmo.2020.02.001 1472-6483/© 2020 Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.
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INTRODUCTION

Of all clinically recognized pregnancies, about 15% end in loss (Nybo Andersen et al., 2000; Rai and Regan, 2006). The great majority of pregnancy losses occur within the first trimester of pregnancy (Baird, 2009; Farr et al., 2007). Aneuploidy of the embryo is an important female age-related genetic cause of pregnancy loss and can be tested in pregnancy loss tissue (Hassold and Hunt, 2001; Rai and Regan, 2006). A proportion of couples (1–3%) not only suffer from one 'sporadic' pregnancy loss but from recurrent pregnancy loss, meaning two or more pregnancy losses (historically three or more losses) (Ford and Schust, 2009). It is believed that other mechanisms could be involved in recurrent pregnancy loss compared with sporadic pregnancy loss (Thangaratinam et al., 2011; van den Boogaard et al., 2011). However, only a few differences can be found between sporadic and recurrent pregnancy loss, for example thyroid autoimmunity and antiphospholipid syndrome (ESHRE Early Pregnancy Guideline Development Group, 2017; van den Boogaard et al., 2011). If other mechanisms than cytogenetic abnormalities play a role in recurrent pregnancy loss, and not in sporadic pregnancy loss, it would be expected that women suffering recurrent pregnancy loss lose more pregnancies without a chromosome aberration (Sullivan et al., 2004). A previous review by the current authors, on prevalence of chromosomal abnormalities, found that the abnormality detection rate of conventional karyotyping of pregnancy loss tissue of women who suffered one pregnancy loss was the same as the abnormality detection rate in pregnancy loss tissue of women who suffered more pregnancy losses. This could suggest that any underlying mechanism that would distinguish sporadic from recurrent pregnancy loss is not likely to be caused by chromosomal abnormalities (van den Berg, 2012).

Genetic testing of pregnancy loss tissue is not recommended by the ESHRE 2017 recurrent pregnancy loss guidelines as routine practice after pregnancy loss. However, the Guideline Development Group stated that genetic testing could be performed for explanatory purposes (Bender Atik et al., 2018; ESHRE Early Pregnancy Guideline Development Group, 2017). Routine testing is not recommended because of the high rates of abnormalities present in pregnancy loss tissue and the low recurrence risk. Even when one partner of the couple is a carrier of a chromosomal aberration, the chances of having a live birth of a healthy child during the next pregnancy are the same compared with couples without carrier status (ESHRE Early Pregnancy Guideline Development Group, 2017; Franssen et al., 2011). Therefore, detection of a chromosomal abnormality in pregnancy loss tissue, whatever the cause, does not seem to have clinical relevance. Still, many clinics offer routine genetic testing of pregnancy loss tissue. With the enhancement of already available testing techniques, and the introduction of new ones, it has been suggested that new or further chromosomal abnormalities may be identified. However, the question is whether chromosomal abnormalities that are identified by new techniques now explain why a couple suffered a pregnancy loss and if so, do these newly found abnormalities influence clinical outcome in future pregnancies or clinical practice? As new testing techniques have been introduced since the previous review (Van den Berg et al., 2012) and already implemented genetic testing techniques have been further developed, this review provides an update on the test results of the techniques that are currently used for genetic testing of pregnancy loss tissue. In addition, by combining the data of the different testing techniques, this review will compare the chromosomal abnormality rates of pregnancy loss tissue from women with a history of recurrent pregnancy loss with that of women who have had only one pregnancy loss.

MATERIALS AND METHODS

The same comprehensive literature search as described by van den Berg et al. (2012) was used to identify articles up to October 2019. A search in PubMed, Embase and CINAHL was conducted to identify articles reporting cytogenetic testing of pregnancy loss tissue published between January 2000 and October 2019. Studies published since 2000 were included, as newer techniques like single nucleotide polymorphism (SNP) array have been used since then and as older studies could create performance bias due to improvement of techniques over time. Search terms (and synonyms) used included 'pregnancy loss', 'nucleic acid hybridization', 'submicroscopic', 'fluorescence in-situ hybridization', 'comparative genomic hybridization', 'next-generation sequencing', 'multiplex ligation-dependent probe amplification', 'single nucleotide polymorphism' and 'quantitative fluorescent polymerase chain reaction'.

Selection of studies

Two reviewers (MS and MW) independently evaluated potentially eligible papers by reading the title and abstract. Subsequently, the full texts of all potentially eligible articles were read and studies were selected that investigated the genetic tests mentioned above. No core outcomes have been developed in this field.

Only original studies were considered and therefore reviews, case reports or case series and editorials were excluded. Only English language full-text articles were included. Articles were excluded when reported results were not of individual testing techniques, but rather a combination of techniques, when one specific gene mutation or only gene mutations of unknown significance were tested, or when not all the desired data could be extracted after the authors were asked for additional information. Techniques were excluded when fewer than three papers had been published on that technique.

