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Importance of QF-PCR method in aborted embryos in comparison with other common relative determination aneuploidies methods

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Abstract

One of the most important method in cytogenetic in order to diagnose the chromosomal abnormalities, is QF-PCR (Quantitative fluorescent polymerase chain reaction).In this way, QF-PCR can be employed in diagnosing the chromosome duplication number by amplification of repeat sequences at polymorphic loci. These repeat sequences are amplified by PCR, and the labelled yields are classified by gel electrophoresis method. Importantly, QF-PCR reaction has been in diagnostic application in many countries and has confirmed to be a robust, cost-effective, and precise rapid prenatal test for many types of common aneuploidies. Special benefits comprise detection of mosaicism, triploidy and maternal cell contamination. So, we try to declare the importance of this technique in comparison with other ones in this review article.

Keywords: QF-PCR, Aneuploidies, Abortion, Chromosomal abnormalities

Introduction

Abortion is the involuntary termination of pregnancy before the twentieth week. Although abortion is a common experience, it is not because it is easy, and it is possible to take steps to treat and prevent it by examining its causes.

Meanwhile, women who experience more than three consecutive miscarriages have recurrent miscarriages. Recurrent miscarriage as a multifactorial disease involves several issues, including immune, anatomical, hormonal, and genetic disorders. In more than 50% of cases, no cause for recurrent miscarriage is identified.

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Disruption of aneuploid chromosomes can be one of the causes of recurrent miscarriage. High-risk pregnancies for chromosomal defects have been studied for two decades by conventional cytogenetic methods. The routine cytogenetic method is very accurate in examining chromosomal defects, but the most important problem of this method is its long duration. Recently, rapid chromosomal screening methods have been developed that detect major abnormalities of certain chromosomes in just one or a few days. These methods include FISH, MLPA, and QF-PCR. Quantitative fluorescence polymerase chain reaction (QF-PCR) is a cheap, fast and reliable method for prenatal diagnosis of aneuploidy on chromosomes 13, 18, 21, X and Y (Table 1). In recent years, quantitative fluorescent technique, PCR or QF-PCR, has been used to rapidly detect chromosomal abnormalities before birth. In this method, short repeats (STRs) or markers on DNA are amplified and marked with fluorescent markers and their value is measured by electrophoresis.

Table 1. QF-PCR efficiency in cytogenetics.

Number of cases detected / number of actual nonmosaic autosomal trisomies and triploidies	Number of cases detected / number of actual non-mosaic sex chromosomal aneuploidies	Sample failure/ not tested or UI, %	Number and type of specimens	False positives for sex chromosomal aneuploidy	False positives for trisomy or triploidy
14/15 1 case of T18 was UI	5/5*	0 / 0	662 AF	0	0
89/89	16/20	0.1 / 2	5097 AF	0	0
437/437	N/A	0.09 / 2.1	7720 (6147AF, 1552 CVS 21 FBS)	N/A	0
16/19 3 cases were UI	0/0	0 / 1.2	1020 AF	0	0
14/14	1/3 Only 2 markers on X chr	0 / 2.5	687 AF	0	0
429/429	NA	2.9	10253AF	NA	0
71/73, 2 cases missed at the beginning when only two markers per chr used.	2/8	0.06 / 0.15	4692 AF	0	0
202/202	14/14	None reported	3854 AF	0	0
110/110	20 / 20	0 / 0.26 for AF	2906 (142 CVS, 2764 AF)	0	0
15/15	4/4	0 / 2.9	576 AF	0	0
1287/1290† 3 cases were UI	265/267†‡ 1 abnormality missed; 1 UI MCC	0.05 / 0.82	37544 AF 4687 CVS	0	0

AF: amniotic fluid; CVS: chorionic villus sampling; chr: chromosome; MCC: maternal cell contamination; FBS: fetal blood sample; UI: uninformative markers.

1-1 Scientific definition of pregnancy

Pregnancy or pregnancy is a condition in which a woman has an embryo or fetus in her womb. Pregnancy is also called the "pregnancy period", which ends with the birth of the baby (delivery). It is noteworthy that the word embryo is assigned during the first 9 weeks after fertilization and the word embryo is assigned from the tenth week to the end of pregnancy. In humans, a normal pregnancy lasts about 38 weeks from fertilization. If the length of this period is calculated from the last menstrual period of a pregnant woman,

the normal amount will be approximately 40 weeks. The developing human sperm in the first weeks of pregnancy is called an embryo and after this period, until the end of pregnancy, it is called an embryo. Humans usually have only one fetus in the womb at a time of pregnancy, although multiple births are not uncommon. The World Health Organization sets a normal pregnancy time of 37 to 42 weeks. In scientific terms, the state of pregnancy is referred to as gravida and to the pregnant female is gravida. A woman who has never been pregnant is called a neoliberal and a woman who becomes pregnant for the first time is called a premiere, and in subsequent pregnancies it is called a multivariate. A woman who has never been pregnant or has not held a fetus for more than 20 weeks is called a neonate. These terms are used to describe a woman's previous pregnancies in her history and to record medical information during pregnancy or other circumstances. In many medical and legal definitions, pregnancy is divided into three parts (trimester). There is the highest risk of miscarriage in the first trimester or first trimester. During the second or second trimester, fetal growth can be assessed, and from the third or second trimester, the fetus can survive outside the uterus. The first trimester of pregnancy refers to the period before the 12th week of pregnancy. Most abortions, embryonic development, and organ building also occurred during this period.

1-1-1 Division of pregnancy

There are generally two types of pregnancy divisions: 1 -Division of the first type. The first 3 months of pregnancy: from the first week to the end of the 14th week of pregnancy. The second trimester of pregnancy: from the 15th week to the end of the 28th week of pregnancy. Third trimester of pregnancy: from week 29 until delivery.

2 -Division of the second type First half of pregnancy: 0-20 weeks.

Second half of pregnancy: 20-40 weeks.

