Review Article

1st trimester miscarriage: four decades of study

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Abstract: Miscarriage is a very common occurrence in humans. This paper sets out to present published data on research that has provided increased understanding of pregnancy failure. Clarification of definitions, exploring the range of failures from preclinical to later pregnancy losses, and the scientific tools employed to find information on the losses have been documented. What is now understood, which tools work best, and the associated limitations are all discussed. Early studies used cytogenetic methods and tissue culture to obtain results. Improvements in laboratory tools such as better tissue culture incubators, inverted microscopes, laminar flow hoods, improvements in culture media, all contributed to obtaining more results for patients. These studies demonstrated the significant contribution of unbalanced chromosomal karyotypes to pregnancy failure. Maternal age as a contributing factor in trisomy was clearly demonstrated. First trimester miscarriage exhibits very high cytogenetic abnormality; in contrast to very low rates in later losses. Combining data across all time periods of pregnancy will affect the significance of chromosomal error in the early pregnancy failures. Cytogenetic methods investigate whole genomes, and are considered to represent the standard against which new methods must be validated. New molecular genetic methods provide the opportunity to examine samples without the necessity of tissue culture. Techniques may be site-specific or whole genome. Fluorescent in situ hybridisation (FISH), comparative genomic hybridisation (CGH), array-based CGH, single nucleotide polymorphism (SNP) detection, quantitative polymerase chain reaction (qPCR), and quantitative fluorescent PCR (QF-PCR), have all been utilised. In comparison studies with classical/conventional cytogenetics, each newer method offers advantages and limitations. At the present time, a combined approach using conventional and molecular methods will elucidate the cause of miscarriage for almost all samples. In a clinical setting this would be optimum.

Keywords: Cytogenetics; 1st trimester miscarriage; molecular cytogenetics; recurrent miscarriage

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Introduction

Human beings suffer very high pregnancy failure rates after conception (1-6). Losses prior to biochemical pregnancy confirmation, using maternal serum markers, such as beta-human chorionic gonadotrophin (ß-hCG) and maternal alpha fetoprotein (AFP), are referred to as "preclinical" (2,7). It has been suggested that one third to one half of zygotes do not mature into blastocysts, and of those that form blastocysts, at least 40% will not implant (8,9). Opitz [1987] (3) estimated that 45% of all conceptions fail. Of these, 80% were pre-clinical losses.

Clinical diagnosis of a pregnancy by recognition of fetal heart movement on ultrasound (7,8,10) can usually be achieved by about 6 weeks postmenstrual or approximately 4 weeks after conception. From the sixth to twelfth week period, a further 10-15% of pregnancies fail (8,11-13). Maternal age has an effect, with losses ranging from 10% for women 20-24 years of age to 51% in those 40-44 (8).

Descriptive terms to delineate different types of spontaneous abortion (SA) were introduced by Robinson in 1975 (13), and have been widely adopted. A blighted ovum is defined as a "*gestational sac but no fetus on ultrasound*". A missed abortion has "*a fetal pole or a fetus in the gestational sac but no fetal heart movement on ultrasound*". A live abortion has "*fetal heart movement demonstrated on ultrasound less than*

a week before the SA". Recommendations to update these terms were proposed in 2005 (7). Technology has provided more sophisticated ways to investigate and measure early pregnancy, so accurate assessment of early development can be quantified. Unification of terminology allows direct comparisons across all research and hospital groups. Accurate measurement of the number of weeks of gestation is critical because there are so many developmental changes in the first trimester. "Miscarriage" is suggested as a more appropriate term than "abortion", to differentiate it from medical or legal abortion.

Pregnancies that abort early show a range of developmental arrest stages. Some pregnancies may only produce a few fetal membranes, others a complete empty sac, or an empty sac with a cord stump. Some may have evidence of an embryo, ranging from properly formed to totally disorganised. There may be fetal development with anything from a well-formed fetus without apparent abnormality, to those with focal malformation, to severely abnormal (4,13-15). Cytogenetic studies of all these stages show some correlation of chromosomal abnormality with the degree of development of fetal material, although a normal karyotype may occur in some cases with very abnormal fetal material. The more severely disorganised the development, and/or developmental delay in relation to estimated fertilization date, the more likely the chance of cytogenetic abnormality. Those better formed, more normal in appearance, or closer in size to estimated gestational dates, tend to be related to cytogenetic alterations more compatible with live birth, i.e., trisomies 13, 18, 21 or sex chromosome abnormalities (numerical or structural) (15). Exceptions may always occur for any cytogenetic alteration, making generalisations difficult. For example, Byrne *et al.* [1985] (4) found severe dysmorphism in fetuses with trisomy 13, 18 and triploidy.

