Expert Review 04

QF-PCR in pregnancy loss analysis

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About the author

Helen White, PhD is a HEFCE-NIHR/CNO Senior Clinical Lecturer at the National Genetics Reference Laboratory (Wessex), Salisbury NHS Foundation Trust and the Faculty of Medicine, University of Southampton, UK. After attaining a BSc in Biology, she trained as a cytogeneticist and completed a PhD at the University of Southampton. In 1997 she moved to the Royal Children's Hospital (Brisbane) to investigate the molecular pathogenesis of dengue fever. In 2000 she joined a European BIOMED concerted action programme network that designed and standardised molecular genetic diagnostic protocols for the detection of T cell receptor and immunoglobulin receptor gene recombinations in lymphoproliferations. Since 2002, her work at NGRL (Wessex) has focused on the development, validation and quality assurance of cytogenetic, molecular cytogenetic and molecular genetic testing for the diagnosis of constitutional and acquired abnormalities. Helen has a strong research interest in non-invasive prenatal testing and is a member of the UK NEQAS/ EMQN Non Invasive Prenatal Diagnosis Specialist Advisory Group and is a co-applicant on the NIHR Programme for Applied Research Grant "Reliable Accurate Prenatal non-Invasive Diagnosis (RAPID) - an integrated project to refine and implement safer antenatal testing".

Introduction

Approximately 10-15% of all clinically recognised pregnancies end in spontaneous miscarriage [1,2]. Chromosome abnormalities are recognised as being a major factor contributing to pregnancy loss and account for about 50% of all spontaneous miscarriages. In those first trimester miscarriages that have a genetic abnormality, 86% have numerical chromosome abnormalities (i.e. trisomies, monosomies and polyploidy), structural abnormalities account for 6% and the remainder (8%) can be attributed to single gene mutations and mosaicism [3]. Identification of these abnormalities can be useful to provide patients with estimated recurrence risks for future pregnancies. The main utility of chromosomal analysis for pregnancy loss samples is to differentiate the less common structural rearrangements where the recurrence risk may be substantial (e.g. one parent is the carrier of a balanced reciprocal or Robertsonian translocation) from the more frequent whole chromosome aneuploidy or polyploidy where the recurrence risks are lower. Non-genetic factors contributing to pregnancy loss include infection, immunological disorders, maternal endocrine imbalances, abnormal uterine anatomy and thrombophilic disorders [4,5].

The referral reasons for pregnancy loss samples will vary in different laboratories but may include; spontaneous loss at any gestational age (with or without congenital abnormalities), termination of pregnancy after an abnormal ultrasound scan or prenatal diagnosis, intrauterine death or stillbirth and neonatal death and possibly samples from recurrent pregnancy loss. Laboratories should have guidelines stating which categories of referral are accepted and the preferred tissues for analysis [6].

Traditionally, karyotype analysis of pregnancy loss samples has been the gold standard method for evaluating the genetic cause of pregnancy loss. Routine cytogenetic analysis requires the successful culture of fetal tissue or chorionic villi and analysis of G-banded chromosome preparations from metaphase cells with a resolution of approximately 5Mb. However, this approach is labour intensive, expensive, requires highly trained technical staff and is often unsuccessful due to the high tissue culture failure rate (10-40%) due to reduced viability in cell culture of poor quality fetal tissue [7]. Tissue cultures from these tissues can also be subject to maternal cell overgrowth and microbial contamination [8].

"Although karyotyping of miscarriage samples is the gold standard it is often unsuccessful due to the high tissue culture failure rate."

Current alternative technologies used

Several alternative methodologies to karyotype analysis have been used to investigate genetic abnormalities in pregnancy loss. These include quantitative fluorescent PCR (QF-PCR), array comparative genomic hybridisation (aCGH) [9-14], interphase fluorescent in situ hybridisation (FISH) [8,15], BACs-on-Beads™ (BoBs) [16-19] and multiplex ligation-dependent probe amplification (MLPA) [20-25] . These techniques do not require the analysis of dividing cells but are instead performed on genomic DNA (excluding FISH) and can be easily implemented in a diagnostic laboratory setting.

Quantitative fluorescent PCR (QF-PCR)

Over the last fifteen years QF-PCR analysis of short tandem repeats (STR) has been used successfully in many laboratories for the rapid diagnosis of prenatal aneuploidy [26-32] and is now being used more widely for the analysis of pregnancy loss samples [10, 20, 33, 34]. See Expert Review 01: An introduction to QF-PCR for more details. For prenatal analysis of the most common viable aneuploidies, multiple chromosome specific polymorphic repeat sequences (short tandem repeats; STRs) located on chromosomes 13, 18, 21, X and Y, which vary in length between individuals, are amplified using fluorescently labelled primers. The PCR amplicons are analysed using an automated genetic analyser capable of 2bp resolution and the representative amount of each allele is quantified by calculating the ratio of the peak height or area using appropriate software. The number of peaks (alleles) detected and allele ratio determine whether a sample is disomic (2 chromosomes present), indicated by the presence of two alleles with a peak height or area ratio of 1:1 or trisomic (3 chromosomes present), indicated by either the presence of three alleles with a 1:1:1 ratio or 2 alleles with a 2:1 or 1:2 ratio. If only one peak is observed the sample is uninformative at that locus and no quantitative information can be obtained (see Figure 1). The assays are robust and tolerant to input of low quantities (1-3ng) of relatively low quality DNA. The test has a rapid turn around time (24 – 48 hours) and is highly automatable.

