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Overview of genetic causes of recurrent miscarriage and the diagnostic approach

Tarek A ATIA

Prince Sattam bin Abdulaziz University, College of Applied Medical Sciences, Medical Laboratory Sciences Department, Al-kharj, Saudi Arabia

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Abstract: Recurring miscarriage (RM) is a frustrating reproductive complication with variable etiology. Numerous genetic defects have been known to play a crucial role in the etiology of RM. Chromosomal abnormalities are frequently detected, while other genetic defects cannot be diagnosed through routine research, such as cryptic chromosomal anomalies, single nucleotide polymorphism, single-gene defect, and gene copy number variation. Diagnostic laboratories have recently used variable advanced techniques to detect potential genetic abnormalities in couples with RM and/or in products of conception. Here we aim to summarize the known genetic causes of RM, with a focus on the new diagnostic techniques. Knowledge of the genetic profile of miscarriages is important for prognosis and potential counseling planning, as well as the prenatal diagnostic strategy in subsequent pregnancies.

Introduction

Human reproduction is a most frustrating process where ~70% of human conceptions fail to achieve viability, and about 50% of all pregnancies are lost before the expected menses (McCoy *et al*., 2015). Recurrent miscarriage (RM) is a common reproductive complication defined by the World Health Organization (WHO) as three or more consecutive fetal losses before the 20th week of gestation, whereas the American College of Obstetricians and Gynecologists defined it as two consecutive miscarriages (Toth *et al*., 2018). RM affects 1-5 % of couples trying to get a child. The incidence of RM could be affected by advanced maternal age and the number of previous abortions (Nybo Andersen and Urhoj, 2017). The risk of abortion in successive pregnancies is related to the previous outcome, as the risk of first abortion is estimated at ~10% for the first pregnancy, ~24% for the second, 26% for the third, and 32% for the fourth pregnancy. It is also suggested that 40% of women with three abortions and 50% of women with four abortions expect a fetal loss in their upcoming pregnancy. Despite thorough diagnostic techniques, in about 50% of affected women, the specific cause of RM remains unexplained (El-Hachem *et al*., 2017).

*Address correspondence to: Tarek A Atia,
t.mohamed@psau.edu.sa

RM etiology is variable, where genetic disorders account for approximately 25% of known causes, and the incidence may be higher in unexplained cases (Molazadeh *et al*., 2014). These disorders could involve the genetic profile of the parent or the fetus, so it is most informative to investigate both the parents and the products of conception. Chromosomal abnormalities represent a large proportion of these genetic disorders, yet researchers reported other significant genetic defects involved in RM etiology, but could not be detected through routine investigation, Fig. 1 summarises the common applicable genetics causes of RM. These include cryptic chromosomal anomalies, gene copy numbers variation, single gene defect, single nucleotide polymorphism, abnormal micro-RNA expression, and many other genetic and epigenetic factors (van den Berg *et al*., 2012).

Chromosomal Disorders

The most common cause of RM was fetal chromosomal abnormalities. It accounts for 50% of all cases in the first trimester, where the majority is *de novo* (Romero *et al*., 2015). However, one partner carries a balanced chromosomal rearrangement in 5% of couples with RM; and the frequency may be higher in with non-viable children (Ly *et al*., 2011). Most autosomal chromosomal aneuploidies are often due to meiotic non-disjunction in oogenesis, while most sex chromosome aneuploidies are due to meiotic spermatogenesis disorders (Szczygiet and Kurpisz, 2001). The sperm

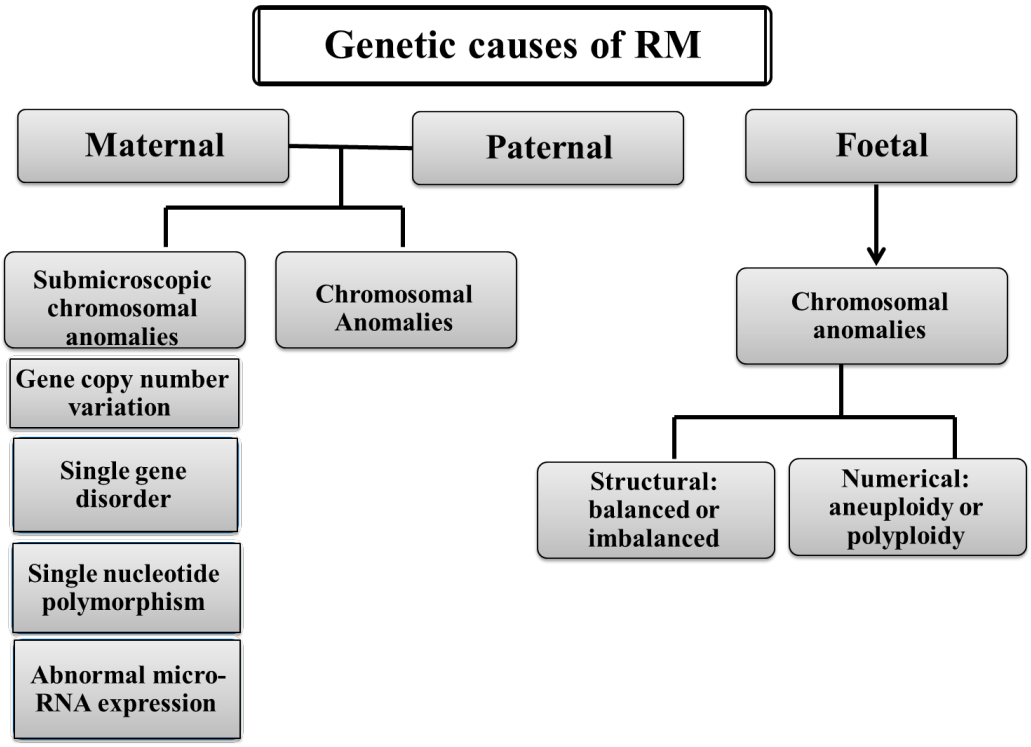

FIGURE 1. Summary of the frequently studied genetic causes of RM.

chromosomal analysis revealed a wide range of abnormalities among healthy individuals, in which numerical anomalies are frequently observed (Chatziparasidou *et al*., 2015).

