CLINICAL AND PROGNOSTIC SIGNIFICANCE OF GENETIC FACTORS IN RECURRENT IN-VITRO FERTILIZATION FAILURES

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SUMMARY

In 1978, a new era has started in the treatment of infertility by the birth of the first baby from a pregnancy achieved by in-vitro fertilization. Following this, healthy pregnancies have been achieved by assisted reproductive techniques such as in-vitro fertilization by an important percentage of the childless couples.

Despite all developments in assisted reproductive techniques, pregnancy rates haven't increased as expected, and unfortunately the rate of implantation success of transferred embryos remained at low levels (15%). Similar to recurrent pregnancy loss in which the etiology is not clear yet and the causes are probably multifactorial, evaluation of patients with recurrent implantation failure is difficult and complex. Genetic risk factors such as genomic rearrangements in the couples and the embryo, sperm DNA damage and imprinting defects have been considered among the causes of recurrent implantation failure.

Genetic screening is an integral part of providing good medical care of patients and families receiving a diagnosis of a genetic disorder. The aim of preconceptional genetic screening is to asses the fertility, to be able to increase success rate of infertility treatments and to detect the healthy carriers who may have a baby with the risk of fatal and/or multiple congenital anomalies. In this review, possible genetic factors associated with recurrent implantation failure are discussed in the light of the current literature.

Key words: *aneuploidy, chromosome aberrations, comparative genomic hybridization, DNA methylation, fertilization in vitro, preimplantation diagnosis*

Journal of Turkish Society of Obstetrics and Gynecology, (J Turk Soc Obstet Gynecol), *2013; Vol: 10, Issue: 3, Pages: 175- 86*

TEKRARLAYAN İVF BAŞARISIZLIKLARINDA GENETİK FAKTÖRLERİN KLİNİK VE PROGNOSTİK ÖNEMİ

ÖZET

1978 yılında in-vitro fertilizasyon sonucu elde edilen bir gebelikten ilk bebeğin doğması sonucunda infertilite tedavisinde yeni bir dönem başlamıştır. Bunu takiben, in-vitro fertilizasyon gibi yardımcı üreme teknikleri sayesinde çocuksuz çiftlerin önemli bir yüzdesi sağlıklı gebelikler elde edebilmişlerdir.

Yardımcı üreme tekniklerindeki tüm gelişmelere rağmen, gebelik hızları beklendiği şekilde artmamış ve ne yazık ki transfer edilen emriyolarda implantasyon başarısı düşük yüzdelerde kalmıştır (%15). Etiyolojisi net olarak bilinmeyen ve muhtemelen multifaktoriel nedenlerden kaynaklanan rekürren gebelik kayıplarına benzer şekilde, tekrarlayan implantasyon başarısızlığı olan hastaların da değerlendirilmesi zor ve komplekstir. Çiftlerde yada embriyoda genomik yeniden düzenlenmeler, sperm DNA hasarı ve imprinting hataları gibi genetik risk faktörleri tekrarlayan implantasyon başarısızlığı nedenleri arasında kabul edilmektedir.

Genetik tarama, genetik bir hastalık tanısı konulan hastalara ve ailelere iyi bir tıbbi bakım verilebilmesi için gerekli

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Received: 28 June 2012, revised: 28 June 2012, accepted: 09 January 2013, online publication: 10 January 2013

DOI ID:10.5505/tjod.2013.85866

bir uygulamadır. Prekonsepsiyonel genetik taramanın amacı fertiliteyi değerlendirmek, infertilite tedavilerinin başarılarını artırabilmek, ve fatal ve/veya çoklu konjenital anomaliler ile etkilenmiş çocuk doğurabilecek sağlıklı taşıyıcıları tespit edebilmektir. Bu derlemede, tekrarlayan implantasyon başarısızlığı ile ilişkili muhtemel genetik faktörler güncel literatür bilgileri ışığında tartışılmıştır.

Anahtar kelimeler: *anöploidi, DNA metilasyonu, karşılaştırmalı genom hibridizasyonu, kromozom aberasyonları, preimplantasyon tanı, tüp bebek,*
Türk Jinekoloji ve Obstetrik Derneği Dergisi, (J Turk Soc Obstet Gynecol), *2013; Cilt: 10, Sayı: 3, Sayfa: 175- 86*

DEFINITION AND IMPORTANCE

Repetitive in-vitro fertilization failure (RIF) is defined as the absence of pregnancy although a good quality embryo has been transferred following at least three successive in-vitro fertilization (IVF)/Intracytoplasmic Sperm Injection (ICSI)-Embryo Transfer (ET) procedures[1,2]. In spite of the evolution of the assisted reproduction techniques, the rate of implantation success is around 15 %[3,4]. The etiology of this situation has not been explained yet and there are probably many reasons for that. That is why the evaluation of the RIF patients is especially difficult and complex.

The analysis of genetic factors in IVF applications is really important for the success of the procedure and for the determination of couple-specific procedural way. The genetic screening planned for these patients should be made during pre-conception period or during the evaluation of the fertility state.

ETHIOLOGY OF RECURRENT IVF FAILURE

RIF is a complex procedure in which factors belonging to the mother, the father and the embryo play a role. The low quality of oocytes and embryo and chromosomal aneuploidy due to the advanced age of the mother are among the main factors.

The IVF success and live born rates decrease with the age of the mother as shown in previous studies (Figure1). Again in mothers, anatomic changes such as endometrial polyps and submucous fibroids, the presence of antithyroid antibodies and antinuclear antibodies that can lead to the development of immune reaction against the embryo may be the cause of RIF or recurrent pregnancy loss (RPL). The presence of genetic abnormalities such as chromosomal anomalies, single gene diseases, multi-factorial diseases and sperm aneuploidies[5,6], suboptimal ovarian stimulation protocols, suboptimal culture conditions, unsuitable embryo transfer technique and embryo-based conditions may affect the success of the implantation[7,8](Figure 2).