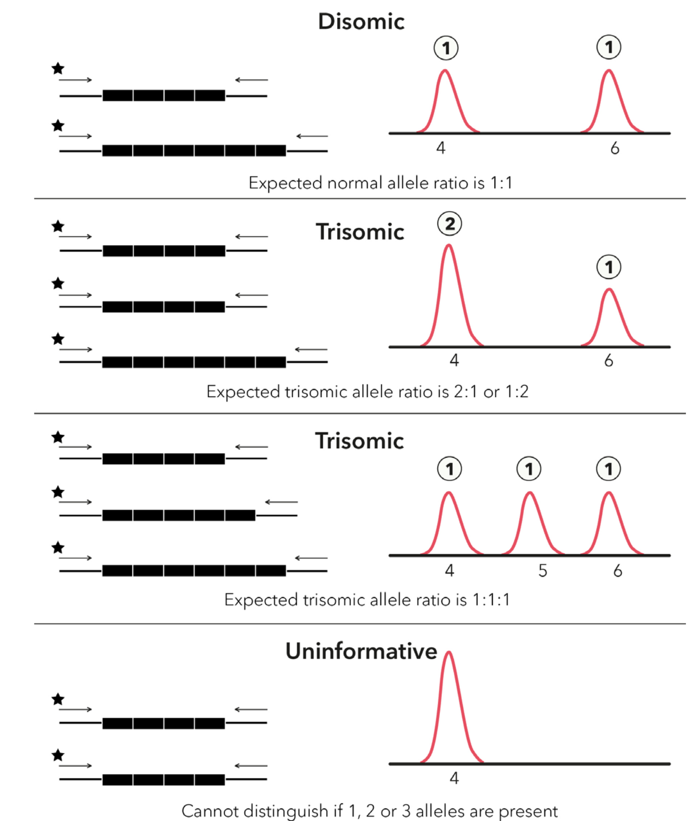

Fig. 1 Interpretation of QF-PCR data. Allele ratios can be disomic (2 alleles with a 1:1 ratio), trisomic (3 alleles with a 2:1/1:2 or 1:1:1 ratio) and uninformative.

The major advantages of the technique are that it is capable of detecting aneuploidies (Figure 2 and Expert Review 01: An introduction to QF-PCR), mosaicism (Figure 3 and Expert Review 02: Detecting mosaicism with QF-PCR) and maternal cell contamination (Figure 4 and Expert Review 03: QF-PCR and maternal cell contamination). However numerical abnormalities on chromosomes that are not targeted by the specific STR primers will not be detected.

QF-PCR analysis in routine practice

QF-PCR is being used increasingly for the analysis of pregnancy loss samples [10, 20, 33, 34] often in conjunction with other techniques e.g. MLPA [20, 35] and aCGH [10, 36]. Several published studies demonstrate the use of different QF-PCR approaches with most studies using QF-PCR as a first line test to identify the common aneuploidies (13, 18, 21, X and Y) and triploidy associated with first and second trimester loss. Samples without an abnormality are then tested using MLPA or aCGH to detect unbalanced rearrangements, aneuploidy of chromosomes not included in the QF-PCR test and other structural rearrangements. As aneuploidy/polyploidy is a less common cause of third trimester pregnancy loss it has been suggested that in these cases aCGH could be used as a first line test for these referrals [10].

"use of an expanded panel that includes STR-markers on chromosomes 15, 16 and 22 should significantly facilitate the detection of aneuploidies."

Autosomal monosomies are not commonly detected in spontaneous pregnancy loss presumably due to high lethality. However trisomy for all chromosomes has been reported with the most rarely seen trisomies reported for chromosomes 1 and 19 [37-41]. The most frequently identified trisomies are those involving chromosomes 15, 16, 21 and 22 which are the major cause of early pregnancy loss and the viable trisomies of chromosomes 13, 18, 21, X and Y which have a higher incidence in later gestation pregnancy loss [15, 41-43].