Embryos with abnormal karyotypes are more likely to be lost due to either implantation failure or other mechanisms interfering with normal placental growth (Koot *et al*., 2012). It has been noted that anomalies of chromosomes can affect placental morphology and histology, as well as certain hormones and protein secretions. In addition, the placenta of chromosomally abnormal pregnancies shows decreased cell proliferation in the vascular smooth muscles, with increased apoptosis, particularly in the villus stromal tissues. This will result in hypoplasia and dysmorphic placental structure with impaired vascular development, resulting in severe retardation of intrauterine fetal growth and subsequent miscarriage (Qumsiyeh *et al*., 2000). In a proportion of couples with RM, subtle chromosomal rearrangements were reported. In these cases, during meiosis, chromosomes fail to pair or segregate properly, leading to gametes that contain an unbalanced chromosomal component that may be lethal to the embryo (Daughtry and Chavez, 2016).

The Spectrum of Chromosomal Abnormalities

Chromosomal aneuploidy

A diploid embryo formation requires a sperm and an ovum containing a haploid set of chromosomes, i.e., one copy of each chromosome. This is produced by a specialized cell division that is known as meiosis. The meiotic errors associated with defective chromosome segregation ensued with sperms or ova result in chromosome aneuploidies. Most of these aneuploidies result mainly from errors in female meiosis, whereas < 5% result from errors in male meiosis (Hassold and Hunt, 2009). In vitro fertilization (IVF) studies have suggested that in combination with advanced maternal age, maternal non-disjunctions and premature centromere divisions in meiosis 1 lead to oocyte aneuploidy. In addition, oocytes in advanced maternal age are more susceptible to meiotic spindle disorders or changes in chiasma, resulting in increased non-disjunction incidence. It has also been suggested by meiotic studies that sex chromosomes and chromosome 21 are more susceptible to non-disjunction (Chiang *et al*., 2012).

Chromosomal aneuploidy is commonly seen in the products of conception, while only 0.6% of newborns are aneuploid (Hassold and Hunt, 2009). Aneuploidy etiology is correlated mainly with advanced maternal age, whereas pathogenesis is variable, and there may be prezygotic (sperm or oocyte) or postzygotic (mitotic or meiotic) errors. However, in normal individuals, 2-3% of sperms and about 20% of oocytes show chromosomal aneuploidy (Čulić *et al*., 2011). Autosomal trisomies are frequently detected and account for up to 60% of all aneuploidies. Trisomy 16 followed by trisomy 22 are, however, more common, while trisomy 1 is rarely detected in the products of conception (Sheth *et al*., 2013). In addition, the only non-mosaic autosomal aneuploidies compatible with life are trisomies 21, 13, and 18. On the other hand, in live-born babies, gonosomal trisomies (XXX, XXY, and XYY) are less commonly observed (Staessen *et al*., 2003). Chromosomal monosomies, on the other hand, are relatively rare, and the only type compatible with life is X-chromosome monosomy. X-chromosome monosomy, however, represents 13% of all miscarried aneuploidies, while it is detected in 0.3% of live-born babies. Indeed, conventional karyotyping can easily detect numerical

chromosome anomalies (Goddijn and Leschot, 2000).

Chromosomal polyploidy

Polyploidy occurs as a triploid (3n = 69) or as a tetraploid (4n = 92). Triploidy is relatively more common than tetraploidy, accounting for about 10% of the early pregnancy loss. Polyploid embryo occurs irrespective of maternal age and may result from an extra haploid set, either maternal or paternal (Filges *et al*., 2015). Dispermy is usually the common cause of triploidy, but it may be due to diploid oocytes caused by maternal meiotic errors. Tetraploidy, on the other hand, accounts for ~2% of early pregnancy loss and is usually caused by post-fertilization errors (Hardy and Hardy, 2015). Studies have revealed a relationship between the origin of chromosomal polyploidy and the feto-placental outcome. A paternally derived extra chromosome set is usually associated with a normally growing fetus and a large cystic placenta, while the maternal origin of polyploidy is associated with severe intrauterine fetal growth retardation (Goddijn and Leschot, 2000).

Structural chromosome anomalies

Structural chromosome anomalies could be balanced if there is no loss or gain of genetic material, or imbalanced. In several human disorders, including recurrent miscarriages, more than 1000 types of constitutional chromosome anomalies have been clinically recognized. Structural chromosome anomalies may disrupt the structure of the gene(s) or alter the gene(s) copy number, resulting in the affected region's genes being haploinsufficient. This, in turn, could affect the normal function(s) of other genes supporting the continuation of pregnancy (Theisen and Shaffer, 2010). Structural chromosome anomalies were detected in approximately 5% of couples with RM, 6% of products of conception, and 0.1% of live births (Priya *et al*., 2018; Pylyp *et al*., 2018). Structural chromosome anomalies could be deletions, duplications, translocations, or inversions, of which only translocations and inversions play a significant role in RM. However, during gametogenesis, most structural chromosomal anomalies were developed *de novo*, while a small proportion was inherited from one parent who had a balanced rearrangement (Goddijn and Leschot, 2000). Conventional karyotyping does not reveal all structural chromosomal anomalies, as cryptic subtelomeric as well as microdeletion and microduplication rearrangements that were noticed in patients with intellectual disabilities (Dawson *et al*., 2002) and couples with RM (Stephenson *et al*., 2002). Y-chromosome microdeletion has also been detected in a proportion of male partners of women experiencing RM (Ghorbian *et al*., 2012). In addition, in some products of conception karyotyped as 46, XX, the Y-chromosomal DNA material was detected by a PCR and cannot be recognized by conventional karyotyping (Bell *et al*., 1999).

Single Gene Disorders

Oocyte fertilization and embryo implantation are highly complicated processes controlled by hundreds of molecules, which in turn could be controlled by multiple genes expression; however, screening of such genes is potentially challenging (Quintero-Ronderos *et al*., 2017). A number of mutated genes, such as those associated with Smith-Lemli-Opitz syndrome, congenital methemoglobinemia, and sickle cell anemia, are known to be involved in RM etiology (Lazarin *et al*., 2017; Kedar *et al*., 2012). Other autosomal dominant mutated genes, such as those associated with myotonic dystrophy, thanatophoric dysplasia, and type II osteogenesis imperfecta, are also involved. Also, a subset of X-linked recessive lethal traits presents in a heterozygous form that is unnoticed in apparently phenotypically normal individuals and may result in RM (Chaithra *et al*., 2011). In addition, strong evidence supported the role of heritable thrombophilic disorders in the pathogenesis of reproductive failure. Genetic defects of the coagulation system such as the mutation of methylene-tetrahydrofolate-reductase (MTHFR) C677 T, factor-V (Leiden) G1691A and factor-II G20210A are common thrombophilic mutations associated with reproductive failure (Aytekin *et al*., 2014; Farahmand *et al*., 2016). Hundreds of heterogeneous genetic mutations have been reported in RM etiology, but these have been investigated individually. Advanced DNA sequence technology, however, allows simultaneous testing of a large number of genes to make a quick and accurate diagnosis (Salk *et al*., 2018).