Some spontaneous losses may be due to underlying medical conditions such as endocrine, immunologic or toxic abnormalities. In other cases anatomical problems such as incompetent cervix, uterine anomalies or serious maternal illness are the cause (2,8,11,16,17).

Repeated miscarriage is the cause of childlessness in 2-5% of reproducing couples (8,18,19). Recurrent miscarriage is defined as two or more spontaneous losses (1). Recurrence risks for patients who have a history of spontaneous fetal loss have been studied in relation to various factors, such as antiphospholipid antibodies, uterine abnormalities, luteal phase defects, diabetes mellitis and thyroid problems (1,8,11). Early literature suggested that some individuals had an increased risk of recurrent chromosomal aneuploidy (20), but later data have not confirmed this (18,21,22). If the cytogenetics is normal in repeated pregnancy loss, there is an increased risk that the next pregnancy will also fail (18,20,22-24). Maternal age is a significant risk factor for repeated miscarriage (21). Mothers aged 20-24 exhibited losses in 8.9% of pregnancies compared with mothers aged 42 who lose more than half their pregnancies, and women 45 or older have a loss risk of 74.7% (25). A study by Choi *et al.* [2014] (26) concluded that there was a statistically significant difference in the rate of cytogenetic abnormalities between sporadic and recurrent miscarriages. Closer examination of the data demonstrated that the sporadic loss group included pregnancies up to 20 weeks. The subset of mothers with sporadic loss of less than 10 weeks gestation exhibited an abnormality rate of 62%, not different from the 64% in the recurrent miscarriage population.

Another identified cause of recurrent abortion is parental constitutional cytogenetic alterations (8). In these cases, the theoretical risk for each pregnancy will be similar. Data from one Japanese study demonstrated 5% of couples with recurrent SA exhibited cytogenetic alterations (27). Of these 639 couples in the study group, 19 carried reciprocal translocations and 9 exhibited Robertsonian translocations. Of the 94 previous pregnancies for these 28 patients, only nine infants were born (27), certainly suggesting a link between the cytogenetic alteration and repeated SA.

Sporadic miscarriage affects one in four women (1,28), and may occur as a single isolated event. Beginning with the studies published by Lauritsen *et al.* [1972] (29) using fluorescent banding techniques and followed by Kajii *et al.* [1973] (30) using Giemsa banding (G-banding), it was possible to identify specific chromosomes associated with early losses. Other research groups elsewhere in the world applied these techniques during the late 1970's and into early 1980's. Cytogenetic examination revealed underlying chromosomal abnormalities in ~50% of fetal samples successfully cultured and karyotyped (5,6,31-34). Research then focused on comparative studies of cytogenetics against other clinical parameters including:

(I) Ultrasound results (10,35-38);
(II) Hormonal profiles (2,39);
(III) Morphology/histology of the aborted tissue (4,14,15,31,40,41);
(IV) Comparative genomic hybridisation (CGH) (42-45);
(V) Array-based CGH compared with conventional cytogenetics and FISH (46);
(VI) Combined array CGH and single nucleotide

polymorphism (SNP) detection (47,48);

(VII) Flow cytometry and CGH (49,50), or array CGH (51);

(VIII) FISH (52-54);

(IX) Quantitative polymerase chain reaction (qPCR) (55,56), and quantitative fluorescent PCR (QF-PCR) (57);

(X) Uniparental disomy (UPD) (58).

None of these studies was able to replace the information provided by the cytogenetics data using the alternative technique. Each of these other methods provided additional information in certain circumstances but individually none were more informative (1). Molecular techniques provide results when tissue culture fails, although the quality of DNA may also be a limitation. Maternal contamination is not isolated to conventional tissue culture, it is also an issue in molecular techniques, where maternal material cannot always be entirely separated during dissection of the sample (1,47). Detection of polyploidy is very difficult with CGH unless SNP analysis or flow cytometry is used in combination or a triploid has both X and Y chromosomes (47,51). Tetraploidy and balanced chromosomal rearrangements where maternal and paternal genomes are equal, cannot be differentiated with SNP microarrays (59).