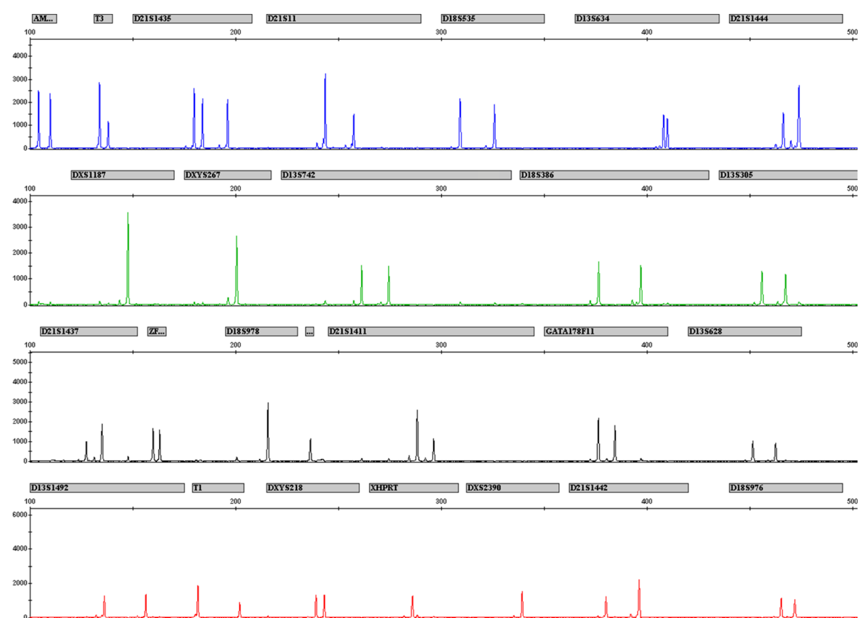

Fig. 2 QF PCR electropherogram showing trisomy 21. The presence of a triallelic result for marker D21S1435 indicates a meiotic non-disjunction event. All other chromosome 21 markers show either a 2:1 (D21S11, D21S1411) or 1:2 (D21S1444, D21S1437, D21S1442) allele ratio consistent with three copies of chromosome 21.

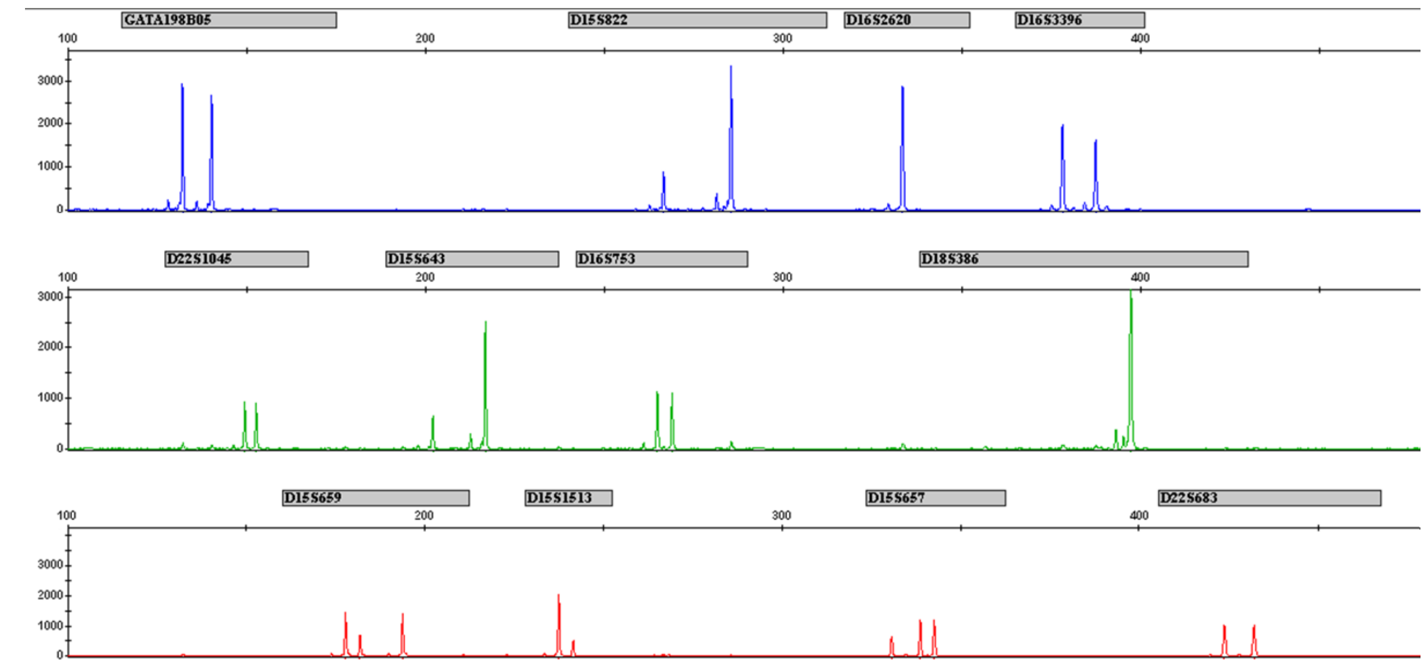

Fig. 3 QF PCR electropherogram showing mosaicism for trisomy 15. The presence of triallelic results for markers D15S643, D15S659, D15S657 indicates a meiotic non-disjunction event followed by a mitotic trisomy rescue event.