1-2 Improper pregnancy

Improper pregnancy remains one of the leading causes of maternal mortality. However, because today, using modern diagnostic methods, it is possible to detect most miscarriages early. Current treatments are more conservative than past treatments. The main focus has shifted from emergency surgery to control hazardous bleeding to medical treatments aimed at avoiding surgery and preserving the anatomy of the reproductive system and maintaining fertility.

1-3 Epidemiology of abnormal pregnancy

The incidence of miscarriage is 1.5-2% of all pregnancies. The rate of miscarriage is higher in blacks and other minorities than in whites in all age groups. This type of pregnancy progressively increases with age in all races and is 3-4 times more likely in women aged 44 to 35 than women aged 15 to 24. In nulliparous women, pregnancies that occur after at least one year without contraception are 2.6 times more likely to be tubular. Additional risks in infertile women are associated with specific therapies. These include reversal of sterilization, tuboplasty, ovulation induction, and IVF. The possibility of tubular implantation predisposes to the hormonal changes that characterize ovulation-stimulating cycles with clomiphene citrate and gonadotropins. About 1.6-1.4% of pregnancies with ovulation induction are abnormal types. In many of these patients, the result of hysterosalpingography is normal and there is no sign of intraoperative tubular pathology. Ovarian hyperstimulation with high estrogen concentrations may play a role in tubal pregnancy. Other predisposing factors include placement of the embryo in the upper part of the uterine cavity, fluid reflux into the fallopian tube, and predisposing tubular factors that prevent the refluxed embryo from returning to the uterine cavity (1).

1-4 Types of miscarriages

The most common types of miscarriages are tubal pregnancies and unusual types include heterotopic pregnancies, abdominal pregnancies, ovarian pregnancies, interstitial pregnancies, cervical pregnancies, and cesarean sections.

1-4-1 Tubular pregnancy

1-4-1-1 Etiology and risk factors

Damage to the fallopian tubes is caused by inflammation, infection, or surgery. Inflammation and infection can cause damage without completely

blocking the tube. Tubal obstruction may be due to salpingitis, incomplete tubal closure, tubal sterilization surgery, incomplete salpingectomy, or congenital atresia of the middle tube. Damage to the mucous membrane of the fallopian tube or fimbriae is responsible for about half of all tubular pregnancies. Tubular diverticula may lead to abnormalities that trap or obstruct the blastocyst. Tubal pregnancy may occur in a blocked tube if the opposite tube is open. In general, 70% of abnormal pregnancies are in the ampulla region, 12% in the ischemic region, 11% in the fimbriae, and 2% in the cornea. Independent risk factors that always indicate the risk of tubal pregnancies include:

1. Previous PID fixed by laparoscopy. 2. Previous tubal pregnancy.

3. Current use of the IUD. 4. Previous tube surgery to treat infertility. 5. Previous abdominal surgery. 6. Sterilization. 7. Diethyl acetylbestrol. 8. Smoking.

1-4-2 Heterotopic pregnancy

Simultaneous pregnancies in two different areas mean implantation. The most common combination is an intrauterine pregnancy and an ectopic pregnancy, most of which are in the fallopian tube.

1-4-3 Abdominal pregnancy

Implantation in the peritoneal cavity, which is referred to as abnormal abdominal pregnancies, is so rare that its estimated incidence is about one case per 10,000 pregnancies and one case per 100 abnormal pregnancies. In this type of pregnancy, implantation usually occurs in areas such as the omentum, the lateral wall of the pelvis, the broad ligament, the cochlea, the spleen, the intestine, the liver, the diaphragm, and the cervix.

1-4-4 Ovarian pregnancy

About 3% of all miscarriages are due to ovarian pregnancy. Symptoms of this type of pregnancy is very similar to the more common types of symptoms in tubal pregnancies. Of course, it should be noted that ovarian pregnancy has specific diagnostic criteria that are mostly academic, which includes the following materials. The ipsilateral tube is intact and is clearly separated from the ovary. Occupation of the ovarian position by the pregnancy sac.

Ovarian ligament attachment of the pregnancy sac to the uterus.

Existence of ovarian tissue in the wall of the pregnancy sac. Treatment in almost all cases is surgery.

1-4-5 Intermediate pregnancy

A maximum of about 2% of abnormal tubular pregnancies are implanted in an interstitial segment within 1-2 cm of the uterine wall. Conventional treatment for interstitial pregnancy has been hysterectomy or corneal resection through laparotomy.

1-4-6 Cervical pregnancy

Cervical pregnancy is a rare type of miscarriage which is occurred in implantation in the duct and cervix. It is somewhat more common in people who become pregnant with ART. The probability of this type of pregnancy was 1 in 1000 pregnancies resulting from IVF. Cervical pregnancy is associated with vaginal bleeding without a door (its classic sign), enlargement (dilation) of the large and soft cervix, and the appearance of bloody or cyanotic, soft and large (hourglass cervix). Common treatments for pregnancy include curettage and hysterectomy, which are used to control bleeding if necessary. But other treatments are performed with the aim of minimizing this risk such as cervical cerclage, intravascular injection of vasopressin, transvaginal ligation of the cervical branches of the uterine artery. Similar methods such as intracervical balloon tamponade and bilateral ligation of the uterine artery or internal iliac artery have been used to control postoperative bleeding.

1-4-7 Pregnancy in cesarean section scar

It accounts for approximately 6% of all miscarriages in women with a history of cesarean section. These abnormal pregnancies are thought to be caused by embryo migration from a defect in a cesarean section scar. Its clinical manifestations are highly variable, ranging from vaginal bleeding with or without pain, to uterine rupture and hemorrhagic shock. The best treatment for it has not yet been determined, and therefore several treatment options such as vaginal resection, laparotomy or laparoscopy, topical injection

of potassium chloride, or treatment with systemic or topical methotrexate are used.