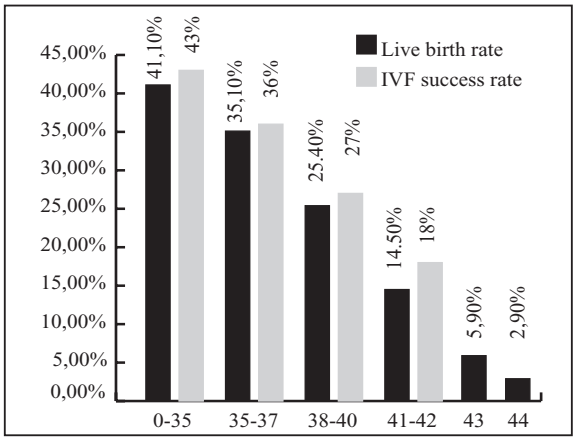

Figure 1: *The relationship between IVF success and live birth rate depending on the age*

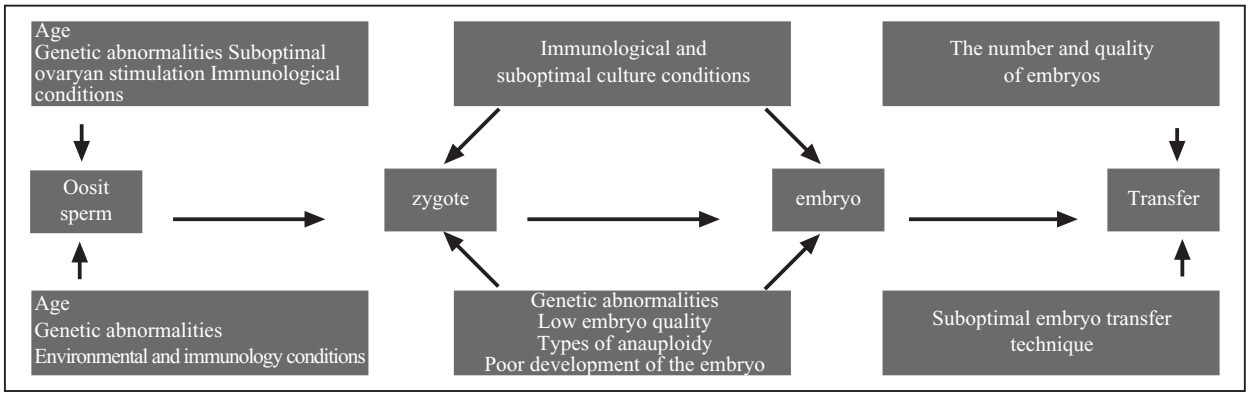

Figure 2: *The main factors affecting the success of implantation.*

1) Genetic factors acting in recurrent IVF failure

a. Chromosomal anomalies:

Previous studies reported that, more than 80% of the pregnancy losses occur in the first trimester and the analysis of the fetus or the abortion material has shown presence of chromosomal anomalies in 53% of the cases[9]. In the same way, high level of chromosomal anomalies has been detected in the embryo of RIF couples using pre-implantation genetic screening (PGS). Among these, the most frequently observed chromosomal anomalies are aneuploidies. In addition, numerous and complex anomalies involving 3 or more chromosomes have also been reported[9,10]. The detection of aneuploid fetus with the analysis of RPL mothers' abortion material in many studies have shown that this risk is higher in mother with advanced age [5]. Independently from the age of the mother, low rates of X chromosome mosaicism observed in FISH analysis performed on RPL mothers' blood and buccal smear using X-alpha satellite probes are also considered as an important risk factor for RPL[11]. Thus, if a mother is at an advanced age or is detected to have mosaic X chromosome monosomy, the couples applying for IVF needs to be submitted to PGS and prenatal diagnosis.

In sperm FISH studies performed on men with normal karyotype and abnormal spermiogram, an increase in numerical chromosome abnormalities and diploid sperm ratio was observed and these observations were reported to be associated with sperm number and motility[12]. In sperm aneuploidy studies, anomalies of sex chromosomes and chromosome number 21 and 22 are were frequently observed[13]. In studies on non-mosaic XXY-Klinefelter cases, sex chromosome disomy rate was 7.69%; this rate was 2.54% in mosaic (XY/XXY) cases and 3.97% in XYY cases[14-16]. Thus, when an IVF is planned due to male factor, sperm aneuploidy should be investigated with sperm FISH studies and the family should be offered PGS and prenatal diagnosis (Figure 3).

In couples with RPL, the frequency of being a carrier for a structural chromosomal anomaly of at least one of the parents varies between 3 and 11%[17]. Among