Conventional cytogenetic studies

The most common cause of first trimester spontaneous loss is sporadic chromosomal error. They may be errors of meiosis, mitosis, or fertilization. Maternal meiotic errors had long been suspected by the relationship between elevated maternal age and increased occurrence of such conditions as Down syndrome (trisomy 21), Edward syndrome (trisomy 18), and Patau syndrome (trisomy 13) (60). Studies on oocytes have provided more data on meiotic nondisjunction in the female (47,61-65). Although super-ovulation employed in in vitro fertilisation (IVF) clinics may lead to additional error (62,63,66) it may also telescope the aging process.

Maternal meiotic errors most commonly lead to autosomal trisomy. Use of molecular methods has provided the opportunity to determine parental origin and stage of meiosis in trisomic miscarriages. Maternal meiosis I (MMI) contributed towards 68% of trisomy 13 cases (67), 64% to 77% in trisomy 21 (68,69), and 90% in trisomy 16 (70). Maternal error (both first and second meiotic division) was demonstrated for 95% of informative cases in trisomy 18 (71). Maternal meiosis II (MMII) errors contributed 16% in trisomy 13 (94% maternal error), 21.5% to 22.2% in trisomy 21 (85.5% maternal) respectively in the above references (67-70). The source of the error could not be specifically determined in the remaining 10% of cases in the trisomy 16 study and were classed as either MMI or MMII (70).

Studies on sperm have demonstrated that paternal meiotic errors also occur (72-74), and have the potential to cause abnormal fertilisation (75,76).

Mitotic error is likely to result in at least two cell lines (mosaicism) in the developing fetus. The degree of mosaicism depends on the timing of the error. If the error occurs very early in the zygote, the percentages of each cell line may be equal. Mitotic error may be either an increase in chromosome number from diploid to trisomy e.g., 46,XX to 47,XX,+8 (77), or it may be a reduction from diploid to monosomy, the most common example being 46,XX or 46,XY to 45,X. Another type of error is due to loss of an additional chromosome (trisomy) to diploidy, termed trisomic zygote rescue (TZR). Imprinting defects in children caused by uniparental disomy are attributed to this. The placental tissue can have a different karyotype from the growing embryo (78,79), while the extra-embryonic membranes and placenta are partially or completely trisomic (80,81). The chromosome involved and the timing of the rescue will determine the success or otherwise of the pregnancy (82). Structural alterations may also occur as a post-zygotic mitotic error. In these cases, there will be a normal cell line as well as one carrying the cytogenetic alteration, once again creating mosaicism.

Triploidy is a result of incorrect ploidy at fertilisation. This occurs when two haploid sets of chromosomes come from one parent, and a third set comes from the other. It may be diandry (two paternal sets) or digyny (two maternal sets) (11). Another source of error and abnormal development is where the first chromosomal replication occurs, but the first mitotic cell division (cytokinesis) does not. This results in a tetraploid nucleus with four haploid sets of chromosomes, being two identical sets from each parent. It represents homozygosity for both parental sets (11). Tetraploidy is generally observed in approximately 2% of first trimester miscarriages, although in some studies it may be reported at higher incidences, due to pooling cases of diploid/ tetraploid mosaicism (57).

The types of cytogenetic alteration are constant across all published series. They include: monosomy of X or 21; triploidy; tetraploidy; single, double or triple trisomy; structural alterations, either balanced or unbalanced; combined monosomy/trisomy; all present in larger

published studies. Normal male and female results may range from less than a 1:1 ratio up to more than 1:2. Abnormality rates also range considerably: 40% up to 76% [50.6% (26); 61% (59); 61% (46); 76% (83); 40% (84); 60% (85); 57% (18); 68% (41); 46% (86); 69.4% (12)]. Ascertainment bias, tissue culture failure and maternal contamination either in the sample or during tissue culture will all affect data sets. Percentages will be affected by the gestational age of the miscarriage samples. Losses in the first trimester exhibit chromosomal error in a reducing scale according to the week of developmental arrest. Preclinical losses between 2 to 6 weeks drop from 78% to 70% chromosomal error (11); in comparison with 48% at 7 weeks falling to 37.5% at twelve weeks (11).