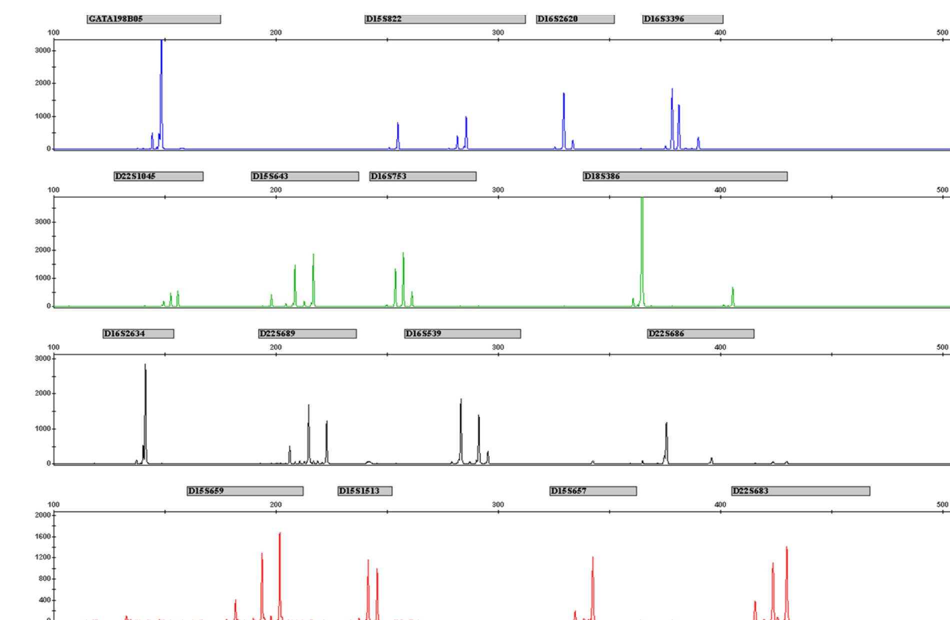

Fig.4 QF PCR electropherogram showing maternal cell contamination. Without analysing a maternal blood sample the fetal and maternal genotypes cannot be accurately assigned. All of the 8 triallelic markers show the classical A+B = C allele ratio pattern consistent with maternal cell contamination.

Therefore use of an expanded panel that includes STR-markers on chromosomes 15, 16 and 22 should significantly facilitate the detection of aneuploidies, particularly in first trimester pregnancy loss samples. To increase the number of abnormal samples detected using QF-PCR, Diego-Alverez et al. [33] used an expanded panel of STRs for chromosomes 2, 7, 13, 15, 16, 18, 21, 22 and X. Using this approach a result was obtained for 94% of samples (where no karyotype result was available) and 36% of cases were shown to have a numerical chromosome abnormality.

Up until March 2013 our laboratory routinely performed full karyotype analysis on all pregnancy loss samples. From April 2011 – April 2012 we received 416 samples and experienced a culture failure rate of 27% (n=112) and detected the abnormalities shown in table 1.

From March 2013 an alternative diagnostic approach was taken and we currently perform QF-PCR for chromosomes 13, 15, 16, 18, 21, 22, X and Y combined with additional aCGH analysis as requested by the referring clinician. The QF-PCR also includes two X/autosome paralogous markers which enable the number of X chromosomes to be calculated relative the number of autosome sequences amplified. This enables monosomy X to be more confidently diagnosed when all X chromosome markers are uninformative [44]. In the first 15 months we received 550 pregnancy loss samples with gestation ranging from 6 weeks to 42 weeks. Types of samples analysed included chorionic villi, cord, skin, amnion and DNA from formalin fixed paraffin embedded (FFPE) biopsies. DNA was extracted from all tissue types using the DNA Mini Kit (QIAGEN). The failure rate dropped to 1.6% (n=9) with 5 samples not being reported due to MCC (4 chorionic villi samples and one severely macerated sample) and the remainder due to poor quality DNA (3 from FFPE material). All samples were analysed in accordance with Association for Clinical Cytogenetics and Clinical Molecular Genetics Society QF-PCR for the diagnosis of aneuploidy Best Practice Guidelines [45] and the frequency of abnormalities detected are shown in table 2. Nineteen aCGH analyses were requested during this period and of these fifteen were normal, confirmation