Single Nucleotide Polymorphism

Single nucleotide polymorphism (SNP) is the most common diffuse genomic variation where a single nucleotide is replaced at a specific position in the DNA. SNPs are the result of mutations that produce base-pair differences between DNA sequences. There are at least 10 million SNPs in the human genome, where they can occur in the coding and non-coding regions, or in between genes. Although the majority of these variations do not alter cellular function and thus have no effect, some SNPs have been discovered to contribute to the development of several diseases, including RM (Robert and Pelletier, 2018). However, advanced genome sequences technology, such as chromosome microarray and next-generation sequencing, allows simultaneous genome-wide screening for SNPs and helps predict pathological forms (Salk *et al*., 2018). Tab. 1 summarizes some studies in which SNPs in specific genes are strongly associated with RM, where the frequency of SNPs in RM cases is significantly higher than in normal cases.

The endothelial nitric oxide synthase (eNOS) gene plays a key role in the uterine and placental angiogenesis and is known to be expressed in the syncytiotrophoblasts and villus blood vessels during pregnancy. SNP of this gene could alter the gene expression resulting in RM (Zhao *et al*., 2019; Azani *et al*., 2017). DNA methylation is important for gametogenesis and pregnancy continuation. An SNP of the DNA methyltransferase 3B (DNMT3B) rs1569686 gene and DNMT3A-448A > G polymorphism of the 3A promoter region were commonly detected in RM (Barišić *et al*., 2017; Liu *et al*., 2017). In addition, the matrix metalloproteinase-2 (MMP2) and matrix metalloproteinase-9 (MMP9) genes are essential for cell proliferation, apoptosis, and angiogenesis. However, the expression and proper activation of these genes in the decidua and extravillous trophoblasts appears to be important for a normal pregnancy. Multiple SNPs have been detected in the promoter regions of the matrix

TABLE 1

Summary of prevalent SNPs studies involving particular genes related to the etiology of RM

Cases	Control	Site of SNP	Gene involved	Author
120	120	• IL-6 C634G • CTLA-4 A 49G	• Interleukin -6 gene • Cytotoxic T-lymphocyte associated protein-4 gene	Nasiri and Rasti, 2016
350	200	• IL-10 C592A • IL-10 C819T	• Interleukin 10 (IL-10)	Zammiti *et al.*, 2006
106	74	• TLR9 G2848A • IL-10 C592A	• Toll-like receptors-9 (TLR9) • Interleukin 10 (IL-10)	Razdaibiedina *et al.*, 2018
200	300	• Leptin C 2549A (rs7799039)	• Leptin • Tumor necrosis factor-alpha	Parveen *et al.*, 2012
300	500	• CTLA4+ A49G CTLA-4 (AT)(n) 3'UTR	• Cytotoxic T-lymphocyte associated protein-4 gene • Tumor necrosis factor-alpha	Gupta *et al.*, 2012
192	201	• eNOS G894T	• Endothelial nitric oxide synthase gene	Zhao *et al.*, 2019; Azani *et al.*, 2017
204	248	• TNF-α T1031C	• Tumor necrosis factor- α	Finan *et al*, 2010
129	116	• MMP2 - C1306 T • MMP9 - C1562 T	• Matrix metalloproteinases	Li *et al*, 2018
120	120	• IL-6 C634G • CTLA-4 A 49G	• Interleukin -6 gene • Cytotoxic T-lymphocyte associated protein-4 gene	Nasiri and Rasti, 2016
350	200	• IL-10 C592A • IL-10 C819T	• Interleukin 10 (IL-10)	Zammiti *et al.*, 2006
106	74	• TLR9 G2848A • IL-10 C592A	• Toll-like receptors-9 (TLR9) • Interleukin 10 (IL-10)	Razdaibiedina *et al.*, 2018
200	300	• Leptin C 2549A (rs7799039)	• Leptin • Tumor necrosis factor-alpha	Parveen *et al.*, 2012
300	500	• CTLA4+ A49G CTLA-4 (AT)(n) 3'UTR	• Cytotoxic T-lymphocyte associated protein-4 gene • Tumor necrosis factor-alpha	Gupta *et al.*, 2012
192	201	• eNOS G894T	• Endothelial nitric oxide synthase gene	Zhao *et al.*, 2019; Azani *et al.*, 2017
204	248	• TNF-α T1031C	• Tumor necrosis factor- α	Finan *et al.*, 2010
129	116	• MMP2 - C1306 T • MMP9 - C1562 T	• Matrix metalloproteinases	Li *et al.*, 2018

metalloproteinases genes in association with RM (Li *et al*., 2018a). The cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) gene has a regulatory function of the immune tolerance of the maternal-fetal interface. SNP of the promoter region and exon-1 is usually associated with abnormal expression of this protein may be the key factor for RM (Li *et al*., 2018b). Anti-inflammatory cytokines such as interleukin-10 (IL-10) and Toll-like receptors are important for embryonic implantation and development. SNPs of these genes may affect their expression and therefore may affect fetal implantation and development resulting in fetal loss (Zammiti *et al*., 2006; Razdaibiedina *et al*., 2018). Additionally, tumor necrosis factor-alpha (TNF-α) is an apoptosis-inducing factor in tumor cells. It is also a primary inflammatory cytokine that helps to maintain the balance of several body processes including coagulation, angiogenesis, and endothelial functions. However, several studies have shown that polymorphisms of the promoter region of the (TNF-α) gene are a genetic risk factor for recurrent fetal loss (Finan *et al*., 2010; Gupta *et al*., 2012; Parveen *et al*., 2012).