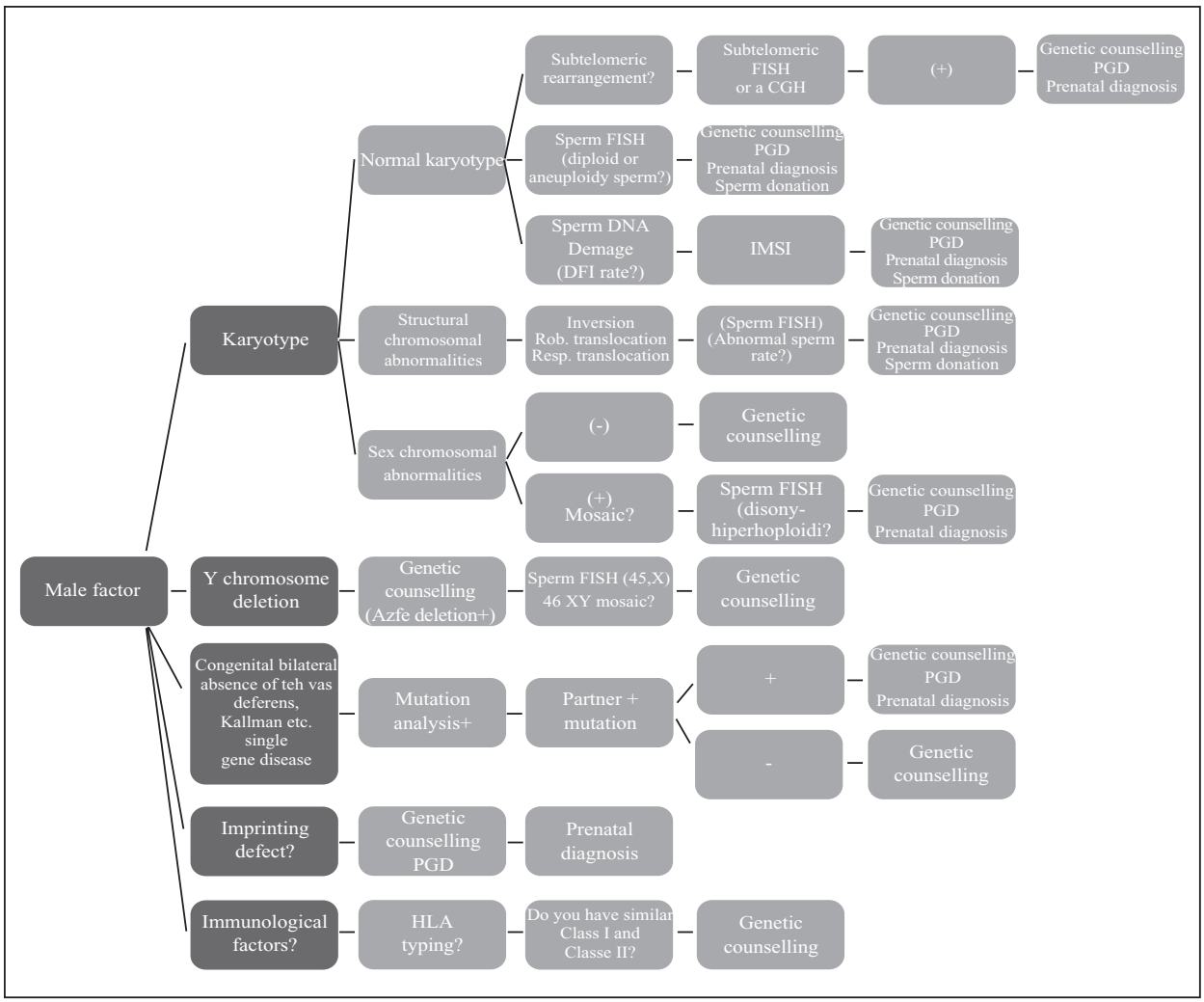

Figure 3: *The Genetic Approch to Planned IVF Application for Male Factors.*

these structural abnormalities, the most frequently observed anomaly is autosomal chromosomal translocations. The frequency of chromosomal translocations is 2.5% in RIF couples, 9.2% in RPL couples and 0.2% in newborns[9,11]. It has been declared that in couples carrying chromosomal anomalies, IVF and pregnancy success rate may decrease and there may be an increased risk of abortion[10]. These couples may also have a fetus with a chromosome anomaly that may lead to congenital anomalies, mental retardation and short life-span[17].

The abnormal sperm ratio varies between 3.4 and 40% in men carrying a Robertsonian translocation and between 47.5 and 81% in those carrying a reciprocal translocation[18]. The patients who are translocation carriers may produce more abnormal embrios than expected in association with abnormal sperm production. Inversions are an other type of structural chromosomal changes[19,20]. They are important since the carrier individuals may develop abnormal gametes. 5-10% of the major chromosomal anomalies detected in RPL couples are pericentric inversions. In men carrying inversions, abnormal spermatocyte frequency varies between 0 and 54.3% according to the length of the inversion and formation of a stich or not by the chromosome[19,20].

Since the rearrangements of the chromosomes affecting the telomeric regions can also produce abnormal gametes, it is reported that these rearrangements may also lead to RIF and RPL. Because of all these reasons, it is recommended to perform pre-implantation genetic diagnosis (PGD) when a structural anomaly is detected in at least one of the patients[21]. (Figure 3,4). Use of more sensitive methods such as subtelomeric FISH or Array-Comparative Genomic Hybridization (a-CGH) for anomalies that cannot be detected by conventional cytogenetic examinations[21,1].

In the recent years, the effect of polymorphisms of heterochromatin region of the chromosomes on reproductive health has been investigated. Even if they do not lead to known genetic diseases, it is thought that these polymorphic changes may be responsible

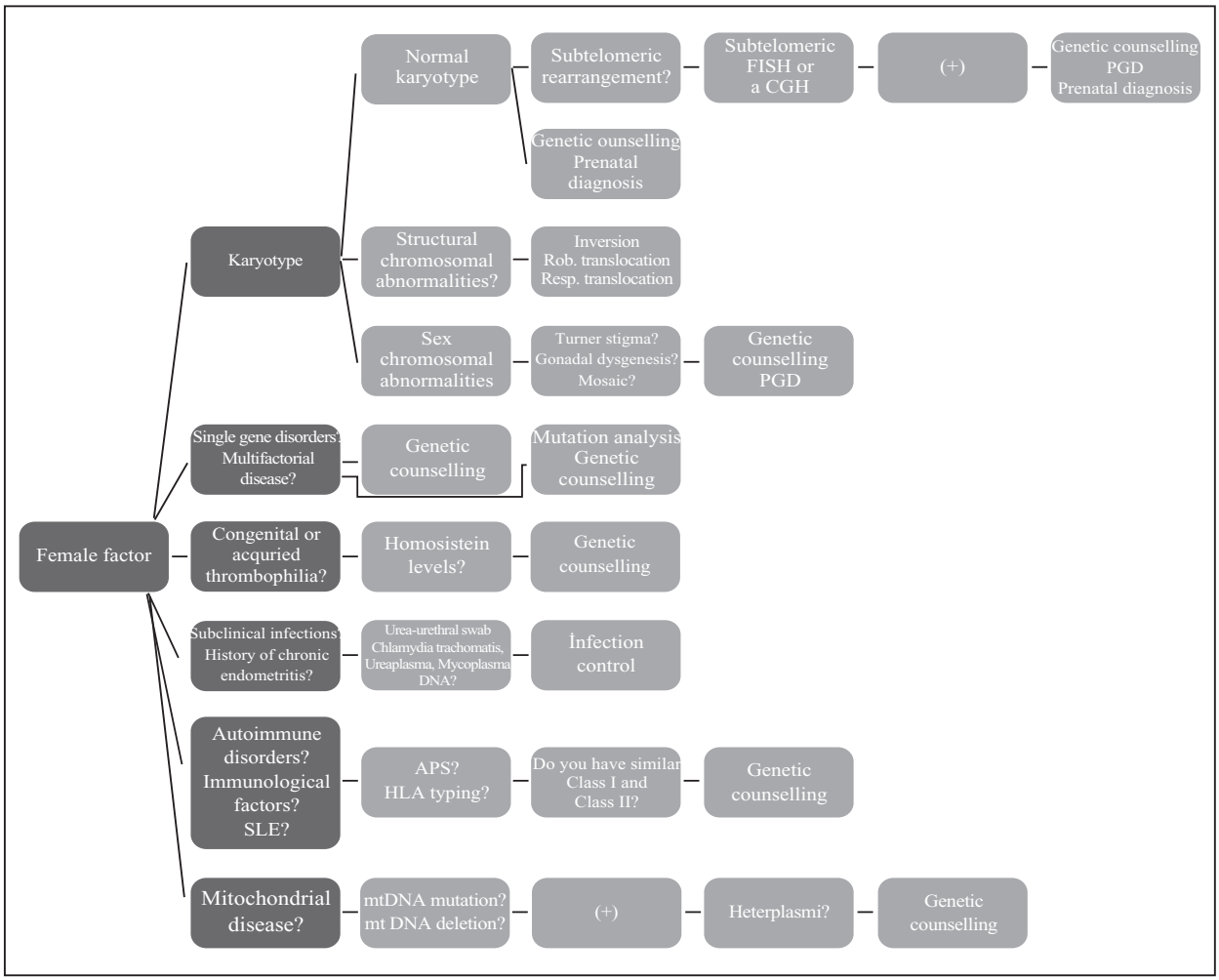

Figure 4: *The Genetic Approch to Planned IVF Application for Female Factors.*

from reproductive failure and be the cause of RPL (17). Increased aneuploidy rates detected by sperm FISH has been reported in male patients with heterochromatin polymorphism[17,22,23].