Results	Number of samples (n=416)	%
Normal male	136	33
Normal female	119	29
Trisomy 4	2 (1 mosaic)	0,5
Trisomy 9	1	0,2
Trisomy 10	1	0,2
Trisomy 13	4 (1 mosaic)	1
Trisomy 14	1	0,2
Trisomy 16	1	0,2
Trisomy 18	4	1
Trisomy 21	11	2,6
Trisomy 22	3	0,7
Triploidy	5	1,2
Monosomy X	8	1,9
Other	46,XY,del(1)(q2?5)[2]/46,XY[28] 46,XX,t(7;21)(p22;q22),inv(12)(q13;q23)/46,XX 45,XY,der(14;22)(q10;q10) 46,XX,t(1;17)(q42.1;p11) 46,Y,der(X)t(X:Y)(p22.3;q11.2)mat 46,XY,t(4;17)(q21;p13) 46,XY,t(2;10)(p22.2;q11.2)mat 45,XX,der(18)t(18;22)(p11.2;q11)dn	1,9
Culture Fails	112	27

Tab. 1 Abnormalities detected by karyotype analysis from April 2011 – April 2012. The assay had an overall detection rate of 12% and a failure rate of 27%.

was provided for the mosaic 22 and monosomy 21 results obtained by QF-PCR and two deletions were detected (del 17q12 de novo, del 16q24.3 mat).

Results	Number of samples (n=550)	%	Gestational age range (weeks)
Normal male	233	42,4	
Normal female	210	38,2	
Trisomy 18	19	3,4	8-21
Monosomy X	15	2,7	8-14
Trisomy 21	14	2,5	7-35
Triploidy	14	2,5	7-20
Trisomy 16	12	2,2	6-12
Trisomy 13	10	1,8	11-21
Trisomy 22	7 (1 mosaic)	1,3	7-10
Trisomy 15	5 (1 mosaic)	0,9	8-12
XYY	1	0,2	16
Monosomy 21	1	0,2	13

Tab. 2 Abnormalities detected by QF-PCR assay for chromsomes 13, 15, 16, 18, 21, 22, X and Y from March 2013 – July 2014. The assay had an overall detection rate of 18% and a failure rate of 1.6%.

Special considerations

Sample types

Samples received for analysis of pregnancy loss should ideally be received within 24 hours of biopsy. Samples are often received by laboratories as products of conception, whole fetuses or biopsy samples that can include skin, placenta and cord from fetuses with a wide gestational range. MCC can be a particular problem when biopsies are taken from placental tissue especially those taken from products of conception. A biopsy of amnion can be taken from the placental surface (near the cord origin) using forceps to lift the thin translucent amniotic membrane free from the chorion and placenta. A placental/chorionic villus biopsy can be taken as near to the umbilicus insertion as possible to maximise the chance of it being fetal in origin. An inverted dissecting microscope can be used to select and clean up the specimen to ensure that it fetal in origin. For skin biopsies it is recommended that they are examined using a dissecting microscope and that any fat is removed using a sterile disposable scalpel (24 weeks gestation and above). Products of conception can be examined with the aid of a dissecting microscope and any fetal parts removed for analysis. Poor quality tissue is often obtained when the fetus is macerated or tissues have undergone severe autolysis and these may present a particular challenge for successful analysis. Occasionally FFPE biopsies (or DNA extracted from FFPE biopsies) are received. These can be analysed successfully but DNA extracted from these samples is often highly degraded and, for PCR based analysis, the higher molecular weight targets will often amplify less efficiently. This can be overcome by using STR designs that target shorter amplicons [46].

Maternal cell contamination (MCC)

Detection of MCC is important to avoid the incorrect reporting of a normal female fetal sample resulting from the inadvertent analysis of maternal cells. When fetal tissues are cultured maternal cell overgrowth can occur. In one study it was shown that as many as 40% of 46,XX fetal karyotypes reported were actually maternal in origin [8]. In uncultured samples MCC is most common in samples of placental origin, in particular chorionic villus. QF-PCR can be a particularly useful technique for detecting the presence of MCC as it gives a very characteristic pattern for markers on all chromosomes tested (figure 4) and can detect MCC at a level of approximately 10%. Additional reading about MCC is available in the Expert Review 02: Detecting mosaicism with QF-PCR, authored by Kathy Mann.

Mosaicism

Mosaicism is defined as the presence of two or more cell populations that have a different genotype and is found in perinatal samples most often in the placenta. Mosaicism can result from a trisomy conception that has occurred following a meiotic non-disjunction event which then undergoes a mitotic rescue event to generate a normal cell line. Alternatively it may occur due to a mitotic non-disjunction event in a normal cell giving rise to a trisomy cell line. QF-PCR analysis will detect a mosaic cell line present at approximately 15% if a triallelic result is present or 20% if only biallelic results are obtained (figure 3 and ref 47). Additional reading about MCC is available in the Expert Review 02: Detecting mosaicism with QF-PCR, authored by Kathy Mann.