1-5 Diagnosis of miscarriage

Diagnosis of miscarriage is associated with many complexities and difficulties due to the wide range of clinical manifestations. The diagnosis and management of a ruptured fallopian tube is very clear. The primary goal is to achieve homeostasis. If abnormal pregnancy can be detected before rupture or irreparable damage to the fallopian tube, then future fertility optimization can be considered. By taking a history and physical examination, patients at risk can be identified, and the likelihood of being diagnosed with a miscarriage before a rupture can be increased (2).

1-6 Methods of assisted reproduction (artificial insemination)

Assisted reproduction methods include all methods that use direct manipulation of oocytes outside the body.

1-6-1 Laboratory method

In vitro fertilization is the first and most common form of auxiliary fertilization today. In standard IVF, 50,000 to 10,000 sperm are placed in a culture medium with an oocyte to fertilize, and then the resulting embryo is transferred to the uterus.

In vitro fertilization, or IVF, is a method in which egg cells are fertilized with sperm in vitro and one or more egg cells are obtained after several stages of "8-cell" or "embryo 5" cell division. "Fasting" is placed in the uterus to allow the fetus to grow normally. In this method, ovulation is first induced in the female and after a suitable number of eggs are obtained, they are cultured in the laboratory. After the eggs mature, they fertilize the egg with the right sperm. There are different methods depending on how the sperm causes the egg to fertilize. For example, if a certain number of sperm are inoculated into a culture medium containing an egg so that sperm with the right motility, shape, and physiology can fertilize the egg, this is called IVF. However, if the appropriate sperm is selected and inoculated into the egg using a microinjection device, this method is called intracytoplasmic injection of sperm (3). Artificial insemination was first performed in the world in 1978 in the United Kingdom by Dr. Robert Edwards, who won the Nobel Prize in Physiology or Medicine in 2010. Louise Brown, the child born of this fertilization, was born on July 25, 1978. Since then, about 5 million children have been born this way in the world.

1-6-2 Cytoplasmic method sperm

Intracytoplasmic injection of sperm, which is widely used today, is performed using sperm isolated from ejaculate or sperms obtained by microsurgical aspiration of sperm from the epididymis or removal of sperm from the testis.

1-6-3 Transfer of sperm and oocyte tubes or transfer of gametes into the fallopian tube

Another ART procedure is gamete intubation, which was introduced in 1984 as a form of IVF and has been a successful alternative to infertile couples with unknown cause or cervical or immunological causes, mild endometriosis, or a few cases of male fertility.

GIFT Placing the sperm and egg combination directly into the fallopian tubes consists of 3 steps:

1. Ovarian stimulation and monitoring: The initial steps of the main ART processes are the same. First, ovarian stimulation is used to create several eggs to increase your chances of successful fertilization. During ovarian stimulation, the ovarian response to hormonal drugs is monitored and egg formation is assessed.

2. Ovulation: In GIFT, eggs are often taken laparoscopically and sperm are prepared in a manner similar to IVF. Oocytes are examined under a microscope to determine their maturity before combining with sperm and transfer to the fallopian tube.

3. Gamete transfer: As soon as the doctor announces that the eggs are ready to transfer, the sperm and egg are placed together in a special catheter. The doctor inserts this catheter with a laparoscope and injects the gametes directly into the fallopian tube.

Thus, the process of fertilization takes place in vivo or in a laboratory setting under normal conditions, like a fertile woman. The developing embryos remain in the fallopian tube and then move into the uterus like a normal pregnancy for implantation.

1-6-4 Transfer of eggs from fertilization of sperm and oocytes or zygote into the fallopian tube

Transfer of eggs from fertilized sperm and oocytes or zygotes into the fallopian tube is another type of assisted reproduction procedure that uses direct manipulation of the oocyte outside the body.

1-6-5 Transfer of multicellular embryo to fallopian tube

In the last three methods, simultaneous laparoscopy is required for transmission. Also, in all ART assisted reproduction methods, the following steps are performed jointly:

Controlled ovarian stimulation is performed using gonadotropins, which follicular growth is monitored by vaginal ultrasound and serum estradiol levels are monitored simultaneously.Prevent LH surge untimely and therefore premature ovulation .Establishment of the final stage of oocyte maturation by hCG injection . Obtaining oocytes.

Fertility of eggs obtained by IVF or ICSI method .In vitro growth and culture of embryos .Supporting the total phase or preparing the endometrium using exogenous progesterone .Transfer the embryo into the uterus and freeze the extra embryos.Assessment of fertility status in the first trimester of fertility.

1-6-6 Folliculogenesis

In an unstimulated cycle, a number of follicles (8 to 9 follicles) in the luteal phase begin their pre-growth cycle. About the middle of the follicular phase of the next cycle, one follicle emerges from them and as the growth of this follicle continues, the growth and development of other follicles in this selected cohort stops. Follicular growth in a non-stimulated cycle with hormonal feedback causes LH surge in the middle of the cycle, which plays a very important role in completing ovarian maturation and ovulation. Progesterone secretion begins before ovulation and increases markedly after ovulation. The secretion of estradiol in the follicular phase also causes the endometrial epithelium to grow and proliferate. The secretion and presence of progesterone is critical for the development of endometrial maturation and stroma to provide a suitable site for implantation in the middle of the secretory phase. If successful implantation occurs, stimulation of the hCG secreted on the corpus luteum will continue to secrete progesterone until the placenta can completely replace it at 8 to 10 weeks of gestation and the placenta will continue to secrete progesterone completely. It should be noted that the first IVF delivery was from an oocyte obtained during a normal, unstimulated menstrual cycle. IVF is still possible with a normal cycle and may also be performed in some elderly patients with low ovarian reserve who have previously failed ovulation-stimulating cycles or who are unable to stimulate ovulation due to complex medical conditions. However, in this method, the rate of non-completion of the cycle is high (25 to 75%, especially due to unplanned ovulation, which eliminates the chance of ovulation). Even if oocyte recycling and fertilization are successful, there will be only one embryo in natural cycles and, of course, there will be little chance of nesting. In these cycles, endogenous HL serum levels should be monitored frequently to prevent premature serum LH and premature release of oocytes. Ovulation is then performed 36 to 40 hours later. GnRH antagonists can also be used to prevent untimely serum LH.