Abnormal Micro-RNA Expression

MicroRNAs (miRNAs) are a group of endogenous, short noncoding molecules that play a role in gene expression. Most of the miRNAs are transcribed from DNA sequences into primary, then precursor, and finally mature, miRNAs (O'Brien *et al*., 2018). It has been reported that a single miRNA can regulate the expression of multiple genes, while different miRNAs can regulate a particular gene (Barchitta *et al*., 2017). Several studies have revealed that miRNAs are involved in variable regulatory pathways associated with various biological and pathological conditions, such as cell growth and differentiation, development, and many human diseases (Wahid *et al*., 2010). Numerous placenta-specific levels of miRNA expression have been identified and their levels of expression could be crucial for fetal growth and development (Hosseini *et al*., 2018). Dysregulated expression of some miRNAs has been reported from compromised pregnancies in the maternal circulating blood and placenta, suggesting that miRNA profiling may be associated with early RM. Tang *et al*. (2016) found 155 miRNAs expressed differently in placental tissues from RM cases, where 98 genes were up-regulated, and 57 genes were down-regulated. Genes of these miRNAs were found to be involved in B-cell receptor, T-cell receptor, and tumor-associated signaling pathways. These genes of differentially expressed miRNAs may help to understand the mechanism of early RM etiology and pathophysiology. In addition, over-expressed miRNAs (microRNA-575) were detected in cases with RM in conjunction with increased villus apoptosis and reduced placental angiogenesis (Xia *et al*., 2017). Several studies also reported differential miRNA expression in adverse pregnancy outcomes (Amin-Beidokhti *et al*., 2017; Yang *et al*., 2018) and concluded that differential miRNA expression levels represent a promising diagnostic biomarker for unexplained RM (Qin *et al*., 2016).

Diagnostic Approach

Karyotyping

Most cytogenetic diagnostic centers routinely use conventional karyotyping to diagnose chromosomal rearrangements in couples with RM or in products of conception. Conventional karyotyping is good for aneuploidy, translocation, large deletion, and duplication; however, cryptic rearrangements of less than two mega-bases (Mb) cannot be detected. Therefore, using another high-resolution technique, from a few kilo-bases (kb) to multiple Mb, such as chromosomal microarray (CMA), can add additional information beyond the scope of conventional G-banding (Dhillon *et al*., 2014). Fig. 2 summarizes the common diagnostic procedures applied to MR cases.

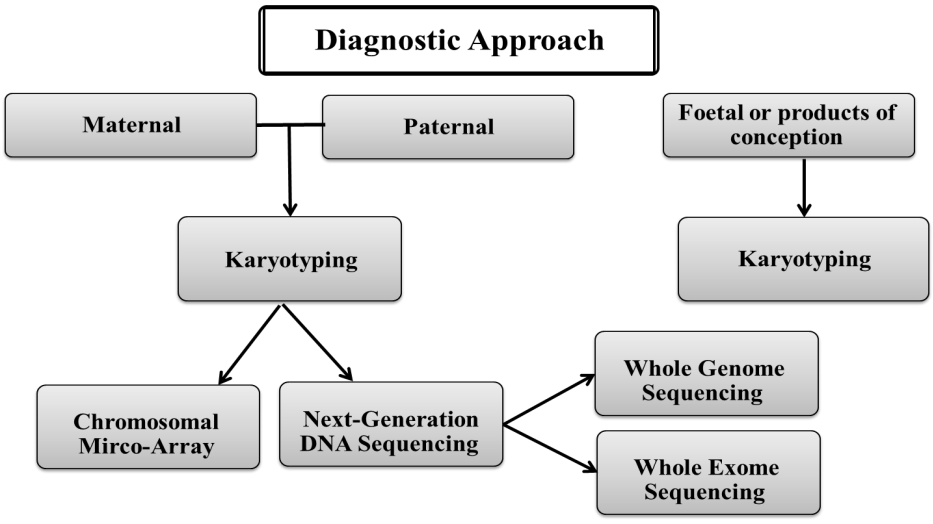

FIGURE 2. Summary of the diagnostic genetic tests often conducted in RM cases.

Chromosomal microarray

Recently, diagnostic centers have started using CMA to evaluate couples with RM, or to analyze the products of conception for an accurate diagnosis. However, two different CMA platforms currently used to diagnose micro-deletion and/or micro-duplication; the array comparative genomic hybridization (aCGH) and the single nucleotide variation (SNV) arrays. CMA increases the diagnostic yield over karyotyping in these conditions and may help in impact clinical management decisions (Sahoo *et al*., 2017; Popescu *et al*., 2018). Array-CGH can detect variants of copy numbers by hybridizing a reference genomic sequence with patient (unknown) sequences labeled with different fluorescent tags to a microarray of DNA fragments; Fig. 3 illustrates the basics of chromosomal microarray technology. If the unknown sample results in a cryptic deletion or duplication of genetic material, the sequence imbalance can easily be detected as a difference in fluorescence intensity (Hayes *et al*., 2013). Array CGH can also detect variants of copy numbers for larger deletions and duplications, including trisomy or monosomy. But, because they are not associated with a fluorescence intensity change, they cannot detect balanced translocations, polyploidy, or sequence inversions (Ahn *et al*., 2016). Single nucleotide polymorphism or variation (SNV) is a variation in the DNA sequence, where a single nucleotide differs in an individual between populations or paired chromosomes. SNV is the most common form of genetic variation in the human genome and could or could not be associated with diseases (Haraksingh and Snyder, 2013). Unlike aCGH, the genomic sample of the patient is directly hybridized to the array platform containing thousands of SNVs in the SNV-based CMA test. The variants of the DNA sequence are diagnosed