Outcomes

Chromosome abnormality detection rate was considered to be the primary outcome. Secondary outcomes were types of abnormalities identified and failure rates. Types of abnormalities included: trisomy, polyploidy, monosomy X, structural abnormalities and other chromosomal abnormalities including: variants of unknown significance, complex or multiple abnormalities, mosaicism, monosomy (not X), uniparental disomy or trisomy of the sex chromosomes. These other chromosome abnormalities were further specified as clinically relevant or clinically irrelevant/clinical relevance unknown. Because female and gestational age are known to influence chromosomal abnormality rates (Carvalho et al., 2010; Jia et al., 2015; Kushnir and Frattarelli, 2009; Soler et al., 2017; Spandorfer et al., 2004), this update also includes the mean female ages and the mean gestational ages at time of pregnancy loss, if available.

Data analysis

Outcomes from individual studies were reported into tables and presented descriptively as proportions and 95% confidence intervals (CI) for all outcomes. Meta-analysis on proportions was carried out when data of more than two studies could be combined using a random effects model. Heterogeneity was assessed using the I^2 statistic. An I^2 value >50% was considered substantial heterogeneity and an I^2 >75% considerable heterogeneity. Pooled proportions were presented with a 95% CI and the I^2 using STATA 14.3 (StataCorp LP, College Station, TX, USA).

RESULTS

Literature searching using the parameters from a previous review (*van den Berg et al., 2012*) identified 525 original papers published between January 2012 and October 2019. After abstract screening, 56 papers remained eligible. Of these, 23 papers did not meet the inclusion criteria and were excluded from the analysis, because the articles were not written in English, did not provide data on individual tests, contained incomplete data or concerned a test technique for which fewer than two articles were identified (next-generation sequencing [NGS] and polymerase chain reaction [PCR]). The remaining 33 original studies were found to fulfil the inclusion criteria. Twenty-two original articles cited in the previous review (*van den Berg et al., 2012*) were also included, giving a total of 55 studies. Most articles identified focused on one of the cytogenetic techniques, rather than comparing them.

Conventional karyotyping

Twenty-nine studies, comprising 18,473 samples, focused on conventional karyotyping and the test results are summarized in TABLE 1. The failure rate due to culture failure or maternal cell contamination was 16% (95% CI 11–23) of all pregnancy loss tissues included. Out of the successful karyotyped samples, 47% (95% CI 43–51) had chromosomal abnormalities. The proportion of abnormalities was divided as follows: trisomies 62% (95% CI 59–66), followed by polyploidies 16% (95% CI 14–17), monosomies X 8% (95% CI 7–10), structural abnormalities 4% (95% CI 3–6) and other chromosomal abnormalities, clinically relevant 3% (95% CI 1–6), clinically irrelevant/unknown clinical relevance 0% (95% CI 0–0).

There was considerable heterogeneity (I^2 = 94.1%) in observed proportions across the studies; the proportion of chromosomal abnormalities varied between 20% (*Halder and Fauzdar, 2006*) and 70% (*Soler et al., 2017*). This might be partly due to differences in female age and the gestational age of products of conception included in the study. Fourteen of the 29 studies presented details on female age and 16 studies had details on gestational age. Reported average female age ranged from 30.0 to 37.2 years in studies and average gestational age from 7.9 to 27.8 weeks.

Array-comparative genomic hybridization (aCGH)

Fifteen studies, entailing 3583 samples, focused on aCGH, and the reported test results are summarized in TABLE 2. aCGH detected chromosomal abnormalities in 48% (95% CI 39–57) of tested samples. The failure rate of aCGH was 2% (95% CI 0–5).

The proportion of abnormalities was divided as follows: trisomies 58% (95% CI 44–71), polyploidies 2% (95% CI 0–5), monosomies X 10% (95% CI 7–14), structural abnormalities 9% (95% CI 3–17) and other chromosomal abnormalities: clinically relevant 9% (95% CI 3–17) and clinically irrelevant or unknown 0.02% (95% CI 0.00–1.08).

Three out of 15 studies identified polyploidy by using aCGH (*Gliem and Aypar, 2017*; *Rosenfeld et al., 2015*; *Shen et al., 2016*). Other studies reporting on polyploidies used fluorescence in-situ hybridization (FISH) or flow cytometry in addition to aCGH (*Li et al., 2017*; *Menten et al., 2009*; *Robberecht et al., 2012*).

The heterogeneity between studies was considerable (I^2 = 94.0%), with proportions of abnormalities ranging from 17% (*Rosenfeld et al., 2015*) to 84% (*Gliem and Aypar, 2017*). The average female age reported varied between 30.8 and 35.7 years, and where the average gestational age was reported, it was from a first trimester pregnancy loss.

SNP array

A total of 5391 pregnancy loss tissues were analysed in ten studies by SNP array, the results of which are summarized in TABLE 3. The failure rates ranged from 0% to 30%, with an overall failure rate of 4% (95% CI 0–13). Sixty per cent (95% CI 58–63) of successfully tested pregnancy loss tissues were classified as abnormal, including trisomies 61% (95% CI 55–67), polyploidies 9% (95% CI 7–11), monosomies X 8% (95% CI 5–11), structural abnormalities 7% (95% CI 5–10) and other abnormalities 9% (95% CI 6–13) (clinically relevant) and 1.27 (95% CI 0.02–3.67) (clinically irrelevant/unknown).

The heterogeneity between the studies was substantial (I^2 = 61.0%). Nine out of ten studies reported the average female age, which ranged from 29.7 years (*Qu et al., 2019*) to 37.2 years (*Lathi et al., 2012*). Gestational age was also reported by nine out of ten studies; two studies reported to only have included first trimester pregnancy losses and seven studies reported the mean gestational age. The mean gestational age ranged from 7.7 weeks (*Levy et al., 2014*) to 27.8 weeks (*Zhu et al., 2016*).