1-6-7 Mild ovarian stimulation

Very mild stimulation with clomiphene citrate from Cycle Syndrome can be done for 5 to 8 days.

In this method, compared to the normal cycle, the rate of cycle cancellation is somewhat less and the number of oocytes obtained and embryos transferred and the rate of pregnancy is higher.

As with the normal cycle, GnRH antagonists are used to prevent untimely serum LH and hCG is used for the final maturation of oocytes. Alternatively, intermittent stimulation of clomiphene citrate and gonadotropin can be used (albeit in small amounts).

It has been shown that stimulation of follicular development is more successful than the use of clomiphene citrate alone. Intermittent use of clomiphene and exogenous gonadotropin in patients with a previous poor response to ovarian stimulation due to stimulation of the endothalamic-pituitary-ovarian endogenous system by clomiphene is a treatment protocol.

1-7 Abortion

Abortion before the start of the 22nd week of pregnancy is called an abortion. The most important sign of abortion is bleeding. If this happens after the first trimester of pregnancy, it is called a late abortion.

1-7-1 Definition of recurrent spontaneous abortions

The occurrence of at least three miscarriages in the first trimester of pregnancy is called recurrent miscarriage. It is defined as a miscarriage more than two or three times before 24 weeks of gestation. By most definitions, it is a fertility defect in 1-5% of patients who experience it. This defect is undoubtedly of multifactorial origin (4). The frequency of these abortions is said to depend on its definition, so its prevalence is estimated at 1-3%. In case of abortions that have been repeated twice without a previous successful pregnancy history. This rate increases. In general, 10-15% of pregnancies end in abortion, although this phenomenon is very rare, it is a very disappointing experience for patients and doctors because usually there is no definite reason and reliable treatment for such abortions. In general, abortion can be considered a natural way to select children with healthy genomes. In fact, after a study by Beau et al. And Hasold et al. On seminal fluid, it was accepted that 50% of abortions originated from chromosomal abnormalities. Also, cytogenetic studies of embryos created by in vitro fertilization (IVF) have shown that only 50% of seemingly normal embryos are chromosomally normal. The risk of developing fetal chromosomal abnormalities gradually decreases during pregnancy until it reaches 1% in newborns. Following this process (until fertility) reveals that most miscarriages occur in the very early stages of pregnancy.

1-7-2 Factors affecting recurrent miscarriage

One of the effective factors in abortion is the age of the mother. Aging reduces the function of the ovaries and the number of healthy eggs and the production of embryos with chromosomal defects (5). The indicator of this correlation is trisomy of chromosome 21 as well as the results of cytogenetic studies of embryos before transfer to the mother. Another factor is the results of previous pregnancies. The risk of miscarriage for young mothers who have no previous history but whose previous pregnancies have been successful is about 30 percent; if all previous pregnancies have failed, it will rise to 50 percent.

1-7-3 -Etiology of recurrent spontaneous abortion

In general, four reasons for spontaneous abortion are given:

1. (Infection (1%)

1. Chromosomal abnormalities (7-50%)

3. Hormonal abnormalities (5-20%)

4. Anatomical abnormalities (5-10%)

But overall, in 80% of cases, immunological abnormalities (autoimmune disorders) and other immunoassays (alloimmune disorders) are involved (6).

1-8 -Genetic factors

1-8-1 -Anoploidy

The most common chromosomal abnormalities are autosomal, polyploid and monosomal X chromosome trisomies. In most trisomies, the effect of maternal age is seen. Anoploids contain most of chromosomes 16 and 18. Triploidy and tetraploidy are the cause of 30% of spontaneous abortions due to chromosomal abnormalities.

Triploid embryos formed by double sperm fertilization usually have the genetic formula "XXX and 69" or "XXY and 69", and tetraploid embryos usually last less than 4 or 5 weeks. Monosomal chromosome (X) is the most common chromosomal abnormality, accounting for 15-20% of all miscarriages. In general, the frequency of chromosomal abnormalities is lower in women under 36 years of age.

1-8-1-1 -Trisomy 21

Down syndrome or trisomy 21 is the most common and well-known chromosomal disorder to date and is by far the most common genetic cause of moderate mental retardation. About 1 in every 800 babies born has Down syndrome, and the incidence is much higher

among live births of mothers aged 35 and older. Two notable features of the population distribution of this disease are noteworthy: increasing maternal age and its specific distribution within families. In 1959, it was discovered that people with Down syndrome have 47 chromosomes, and the extra member is a small acrocentric chromosome, hereinafter referred to as chromosome 21.

Down syndrome can be diagnosed at birth or shortly thereafter by dysmorphic features (varying among patients) that produce distinct phenotypes. In about 95% of all patients, Down syndrome is caused by trisomy on chromosome 21, which results from the meiosis of chromosome 21 pairs not being separated. As mentioned earlier, the risk of having a child with trisomy 21 increases with increasing maternal age, especially in those over 30 years of age. The meiotic error responsible for causing trisomy 21 usually occurs during our meiosis (about 90% of cases), and mostly in meiosis I, but about 10% of cases of the disease occur in paternal meiosis, usually in paternal meiosis II.

1-8-1-2 -Trisomy 18

Trisomy 18 features always include mental retardation and growth retardation and often include severe heart deformity. The incidence of this condition in live infants is about 1 in 7,500 births. The incidence of trisomy 18 is much higher at fertilization, but 95% of all pregnancy products with trisomy 18 miscarry spontaneously. Postnatal survival is also low, and survival of more than a few months is rare. At least 60% of patients are female, which is probably due to their preferential survival.