Summary

A genetic diagnosis for pregnancy loss can provide important information for future reproductive advice and may also have emotional benefit to the couple concerned. Although karyotyping of miscarriage samples is the gold standard method for analysis this approach is labour intensive, expensive, requires highly trained technical staff and is often unsuccessful due to the high tissue culture failure rate. Consequently several different molecular methods, that do not require the culture of dividing cells, have been proposed to overcome the limits of cytogenetic analysis: these include aCGH, FISH, MLPA, BoBs and QF-PCR. These methodologies have distinct advantages and disadvantages and currently none of the techniques have been shown to be suitable to be used as a stand alone test. Many studies have shown that a combination of testing strategies can be used to obtain a successful diagnosis. Most focus on the use of QF-PCR (or FISH) as a first line test to detect samples with aneuploidy or triploidy (with or without mosaicism) and can be used to exclude the presence of MCC. The use of extended panels of STR markers to include chromosomes 15, 16 and 22 will improve detection rates for earlier gestational age samples. Where no numerical abnormality is detected samples can then be reflex tested using additional techniques as required to detect other chromosome aneuploidies and unbalanced rearrangements. The type of combined strategy implemented by laboratories will differ depending on the referral categories for samples accepted, equipment and staffing structures available and local cost benefit analysis. These approaches are likely to replace conventional cytogenetic analysis and provide a successful, efficient and good quality diagnostic service for the genetic analysis of pregnancy loss samples.

"A genetic diagnosis for pregnancy loss can provide important information for future reproductive advice and may also have emotional benefit to the couple concerned"

References

1. Gardener RJM and Sutherland GR (2004) Chromosome Abnormalities and Genetic Counseling (3rd Edition), Oxford Univeristy Press: New York.
2. Warren JE, Silver RM. Genetics of pregnancy loss. Clin Obstet Gynecol. 2008 Mar;51(1):84-95.
3. Goddijn M, Leschot NJ. Genetic aspects of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol. 2000 Oct;14(5):855-65
4. Carp H, Toder V, Aviram A, Daniely M, Mashiach S, Barkai G. Karyotype of the abortus in recurrent miscarriage. Fertil Steril. 2001 Apr;75(4):678-82.
5. Sullivan AE, Silver RM, LaCoursiere DY, Porter TF, Branch DW. Recurrent fetal aneuploidy and recurrent miscarriage. Obstet Gynecol. 2004 Oct;104(4):784-8.
6. Association for Clinical Cytogenetics Solid Tissue Best Practice Guidelines (2010) v1.00) http://www.acgs.uk.com/quality-committee/best-practice-guidelines/
7. Lomax B, Tang S, Separovic E, Phillips D, Hillard E, Thomson T, Kalousek DK. Comparative genomic hybridization in combination with flow cytometry improves results of cytogenetic analysis of spontaneous abortions. Am J Hum Genet. 2000 May;66(5):1516-21
8. Jobanputra V, Esteves C, Sobrino A, Brown S, Kline J, Warburton D. Using FISH to increase the yield and accuracy of karyotypes from spontaneous abortion specimens. Prenat Diagn. 2011 Aug;31(8):755-9.
9. Levy B, Sigurjonsson S, Pettersen B, Maisenbacher MK, Hall MP, Demko Z, Lathi RB, Tao R, Aggarwal V, Rabinowitz M. Genomic Imbalance in Products of Conception: Single-Nucleotide Polymorphism Chromosomal Microarray Analysis. Obstet Gynecol. 2014 Aug;124(2, PART 1):202-209.
10. Kooper AJ, Faas BH, Feenstra I, de Leeuw N, Smeets DF. Best diagnostic approach for the genetic evaluation of fetuses after intrauterine death in first, second or third trimester: QF-PCR, karyotyping and/or genome wide SNP array analysis. Mol Cytogenet. 2014 Jan 16;7(1):6
11. Bug S, Solfrank B, Schmitz F, Pricelius J, Stecher M, Craig A, Botcherby M, Nevinny-Stickel-Hinzpeter C. Diagnostic utility of novel combined arrays for genome-wide simultaneous detection of aneuploidy and uniparental isodisomy in losses of pregnancy. Mol Cytogenet. 2014 Jun 24;7:43.
12. Schaeffer AJ, Chung J, Heretis K, Wong A, Ledbetter DH, Lese Martin C. Comparative genomic hybridization-array analysis enhances the detection of aneuploidies and submicroscopic imbalances in spontaneous miscarriages. Am J Hum Genet. 2004 Jun;74(6):1168-74.
13. Wang BT, Chong TP, Boyar FZ, Kopita KA, Ross LP, El-Naggar MM, Sahoo T, Wang JC, Hemmat M, Haddadin MH, Owen R, Anguiano AL. Abnormalities in spontaneous abortions detected by G-banding and chromosomal microarray analysis (CMA) at a national reference laboratory. Mol Cytogenet. 2014 May 22;7:33.
14. Dhillon RK1, Hillman SC, Morris RK, McMullan D, Williams D, Coomarasamy A, Kilby MD. Additional information from chromosomal microarray analysis (CMA) over conventional karyotyping when diagnosing chromosomal abnormalities in miscarriage: a systematic review and meta-analysis. BJOG. 2014 Jan;121(1):11-21.
15. Shearer BM, Thorland EC, Carlson AW, Jalal SM, Ketterling RP. Reflex fluorescent in situ hybridization testing for unsuccessful product of conception cultures: a retrospective analysis of 5555 samples attempted by conventional cytogenetics and fluorescent in situ hybridization. Genet Med. 2011 Jun;13(6):545-52
16. Baxter L, Adayapalam N. A comparative study of standard cytogenetic evaluation and molecular karyotyping for products of conception. Diagn Mol Pathol. 2013 Dec;22(4):228-35.