Losses between 12 and 22 weeks constitute approximately 4% of pregnancy outcomes, and less than 4% of these exhibit chromosomal errors (8). Ascending infection has been identified in 85% of recent demises in one study of second trimester losses (11). Fetal structural abnormalities constituted 7.6%, of which half were suspected or confirmed to be chromosomal abnormalities (11).

In these later losses monosomy X, and trisomy of chromosomes 13, 18 and 21 may be identified. Pooling these cases with first trimester losses will reduce the incidence of chromosomal error in a study population. Jenderny [2014] (57) included cases from 7 to 34 weeks gestation, and reported a 61% abnormality rate. In comparison, studies where samples were all from first trimester loss (prior to 12 weeks gestation) reported an abnormality rate of 76% (83).

Of the specific types of abnormalities, monosomy X is the most frequent in first trimester spontaneous losses and has been reported in 8.6% of cases (2). It appears to be unaffected by maternal age, although one report suggested that it was inversely related (87). Only 1% of monosomy X conceptuses survive to term (11,88).

Autosomal trisomy is the largest group representing 20% of the total population of first trimester spontaneous miscarriages successfully cultured and examined cytogenetically. Trisomy of chromosomes 19 and 1 are the most rare. Trisomy 19 has been given a risk figure of 0.01% by Simpson [1990] (2). There is little direct evidence of trisomy 19 in the literature. There are two reports of mosaic trisomy 19 in term births (89,90). There are only three reports of trisomy 1 in the literature at present. The first case was an 8 cell pre-embryo, which may not have survived in utero (91), while the second was observed in a SA lost at 8-9 weeks post last menstrual period (LMP) (92). This particular case had no evidence of a fetal pole, although implantation and fetal sac formation were documented. The latest case of trisomy 1 was the result of an IVF pregnancy that initially implanted, but at 42 days post-fertilisation no evidence of a fetal heart-beat could be detected (93).

Chromosome 16 is the most common trisomy in SA, occurring in more than 7% of cases (2,5,11,26,31). This trisomy is not compatible with life and has not been documented in a full term birth. Trisomy 22 is the next most common (2,5,11,26,31). This is observed in rare cases at birth, but survival is very brief. Trisomy 21 is only slightly less common, followed by trisomy 15, trisomy 18, and trisomy 2 (2,5,11,26,31). Trisomy for either 15 or 2 is not observed at term, but both +18 and +13 can survive with severe birth defects (11).

The only autosomal monosomy observed is for chromosome 21, and this is a very rare event. A recent study reported seven cases in a single cohort, including one with mosaicism for a cell line containing monosomy for both X and 21 (94). One case has been presented in the literature where FISH was utilised to confirm the monosomy 21 (95) while a second case was investigated by molecular analysis (96). Fourteen cases of monosomy 21 among 2040 abnormal results were reported in a large study where both cytogenetics and FISH were utilised, although FISH was only used in three cases (52).

Triploidy involves three full haploid sets of chromosomes (i.e., 3n), and occurs in nearly 8% of SA samples. The most common arrangements are 69,XXY (4.0% of the study population) followed by 69,XXX (2.7%) (2). Triploidy has been documented in 0.6% of stillborns and 0.002% of liveborns (97). According to Wang [1999] (98), more than 50 cases of apparently nonmosaic triploidy have been documented in the literature. One case of a child investigated at 8 and still alive at 13 has been reported (99). This child was a diploid/triploid mosaic with triploidy being of maternal origin.

Advanced maternal age

Across all the studies, advancing maternal age was linked to the increased incidence of single (2,60,100-102), double (31,85,100,101,103-106) and triple trisomy (85,104,107,108). Trisomic conceptuses account for as many as 30% of spontaneous losses for women of 40 or older (60,109). Maternal age appears to have little effect on the occurrence of polyploidy (triploidy and tetraploidy), and possibly a reverse effect on monosomy X (60,102,110). Structural errors may be sporadic or familial. Inherited

unbalanced structural alterations from phenotypically normal carrier parents will be independent of maternal age, with a constant unchanging risk for each conception.