1-8-1-3 -Trisomy 13

The incidence of trisomy 13 is about 1 in every 15,000 to 20,000 births. Trisomy 13 is clinically severe, with about half of patients dying within the first month of life. Like many other trisomies, these patients are associated with the older age of the mother, and the extra chromosome is usually caused by the phenomenon of segregation in maternal meiosis I. Determining the karyotype of infected infants or fetuses is required for clinical confirmation. About 20% of cases result from an unbalanced displacement (7).

1-8-2 -Abnormalities in chromosome structure

The most common change in chromosome structure is displacement. Cytogenetic studies show that the frequency of this abnormality is 3-5% in sick couples and almost twice as high in women as in men. Abortion and fetal abnormalities depend on the size, location and type of change in chromosome structure. When a couple has these types of abnormalities, their fetus is 5 to 5 percent more likely to be infected (8).

1-8-3 -Multigenic factors

Single or multifactorial factors influencing the reproductive process (which are rarely detected) can cause miscarriage. For example, asymmetric deactivation of the X chromosome, which results from 90% deactivation of one parent-specific allele, is more common in mothers with spontaneous abortions.

1-8-4 -Anatomical abnormalities of the uterus

Undoubtedly, anatomical abnormalities of the uterus are one of the factors influencing abortion (in the first trimester). Evidence has been shown that surgery (to correct these disorders) is usually of little success.

1-8-5 -Environmental factors and life habits

Consumption of 5 or more units of alcohol per week and 375 mg or more of caffeine per day during pregnancy increases the rate of miscarriage. Also, there is a weak link between smoking and miscarriage. Heavy metals (such as lead and mercury), organic solvents, and radioactive ionizing radiation, which are considered environmental teratogens, increase the rate of miscarriage. Couples' jobs, environmental pollution, and smoking can reduce sperm quality and cause miscarriage. Couples' jobs, environmental pollution, and smoking can reduce sperm quality and miscarriage early in pregnancy (9).

1-8-6 -Hormonal abnormalities

Maternal hormonal disorders (diabetes and thyroid failure) are effective in causing miscarriage. High maternal hemoglobin levels in the first trimester of pregnancy increase the risk of miscarriage (10). Controlled diabetes does not increase the risk of miscarriage (11), but thyroid disorders increase the risk of miscarriage (12). Reports of progesterone deficiency

have been implicated in abortion. Some researchers believe that progesterone deficiency may, in some cases - not always - be found to be beneficial.

In human placental gonadotropin deficiency, administration of this substance is not always beneficial(13).

Polycystic ovary syndrome (PCOS) is associated with miscarriage. The hallmark of this disease is excessive secretion of LH hormone, which is considered as one of the causes of abortion, but reducing LH secretion (by inhibiting the pituitary gland) has little effect on preventing abortion (8)).

In 25-30% of women with RSA, there is a history of delay of more than 12 months before pregnancy, the most common cause of which is an abnormality in the ovary (10).

Hyperprolactinemia has been reported in some sources as a factor in increasing abortion, but there are insufficient reasons for this (14).

1-8-7 -Immunological abnormalities

When ionological abnormalities cause abortion, the probability of a successful pregnancy of the mother from three abortions is 30%; this probability decreases to 25% after 4 miscarriages and to 5% after 5 miscarriages (15) (Figure 1).

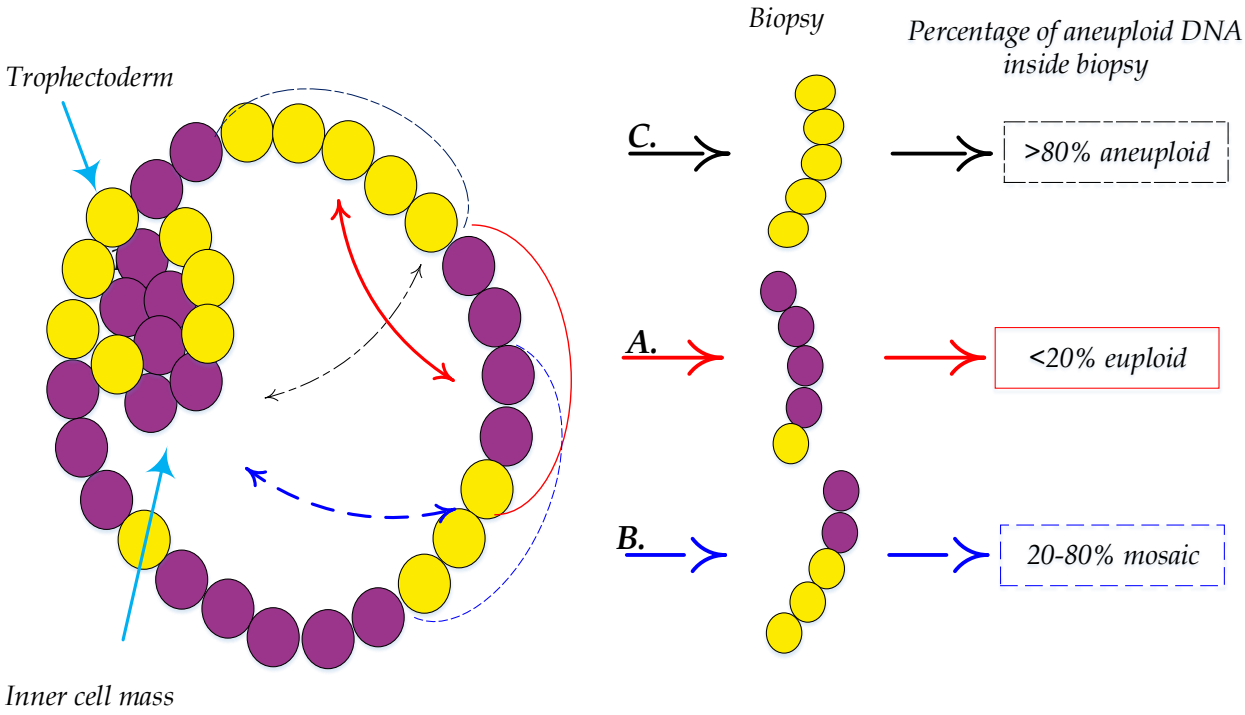

Figure 1 . Percentage of aneuploidy DNA inside biopsy for diagnosis.