FISH

Results of the FISH technique are shown in TABLE 4, including the probes used. The overall failure rate was 1% (95% CI 0–4). The chromosomal anomaly detection rate was 38% (95% CI 28–49). FISH as used in these studies does not detect structural abnormalities. The proportions of found abnormalities were further divided as follows: trisomies 60% (95% CI 56–64), polyploidies 19% (95% CI 16–23), monosomies X 13% (95% CI 9–17) and other abnormalities 5% (95% CI 2–8) (clinically relevant) and 0.05% (95% CI 0.00–0.46) clinically irrelevant/unknown.

Again, there was considerable heterogeneity between the studies (I^2 = 96.7%). Only four out of nine studies reported on gestational age, of which only *Haoud et al. (2014)* included pregnancy loss tissues of all trimesters. Five studies reported on female age, which ranged from 31.0 (*An et al., 2015*) to 34.7 years (*Russo et al., 2016*). All studies included probes for chromosomes 13, 18, 21, X and Y. In addition, some studies included probes on chromosomes 1, 5, 14, 15, 16, 19 and 22.

Multiplex ligation-dependent probe amplification (MLPA)

Eight studies focused on MLPA. The results of the studies, including probes used, are shown in TABLE 5. MLPA testing failed in 5% (95% CI 0–14) of cases

TABLE 1 REPORTED RESULTS FROM ARTICLES USING CONVENTIONAL KARYOTYPING

Study	Total number of samples	Failures	Successful tested		Trisomy	Polyploidy	Monosomy X	Structural abnormalities	Others		Mean age	Mean GA
			Normal	Abnormal					Clinically relevant	Clinically irrelevant/unknown		
	n	*n* (%)	*n* (%)	*n* (%)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)		
Soler et al., 2017	1119	108 (10)	300 (30)	711 (70)	459 (65)	103 (14)	74 (10)	37 (5)	35 (5)	3 (0)	NR	1st trimester
Pylyp et al., 2018	1000	0 (0)	499 (50)	501 (50)	290 (58)	110 (22)	38 (8)	35 (7)	25 (5)	3 (1)	33.7	7.9
Zhu et al., 2016	12	1 (8)	7 (64)	4 (36)	3 (75)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	NR	27.8
Zhou et al., 2016	1180	179 (15)	513 (51)	488 (49)	291 (60)	88 (18)	63 (13)	24 (5)	20 (4)	2 (0)	36.6	NR
Lin et al., 2015	155	22 (14)	49 (37)	84 (63)	NR	NR	NR	NR	NR	NR	32.2	9.6
Wang et al., 2014	5457	1365 (25)	2220 (54)	1872 (46)	1236 (66)	337 (18)	243 (13)	37 (2)	19 (1)	0 (0)	NR	NR
Jenderny, 2014	534	144 (27)	153 (39)	237 (61)	111 (47)	54 (23)	16 (7)	10 (4)	46 (19)	0 (0)	NR	<34
Chang et al., 2013	100	34 (34)	46 (70)	20 (30)	11 (55)	3 (15)	3 (15)	1 (5)	0 (0)	2 (10)	33.2	8
Gao et al., 2012	100	14 (14)	39 (45)	47 (55)	40 (85)	3 (6)	2 (4)	1 (2)	1 (2)	0 (0)	32	1st trimester
Lathi et al., 2012	30	0 (0)	10 (33)	20 (67)	16 (80)	2 (10)	0 (0)	2 (10)	0 (0)	0 (0)	37.2	8.8
Shearer et al., 2011	5555	1033 (19)	2686 (59)	1836 (41)	1074 (58)	278 (15)	260 (14)	135 (7)	89 (5)	0 (0)	NR	NR
Carvalho et al., 2010	489	161 (33)	243 (74)	85 (26)	47 (55)	19 (22)	8 (9)	5 (6)	1 (1)	5 (6)	30	21.5
Deshpande et al., 2010	20	0 (0)	12 (60)	8 (40)	2 (25)	0 (0)	1 (13)	3 (38)	2 (25)	0 (0)	NR	NR
Robberecht et al., 2009	103	26 (25)	55 (71)	22 (29)	10 (45)	5 (23)	6 (27)	1 (5)	0 (0)	0 (0)	NR	NR
Menten et al., 2009	100	28 (28)	55 (76)	17 (24)	10 (59)	3 (18)	2 (12)	2 (12)	0 (0)	0 (0)	NR	NR
Zhang et al., 2009	115	23 (20)	37 (40)	55 (60)	36 (65)	8 (15)	5 (9)	2 (4)	4 (7)	0 (0)	NR	NR
Doria et al., 2009	232	59 (25)	107 (62)	66 (38)[a]	36 (55)	13 (20)	6 (9)	5 (8)	15 (23)	0 (0)	32.1	All trimesters
Diego-Alvarez et al., 2005	221	119 (54)	62 (61)	40 (39)	24 (60)	5 (13)	6 (15)	1 (3)	4 (10)	0 (0)	NR	6–24
Bruno et al., 2006	78	11 (14)	38 (57)	29 (43)	17 (59)	3 (10)	2 (7)	7 (24)	0 (0)	0 (0)	NR	22
Hu et al., 2006	38	7 (18)	15 (48)	16 (52)	12 (75)	2 (13)	2 (13)	0 (0)	0 (0)	0 (0)	22–47	7–13
Halder and Fauzdar, 2006	33	18 (55)	12 (80)	3 (20)	1 (33)	1 (33)	0 (0)	0 (0)	1 (33)	0 (0)	NR	NR
Schaeffer et al., 2004	41	0 (0)	25 (61)	16 (39)	13 (81)	1 (6)	1 (6)	1 (6)	0 (0)	0 (0)	NR	NR
Sullivan et al., 2004	285	30 (11)	168 (66)	87 (34)[a]	56 (64)	18 (21)	8 (9)[b]	4 (5)	0 (0)	0 (0)	31	NR
Jobanputra et al., 2002	57	5 (9)	22 (42)	30 (58)	17 (57)	6 (20)	2 (7)	0 (0)	5 (17)	0 (0)	NR	<9
Stephenson et al., 2002	472	52 (11)	225 (54)	195 (46)	121 (62)	37 (19)	18 (9)	8 (4)	10 (5)	1 (1)	34.3	<20
Tabet et al., 2001	21	0 (0)	10 (48)	11 (52)	6 (55)	1 (9)	1 (9)	3 (27)	0 (0)	0 (0)	NR	NR
Carp et al., 2001	167	42 (25)	89 (71)	36 (29)	24 (67)	5 (14)	5 (14)	2 (6)	0 (0)	0 (0)	31.6	<20
Lomax et al., 2000	301	48 (16)	98 (39)	155 (61)	111 (72)	25 (16)	12 (8)	7 (5)	0 (0)	0 (0)	Mostly advanced maternal age	
Ogasawara et al., 2000	458	224 (49)	114 (49)	120 (51)	63 (53)	18 (15)	5 (4)	0 (0)	34 (28)[c]	NR	29.4–34.4	NR
Estimated proportion[d]		16	55	47	62	16	8	4	3	0		
95% CI		12–21	48–62	43–51	59–66	14–17	7–10	3–6	1–6	0–0		