The mode of action of heterochromatin changes on mechanisms of sperm production are not known yet, however, with the detailed analysis of the changes that are suspected to be normal variants, how these interact with the genes acting in sperm production will be clarified.

b. Y Chromosome Microdeletion:

Lower fertilization and pregnancy rates have been reported in cases with Y chromosome deletion. Y microdeletion is mostly de novo in patients[24]. The deletions are mostly localized on the long arm of Y chromosome (Yq11). The locus of deletion on Y chromosome is important for its effect on spermatogenesis. Y chromosome microdeletion is observed at a rate of 10-15% in patients with azospermia or severe oligozoospermia[20,24]. AZFc deletion has been detected in 16% of azospermia patients and in 5% of oligozoospermia patients. The frequency of AZFc deletion in the population is supposed to be 1/4000[20]. AZFb deletions are observed in 2% of the patients[18,21].

In couples who are fertile but who present RPL, Y chromosome deletions may have an etiological role. In a study about the interaction of RPL and proximal AZFc deletion, 14(82%) the of 17 patients with RPL were found to have a deletion in proximal AZFc region of the Y chromosome, while no deletion has been observed in patients having live birth[20,23,24].

In addition to that, 45,X/46,XY mosaicism may be detected in the gonads of the father with an AZFc deletion as a result of loss of Y chromosome. This way, monosomic embryo for X may be produced by ICSI or 45,X/46,XY mosaicism may occur due to unstable Y chromosome transferred to the male embryo at early stages. Because of this mosaicism, birth of a male baby with ambiguous external genitalia or mixed gonadal disgenesis has been reported[24]. It has been recommended to perform Y chromosome microdeletion test in addition to karyotype analysis to IVF candidates depending on the indication before IVF/ICSI procedures.

c. Sperm DNA Damage:

Tests including sperm number, morphology and motility that indirectly evaluate male infertility are widely used in fertility clinics. In last years, the detection of high sperm DNA damage in patients with abnormal semen parameters brought up new approaches[25]. Sperm DNA damage may develop with different mechanisms such as abnormal chromatin packing, reactive oxygen species (ROS) and sperm apoptosis[26]. The potential effect of sperm DNA integrity on male infertility is unknown. Damaged sperm that cannot be repaired enter the apoptosis process and lose their fertile capacity [27]. DNA damage occurring in male germ cells with increasing age of the father are repaired by DNA repair mechanisms under normal conditions and reproduction/fertilization pursue[28]. However, as sperm DNA damages have been detected in 8% of the patients with normal semen parameters, their effect on IVF and ICSI results are still discussed.

It has been demonstrated that the oocyte may compensate sperm DNA fragmentation in cases with a DNA fragmentation index (DFI) up to 30% which is measured using Sperm Chromatin Structure Analysis (SCSA) and shows the level of DNA denaturation[29]. In fertile couples with a DFI<30%, the possibility to get pregnant by ICSI and IVF is reported to be 2 times higher[30]. However, in patients with DNA fragmentation >30%, even if the first 3-days of embryo development will not be affected, healthy blastocyte development and pregnancy will not follow[31,32]. In a study of Bungum et al. including 387 intrauterine insemination (IUI) cycles with DFI >%30, which is the largest series up to date, biochemical pregnancy, clinical pregnancy and birth rate have been found to be significantly low in patients[28]. However, in studies about this topic, the correlation detected between DFI and sperm aneuploidy is contradictory. In two studies about the relation between DFI and sperm aneuploidy, a positive relation has been observed, while this has not been observed in a different study performed in the recent years. In these three studies, embryo aneuploidies have not been evaluated[33]. In another prospective randomized study of Balaban et al., implantation and clinical pregnancy rates of intracytoplasmic injection of morphologically selected sperm (IMSI) cases was compared with that of ICSI cases. As a result, the rates of 19.5% and 54.0% observed in ICSI have respectively increased to 28.9%

and 54.0% with IMSI[34]. Thus, routine IMSI have been recommended for couples with five or more RIF[34].

Problems such as production of embryo with high aneuploidy rate, early pregnancy losses, risk of metabolic diseases associated with epigenetic modifications, childhood cancers are still subjects of investigations. The relation between damaged sperm DNA used in ICSI and the potential effects on the child is not clear, further studies are required. Further larger studies are required to detect the mechanisms of occurrence of sperm DNA damage and their effects on IVF success.

d. Single Gene Disease and Multifactorial Diseases:

Single gene diseases may lead to RPL and IVF failures especially in patients with family history. These diseases are supposed to affect pregnancy losses in two ways. The first one is the development of a disease in the fetus that can be incompatible with life, while the other one is the possibility that single gene disease present in mother adversely affects the pregnancy. Single gene diseases such as sickle cell anemia, alpha thalassemia, Zelweger disease and glutaric aciduria for which the incidence increases in consanguineous marriage may lead to pregnancy losses[23]. According to the data of PGD Consortium of 2012 of the European Society of Reproduction and Embryology (ESHRE), 4733 cycles have been performed for single gene diseases in the last ten years. According to this report, PGD have been mostly performed for cystic fibrosis, sickle cell anemia and spinal muscular dystrophies. For autosomal dominant diseases, PGD has been mostly used for myotonic dystrophy[36].

Hereditary recessive defects of X chromosome may be lethal for the male fetus in intrauterine environment. In a study about the X-linked lethal genes in women with RPL, a higher amount of shifted X inactivation pattern compared to the control group has been observed [22,23]. X chromosome inactivation in a certain region in more than 90% of the cells has been highlighted. Same results have been obtained in leucocytes, oral mucosa cells and muscle biopsies. The locus with genetic defects has been mapped on Xq28 region [23].