Culture success rates and maternal contamination

The availability of fetal tissue in a sample is the first step towards obtaining an explanation of fetal demise. Historically, success rates of culture of material from abortuses have varied widely from a possible low of around 37% (2,517 from 6,842 received) (100), to a high of 95.1% (609 from 640) (111). Successful tissue culture, followed by harvesting to obtain metaphase spreads, G-banding and analysing all contribute to providing a meaningful result to patients, families and doctors. Overgrowth of maternal cells can affect the identification of a result, and has hampered elucidation of the true cause of first trimester miscarriage. Early studies apparently did not clearly differentiate maternal from fetal tissues and these studies report a marked bias towards normal female (87). An early method of estimation to eliminate bias was to take the number of normal female results equivalent to normal male results and then disregard the excess female data (112). A retrospective study of 34 cases with "normal female" results identified male material in five cases (113). Some studies used direct preparations of chorionic villus to analyse dividing cells, obtained from the freshly collected tissue. These studies reported much higher abnormality rates, and relied on the premise that the maternal decidua would not contain spontaneously dividing cells, while the fetal villi would (12,114,115).

Identification of fetal material in a sample collected for cytogenetic studies has been problematic for many organisations. Samples collected during dilation and curettage (D and C) consist of everything in the uterus. The actual evacuation procedure may provide the fetal tissue intact, in which case identification is straightforward. On other occasions, fetal tissue may disintegrate, making dissection of fetal membranes and villi more complex and time-consuming. On some occasions, the conception may have been expelled prior to the patient presenting for examination. Any tissue collected will only represent maternal decidua.

Molecular tools

Conventional cytogenetics is hampered by maternal cell contamination, tissue culture failure and occasionally, very poor chromosome morphology. The lower limit of observation at the light microscope is 3-5 Mb. Each of these limitations results in an inability to correctly identify the fetal karyotype.

Molecular techniques have provided different tools to study SA. Cytogenetic studies are whole genome investigations. Molecular tools may be site-specific (FISH, MLPA, QF-PCR) or whole genome (CGH, array-CGH, next generation sequencing). Earlier molecular methods also required metaphase spreads (CGH), whereas the newer ones only require good quality DNA; thus dispensing with the need for tissue culture. When cytogenetic studies have been successful, the newer techniques may be of limited additional clinical use, as they only identify a very small number of submicroscopic changes of questionable significance (1,116). However, when tissue culture fails, molecular techniques are very useful, although it is important to understand the limitations of each tool (52,117).

With the advent of molecular technology, it has become possible to reliably differentiate fetal tissue from maternal (52,59). Lathi and team (59) applied a SNP microarray to delineate fetal tissue. In addition it was considered to be reliable enough to detect 25% fetal tissue in a predominantly maternal sample. The technology is limited by an inability to detect balanced cytogenetic alterations or tetraploidy where the two parental contributions are equal. The multi-centre study reported 22% of 1,222 cases were affected by maternal cell contamination. Removing these from the data set raised the abnormality rate from 48% to 62%.

Fluorescent in situ hybridisation (FISH), MLPA and QF-PCR

Site-specific tools such as FISH are most applicable where culture was unsuccessful. It is useful to confirm a cytogenetic result when chromosomal morphology is poor. Probe sets must be designed to cover the majority of abnormalities detected in products of conceptions (POCs), otherwise only a limited number of alterations will be detected (1,53). FISH will detect polyploidy. It may also be useful in cases of aneuploidy or male conceptions when maternal cells have overgrown the fetal tissue.

MLPA is also a targeted approach to analysis, with probe sets designed to detect a specific subgroup of abnormalities. It cannot detect polyploidy. It has been used to detect alterations where tissue culture failed, although it too may be hampered by poor quality DNA (1,117).

QF-PCR uses polymorphic DNA sequences to

determine the presence of different alleles. These sequences are referred to as small tandem repeat (STR) markers (microsatellites). It was originally introduced for prenatal studies of ongoing pregnancies, utilising loci on chromosomes 13, 18, 21 and the sex chromosomes. The methodology was utilised with an expanded set of microsatellites to investigate POCs in 2005. Additional probes included STRs on 2, 7, 15 and 16. In this study, there were discrepancies in 8 out of 89 cases examined by both traditional karyotyping and QF-PCR. This included five cases that were male by QF-PCR and female by cytogenetic analysis. The other three cases were normal female by QF-PCR and aneuploid by cytogenetic analysis (118).