Studies included in the conventional karyotyping analysis. Including mean age and GA of included women, failure rates, abnormality rates of successfully tested specimen and proportion of trisomy, monosomy X, structural abnormalities and other abnormalities, specified as clinically relevant, or clinically irrelevant/relevance unknown.

[a] Total number of reported results does not correspond with the total number of successfully karyotyped specimens. The numbers shown here represent number shown in article.

[b] Total number of monosomy (not specified as X).

[c] Unable to specify as clinically relevant or not.

[d] Calculated by meta-analysis.

CI = confidence interval; GA = gestational age; NR = not reported.

TABLE 2 REPORTED RESULTS FROM ARTICLES USING ACGH

Study	Total number of samples	Failures	Successful tested		Trisomy	Polyploidy	Monosomy X	Structural abnormalities	Others		Mean age	Mean GA
			Normal	Abnormal					Clinically relevant	Clinically irrelevant/ unknown		
	n	*n* (%)	*n* (%)	*n* (%)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)		
Li et al., 2017	2172	0 (0)	1196 (55)	976 (45)	796 (82)	8 (1)[a]	116 (12)	4 (0)	50 (5)	2 (0)	32.2	NR
Gliem and Aypar, 2017	25	0 (0)	4 (16)	21 (84)	12 (57)	5 (24)	2 (10)	0 (0)	2 (10)	0 (0)	NR	NR
Chen et al., 2017	98	0 (0)	49 (50)	49 (50)	36 (73)	0 (0)	9 (18)	3 (6)	1 (2)	0 (0)	31.6	10.2
Ozawa et al., 2016	15	0 (0)	5 (33)	10 (67)	7 (70)	0 (0)	0 (0)	1 (10)	2 (20)	0 (0)	35.7	7.9
Shen et al., 2016	256	0 (0)	125 (49)	131 (51)	115 (88)	9 (7)	0 (0)	7 (5)	0 (0)	0 (0)	30.8	NR
Rosenfeld et al., 2015	535	20 (4)	425 (83)	90 (17)	21 (23)	1 (1)	6 (7)	29 (32)	33 (37)	0 (0)	NR	All trimesters
Kudesia et al., 2014	20	4 (20)	8 (50)	8 (50)	3 (38)	0 (0)	0 (0)	2 (25)	1 (13)	2 (25)	33	8
Robberecht et al., 2012	51	19 (37)	11 (34)	21 (66)	9 (43)	7 (33)	4 (19)	1 (5)	0 (0)	0 (0)	31.7	7.5
Gao et al., 2012	100	0 (0)	43 (43)	57 (57)	48 (84)	0 (0)	4 (7)	3 (5)	2 (4)	0 (0)	32	NR
Deshpande et al., 2010	20	0 (0)	12 (60)	8 (40)	3 (38)	0 (0)	0 (0)	3 (38)	2 (25)	0 (0)	NR	NR
Zhang et al., 2009	21	0 (0)	8 (38)	13 (62)	6 (46)	0 (0)	2 (15)	5 (38)	0 (0)	0 (0)	NR	NR
Robberecht et al., 2009	103	12 (12)	65 (71)	26 (29)	13 (50)	0 (0)[a]	8 (31)	0 (0)	2 (8)	3 (12)	NR	NR
Menten et al., 2009	100	2 (2)	72 (73)	26 (27)	15 (58)	3 (12)[a]	5 (19)	3 (12)	0 (0)	0 (0)	NR	NR
Benkhalifa et al., 2005	26	0 (0)	11 (42)	15 (58)	4 (27)	0 (0)	1 (7)	2 (13)	7 (47)	1 (7)	NR	9–11
Schaeffer et al., 2004	41	0 (0)	24 (59)	17 (41)	10 (59)	0 (0)	1 (6)	2 (12)	3 (18)	1 (6)	NR	NR
Estimated proportion[b]		2	52	48	58	2	10	9	9	0		
95% CI		0–5	43–61	39–57	44–71	0–5	7–14	3–18	3–17	0–1		