Nowadays, PGD is mostly performed for Fragile X syndrome, Duchenne muscular dystrophy (DMD) and hemophilia among X chromosome-associated diseases [36]. These situations that can directly lead to fetal death should be analyzed before IVF applications.

e. Polymorphic Modifications:

According to the data obtained from studies, mutations and polymorphisms in some genes such as p53, HLA-G, VEGF and IL-1RN play a genetic role for RIF after IVF. Sipak-Szmigiel et al. have studied the existence of a relation between gene polymorphism of HLA-G antigen expressed in trophoblast cells of the fetus and RIF. This antigen which plays an important role in pregnancy physiology participates to the formation of maternal immune system during pregnancy. As the antigen may also be detected in some blastocysts, it may have a role in implantation. Previous studies have shown that HLA-G gene polymorphism may lead to preclampsia, RPL and RIF[37]. In the study of Sipak-Szmigiel et al., a relation between HLA-G gene polymorphism and increased RIF risk has been established[37]. Goodman et al. have shown in a study that -1154A/A polymorphism of VEGF gene which is the best characterized regulator of angiogenesis may be a factor of RIF[38]. In a study of Goodman et al., the Pro 72 polymorphism of the p53 gene that is supposed to act in fertility and to regulate reproduction, the 4G/4G polymorphism of the PAI gene which encodes proteins that have a role in coagulation and 1154A/A polymorphism of the VEGF gene were reported to have a relation with RIF[39].

Using all these data, it has been proposed that polymorphisms may play a role in RIF development and that it will be useful to prepare a test panel to determine these gene polymorphisms to detect women who will be under risk of RIF after IVF.

f. Mitochondrial Modifications:

Rearrangements, long region deletions and point mutations in mtDNA genome are important for sperm motility and morphology. It has been demonstrated that genetic modifications in sperm mtDNA affect sperm function and thus the normal fertilization[40]. An increase is observed in abnormal sperm and sperm mtDNA in infertile men and this increase creates serious problems during ICSI. The total loss of mitochondrial rRNA or tRNA may lead to the arrest of mitochondrial protein synthesis and affect early embryonic development and thus may provoke fetal death[55,56]. Also, an increase of defective mtDNA in

ovarian dysfunction that occurs with advanced age of the mother has been described in literature[40].

g. Methylation-Imprinting Defects:

The methylation of germ cell genome is important both for normal spermatogenesis and embryo development following fertilization. The major epigenetic regulator for the cellular genome to be able to function is reported to be DNA methylation. It has been observed that genome imprinting defects lead to hypospermatogenesis and that oligozoospermic men transfer this imprinting defects to their children[41].

Due to the methylation of sperm and oocyte during fertilization, transcription is not possible. But chromatin DNA reorganizes by making modifications in methylation state to permit the somatic development of the embryo after it becomes diploid. In that purpose, the genome obtained from the father undergoes demethylation within few hours after fertilization while the genome obtained from the mother undergoes a passive demethylation process following the two-cell embryo period. After the development of morula and blastocyst, the two genomes have undergone equal demethylation and then new methylation occurs[42]. Benchaib et al. have shown in a study that methylation defects may lead to abnormal fetal development, diseases such as Angelman syndrome and Beckwith-Wiedemann syndrome and that pregnancy success increases with the increase of methylation[42,43].

h) Immunologic Factors and thrombophilias

Nowadays, effects of abnormalities in local immune functions at the maternal and fetal surfaces on implantation success are an emerging issue[44]. In last years, studies in immunology have shown that 80% of the unexplained abortions may be associated with immunologic factors. Alloimmune defects, cytotoxic antibodies, "natural killer" cells function and dispersion anomalies constituting an abnormal maternal immune response against fetal and placental antigens are among immunologic factors that can affect IVF success[44]. Most of these may be prevented using new treatment methods[7]. If all the tests performed on the couples before IVF are normal, immunologic factors that may affect the implantation should be taken into consideration. For this, the first thing to do is to observe immunologic reactions with a lymphocyte cross-match test of the couples. If there is no reaction against male antigen in women, they may have similar human leukocyte antigens (HLA). As this similarity has been proposed as a cause of RPL and RIF, analysis of class I and class II HLA of the couples and treatment with high dose IV immunoglobulin has been recommended in the literature[46].

It has been declared that hereditary and acquired thrombophilias create local micro thrombi in maternal vascular structure on the implantation surface and affect the microcirculation, thus leading to RIF and early pregnancy losses[47]. Antiphospholipid antibody syndrome (AFS) which is one of the causes of acquired thrombophilias, is an autoimmune pathology leading to fetal death and RPL due to arterial and venous thrombosis. Antiphospholipid antibodies are observed in 14% of the total population and in 30-50% of patients with systemic lupus eritamatosis (SLE). Besides, temporary antiphospholipid antibodies may be detected in Syphilis, Lyme disease, in frequent viral and mycoplasma infections as well as with the use of drugs such as chlorpromazine, clonidine, phenytoin, procainamide. But in these situations, thrombosis does not develop[48].

Studies have shown that, almost all of the fetal deaths observed in women with SLE were associated with AFS (lupus anticoagulant-LA and anticardiolipin antibodies-ACA)[47,48]. These antibodies are the most sensitive indicators in fetal distress and death. Besides the clinical criteria, demonstration of medium or high levels of ACA (IgG or IgM) constitute an important laboratory diagnostic criteria. In spite of this relation between AFS and RPL, American Society of Reproductive Medicine (ASRM) has reported in 2008 that AFS does not affect IVF success[49]. In this report, 16 review articles and 2053 cases have been investigated bot individually and globally and no statistically significant difference has been observed between AFS and IVF success[49].

In literature, there are at least 694 studies about the effect of hereditary thrombophilic gene mutations on RPL and IVF success[50]. Among these, when review articles, meta analyses and case presentations are considered, it has been observed that at least 6092 cases have been studied. Case control studies showed that, when more than one AFS antibody have been detected, the risk of IVF failure is three times the normal value[50].

Cohort studies showed no relation between AFS and live births and pregnancy rate[50]. In eight case control studies, the success of IVF decreased three times compared to normal conditions in patients with Factor V Leiden mutation[50]. This relation has not been established in two cohort studies. The homozygote mutation of methylene tetrahydropholate reductase (MTHFR) gene was demonstrated to cause hyperhomocystinemia leading to defects in chorion villous vascularization, increasing the risk of early pregnancy loss[51,52]. This mutation also increases the NTD risk in the fetus up to 1.9 times[51]. In spite of all fetal effects of MTHFR mutation that can affect folate metabolism, its relation with RIF has not been proved. In a recent study of Laanpere et al., it has been proposed that a heterozygote modification of gene working in folate metabolism causes a decrease in ovarian stimulation and IVF success compared to the control group[50].