Chromosomal and array CGH

Chromosomal CGH requires metaphase spreads to compare the fetal (POC) with reference DNA from a chromosomally normal individual. It would be most useful in samples where chromosomal morphology is too poor, or in identifying marker chromosomes.

Array-CGH will identify sub-microscopic alterations, referred to as copy number variants (CNVs). There are now extensive databases listing benign CNVs. In the event of identifying a new CNV it is also relevant to test parents for inheritance. Six of seven studies using array-CGH in the review by van den Berg *et al.*, [2012] (1), did not investigate the parents. As a result the CNVs would be classified as "of unknown significance", which may not be useful to patients and clinicians.

As a clinical tool, array-CGH has limitations. It cannot detect balanced structural alterations, female triploidy or tetraploidy. Use of flow cytometry in addition to aCGH, should identify the additional 8-10% of samples with polyploidy as demonstrated by Menten *et al.* [2009] (51).

A combined approach to study of first trimester miscarriage samples is most likely to overcome limitations imposed by a single technique. Molecular approaches provide faster turn-around times and higher resolution, yet were not able to detect more abnormalities than conventional karyotyping. They come at a much higher cost-per-test (1,45), and identification of polyploidy or balanced chromosomal rearrangements cannot be achieved without additional tools (46,47,51,52,117). In cases of failed tissue culture, aCGH is likely to be of benefit unless the miscarriage is due to polyploidy (45). FISH would detect the polyploidy samples. Where only preserved tissue is available, the molecular tools can be utilised (119). This group was able to obtain results in 79% of cases. In this particular study reflex microsatellite analysis was used to detect maternal cell contamination in the 46,XX cases identified by aCGH (119).

aCGH plus SNP has been successfully utilised to detect a pure molar pregnancy (47). The methodology was applied to detect loss/absence of heterozygosity. A pure mole is comprised of two copies of the paternal genome, and nullisomic for the maternal chromosomes. These pregnancies are important to correctly identify due to the clinical implications of persistent trophoblastic disease.

A recent review by van den Berg *et al.* [2012] (1) stated that across all the platforms conventional chromosomal abnormality never exceeded 50%. In understanding the current data and placing these in perspective with older publications, several issues require consideration. Since the application of molecular tools, there has been a change in data analysis. Traditionally using conventional cytogenetics, abnormality rates were ascertained according to the number of successfully karyotyped samples from tissue culture. In contrast, use of molecular tools in theory should not be limited by culture success, so every sample examined should be included. Therefore, current practice, when comparing conventional cytogenetics against any molecular method, every sample has been included. Success rates for conventional cytogenetics would then be lower than traditionally reported. In addition, many studies have included miscarriage samples up to 34 weeks gestation (55,57). As the abnormality rate after 12 weeks is very low, the data will be diluted. Therefore there is a risk that study of POCs may be dismissed as a robust clinical tool (1). As an example, the study by Diego-Alvarez *et al.* [2005] (118) included 160 samples. Gestational weeks ranged between 4 and 24. Twelve samples were rejected for tissue culture, of which 94 were successfully karyotyped (63.5%), and 35% were abnormal. Molecular results were obtained for 151 of 160 (94%). This compares with the study by Shearer *et al.* [2011] (52), where gestational age was unknown, tissue culture was successful in 80% of samples, and FISH was considered successful in 95% of samples. However in this second study, abnormality rates were 52% and 25% respectively (52). The researchers then pooled these data sets to arrive at a final abnormality rate of 47% for successfully analysed samples (52). The study included fetal tissue, indicative of later gestation, thus consistent with a low abnormality rate of 17%. FISH is generally utilised with a restricted panel of probes. Chromosome 15 was not

included in their panel, yet it is one of the more common trisomies. When the abnormality rate is much lower than that for routine cytogenetics being run in parellel, the probe set has been inadequate to detect the abnormality.

A recent review of the literature (114) provided a comprehensive overview of the publications where both conventional cytogenetics and microarray were utilised on the same samples. These were analysed in depth to determine which methodology was the most appropriate platform to study miscarriages. Nine publications of the thirty-three that met the initial eligibility criteria were included in the final analysis; and the resultant pooled sample included 314 cases. Concordance of the two techniques was 86%. The data set was reduced to 233 as four studies only tested the cytogenetically normal population with microarray. They were therefore excluded from further study. Of the final five publications, microarray identified 13% additional abnormalities not detected by conventional cytogenetics; whereas cytogenetics identified 3% not detected by microarray (114).