Studies included in the aCGH analysis. Including mean age and GA of included women, failure rates, abnormality rates of successfully tested specimen and proportion of trisomy, monosomy X, structural abnormalities and other abnormalities, specified as clinically relevant, or clinically irrelevant/relevance unknown.

[a] Polyploidy detected with additional technique.

[b] Calculated by meta-analysis.

aCGH = array-comparative genomic hybridization; CI = confidence interval; GA = gestational age; NR = not reported.

TABLE 3 REPORTED RESULTS FROM ARTICLES USING SNP ARRAY

Study	Total number of samples	Failures	Successful tested		Trisomy	Polyploidy	Monosomy X	Structural abnormalities	Others		Mean age	Mean GA
			Normal	Abnormal					Clinically relevant	Clinically irrelevant/ unknown		
	n	*n* (%)	*n* (%)	*n* (%)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of ab-normal)	*n* (% of abnormal)	*n* (% of abnormal)		
Qu et al., 2019	484	16 (3)	178 (38)	290 (62)	150 (52)	35 (12)	42 (14)	21 (7)	26 (9)	16 (6)	29.7	9.4
Li et al., 2018	1493	0 (0)	550 (37)	943 (63)	597 (63)	63 (7)	52 (6)	72 (8)	159 (17)	0 (0)	32.1	NR
Wang et al., 2017	551	16 (3)	225 (42)	310 (58)	164 (53)	40 (13)	35 (11)	31 (10)	25 (8)	15 (5)	30.7	9.8
Zhang et al., 2016	60	0 (0)	37 (62)	23 (38)	8 (35)	2 (9)	3 (13)	3 (13)	4 (17)	3 (13)	29.9	17.2
Zhu et al., 2016	83	3 (4)	38 (53)	42 (48)	27 (64)	4 (10)	4 (10)	7 (17)	0 (0)	0 (0)	NR	27.8
Maslow et al., 2015	62	18 (29)	19 (43)	25 (57)	18 (72)	1 (4)	4 (16)	0 (0)	1 (4)	1 (4)	34.2	1st trimester
Lin et al., 2015	155	3 (2)	55 (36)	97 (64)	63 (65)	6 (6)	11 (11)	3 (3)	14 (14)	0 (0)	32.2	9.6
Levy et al., 2014	2392	531 (22)	715 (38)	1146 (62)	794 (70)	118 (10)	53 (5)	50 (4)	110 (10)	21 (2)	36.2	7.7
Li et al., 2013	81	0 (0)	25 (31)	56 (69)	35 (63)	3 (5)	2 (4)	12 (21)	4 (7)	0 (0)	34.2	NR
Lathi et al., 2012	30	1 (3)	11 (38)	18 (62)	14 (78)	1 (6)	0 (0)	1 (6)	2 (11)	0 (0)	37.2	NR
Estimated proportion[a]		4	40	60	61	9	8	7	9	1		
95% CI		0–13	37–43	58–63	55–67	7–11	5–11	5–10	6–13	0–4		

Studies included in the SNP array analysis. Including mean age and GA of included women, failure rates, abnormality rates of successfully tested specimen and proportion of trisomy, monosomy X, structural abnormalities and other abnormalities, specified as clinically relevant, or clinically irrelevant/relevance unknown.

[a] Calculated by meta-analysis.

CI = confidence interval; GA = gestational age; NR = not reported; SNP = single nucleotide polymorphism.