1-9 -Polymerase chain reaction

Polymerase chain reaction is a laboratory technique that allows the amplification of a specific piece of DNA that exists between two known sequences. DNA amplification occurs after the primer is attached to the end of specific sequences of the template DNA. The polymerase chain reaction technique was developed in 1985 to determine the sequence of DNA strands by Kerry Mollis. Kerry Mollis was awarded the Nobel Prize in 1993 for his invention of PCR (Figure 2).

In this technique, DNA complement strands are synthesized using dNTPs in the presence of the polymerase enzyme. The resulting two strands of DNA can be separated by heat, and then the temperature must be adjusted to allow the primers to adhere. DNA elongation is performed using DNA polymerase at the optimum temperature for enzyme activity. Repeating the steps of opening two DNA strands, attaching primers, and lengthening is called a PCR cycle. Each newly made DNA strand is used in the next cycle as a new pattern strand, and the target DNA fragment is

made from it. The first cycle of PCR is performed on the initial pattern and will continue as long as the polymerase enzyme is active or until the start of the next cycle. In the second PCR cycle, the strands are made of a certain length that is limited to two primers. From the fourth cycle onwards, the DNA sequence is amplified exponentially, so the number of final copies of the target sequence is defined by the formula (2n-2n) X. In this formula, n is the number of bicycles and 2n is the initial product of the first and second cycles, which have certain lengths, and X is the number of copies of the original pattern. If we assume that the work of this PCR is 100%, the DNA of the template will be multiplied 220 times. It should be noted that the performance of PCR varies depending on the type of template DNA and the optimization conditions. The target sequence in PCR also includes two-end primers. Exponential amplification of target DNA does not occur indefinitely, and factors prevent maximizing efficiency in each cycle. The effect of these factors is greater in the end cycles. For example, the Baghdad DNA sequence is amplified 106 times from 25-30 cycles of PCR, and the ratio of enzyme to DNA decreases due to the increase in the DNA molarity of the template. The activity of enzymes is also reduced by thermal decomposition. On the other hand, the high concentration of pattern filaments causes the filaments to stick together and compete with the primer adhesion.

Two factors have contributed to the development of PCR:

A) The use of DNA polymerase stable enzymes at high temperatures

B) The use of devices that automatically generate temperature cycles.

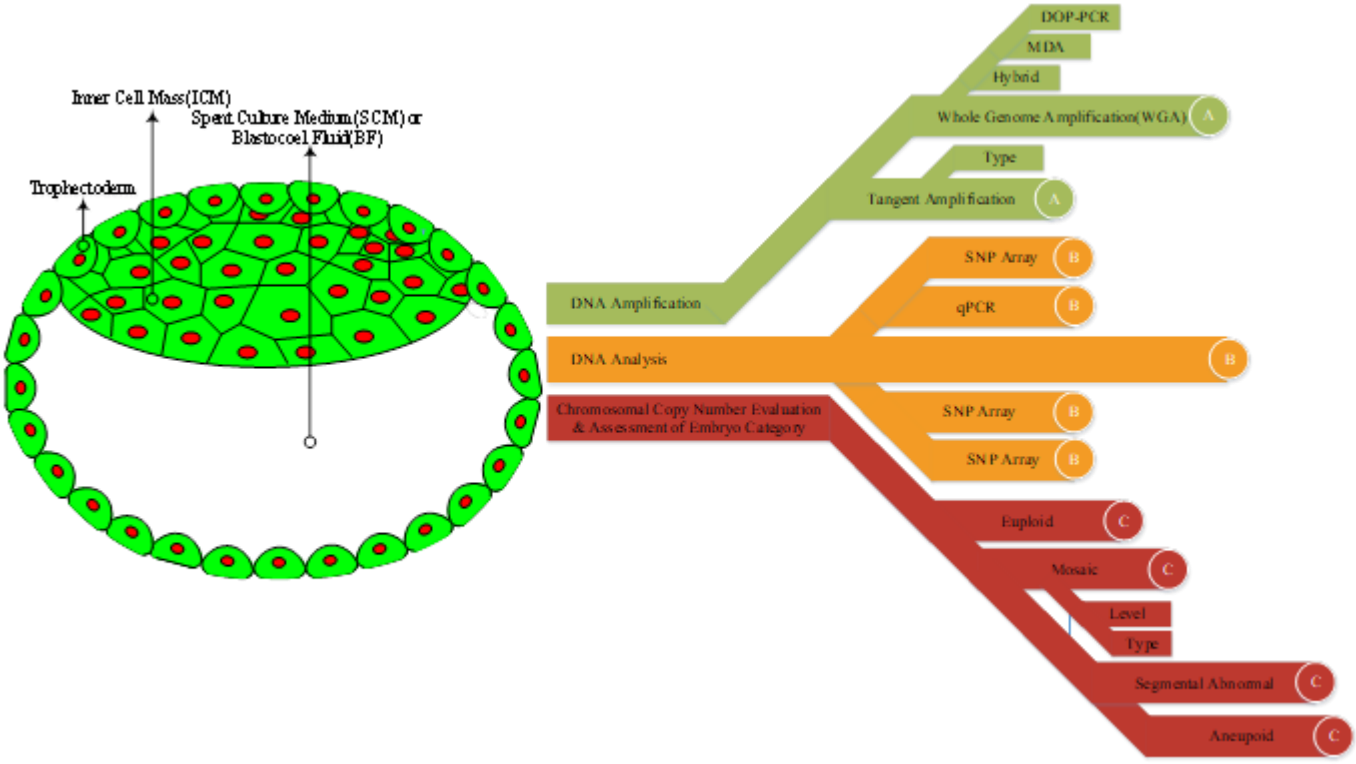

Figure 2. DNA amplification, DNA analysis alongside with chromosomal copy number evaluation and assessment of embryo category in some types of PCR for diagnosis.