Even if there are many people thinking that thrombophilia in fetus may increase the risk of pregnancy loss, there is no clear proof about this issue yet[53]. There are few cases of pregnancy loss, early birth and cerebral palsy defined in literature[54].

Multiple thrombophilic gene mutation rate is 74% in women with RPL and 20% in control group[52]. Based on these data, it is possible to declare that thrombophilia screening will be useful in RIF patients before IVF/ICSI procedures (Figure 4).

2) Subclinical Infections:

Chlamydia trachomatis, Ureoplasma and Mycoplasma, Toxoplasma gondii, Listeria Monocytogenes, Herpes virus and Cytomegalovirus infections may cause RPL[55]. However, the data about cervicovaginal infections as a cause of early pregnancy losses are insufficient and contradictory[56].

Kamiyama et al. have evaluated the existence of a relation between subclinical upper genital tract infections and IVF-ET failure; no pregnancy has been observed in 38 patients with menstrual blood endotoxin concentration higher than a threshold value while pregnancy has been detected in 1/3 of the patients with an endotoxin concentration within accepted limits[57]. They proposed that early colonization of the endometrium by Gram negative bacteria affects the implantation or triggers early spontaneous loss thus causing IVF-ET failure. Based on these results, Romero et al. also supported the potential role of genital canal infections on IVF-ET failure in a review article[58].

With these information, even if screening for these infections before IVF seems to be useful in a patient who does not have clinic infection findings or is not under risk of sexually transmitted diseases, there are not enough studies proving the necessity to perform it in routine practice. On the other side, routine screening for HIV, hepatitis B, hepatitis C infections should be performed before IVF procedure. This is important for the protection of the fetus obtained by IVF as well as members of the the clinical and laboratory team and to prevent cross contamination between frozen embryos.

PREIMPLANTATION GENETIC DIAGNOSIS AND ARRAY CGH APPLICATION IN IVF FAILURES

In a report published by ESHRE PGD Consortium, more than 27 000 cycles from 39 different centers have been performed; of these, 61% were for aneuploidy, 17% were for single gene diseases, 16% were for chromosomal anomalies, 4% were for X-associated diseases and 2% were for social indications[36].

PGS is a technique used to chose and transfer the best embryo obtained by IVF by evaluating aneuploidies of the embryo. One of the main reasons to use PGS is to increase implantation success in IVF centers or to decrease pregnancy losses[36]. PGS technique should especially be used for patients with advanced maternal age or couples with RPL or RIF.

In previous studies, pregnancy rate per oocyte increased from 29% to 38% and the abortion rate in carrier parents decreased from 92% to 12.5% after PGS application[59]. FISH technique has been frequently used for aneuploidy and translocation screening. With the FISH technique, the analysis of limited number of chromosome, the debatable results, the use of unsuitable biopsy and fixation techniques lead to different results[60]. As an alternative to FISH technique, aCGH technique permits the analysis of all chromosomes in the embryo. This technique may be used indications such as advanced maternal age, RPL, RIF, severe male factor and translocation and inversion carriers. 30-40% of chromosome deficiencies determined by aCGH cannot be determined by FISH technique[60,61].

Different from PGS, PGD is based on a selective

transfer of embryo in couples carrying genetic diseases such as structural chromosomal rearrangements like translocations, familial genetic diseases or carriers of genetic diseases. By this way, it is aimed to increase the chance of the couples for having a healthy children and to decrease the amount of pregnancy terminations due to medical indications.

Due to frequent consanguineous marriages in our country, it is important to perform PGD in autosomal recessive diseases such as beta-thalassemia. PGD applications are now developed for autosomal dominant diseases such as myotonic dystrophy, Huntington disease, and Marfan syndrome. PGD application frequency for autosomal dominant diseases is reported to be lower compared to that for autosomal recessive diseases[36]. However, we suppose that PGD applications will increase in the near future since the risk of passage of the autosomal dominant diseases to the embryo is higher than recessive diseases[36].

Even if PGD procedure seems to be advantageous for IVF applications, it also leads to problematic situations. The procedure is very expensive especially in countries such as England where prenatal diagnosis is endorsed by the government but the PGD is not. The most difficult part of PGD for fertile couples is the necessity to undergo IVF. Also, the possibility to have false positive or negative results is one of the disadvantages which should also be mentioned during genetic counseling. The other disadvantage of the PGD is the ethic apprehension it brings. Use of PGD application in hereditary cancers without a penetrance of 100%, in late onset genetic diseases such as Huntington disease and in situations like non-fatal hearing loss may lead to ethical apprehension[36,62].

Single nucleotide polymorphism-copy number variation (SNP-CNV) technique that permits the detection of structural chromosomal abnormalities such as microdeletions, duplications uniparental disomies and marker chromosomes besides the numerical chromosome abnormalities, has also gained popularity in the last years. In this technique based on oligo aCGH, approximately 250.000 genomic data may be analyzed at once. Following whole genome amplification on one blastomer, 250.000 SNP regions may be analyzed [61]. Even if the recent data show that implantation and pregnancy success increased following microarray tests, the results may not be evaluated correctly and should be supported with long-term and comprehensive bioinformatic studies[61].Bacause of the expectations and unanswered questions, array-SNP applications lead to anxiety, but they also cause some concerns because of the present problems in healthy evaluation of some data and the ethical dilemma. Even if all these studies performed at pre-implantation stage give rapid results, they are still laborious and difficult.

The accreditation of PGD laboratories with ISO 15189 or its equivalent and the data sharing by PGD researchers in scientific meeting will play an important role. The organization of an educational programs for the association of cytogenetic and molecular genetics disciplines will help develop this technique[63].

GENETIC EVALUATION

As a result; the utilization of genetic diagnosis before, during and after the application of assisted reproductive techniques is a part of good clinic practice. These tests will permit right diagnosis and suitable genetic counseling. During the process of decision about the tests to perform, the evaluation of family history in addition to clinic findings will be more beneficial for the patient.

In genetic screening for reproductive health, the aim should be the detection of healthy carriers who may give birth to a baby with fatal and/or severe anomalies. The cost and the efficiency of the test for the detection of the etiological cause should be taken into consideration while deciding which test will be performed on RIF couples. Tests performed for genetic screening should be able to provide correct information about their reproductive health to individuals or couples with a disease to facilitate their decision making process.

REFERENCES

1. Ly KD, Aziz N, Safi J and Agarwal A. Evidence-Based Management of Infertile Couples with Repeated Implantation Failure Following IVF. Current Women's Health Reviews 2010; 6: 200- 18.
2. Macklin VM. Ovarian Stimulation and ovulation induction. Fertil Steril 2001; 75: 88- 91.
3. ASRM/SART Registry. Assisted reproductive technology in the United States: Results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive

Technology Registry. Fertil Steril 2000; 72: 641.