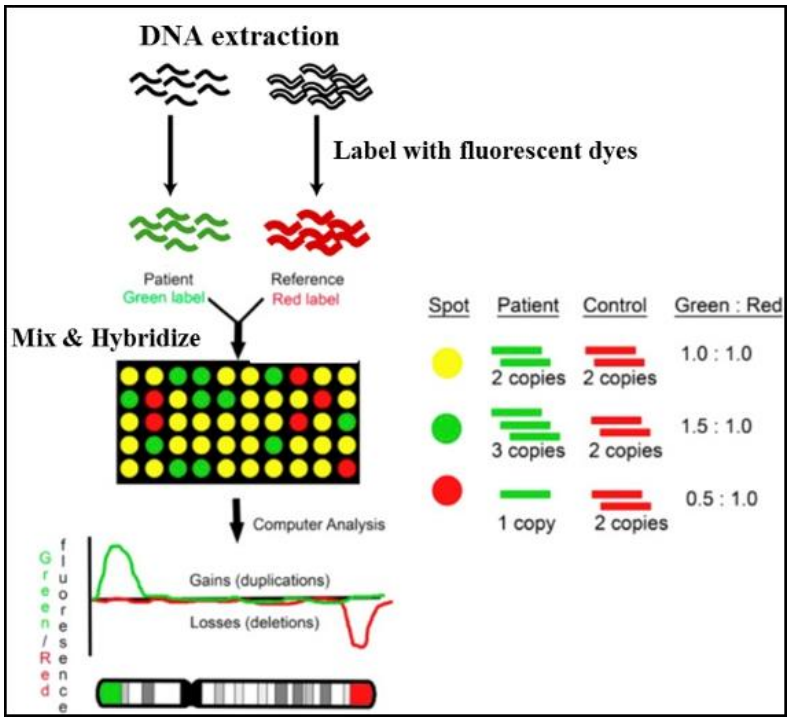

FIGURE 3. Illustration of chromosome micro-array, where a normal genomic DNA and the test DNA are labelled with different fluorescent dye color. Equal amounts of the two DNA samples are mixed, hybridized, and tested for gain or loss of the genetic material (Dugoff *et al*., 2016).

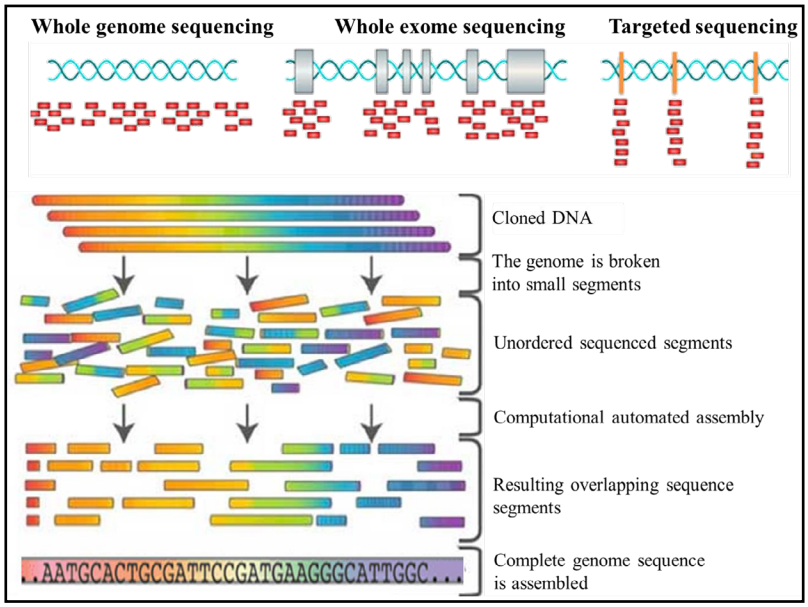

FIGURE 4. Illustration of the whole genome, whole exome and targeted gene/s sequencing. First, the genomic DNA is extracted, fragmented into small pieces, cloned, amplified and sequenced separately. After that, assemble ordered sequencing (GenomixLAB, 2016; Commins *et al*., 2016).

directly by the signal intensity (Cheung and Bi, 2018). SNV-based CMA can detect other genomic aberrations rather than cryptic rearrangements, such as genomic homozygosity and uniparental disomy. Generally speaking, CMA has higher analytical accuracy to detect copy number variants in more than 95% of cases (Papenhausen *et al*., 2011).

Next-generation DNA sequencing

The human genome includes approximately 20000 genes, representing about 2% of the entire genome. The remaining DNA sequences, however, can regulate these genes' function or expression through different pathways, and their screening is challenging (Chi, 2016). Advances in DNA sequencing technology have made it possible to screen a large scale of DNA that extends to the entire genome sequencing, with low cost, greater confidence, and potential advantages over the classic approaches in which genes are screened individually. This will help identify numerous genetic mutations to provide the diagnosis of several unexplained disorders. This strategy offers genomic sequencing-based tests such as next-generation DNA sequences (NGS), with its subsequent variation that includes whole exomes (all exons), whole genomes, and analysis of multigene panels (Liu *et al*., 2012; Quintero-Ronderos *et al*., 2017). Fig. 4 demonstrated the basics of the whole genome, whole exome, and the targeted genes/DNA sequences. Not only can NGS diagnose submicroscopic chromosomal rearrangement that cannot be detected by aCGH, but it can also detect variations in the DNA and RNA sequence. NGS can also detect epigenetic variants that contribute to genomic expression or regulations that may affect the outcome of pregnancy (Haraksingh and Snyder, 2013).

Whole exome sequencing (WES) determines with high accuracy the order of nucleotides in the coding DNA sequences. WES analyzes the exons or coding regions of thousands of genes simultaneously, approximately 180000 exons transcribed into mature RNA, the element of interpretable mutations associated with clinical disorders (Schwarze *et al*., 2018). Numerous studies have used WES to investigate families with RM and the product of conceptions. Studies have found several deleterious gene mutations, copy number variations, SNVs, and small insertions and deletions that can affect the motive pathways of placental functions, embryo implantation, and coagulation (Bao *et al*., 2014; Qiao *et al*., 2016). On the other hand, whole genome sequencing (WGS) determines the entire genome sequences including sequences of coding and non-coding regions. This will help detect variable genetic anomalies such as single nucleotide variants, deletions, insertions, and copy number variants (Ekblom and Wolf, 2014). WGS, however, misses some sequential genomic regions, such as regions with high GC content, highly repetitive sequences, centromeres, and telomeres (Quintero-Ronderos and Laissue, 2019). WGS has become an established method for studying human genetic variation in various diseases, including RM (Nagirnaja *et al*., 2014).