1-9-1 -Factors affecting the success of PCR

1-9-1-1- Incubation of two strands of DNA

Separation of two DNA strands takes place at a temperature of 92-100 ° C. Rising temperatures cause DNA damage. This affects the reproducibility of the results. Therefore, high temperatures should be avoided. PCR is typically used to separate two strands of DNA at temperatures between 95-92 ° C, but the nucleation temperature must be based on different DNA patterns (if the target DNA is in the

heterochromatin region to separate DNA strands require higher temperatures).

1-9-1-2 -Sticking primers

Calculating the adhesion temperature of primers is a starting point and an important factor in PCR optimization. If a single strand of DNA is slowly cooled, the strands that complement each other in terms of the play sequence are connected in a specific way. In the PCR process, after priming the template DNA, each primer must be optimized practically by repeating the experiments.

1-9-1-3 -Elongation of DNA strand

Supplemental strand elongation begins with 'OH3' at 72 ° C, which is the best temperature for Taq DNA polymerase. The development of new strands depends on the activity of the DNA polymerase enzyme. This enzyme was first extracted from the bacterium Termus aquaticus belonging to the hot spring in Yellowstone, which simplified and automated PCR. This enzyme is most active at 72 ° C.

If the amplified DNA fragment is large, the amplification time can be increased. But in most cases, two minutes is enough. Factors such as divalent cations (such as Mg2 +) are very effective in the activity of this enzyme. The PCR process creates a new copy of each DNA strand molecule, which is actually the target region. Each new version can be rewritten and produced in a similar cycle.

1-9-1-4 -Number of cycles

The number of cycles is usually considered to be between 25-35 and increases with. Also, due to the decrease in enzyme activity, the number of cycles is not selected more than 40 cycles.

1-9-2 -Types of PCR techniques

1-9-2-1 -ARMS technique

The shaky mutation amplification system, or ARMS, is a simple and rapid way to detect point mutations, restricted fragment length polymorphisms (RFLPs), small deletions or additions in the DNA molecule sequence. In this method, the reaction is performed in two separate tubes, one of which contains mutated type primers and the other contains normal type primers. If amplification occurs in a tube containing a mutated primer, mutation has occurred in the target DNA, and amplification in a tube containing a normal primer indicates that no mutation has occurred (16).

1-9-2-2 -Nested-PCR technique

In this method, two pairs of primers are used to increase the sensitivity of PCR. First, with a pair of first primers over 15-30 cycles, specific pieces of target DNA are amplified. Then the resulting PCR product is transferred to another tube and used as a template and is performed by the second pair of primers in the second stage of PCR (17).

1-9-2-3 -RT-PCR technique

The primary pattern in RT-PCR is a single-stranded RNA molecule. Since DNA polymerase is not able to use RNA as a template, another step has been added to PCR. During this stage, using the reverse transcriptase enzyme, from the RNA pattern, its complement CDNA is synthesized and amplified by PCR technique (18).

1-9-2-4 -Multiplex-PCR technique

In this method, several pairs of specific primers are used for different purposes. In clinical microbiology, using this method, it is possible to identify several disease agents in a sample at the same time and to diagnose mixed infections. It is a type of PCR reaction in which two or more sites of the target sequence are amplified simultaneously in a PCR reaction. Separate primers are designed for each target DNA fragment. The PCR product contains a mixture of parts of different lengths that can be differentiated by electrophoresis on agarose gel (19).

1-9-2-5 -QF-PCR technique

Quantitative fluorescent polymerase chain reaction has been used in diagnostics in the UK for over 10 years and has proven to be a fast, cost-effective, robust and accurate prenatal test for common aneuploidies. Its specific benefits include the diagnosis of triploidy, mosaicism of the stem cell infection.

QF-PCR can be used to detect copying of chromosomal numbers by incremental duplicate sequences at polymorphic sites. These duplicate sequences are

amplified by PCR and the labeled products are separated by gel electrophoresis.

QF-PCR was introduced to the UK National Health Service as a valid diagnostic test in 2000 (20) and then to other UK genetic centers (21) and privately in the UK and Europe. Introduced (21). Primer pairs for polymorphic loci (markers) together led to a rapid, efficient, and inexpensive diagnostic test for trisomy 13, 18, and 21 aneuploidies and sex and triploid chromosomes, which are now available in commercial kits from a number of different companies. Sometimes it is not possible to spend this time for various reasons, such as the anxiety of parents, especially the pregnant mother, or the lack of time to receive results before the end of the legal opportunity to terminate the pregnancy. If one of the families wants to get results faster due to high anxiety and also the risk that threatens the pregnancy, one of the most common chromosomal abnormalities - including aneuploidy (number abnormalities) of chromosomes 13, 18, 21, X or Y - has been specifically identified. QF-PCR technique can be used to accelerate the presentation of results (Figure 3). In this case, the anxiety caused by long-term waiting will be reduced. Due to the high sensitivity and specificity of this test and its relative equality of accuracy with chromosomal culture for the diagnosis of common anoploids, the answer to the family is provided with high confidence.

Also, in cases where the result of screening or ultrasound clearly raises the risk of a number of abnormalities on one of these chromosomes and the gestational age is more than 17 weeks, there is enough time to obtain a termination permit after the chromosomal culture results are ready. will not be. It should be noted that the termination permit of the infected fetus is issued only until the end of the 18th week of pregnancy (18 weeks and 6 days). In such cases, the QF-PCR technique will be helpful.