TABLE 4 REPORTED RESULTS FROM ARTICLES USING FISH

Study	Total number of samples	Failures	Successful tested		Trisomy	Polyploidy	Monosomy X	Structural abnormalities	Others		Mean age	Mean GA	Chromosome probes used
			Normal	Abnormal					Clinically relevant	Clinically irrelevant/ unknown			
	n	*n* (%)	*n* (%)	*n* (%)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)				
Gliem and Aypar, 2017	25	0 (0)	5 (20)	20 (80)	12 (60)	6 (30)	2 (10)	0 (0)	0 (0)	0 (0)	NR	NR	13, 15, 16, 18, 21, 22, X, Y
Russo et al., 2016	855	0 (0)	425 (50)	430 (50)	258 (60)	100 (23)	60 (14)	0 (0)	7 (2)	5 (1)	34.7	NR	13, 15, 16, 18, 21, 22, X, Y
An et al., 2015	144	0 (0)	93 (65)	51 (35)	28 (55)	8 (16)	12 (24)	0 (0)	3 (6)	0 (0)	31	6–12	13, 16, 18, 21, 22, X, Y
Jia et al., 2015	832	0 (0)	464 (56)	368 (44)	229 (62)	58 (16)	57 (15)	0 (0)	20 (5)	4 (1)	NR	NR	13, 16, 18, 21, 22, X, Y
Haoud et al., 2014	151	26 (21)	115 (92)	10 (8)	5 (50)	3 (30)	1 (10)	0 (0)	1 (10)	0 (0)	NR	All trimesters	13, 18, 21, X, Y
Chang et al., 2013	101	0 (0)	54 (53)	47 (47)	32 (68)	10 (21)	5 (11)	0 (0)	0 (0)	0 (0)	33.2	8	13, 16, 18, 21, 22, X, Y
Gao et al., 2012	100	0 (0)	58 (58)	42 (42)	31 (74)	4 (10)	4 (10)	0 (0)	3 (7)	0 (0)	32	NR	13, 16, 18, 21, 22, X, Y
Shearer et al., 2011	943	63 (7)	676 (77)	204 (23)	108 (53)	48 (24)	43 (21)	0 (0)	5 (2)	0 (0)	NR	NR	13, 16, 18, 21, 22, X, Y
Jobanputra et al., 2011	109	0 (0)	86 (79)	23 (21)	13 (57)	4 (17)	2 (9)	0 (0)	4 (17)	0 (0)	34.4	NR	13, 15, 16, 18, 21, 22, X, Y
Lebedev et al., 2004	60	0 (0)	28 (47)	32 (53)	16 (50)	6 (19)	0 (0)	0 (0)	9 (28)	1 (3)	NR	5–12	1,5, 13–16, 18, 19, 21, 22, X, Y
Estimated proportion[a]		1	62	38	60	19	13	0	5	0			
95% CI		0–4	51–72	28–49	56–64	16–23	9–17	0–0	2–8	0–0			

Studies included in the FISH analysis. Including mean age and GA of included women, chromosome probes used, failure rates, abnormality rates of successfully tested specimen and proportion of trisomy, monosomy X, structural abnormalities and other abnormalities, specified as clinically relevant, or clinically irrelevant/relevance unknown.

[a] Calculated by meta-analysis.

CI = confidence interval; FISH = fluorescence in-situ hybridization; GA = gestational age; NR = not reported.

TABLE 5 REPORTED RESULTS FROM ARTICLES USING MLPA

Study	Total number of samples	Failures	Successful tested		Trisomy	Polyploidy	Monosomy X	Structural abnormalities	Others		Mean age	Mean GA	MLPA kit used
			Normal	Abnormal					Clinically relevant	Clinically irrelevant/ unknown			
	n	*n* (%)	*n* (%)	*n* (%)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of abnormal)	*n* (% of ab-normal)	*n* (% of abnormal)				
Chen et al., 2017	98	0 (0)	49 (50)	49 (50)	36 (73)	0 (0)	9 (18)	3 (6)	1 (2)	0 (0)	31.6	10.2	Aneuploidy (24 chromosomes)
Zimowski et al., 2016	181	5 (3)	103 (59)	73 (41)	40 (55)	3 (4)[a]	14 (19)	13 (18)	3 (4)	0 (0)	33.3	9	Subtelomeric and subcentromeric probe kits
Saxena et al., 2016	90	23 (26)	55 (82)	12 (18)	6 (50)	0 (0)	2 (17)	3 (25)	1 (8)	0 (0)	30.7	9.8	Subtelomeric probe kits
Tekcan et al., 2015	43	12 (28)	19 (61)	12 (39)	3 (25)	5 (42)[a]	2 (17)	2 (17)	0 (0)	0 (0)	33.6	9.2	Subtelomeric probe kits
Haoud et al., 2014	63	6 (10)	55 (96)	2 (4)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	NR	NR	Subtelomeric regions and targeted locus probe kits
Carvalho et al., 2010	489	0 (0)	451 (92)	38 (8)	21 (55)	4 (11)[a]	11 (29)	0 (0)	1 (3)	1 (3)	30	21.5	Aneuploidy (chromosomes 13, 18, 21, X, Y)
Deshpande et al., 2010	20	0 (0)	16 (80)	4 (20)	3 (75)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	NR	NR	Subtelomeric probe kits
Bruno et al., 2006	78	4 (5)	46 (62)	28 (38)	20 (71)	0 (0)	2 (7)	5 (18)	1 (4)[a]	0 (0)	NR	22	Subtelomeric probe kits
Estimated proportion[c]		5	75	25	62	2	16	7	1	0			
95% CI		0–14	58–88	12–42	46–76	0–9	8–26	1–17	0–3	0–0			

Studies included in the MLPA analysis. Including mean age and GA of included women, MLPA kit used, failure rates, abnormality rates of successfully tested specimen and proportion of trisomy, monosomy X, structural abnormalities and other abnormalities, specified as clinically relevant, or clinically irrelevant/relevance unknown.

[a] Polyploidy detected with additional technique.[b]Used high-throughput ligation-dependent probe amplification (HLPA).

[c] Calculated by meta-analysis.