Conclusion and Summary

Knowledge of pathogenesis of miscarriage is important for the prognosis and planning of prenatal diagnosis in subsequent pregnancies, as well as for appropriate genetic counseling. Karyotyping is routinely used to investigate the product of conception and RM cases, but it cannot detect cryptic or submicroscopic genetic aberrations. However, new diagnostic methods such as chromosomal microarray and next-generation DNA sequencing allow accurate genetic diagnosis, especially in unexplained cases with RM. The technique of chromosomal microarray allows simultaneous investigation for submicroscopic chromosome anomalies, SNP, and gene copy number variation. Whereas next-generation DNA sequencing allows sequencing of coding DNA regions (whole exome sequence) or even sequencing of coding and non-coding DNA regions (whole genome sequence). Whole exome sequencing may be an efficient diagnostic technique for unexplained cases of RM. Indeed, some non-coding regions of the genome can play significant functions in gene expression, so after using exome sequencing, still a proportion of patients without a diagnosis. This will increase the value of the whole genome sequencing as a diagnostic method. A debate has arisen about the benefits of each technique as a whole genome and exome sequencing as a better molecular diagnostic method in research and clinical practice. Researchers, however, prefer the whole genome, while clinicians are more interested in exome sequences due to the time-consuming, the cost, and difficulties with result interpretation.

References

Ahn JW, Coldwell M, Bint S, Mackie Ogilvie M (2015). Array comparative genomic hybridization (Array CGH) for detection of genomic copy number variants. *JoVE: Journal of Visualized Experiments* **96**: e51718.

Amin-Beidokhti M, Mirfakhraie R, Zare-Karizic S, Karamoddin F (2017). The role of parental microRNA alleles in recurrent pregnancy loss: an association study. *Reproductive BioMedicine Online* **34**: 325-330.

Aytekin E, Ergun SG, Ali M (2014). Evaluation of GenoFlow thrombophilia array test kit in its detection of mutations in factor V Leiden (G1691A), Prothrombin G20210A, MTHFR C677T and A1298C in blood samples from 113 Turkish female patients. *Genetic Testing and Molecular Biomarkers* **18**: 717-721.

Azani A, Hosseinzadeh A, Azadkhah R, Zonouzi AAP, Zonouzi AP, Aftabi Y, Khani H, Heidary L, Danaii S, Bargahi N, Pouladi N, Hosseini SM (2017). Association of endothelial nitric oxide synthase gene variants (-786 T > C, intron 4 b/a VNTR and 894 G > T) with idiopathic recurrent pregnancy loss: a case-control study with haplotype and *in silico* analysis. *European Journal of Obstetrics & Gynecology and Reproductive Biology* **215**: 93-100.

Bao R, Huang L, Andrade J, Tan W, Kibbe WA, Jiang H, Feng G (2014). Review of current methods, applications, and data management for the bioinformatics analysis of whole exome sequencing. *Cancer Informatics* **13**: 67-82.

Barchitta M, Maugeri A, Quattrocchi A, Agrifoglio O, Agodi A (2017). The role of miRNAs as biomarkers for pregnancy outcomes: a comprehensive review. *International Journal of Genomics* **2017**: 8067972.

Barišić A, Pereza N, Hodžić A, Ostojić S, Peterlin B (2017). A single nucleotide polymorphism of DNA methyltransferase 3B gene is a risk factor for recurrent spontaneous abortion.

American Journal of Reproductive Immunology **78**: e12765.

Bell KA, Van Deerlin PG, Haddad BR, Feinberg RF (1999). Cytogenetic diagnosis of "normal 46, XX" karyotypes in spontaneous abortions frequently may be misleading. *Fertility and Sterility* **71**: 334-341.

Chaithra PT, Malini SS, Kumar CS (2011). An overview of genetic and molecular factors responsible for recurrent pregnancy loss. *International Journal of Human Genetics* **11**: 217-225.

Chatziparasidou A, Christoforidis N, Samolada G, Nijs M (2015). Sperm aneuploidy in infertile male patients: a systematic review of the literature. *Andrologia* **47**: 847-860.

Cheung SW, Bi W (2018). Novel applications of array comparative genomic hybridization in molecular diagnostics. *Expert Review of Molecular Diagnostics* **18**: 531-542.

Chi KR (2016). The dark side of the human genome. *Nature* **538**: 275-277.

Chiang T, Schultz RM, Lampson MA (2012). Meiotic origins of maternal age-related aneuploidy. *Biology of Reproduction* **86**: 1-7.

Commins J, Toft C, Fares MA (2016). CC BY-SA 2.5, via Wikimedia Commons. Whole genome shotgun sequencing.

Čulić V, Lozić B, Kuzmić-Prusac I, Mijaljica G, Pavelić J (2011). Full trisomy 5 in a sample of spontaneous abortion and arias stella reaction. *Medical Science Monitor* **17**: CS116-CS119.

Daughtry BL, Chavez SL (2016). Chromosomal instability in mammalian pre-implantation embryos: potential causes, detection methods, and clinical consequences. *Cell and Tissue Research* **363**: 201-225.

Dawson AJ, Putnam S, Schultz J, Riordan D, Prasad C, Greenberg CR, Chodirker BN, Mhanni AA, Chudley AE (2002). Cryptic chromosome rearrangements detected by subtelomere assay in patients with mental retardation and dysmorphic features. *Clinical Genetics* **62**: 488-494.

Dhillon RK, Hillman SC, Morris RK, McMullan D, Williams D, Coomarasamy A, Kilby MD (2014). Additional information from chromosomal microarray analysis (CMA) over conventional karyotyping when diagnosing chromosomal abnormalities in miscarriage: a systematic review and meta-analysis. *BJOG* **121**: 11-21.

Dugoff L, Norton ME, Kuller JA (2016). The use of chromosomal microarray for prenatal diagnosis. *American Journal of Obstetrics and Gynecology* **215**: B2-B9.

El-Hachem H, Crepaux V, May-Panloup P, Descamps P, Legendre G, Bouet PE (2017). Recurrent pregnancy loss: current perspectives. *International Journal of Women's Health* **9**: 331-345.

Ekblom R, Wolf JBW (2014). A field guide to whole-genome sequencing, assembly and annotation. *Evolutionary Applications* **7**: 1026-1042.

Farahmand K, Totonchi M, Hashemi M, Sabet FR, Kalantari H, Gourabi H, Meybodi AM (2016). Thrombophilic genes alterations as risk factor for recurrent pregnancy loss. *Journal of Maternal-Fetal & Neonatal Medicine* **29**: 1269-1273.

Filges I, Manokhina I, Peñaherrera MS, McFadden DE, Louie K, Nosova E, Friedman JM, Robinson WP (2015). Recurrent triploidy due to a failure to complete maternal meiosis II: whole-exome sequencing reveals candidate variants. *Molecular Human Reproduction* **21**: 339-346.