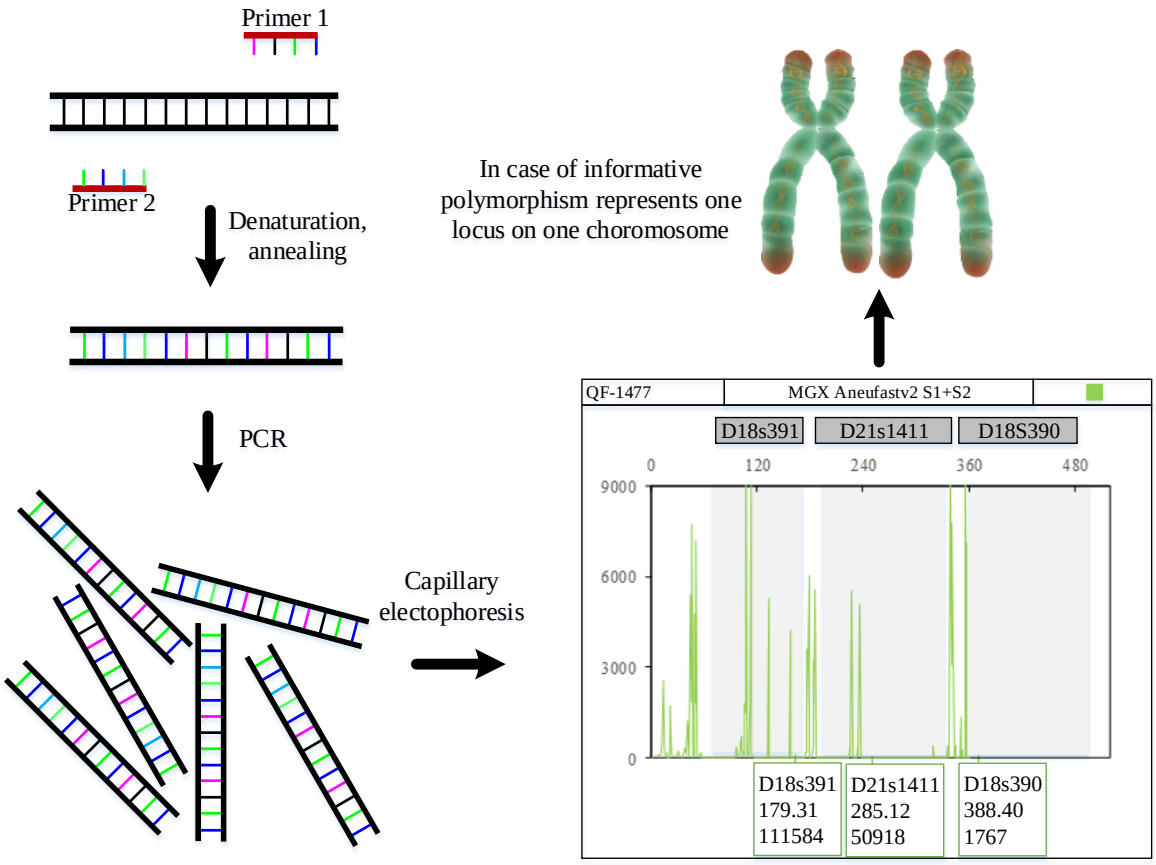

Figure 3. Position and arrangement primer, denaturation, annealing duration PCR function.

1-9-2-5-1 -QF-PCR as an independent test

Performing QF-PCR as a rapid initial test for aneuploidies raises questions about the benefits of this program with complete karyotype analysis that shows no signs of non-trisomy disorders. This was first possible in 2001 (22) Has been proposed and has since been widely considered with a number of retrospective reviews published. (23); (24). These reviews generally organized the karyotype test results form for prenatal samples and the number and nature of abnormal results that could not be detected by QF-PCR alone. Overall, this review suggests that the clinical significance of the prevalence of non-trisomic chromosome disorders in women at risk of trisomy is about 0.07%, close to the prevalence in the general population (25, 26).

However, with the higher resolution testing currently available, the prevalence of detectable non-trisomic disorders will be significantly higher in the general population.

Since then, two models for performing QF-PCR have been introduced as an independent test.

Model 1: Implemented by the Karolinska Institute in Stockholm, which gives women who were not at increased risk for non-trisomic chromosomal abnormalities the right to choose a rapid test for trisomies or a complete chromosome analysis, but not both.

Model 2: Includes QF-PCR testing of whole pregnancy specimens, regardless of referral, but sets low criteria for complete karyotyping of a subset of specimens. Introduced in the UK, the model is funded by the London Commission. With the imminent introduction of highly sensitive non-invasive screening for Down syndrome, the number of women who will have invasive testing will be reduced. For those at high risk for Down syndrome, invasive testing to confirm screening results will be a pre-term requirement, and QF-PCR should be considered as the method of choice for this rapid confirmation. Women at low risk for noninvasive screening will be identified later for invasive testing for fetal abnormalities in ultrasound screening.

For these women, an initial QF-PCR test, in the absence of a trisomy, must first perform a very expensive whole genome test, whether G-chromosome analysis or comparative genome array hybridization.

Therefore, QF-PCR will continue to play a key role in prenatal diagnosis in the future.

Conclusion

The benefits of QF-PCR are greater than other rapid aneuploidy diagnostic approaches. An important point is the significant difference in strategy and performance between other molecularly based methods for their inability to detect triploids. For triploid specimens, chromosome comparison analysis by MLPA, BAC, and comparison of genomic array hybrids may result in a natural diploid or MCC. Another important clinical advantage of QF-PCR is the ability to identify other cell lines at 10% MCC levels in female embryo samples and 20% (mosaicism) (26). It has been proven that despite all types of samples, this service is an affordable service and can receive results 6 hours after receiving the sample.

Author contribution

KB, MA, AAS wrote the manuscript, revised and conducted this study. All authors read the final edited version of the manuscript.

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Ethical Considerations

There are no ethical problems for this present review article.

Conflict of interest

The authors declare no potential conflicts of interest.

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