4. European Society of Human Reproduction and Embryology (ESHRE). The European İVF-monitoring programme (EIM), for ESHRE. Assisted reproductive technology in Europe, 1998. Results generated from European Registers by ESHRE. Hum Reprod 2001; 16: 2459.
5. Dewald GW, Michels VV. Recurrent Miscarriages: Cytogenetic Causes and Genetic Counseling of Affected Families. Clinical Obstetrics and Gynecology 1986; 29: 865- 83.
6. Chandley AC. Chromosome anomalies and Y chromosome microdeletions as causal factors in male infertility. Hum Reprod 1998; 13: 45- 50.
7. Centers for Disease Control and Prevention, American Society for Reproductive Medicine. 2001 assisted reproductive technology success rates, U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA, 2003. 2000; 72: 641.
8. Bolarinde O, Tin-Chiu L. İn-vitro fertilizasyonu takiben implantasyon başarısızlığı. Current Opinion in Obstetrics and Gynecology (Türkçe Baskı) 2006; 18: 440- 5.
9. Hassold T, Chiu D. Maternal age-spesific rates of numerical chromosome abnormalities with special reference to trisomy. Hum Genet 1985; 70: 11- 7.
10. Delhanty J, Griffin D, Handyside A. Detection of aneuploidy and chromosomal mosaicism in human embryos during preimplantation sex determination by fluorescent in situ hybridisation, (FISH). Hum Mol Genet 2 1993; 1183- 5.
11. Ishikawa M, Hidaka E, Wakui K, Nakayama K, Takagi Y, Fukushima Y, Katsuyama T. Habitual abortion and high frequency of low frequent X chromosome monosomy mosaicism: detection by interphase FISH analysis of buccal mucosa cells and lymphocytes Rinsho Byori 2000; 48(10): 955- 9.
12. Bronet F, Martınez E, Gaytan M, Linan A, Cernuda D, Ariza M. et al. Sperm DNA fragmentation index does not correlate with the sperm or embryo aneuploidy rate in recurrent miscarriage or implantation failure patients. Human Reproduction. 2012; 1- 8.
13. Vidal F, Blanco J, Egozcue J. Chromosomal abnormalities in sperm. Mol Cell Endocrinol 2001; 22; 183: 51- 4.
14. Egozcue S, Blanco J, Vidal F, Egozcue J. Diploid sperm and the origin of triploidy. Hum Reprod 2002; 17: 5- 7.
15. Jaarola M, Martin, RH, Ashley, T. Direct evidence for suppression of recombination within two pericentric inversions in humans: a new sperm-FISH technique. Am J Hum Genet 1998; 63: 218- 24.
16. Ishikawa M, Hidaka E, Wakui K, Nakayama K, Takagi Y, Fukushima Y, KatsuyamaT. Habitual abortion and high frequency of low frequent X chromosome monosomy mosaicism: detection by interphase FISH analysis of buccal mucosa cells and lymphocytes Rinsho Byori 2000 Oct; 48(10): 955- 9.
17. Durak B, Yesil M, et al. Is recurrent abortion an indication for subtelomeric region analysis? Turkiye Klinikleri J Med Sci 2010; 30: 1465- 8.
18. Jaarola M, Martin RH, Ashley T. Direct evidence for suppression of recombination within two pericentric inversions in humans: a new sperm-FISH technique. Am J Hum Genet 1998; 63: 218- 24.
19. Dohle GR, Diemer T, Giwercman A et al. Guidelines on Male Infertility. European Association of Urology 2010; 4: 14- 6.
20. Pang MG, Kim YJ, Lee SH, Kim CK. The high incidence of meiotic errors increases with decreased sperm count in severe male factor infertilities. Human Reproduction 2005; 20: 1688- 94.
21. Harper JC, Sengupta SB. Preimplantation genetic diagnosis: state of the ART 2011. Hum Genet 2012; 131: 175- 86.
22. Mercy YL, Robin L, Bennett, Devki SS, et al. Genetic Evaluation and Counseling of Couples with Recurrent Miscarriage: Recommendations of the National Society of Genetic Counselors. Journal of Genetic Counseling 2005; 14: 3.
23. Hoffman, Eric P. Screening test for the lethal genetic trait of recurrent spontaneous pregnancy loss.
24. Siffroi JP, Bourhis CL, Krausz C, Barbaux S, Quintana-Murci L, Kanafani S, et al. Sex chromosome mosaicism in males carrying Y chromosome long arm deletions. Hum Reprod 2000; 15: 2559- 62.
25. Zini A, Bielecki R, Phang D, Zenzes MT. Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril 2001; 75: 674- 7.
26. Gorczyca W, Traganos F, Jesionowska H, Darzynkiewicz Z. Presence of DNA strand breaks and increased sensitivity of DNA in situ to denaturation in abnormal human sperm cells: analogy to apoptosis of somatic cells. Exp Cell Res 1993; 207: 202- 5.
27. Alvarez JG. The predictive value of sperm chromatin structure assay. Hum Reprod 2005; 28: 2365- 7.
28. Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod 2007; 22: 174- 9.
29. Regan L, Rai R. Thrombophilia and pregnancy loss. J Reprod Immunol 2002; 55: 163.
30. Duran EH, Morshedi M, Taylor S, Oehninger S. Sperm DNA

quality predicts intrauterine insemination outcome: a prospective cohort study. Hum Reprod 2002; 17: 3122- 8.