Finan RR, Al-Irhayim Z, Mustafa FE, Al-Zaman I, Mohammed FA, Al-Khateeb GM, Madan S, Issa AA, Almawi WY (2010). Tumor necrosis factor-alpha polymorphisms in women with idiopathic recurrent miscarriage. *Journal of Reproductive Immunology* **84**: 186-192.

Genomix LAB (2016). Advanced Genomics Sdn Bhd (1181235-D).

Ghorbian S, Saliminejad K, Sadeghi MR, Javadi GhR, Kamali K, Amirjannati N, Bahreini F, Edalatkhah H, Khorram Khorshid HR (2012). The association between Y-chromosome microdeletion and recurrent pregnancy loss. *Iranian Red Crescent Medical Journal* **14**: 358-362.

Goddijn M, Leschot NJ (2000). Genetic aspects of miscarriage. *Best Practice & Research Clinical Obstetrics & Gynaecology* **14**: 855-865.

Gupta R, Prakash S, Parveen F, Agrawal S (2012). Association of CTLA-4 and TNF-α polymorphism with recurrent miscarriage among North Indian women. *Cytokine* **60**: 456-462.

Haraksingh RR, Snyder MP (2013). Impacts of variation in the human genome on gene regulation. *Journal of Molecular Biology* **425**: 3970-3977.

Hardy K, Hardy PJ (2015). 1st trimester miscarriage: four decades of study. *Translational Pediatrics* **4**: 189-200.

Hassold T, Hunt P (2009). Maternal age and chromosomally abnormal pregnancies: what we know and what we wish we knew. *Current Opinion in Pediatrics* **21**: 703-708.

Hayes JL, Tzika A, Thygesen H, Berri S, Wood HM, Hewitt S, Pendlebury M, Coates A, Willoughby L, Watson CM, Rabbitts P, Roberts P, Taylor GR (2013). Diagnosis of copy number variation by Illumina next generation sequencing is comparable in performance to oligonucleotide array comparative genomic hybridisation. *Genomics* **102**: 174-181.

Hosseini MK, Gunel T, Gumusoglu E, Benian A, Aydinli K (2018). MicroRNA expression profiling in placenta and maternal plasma in early pregnancy loss. *Molecular Medicine Reports* **17**: 4941-4952.

Kedar P, Warang P, Ghosh K, Colah R (2012). Recessive congenital methemoglobinemia due to NADH cytochrome b5 reductase deficiency associated with recurrent early pregnancy loss (REPL) in an Indian family. *Annals of Hematology* **91**: 1985-1986.

Koot YEM, Teklenburg G, Salker MS, Brosens JJ, Macklon NS (2012). Molecular aspects of implantation failure. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* **1822**: 1943-1950.

Lazarin GA, Haque IS, Evans EA, Goldberg JD (2017). Smith-Lemli-Opitz syndrome carrier frequency and estimates of *in utero* mortality rates. *Prenatal Diagnosis* **37**: 350-355.

Li L, Liu J, Qin S, Li R (2018). The association of polymorphisms in promoter region of *MMP2* and *MMP9* with recurrent spontaneous abortion risk in Chinese population. *Medicine (Baltimore)* **97**: e12561.

Li L, Liu J, Qin S, Li R (2018). Correlation between CTLA4 genetic polymorphisms, its serum protein level and the susceptibility to recurrent spontaneous abortion: a case-control study. *Medicine (Baltimore)* **97**: e12754.

Liu L, Li Y, Li S, Hu N, He Y, Pong R, Lin D, Lu L, Law M (2012). Comparison of next-generation sequencing systems. *Journal of Biomedicine and Biotechnology* **2012**: 251364.

Liu Y, Zheng H, Guo P, Feng S, Zhou X, Ye D, Chen X, Chen S (2017). DNA methyltransferase 3A promoter polymorphism is associated with the risk of human spontaneous abortion after assisted reproduction techniques and natural conception. *Journal of Assisted Reproduction and Genetics* **34**: 245-252.

Ly KD, Agarwal A, Nagy ZP (2011). Preimplantation genetic

screening: does it help or hinder IVF treatment and what is the role of the embryo? *Journal of Assisted Reproduction Genetics* **28**: 833.

McCoy RC, Demko ZP, Ryan A, Banjevic M, Hill M, Sigurjonsson S, Rabinowitz M, Petrov DA (2015). Evidence of selection against complex mitotic-origin aneuploidy during preimplantation development. *PLoS Genetics* **11**: e1005601.

Molazadeh M, Karimzadeh H, Azizi MR (2014). Prevalence and clinical significance of antinuclear antibodies in Iranian women with unexplained recurrent miscarriage. *Iranian Journal of Reproductive Medicine* **12**: 221-226.

Nagirnaja L, Palta P, Kasak L, Rull K, Christiansen OB, Nielsen HS, Steffensen R, Esko T, Remm M, Laan M (2014). Structural genomic variation as risk factor for idiopathic recurrent miscarriage. *Human Mutation* **35**: 972-982.

Nasiri M, Rasti Z (2016). CTLA-4 and IL-6 gene polymorphisms: risk factors for recurrent pregnancy loss. *Human Immunology* **77**: 1271-1274.

Nybo Andersen AM, Urhoj SK (2017). Is advanced paternal age a health risk for the offspring? *Fertility and Sterility* **107**: 312-318.

O'Brien J, Hayder H, Zayed Y, Peng C (2018). Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. *Frontiers in Endocrinology* **8**: 204.

Papenhausen P, Schwartz S, Risheg H, Keitges E, Gadi I, Burnside RD, Jaswaney V, Pappas J, Pasion R, Friedman K, Tepperberg J (2011). UPD detection using homozygosity profiling with a SNP genotyping microarray. *American Journal of Medical Genetics* **155**: 757-768.

Parveen F, Agrawal S (2012). A Study of forty-seven single nucleotide polymorphisms among recurrent miscarriage using classification and regression tree analysis. *American Journal of Reproductive Immunology* **70**: 529-537.

Popescu F, Jaslow CR, Kutteh WH (2018). Recurrent pregnancy loss evaluation combined with 24-chromosome microarray of miscarriage tissue provides a probable or definite cause of pregnancy loss in over 90% of patients. *Human Reproduction* **33**: 579-587.