17. Sheath KL, Duffy L, Asquith P, Love DR, George AM. Bacterial artificial chromosomes (BACs)-on-Beads™ as a diagnostic platform for the rapid aneuploidy screening of products of conception. Mol Med Rep. 2013 Aug;8(2):650-4.

18. Grati FR, Gomes DM, Ganesamoorthy D, Marcato L, De Toffol S, Blondeel E, Malvestiti F, Loeuillet L, Ruggeri AM, Wainer R, Maggi F, Aboura A, Dupont C, Tabet AC, Guimiot F, Slater HR, Simoni G, Vialard F. Application of a new molecular technique for the genetic evaluation of products of conception. Prenat Diagn. 2013 Jan;33(1):32-41

19. Paxton CN, Brothman AR, Geiersbach KB. Rapid aneusomy detection in products of conception using the KaryoLite™ BACs-on-Beads™ assay. Prenat Diagn. 2013 Jan;33(1):25-31

20. Donaghue C, Mann K, Docherty Z, Mazzaschi R, Fear C, Ogilvie C.Combined QF-PCR and MLPA molecular analysis of miscarriage products: an efficient and robust alternative to karyotype analysis. Prenat Diagn. 2010 Feb;30(2):133-7.

21. Carvalho B, Dória S, Ramalho C, Brandão O, Sousa M, Matias A, Barros A, Carvalho F.Aneuploidies detection in miscarriages and fetal deaths using multiplex ligation-dependent probe amplification: an alternative for speeding up results? Eur J Obstet Gynecol Reprod Biol. 2010 Dec;153(2):151-5.

22. Caramins MC, Saville T, Shakeshaft R, Mullan GL, Miller B, Yip MY, Buckley MF. A comparison of molecular and cytogenetic techniques for the diagnosis of pregnancy loss. Genet Med. 2011 Jan;13(1):46-51.

23. Bruno DL, Burgess T, Ren H, Nouri S, Pertile MD, Francis DI, Norris F, Kenney BK, Schouten J, Andy Choo KH, Slater HR. High-throughput analysis of chromosome abnormality in spontaneous miscarriage using an MLPA subtelomere assay with an ancillary FISH test for polyploidy. Am J Med Genet A. 2006 Dec 15;140(24):2786-93

24. Diego-Alvarez D, Rodriguez de Alba M, Cardero-Merlo R, Diaz-Recasens J, Ayuso C, Ramos C, Lorda-Sanchez I. MLPA as a screening method of aneuploidy and unbalanced chromosomal rearrangements in spontaneous miscarriages. Prenat Diagn. 2007 Aug;27(8):765-71.

25. Deshpande M, Harper J, Holloway M, Palmer R, Wang R. Evaluation of array comparative genomic hybridization for genetic analysis of chorionic villus sampling from pregnancy loss in comparison to karyotyping and multiplex ligation-dependent prob amplification. Genet Test Mol Biomarkers. 2010 Jun;14(3):421-4.

26. Verma L, Macdonald F, Leedham P, Mcconachie M, Dhanjal S, Hulten M (1998). Rapid and simple prenatal DNA diagnosis of Down's syndrome. Lancet 352(9121): 9-12.

27. Pertl B, Kopp S, Kroisel P M, Tului L, Brambati B, Adinolfi M (1999) Rapid detection of chromosome aneuploidies by quantitative fluorescence PCR: first application on 247 chorionic villus samples. J Med Genet 36(4): 300-3.

28. Schmidt W, Jenderny J, Hecher K, Hackeloer B J, Kerber S, Kochhan L, Held K R (2000) Detection of aneuploidy in chromosomes X, Y, 13, 18 and 21 by QF-PCR in 662 selected pregnancies at risk. Mol Hum Reprod 6(9): 855-60.