CI = confidence interval; GA = gestational age; MLPA = multiplex ligation-dependent probe amplification; NR = not reported.

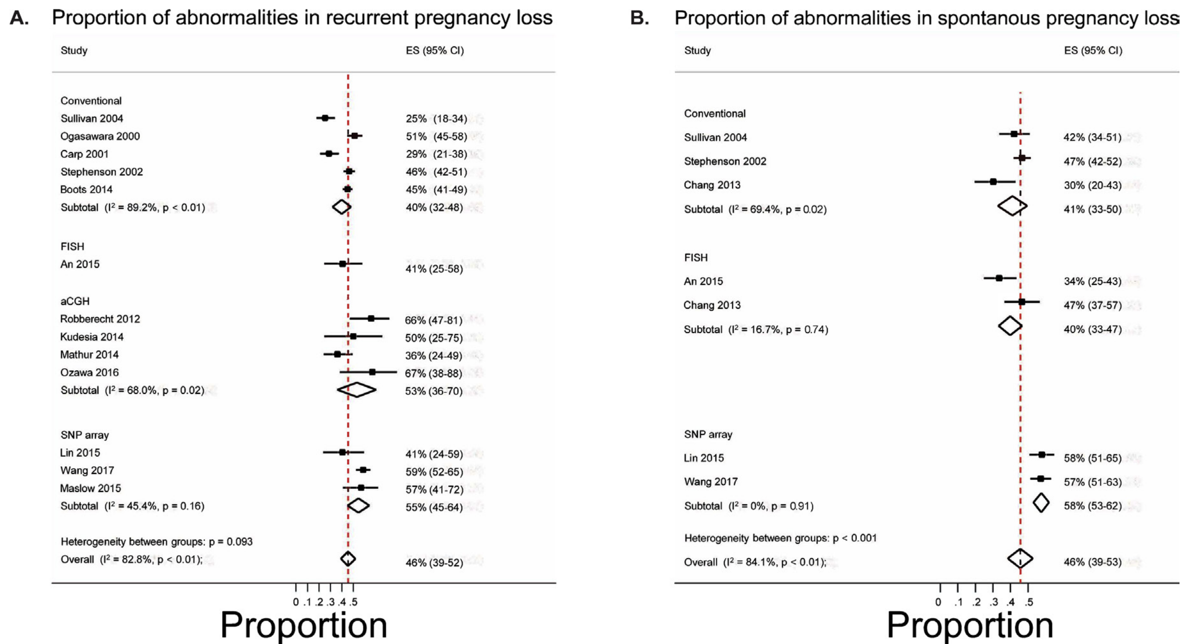

FIGURE 1 Forest plots of proportion of abnormalities within successfully tested products of conception. (A) Pregnancy loss tissue of women with a history of recurrent pregnancy loss. (B) Pregnancy loss tissue of women without a history of recurrent pregnancy loss (after a sporadic loss). aCGH = array-comparative genomic hybridization; CI = confidence interval; ES = estimated proportion; FISH = fluorescence in-situ hybridization; SNP = single nucleotide polymorphism.

studied. The abnormality rate was 25% (95% CI 12–42). MLPA cannot detect polyploidies, so other techniques were used to detect these. The detected abnormalities reported in the studies were: 62% (95% CI 46–76) trisomies, 2% (95% CI 0–9) polyploidies, 16% (95% CI 8–26) monosomy X, 7% (95% CI 1–17) structural abnormalities and 1% (95% CI 0–3) other abnormalities that were considered clinically relevant and 0 (95% CI 0–0) that were considered clinically irrelevant or of which the clinical relevance was unknown.

The heterogeneity between studies was considerable (I^2 = 96.0%), most likely due to the different MLPA probe kits used. The range in average age for the five out of eight studies where data were available was 30.0 (*Carvalho et al., 2010*) to 33.6 years (*Tekcan et al., 2015*). Six studies reported gestational age, ranging from 9 (*Zimowski et al., 2016*) to 22 weeks (*Bruno et al., 2006*). Two studies reported mean gestational ages of >20 weeks (*Bruno et al., 2006*; *Carvalho et al., 2010*).

Recurrent versus sporadic pregnancy loss

If test results were reported specifically on women suffering from either recurrent (two or more) or sporadic pregnancy loss, the detection rates were collected in this study for subgroup analysis. Data were collected on all the different techniques included in this study. FIGURE 1 shows the proportion of abnormalities observed in recurrent (FIGURE 1A) and spontaneous (FIGURE 1B) pregnancy loss analyses. Seven studies were included that reported on sporadic pregnancy loss. Chromosomal abnormalities were identified in 46% of sporadic pregnancy loss tissue samples (95% CI 39–53) and in 46% (95% CI 39–52) of recurrent pregnancy loss tissue samples. When comparing the different genetic tests between sporadic and recurrent pregnancy loss tissue, overlapping 95% CI boundaries were observed, showing that there is no statistically significant difference between the prevalence of chromosomal abnormalities between tissue from sporadic and recurrent pregnancy loss.