However, examination of the final subset is interesting. One case was assigned to microarray because the cytogenetic result (93,XXYY,+22) was considered to be artefact. As the tetraploidy also exhibited a single extra copy of chromosome 22, it is more likely to represent the true karyotype of the conception. Additionally, if the tetraploid state had arisen in tissue culture, there should have been two additional copies of 22. A diploid cell line should also have been identified cytogenetically.

There were four cases exhibiting mosaicism of an abnormal chromosomal alteration with a normal female karyotype; two were detected by microarray and two by conventional cytogenetics. These are all most likely to represent maternal contamination.

Where results were discrepant, two cases were identified by conventional cytogenetics, and six were detected by microarray. Four cases with discrepancies of mosaicism identified by microarray and undetected by conventional cytogenetics included: mosaic trisomy 13 on array, but full trisomy 13 with cytogenetics; mosaic isochromosome 7p on array, full isochromosome 7p with cytogenetics; trisomy 20 as well as trisomy 21with array, only trisomy 21 with conventional cytogenetics; mosaicism for trisomy 9 and 22 on array, only trisomy 22 with cytogenetics. The array results would not change counselling and clinical management for these patients.

The four remaining cases included two cases identified by conventional cytogenetics: a marker chromosome, and deletion of 5p14; and two cases identified by microarray: a deletion of 9p21and a duplication of 15q. These four cases would require further parental studies.

In conclusion this review of the literature demonstrated four cases that would require further parental investigation; one identified by conventional cytogenetics, one identified by both methods, and two by microarray. Of all the cases analysed, microarray would have only detected two additional cases over the conventional cytogenetic studies, representing only 0.86% improvement in diagnosis; but would have missed one case found by routine karyotyping (0.43%).

If microarray is introduced into a routine clinical setting, the detection rate of abnormalities may not be as robust as reported from a research study. Research studies focus on optimum samples to obtain results; rejecting suboptimal samples from the study population (115). Samples that would fail in tissue culture, may also fail by molecular methods due to the inability to obtain sufficient good quality DNA (51). Routine clinical samples are often very bloodstained, may only contain maternal decidua, and maternal invasion of a failed pregnancy may have occurred (giving a false normal female result). With the best efforts of technical staff, maternal contamination cannot be entirely eliminated (47,51,57,115). One study identified more than 10% of their samples contaminated by maternal DNA. By reducing the detection threshold for aCGH, maternal cell contamination was minimised (46). The attention to detail and the focused interest of referring clinicians and laboratory personnel are often beyond the resources of many clinical laboratories.

Finding a cause for failure of a pregnancy assists patients and their clinicians. Each country sets guidelines for testing samples, and these differ. Under the Australian Medicare programme, clinicians have the opportunity to refer samples for testing. The European Society of Obstetrics and Gynaecology recommends that samples are only tested within research studies. The Royal College of Obstetricians and Gynaecologists (RCOG) recommends testing; in contrast to the Dutch Society of Obstetrics and gynaecology (NVOC) which recommends no analysis (1).

Concluding remarks

Conventional cytogenetics has provided a very robust platform to understand first trimester miscarriage. It has demonstrated the strong contribution of chromosomal error to fetal loss. Molecular methods offer the opportunity to

overcome the shortfalls associated with samples that cannot be examined by tissue culture. As microarray and cytogenetics provide equivalent results on successful cases, a combined approach to study these samples may optimise identification of cause of pregnancy failure. Array would be the most likely tool when culture failure occurs; keeping in mind that at least two techniques would be required: for example array and FISH (53), or array and flow cytometry (51). Each laboratory may choose according to cost-benefit for their particular client and patient population.

Maternal cell contamination, a constant question for conventional cytogenetics, can also be identified. Molecular techniques that identify the parent have been instrumental in elucidating many of the mechanisms underlying consequent genetic error: maternal contribution to trisomy (either MMI or MMII); the presence of both parents in triploidy; two paternal genomes and no maternal contribution in pure moles; postzygotic mitotic errors in tetraploidy and rare cases of trisomies have all been clarified with molecular tools. Each step takes us closer to true understanding.

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Footnote

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