29. Cirigliano V, Ejarque M, Canadas M P, Lloveras E, Plaja A, Perez M M, Fuster C, Egozcue J (2001) Clinical application of multiplex quantitative fluorescent polymerase chain reaction (QF-PCR) for the rapid prenatal detection of common chromosome aneuploidies. Mol Hum Reprod 7(10): 1001-6.

30. Levett L J, Liddle, S Meredith R (2001) A large-scale evaluation of amnio-PCR for the rapid prenatal diagnosis of fetal trisomy. Ultrasound Obstet Gynecol 17(2): 115-8.

31. Mann K, Fox S P, Abbs S J, Yau S C, Scriven P N, Docherty Z, Ogilvie C M (2001) Development and implementation of a new rapid aneuploidy diagnostic service within the UK National Health Service and implications for the future of prenatal diagnosis. Lancet 358(9287): 1057-61.

32. Mann K, Ogilvie CM. QF-PCR: application, overview and review of the literature. Prenat Diagn. 2012 Apr;32(4):309-14.

33. Diego-Alvarez D, Garcia-Hoyos M, Trujillo MJ, Gonzalez-Gonzalez C, Rodriguez de Alba M, Ayuso C, Ramos-Corrales C, Lorda-Sanchez I. Application of quantitative fluorescent PCR with short tandem repeat markers to the study of aneuploidies in spontaneous miscarriages. Hum Reprod. 2005 May;20(5):1235-43

34. Zou G, Zhang J, Li XW, He L, He G, Duan T. Quantitative fluorescent polymerase chain reaction to detect chromosomal anomalies in spontaneous abortion. Int J Gynaecol Obstet. 2008 Dec;103(3):237-40

35. McClelland LS, Allen SK, Larkins SA, Hamilton SJ, Marton T, Cox PM, Hargitai B, Johnston EH, Morgan C, Hardy G. Implementation and experience of an alternative QF-PCR and MLPA diagnostic strategy to detect chromosomal abnormalities in fetal and neonatal pathology samples. Pediatr Dev Pathol. 2011 Nov-Dec;14(6):460-8.

36. Morgen EK1, Maire G, Kolomietz E. A clinical algorithm for efficient, high-resolution cytogenomic analysis of uncultured perinatal tissue samples. Eur J Med Genet. 2012 Aug-Sep;55(8-9):446-54.

37. Dunn TM, Grunfeld L, Kardon NB. Trisomy 1 in a clinically recognized IVF pregnancy. Am J Med Genet. 2001 Mar 1;99(2):152-3.

38. Hanna JS1, Shires P, Matile G. Trisomy 1 in a clinically recognized pregnancy. Am J Med Genet. 1997 Jan 10;68(1):98.

39. Hassold T, Chen N, Funkhouser J, Jooss T, Manuel B, Matsuura J, Matsuyama A, Wilson C, Yamane JA, Jacobs PA. A cytogenetic study of 1000 spontaneous abortions. Ann Hum Genet. 1980 Oct;44(Pt 2):151-78.

40. Fritz B, Hallermann C, Olert J, Fuchs B, Bruns M, Aslan M, Schmidt S, Coerdt W, Müntefering H, Rehder H. Cytogenetic analyses of culture failures by comparative genomic hybridisation (CGH)-Re-evaluation of chromosome aberration rates in early spontaneous abortions. Eur J Hum Genet.

41. Menasha J1, Levy B, Hirschhorn K, Kardon NB. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. Genet Med. 2005 Apr;7(4):251-63.

42. Stephenson MD, Awartani KA, Robinson WP. Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. Hum Reprod. 2002 Feb;17(2):446-51.

43. Jenderny J. Chromosome aberrations in a large series of spontaneous miscarriages in the German population and review of the literature. Mol Cytogenet. 2014 Jun 5;7:38.

44. Deutsch S, Choudhury U, Merla G, Howald C, Sylvan A, Antonarakis SE. Detection of aneuploidies by paralogous sequence quantification. J Med Genet. 2004 Dec;41(12):908-15.

45. Association for Clinical Cytogenetics and Clinical Molecular Genetics Society QF-PCR for the diagnosis of aneuploidy Best Practice Guidelines (2012) v3.01 http://www.acgs.uk.com/quality-committee/best-practice-guidelines/

46. Furtado LV, Jama MA, Paxton CN, Wilson AA, Gardiner AE, Lyon E, Geiersbach KB. Aneuploidy detection in paraffin embedded tissue from products of conception by mini-STR genotyping. Fetal Pediatr Pathol. 2013 Apr;32(2):133-50.

47. Donaghue C, Mann K, Docherty Z, Ogilvie CM. Detection of mosaicism for primary trisomies in prenatal samples by QF-PCR and karyotype analysis. Prenat Diagn. 2005 Jan;25(1):65-72.

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