DISCUSSION

This was a meta-analysis examining the prevalence of failure rates, abnormality detection rates and percentages of trisomy, monosomy X, structural abnormalities and other abnormalities detected by conventional karyotyping, aCGH, SNP array, FISH and MLPA. Some studies compared different testing techniques (*Benkhalifa et al., 2005*; *Chang et al., 2013*; *Gao et al., 2012*; *Gliem and Aypar, 2017*; *Lathi et al., 2012*; *Menten et al., 2009*; *Robberecht et al., 2009*; *Schaeffer et al., 2004*; *Zhang et al., 2009*; *Zhu et al., 2016*) and highlighted the various (dis)advantages of the techniques: conventional karyotyping has a high failure rate, aCGH and SNP array have a high detection rate but can also identify clinically irrelevant findings and FISH and MLPA are limited by the probes they use. In addition, the abnormality detection rates of women that suffered a sporadic pregnancy loss were compared with those of women who suffered recurrent pregnancy loss. In nearly half of the pregnancy losses, chromosomal abnormalities were identified. The percentage of detected abnormalities was comparable in women that suffered from a sporadic pregnancy loss and those who suffered recurrent pregnancy loss.

This is the most complete meta-analysis to date on testing techniques currently used in clinical practice, including studies published between January 2000 and October 2019. Apart from SNP array, the degree of heterogeneity between the studies was considered to be serious. This may have to do with the selection of patients in the studies and might also

be due to a time effect, considering the improvement in techniques over the years, the differences in mean maternal and gestational age of the studies included and the different probes used in FISH and MLPA. Unfortunately, the majority of included articles did not report on female or gestational age, which is a limitation of this study. Because many studies did not report on mean female age or gestational age, it was decided not to exclude studies from the analysis based on those parameters. As only a few studies compared different techniques, no conclusions were drawn on which technique prevails.

Cytogenetic testing of pregnancy loss tissue is still being carried out, even though routine testing is not recommended by the ESHRE recurrent pregnancy loss guidelines (ESHRE Early Pregnancy Guideline Development Group, 2017). Testing for chromosomal aberrations does not provide clinical benefit. First, the chances of having a pregnancy loss due to chromosomal abnormalities are high, ranging from 8.9% in women under 24 years to 74.7% in women of 45 years of age (Nybo Andersen et al., 2000; van Leeuwen et al., 2013). Second, the chances of having the same chromosomal anomaly during the next pregnancy is low and most cytogenetic abnormalities in pregnancy loss tissue occur only sporadically. Carrier status of chromosomal aberrations in couples suffering pregnancy loss is rarely found (Practice Committee of the American Society for Reproductive Medicine, 2012). On top of that, in cases where one partner of the couple turns out to be a carrier of a genetic abnormality (for example a balanced rearrangement), preimplantation genetic testing does not increase the chances of (a healthy) live-born (Franssen et al., 2011; Hirshfeld-Cytron et al., 2011).

A reason for cytogenetic testing of pregnancy loss tissue still being done frequently could be the expectation that detection rates have been improved or that novel techniques identify new abnormalities. The chromosomal abnormality detecting rate of conventional karyotyping reported in this meta-analysis was comparable to the detection rates described in 2000 and 2012 (Goddijn and Leschot, 2000; van den Berg et al., 2012). Furthermore, the percentage of abnormalities found in pregnancy loss tissue did not differ between the sporadic and recurrent pregnancy loss group when different testing techniques were combined (van den Berg et al., 2012). Consequently, even with newer techniques, there is no evidence that the aetiology of recurrent pregnancy loss differs from sporadic pregnancy loss regarding chromosomal abnormalities.

Suffering pregnancy loss is an emotional burden for women and, although to a lesser extent, also for their partners (Cumming et al., 2007; Royal College of Obstetricians & Gynaecologists, 2011). Gaining more insight into the cause of pregnancy loss could help the patient understand why the miscarriage may have occurred and might help with processing the grief of their loss. Consequently, it is understandable that the advice to not test for chromosomal abnormalities routinely is difficult to implement in clinical practice. Possibly more attention needs to be given to prevention of miscarriages. A large trial suggests that administering progesterone to pregnant women with early pregnancy bleeding and a history of miscarriage could prevent a later pregnancy loss and increase the chance of having a live birth (Coomarasamy et al., 2019). The present review clarifies how common chromosomal abnormalities in pregnancy loss tissues actually are and so could help in a better understanding or closure for the couple without doing the actual test.

There will still be indications for non-routine testing, for example a positive family history or a previous live birth within the family with a chromosomal aberration. Still, in these cases, it is preferred to screen the couple's carrier status instead of the pregnancy loss tissue (Franssen et al., 2005). When deciding whether non-routine genetic testing of the pregnancy loss is desired, it is advisable to make a distinction between early and late pregnancy losses (after 20 weeks of pregnancy, i.e. intrauterine death). Autopsy could be considered because understanding the cause of death can be helpful in coping with the loss.

In nearly half of pregnancy losses, chromosomal abnormalities can be identified in both recurrent and sporadic pregnancy loss tissue. However, the identification of abnormalities does not change subsequent (clinical) practice and, in case of negative family history, the chances of recurrence are low. Therefore, routine testing of pregnancy loss tissue in order to increase the chances of live birth should not be done (Carp et al., 2001; Warburton et al., 2004). DNA testing of the pregnancy loss tissue could be considered as part of the autopsy of the fetus in late pregnancy losses. When doing so, the different characteristics of the testing techniques need to be taken into account to understand what percentage and type of abnormalities can be detected using a specific testing technique.

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