31. Liu CH, Tsao HM, Cheng TC, Wu HM, Huang CC, Chen CI, Lin DP, Lee MS. DNA fragmentation, mitochondrial dysfunction and chromosomal aneuploidy in the spermatozoa of oligoasthenoteratozoospermic males. J Assist Reprod Genet 2004; 21: 119- 26.
32. Muriel L, Goyanes V, Segrelles E, Gosa´lvez J, Alvarez JG, Ferna´ndez JL.Increased Aneuploidy rate in sperm with fragmented DNA as determined by the sperm chromatin dispersion (SCD) test and fish analysis. J Androl 2007; 28: 38- 49.
33. Balasuriya A, Speyer B, Serhal P, Doshi A, Harper JC. Sperm chromatin dispersion test in the assessment of DNA fragmentation and aneuploidy in human spermatozoa. Reprod Biomed Online 2011; 22: 428- 36.
34. Bronet F, Martınez E, Gaytan M, Linan A, Cernuda D, Ariza, Nogales M. at al. Sperm DNA fragmentation index does not correlate with the sperm or embryo aneuploidy rate in recurrent miscarriage or implantation failure patients Human Reproduction 2012; Vol.00, No.0 pp. 1- 8.
35. Balaban B, Yakin K, Alatas C, Oktem O, Isiklar A, Urman B. Clinical outcome of intracytoplasmic injection of spermatozoa morphologically selected under high magnification: a prospective randomized study. Reprod Biomed Online 2011; 22: 472- 6.
36. Duran EH, Morshedi M, Taylor S, Oehninger S. Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. Hum Reprod 2002; 17: 3122- 8.
37. Harper JC, Wilton L, Traeger-Synodinos J, Goossens V, Moutou C, Sengupta SB, Pehlivan Budak T, Renwick P, De Rycke M, Geraedts JP, Harton G.The ESHRE PGD Consortium: 10 years of data collection. Hum Reprod Update 2012; 18(3): 234- 47.
38. Sipak-Szmigiel O, Cybulski C. HLA-G polymorphism and in vitro fertilization failure in a Polish population. Tissue Antigens 2009; 73: 348- 52.
39. Goodman C, Jeyendran RS. Vascular endothelial growth factor gene polymorphism and implantation failure. Reprod Biomed Online 2008; 16: 720- 3.
40. Goodman C, Jeyendran RS. P53 tumor suppressor factor, plasminogen activator inhibitor, and vascular endothelial growth factor gene polymorphisms and recurrent implantation failure. Fertil Steril 2009; 92: 494- 8.
41. Sanchez CD, Ruiz-Pesini E, Lapen˜a, AC, Montoya J, Pe´rez-MartosA, Enr_´quez, JA, Lo´pez-Pe´rez MJ. Mitochondrial DNA Content of Human Spermatozoa. Biology of Reproduction 2003; 68: 180.
42. Marques CJ, Carvalho F, Sousa M, Barros A. Genomic imprinting in disruptive spermatogenesis. Lancet 2004; 22: 1700- 2.
43. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. Science 2001; 293: 1089- 93.
44. Benchaib M, Ajina M, Lornage J, Niveleau A, Durand P, Guerin JF. Quantitation by image analysis of global DNA methylation in human spermatozoa and its prognostic value in in vitro fertilization: a preliminary study. Fertil Steril 2003; 80: 947- 53.
45. Ogasawara M, Aoki K, Hayashi Y. A prospective study on pregnancy risk of antiphospholipid antibodies in association with systemic lupus erythematosus. J Reprod Immunol 1995; 28: 159.
46. Lachapelle MH, Miron P, Hemmings R, Roy DC. Endometrial T, B, and NK cells in patients with recurrent spontaneous abortion. Altered profile and pregnancy outcome. J Immunol 1996; 156: 4027.
47. Simon A, Laufe N. Repeated implantation failure: clinical approach Fertility and Sterility 2012; 97, 15- 0282.
48. Ogasawara M, Aoki K, Hayashi Y. A prospective study on pregnancy risk of antiphospholipid antibodies in association with systemic lupus erythematosus. J Reprod Immunol 1995; 28: 159.
49. Sauer R, Roussev R, Jeyendran RS, Coulam CB. Prevalence of antiphospholipid antibodies among women experiencing unexplained infertility and recurrent implantation failure. Fertil Steril 2010; 1; 93: 2441- 3.
50. Practice Committee of the ASRM. Anti-phospholipid antibodies do not affect IVF success. Practice Committee of the ASRM. Fertil Steril 2008; 90: S172- 3.
51. Laanpere M, Altmäe S, Kaart T, Stavreus-Evers A, Nilsson TK, Salumets A. Folate-metabolizing gene variants and pregnancy outcome of IVF. Reprod Biomed Online. 2011; 22(6): 603- 14.
52. Rey E, Kahn SR, David M and Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet 2003; 361: 901- 08.
53. Coulam CB, Jeyendran RS. Multiple thrombophilic gene mutations rather than specific gene mutations are risk factors for recurrent miscarriage. American Journal of Reproductive Immunology 2006; 12: 322- 7.
54. Tranquilli AL, Saccucci F, Giannubilo SR, Cecati M, Nocchi L, Lorenzi S, Emanuelli M. Unexplained fetal loss: the fetal side of thrombophilia. Fertil Steril. 2009.
55. von Kries R, Junker R, Oberle D, Kosch A, Nowak-Göttl U.Foetal growth restriction in children with prothrombotic risk factors. Thromb Haemost. 2001; 86(4): 1012- 6.

56. Witkin SS, Kligman I, Grifo JA, Rosenwaks Z. Ureaplasma urealyticum and Mycoplasma hominis detected by the Polymerase Chain Reaction in the cervices of women undergoing in vitro fertilization: Prevalence and Consequences. Journal of Assisted Reproduction and Genetics 1995; 12(9): 610- 4.
57. Sermon K, Steirteghem AV, Liebaers I. Preimplantation genetic diagnosis. Lancet 2004; 363: 1633- 41.
58. Kamiyama S, Teruya Y, Nohara M, Kanazawa K. Impact of detection of bacterial endotoxin in menstrual effluent on the pregnancy rate in in vitro fertilization and embryo transfer. Fertil Steril 2004; 82: 788- 92.
59. Romero R, Espinoza J, Mazor M.Can endometrial infection/ inflammation explain implantation failure, spontaneous abortion, and preterm birth after in vitro fertilization? Fertil Steril. 2004; 82(4): 799- 804.
60. Abdelhadi I, Colls P, Sandalinas M, Escudero T, Munn. S. Preimplantation genetic diagnosis of numerical abnormalities for 13 chromosomes. Reprod Biomed Online 2003; 6: 226- 31.
61. Trussler JL, Pickering S, Ogilvie CM. Investigation of chromosomal imbalance in human embryos using Comparative Genomic Hybridization. Reprod Biomed Online 2004; 80: 860- 8.
62. Bisignano A, Wells D, Harton G, Munné S. Reply: PGD and aneuploidy screening for 24 chromosomes by genome-wide SNP analysis: a responsible path towards greater utility. Reproductive BioMedicine Online 2012; 24: 4- 5.
63. European Society of Human Reproduction and Embryology (ESHRE). The European İVF-monitoring programme (EIM), for ESHRE. Assisted reproductive technology in Europe, 1998. Results generated from European Registers by ESHRE. Hum Reprod 2001; 16: 2459.
64. Harper JC, Harton G (2010) The use of arrays in preimplantation genetic diagnosis and screening. Fertil Steril 94(4): 1173- 7.