Priya PK, Mishra VV, Roy P, Patel H (2018). A study on balanced chromosomal translocations in couples with recurrent pregnancy loss. *Journal of Human Reproductive Sciences* **11**: 337-342.

Pylyp LY, Spynenko LO, Verhoglyad NV, Mishenko AO, Mykytenko DO, Zukin VD (2018). Chromosomal abnormalities in products of conception of first-trimester miscarriages detected by conventional cytogenetic analysis: a review of 1000 cases. *Journal of Assisted Reproduction Genetics* **35**: 265-271.

Qiao Y, Wen J, Tang F, Martell S, Shomer N, Leung PCK, Stephenson MD, Rajcan-Separovic E (2016). Whole exome sequencing in recurrent early pregnancy loss. *Molecular Human Reproduction* **22**: 364-372.

Qin W, Tang Y, Yang N, Wei X, Wu J (2016). Potential role of circulating microRNAs as a biomarker for unexplained recurrent spontaneous abortion. *Fertility and Sterility* **105**: 1247-1254.

Quintero-Ronderos P, Laissue P (2019). Genetic variants contributing to early recurrent pregnancy loss etiology identified by sequencing approaches. *Reproductive Sciences* **17**.

Quintero-Ronderos P, Mercier E, Fukuda M, González R, Suárez CF, Patarroyo MA, Vaiman D, Gris JC, Laissue P (2017). Novel genes and mutations in patients affected by recurrent pregnancy loss. *PLoS One* **12**: e0186149.

Qumsiyeh MB, Kim KR, Ahmed MN, Bradford W (2000). Cytogenetics and mechanisms of spontaneous abortions: increased apoptosis and decreased cell proliferation in chromosomally abnormal villi. *Cytogenetics and Cell Genetics* **88**: 230-235.

Razdaibiedina A, Khobzey M, Tkachenko V, Vorobiova I (2018). Effects of single-nucleotide polymorphisms in cytokine, Toll-like receptor, and progesterone receptor genes on risk of miscarriage. *Obstetrics and Gynecology International* **2018**: 9272749.

Robert F, Pelletier J (2018). Exploring the impact of single-nucleotide polymorphisms on translation. *Frontiers in Genetics* **9**: 507.

Romero ST, Geiersbach KB, Paxton CN, Rose NC, Schisterman EF, Branch DW, Silver RM (2015). Differentiation of genetic abnormalities in early pregnancy loss. *Ultrasound in Obstetrics & Gynecology* **45**: 89-94.

Sahoo T, Dzidic N, Strecker MN, Commander S, Travis MK, Doherty C, Tyson RW, Mendoza AE, Stephenson M, Dise CA, Benito CW, Ziadie MS, Hovanes K (2017). Comprehensive genetic analysis of pregnancy loss by chromosomal microarrays: outcomes, benefits, and challenges. *Genetics in Medicine* **19**: 83-89.

Salk JJ, Schmitt MW, Loeb LA (2018). Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations. *Nature Reviews Genetics* **19**: 269-285.

Schwarze K, Buchanan J, Taylor JC, Wordsworth S (2018). Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. *Genetics in Medicine* **20**: 1122-1130.

Sheth FJ, Liehr T, Kumari P, Akinde R, Sheth HJ, Sheth JJ (2013). Chromosomal abnormalities in couples with repeated fetal loss: an Indian retrospective study. *Indian Journal of Human Genetics* **19**: 415-422.

Staessen C, Tournaye H, Van Assche E, Michiels A, Van Landuyt L, Devroey P, Liebaers I, Van Steirteghem A (2003). PGD in 47, XXY Klinefelter's syndrome patients. *Human Reproduction Update* **9**: 319-330.

Stephenson MD, Awartani KA, Robinson WP (2002). Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. *Human Reproduction* **17**: 446-451.

Szczygiet M, Kurpisz M (2001). Chromosomal anomalies in human gametes and pre-implantation embryos, and their potential effect on reproduction. *Andrologia* **33**: 249-265.

Tang L, Gao C, Gao L, Cui Y, Liu J (2016). Expression profile of micro-RNAs and functional annotation analysis of their targets in human chorionic villi from early recurrent miscarriage. *Gene* **576**: 366-371.

Theisen A, Shaffer LG (2010). Disorders caused by chromosome abnormalities. *The Application of Clinical Genetics* **3**: 159-174.

Toth B, Würfel W, Bohlmann M, Zschocke J, Rudnik-Schöneborn S, Nawroth F, Schleußner E, Rogenhofer N, Wischmann T, von Wolff M, Hancke K, von Otte S, Kuon R, Feil K, Tempfer C (2018). Recurrent miscarriage: diagnostic and therapeutic procedures. Guideline of the DGGG, OEGGG and SGGG (S2k-Level, AWMF Registry Number 015/050). *Geburtshilfe Frauenheilkd* **78**: 364-381.

van den Berg MMJ, van Maarle MC, van Wely M, Goddijn M (2012). Genetics of early miscarriage. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* **1822**: 1951-1959.

Wahid F, Shehzad A, Khan T, Kim YY (2010). MicroRNAs: synthesis, mechanism, function, and recent clinical trials. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* **1803**: 1231-1243.

Xia S, Zhen Y, Ma H, Wang A (2017). Abnormal expression of microRNA-575 leads to missed abortion through regulating apoptosis and angiogenesis. *Experimental and Therapeutic Medicine* **14**: 3993-4000.

Yang Q, Gu WW, Gu Y, Yan NN, Mao YY, Zhen XX, Wang JM, Yang J, Shi HJ, Zhang X, Wang J (2018). Association of the peripheral blood levels of circulating microRNAs with both recurrent miscarriage and the outcomes of embryo transfer in an in vitro fertilization process. *Journal of Translational Medicine* **16**: 186.

Zammiti W, Mtiraoui N, Cochery-Nouvellon E, Mahjoub T, Almawi WY, Gris JC (2006). Association of -592C/A, -819C/T and -1082A/G interleukin-10 promoter polymorphisms with idiopathic recurrent spontaneous abortion. *Molecular Human Reproduction* **12**: 771-776.

Zhao X, Li Q, Yu F, Lin L, Yin W, Li J, Feng X (2019). Gene polymorphism associated with endothelial nitric oxide synthase (4VNTR, G894T, C786T) and unexplained recurrent spontaneous abortion risk. A meta-analysis. *Medicine (Baltimore)* **98**: e14175.