

## Reproductive Genetic Counseling in Genomic Era

**Ashutosh Halder\***

*Department of Reproductive Biology, AIIMS, New Delhi, India*

**\*Corresponding Author:** Dr. Ashutosh Halder, Professor, Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi 110029, India.

**Received:** August 08, 2015; **Published:** October 09, 2015

### Abstract

Reproductive genetics counseling is an important branch of medicine as it involves in preventing as well as predicting a disorder in a family. It is becoming more important now due to advancement of genomic medicine. Advances in genomic technologies created opportunity to predict, prevent and diagnose precisely more and more disorders, often noninvasive as well as rapid way. This review on reproductive genetic counseling in genomic era will describe briefly reproductive genetic counseling in infertility practice and obstetric practice. Due attention will be given to newer technologies with its potential in predictive, preventive and precision medicine. This review will analyze how to overcome various difficulties, dangers of transmitting disorders to offspring and how to prevent transmissions. Review will also highlight on conditions those can be predictable, preventable or early diagnosis possible using high throughput genomic platforms viz., next generation sequencing, DNA microarray, global methylation array, etc besides information on genesis, technologies and counseling.

**Keywords:** *Reproductive Genetic; Genomic Era; Genetic counseling; In vitro fertilization*

### Introduction

The branch of reproductive genetic counselling has become increasingly important in the practice of medicine due to advancement of genomic medicine and genomic technologies. Newer genomic technologies can be incorporated in practice of reproductive medicine for precision diagnosis, prevention as well as prediction. Genetics has a role in virtually every disorder, hence, every discipline of Medical Sciences, in causation, predisposition, susceptibility, modulation & treatment response. It is estimated that each of us carries at least 20 harmful genes [1]. About 1 in 13 conception results in a conceptus with a chromosomal abnormality [2], whereas about 50% of first trimester abortions are associated with chromosomal defects [3]. Approximately 0.2% baby born with balanced structural chromosome rearrangement and these have implications on reproduction later in life [1]. Between 5.6% and 11.5% of stillbirths and neonatal deaths are associated with chromosome defects [4]. Three to four percent of all births are associated with a major congenital malformation or genetic disorders, a rate that doubles by 8 years of age, given consideration of late appearing/diagnosed genetic disorders [5]. Genetics also contributes infertility in approximately 15% and anticipated responsible for most of idiopathic cases, which is over 50%. This statistics indicates that no scientific field has had more genetic impact on the practice than reproductive process.

An understanding of genetics is important for all reproductive specialists (obstetrics, gynaecology, andrology, reproductive endocrinology & reproductive genetics), particularly in molecular and precision medicine era on the background of immersing high throughput genomic/omics technologies. It is also important to know reproductive genetic counselling, in particular knowledge on risk and how to prevent genetic disorders in offspring. This is essential, as this will protect reproductive specialist from medico-legal consequences due to failure of prevention of genetic disorders in offspring. As scientific knowledge and medicine advance so do the expectations of the public. Advances in molecular biology/technology (next generation sequencing and microarray), introduction of non-invasive prenatal screening/testing (NIPT), preimplantation genetic screening/diagnosis, etc has helped both drive and meet public expectations. Furthermore,

**Citation:** Ashutosh Halder. "Reproductive Genetic Counseling in Genomic Era". *EC Gynaecology* 2.1 (2015): 132-148.

rapid dissemination of genetic information, especially through electronic media, has effects in daily reproductive care. Genetic testing is now commonplace in all specialties. Genetic counselling is progressively becoming an integral part of reproductive medicine practice [6]. Historically couples at risk were given information regarding their reproductive chances of producing an affected offspring. Such couple then had the option of either taking the chance or not reproducing at all. The advent of preimplantation & prenatal diagnosis has allowed these couple the option of having unaffected offspring and this has immensely increased the scope of genetic counselling in reproductive practice especially in men with previous infertility and requiring assisted reproductive technologies (ART) in particular *in vitro* fertilization (IVF) and intra cytoplasmic sperm injection (ICSI) as these may increase transmission of genetic disorders in offspring (Yq micro deletion, chromosomal abnormality, cystic fibrosis, unbalanced translocation, pathogenic copy number variations/CNVs, etc) or couples with carrier state with recessive monogenic disorder or affected person with dominant monogenic disorders. Moreover, progress has been made in population screening test both to identify couple at increased risk for having offspring affected with genetic disorder as well as to screen for abnormal fetuses. This has further extended the need for genetic counselling to every pregnant woman. Reproductive genetic counselling is also important to prevent future risk on medico legal problems (law suits) between reproductive specialist and patient. Although it is impossible to know all aspects of genetics, basic knowledge of certain topics in particular genetic counselling to prevent birth of an abnormal baby, male child with infertility and its medico-legal consequence due to failure of prevention or prediction is must for all reproductive specialists in coming years. Furthermore, we are gradually experiencing newer group of disorders as genomic disorders where multiple genes across genome are involved as with multiple malformations. This review will discuss the genetic diseases in brief along with indications, utility and importance of genetic counselling as well as potential risks, if not properly done in areas of reproductive medicine practice.

### Genetic Disorders

Genetic disorders could be monogenic (Mendelian inheritance), polygenic multi-factorial (many genes interacting with environmental factors), chromosomal (including microdeletion/duplications), mitochondrial, genomic disorders (copy number variations) and epigenomic disorders. Monogenic disorders could be autosomal or sex linked and expression may be dominant or recessive. Autosomal dominant monogenic disorder is caused by single mutated gene. Expressivity and severity of the condition vary within families e.g. neurofibromatosis. A carrier/patient of a dominant gene has a 50% chance of transmitting the mutated gene to offspring. Both sexes are equally at risk. Isolated case in a family may represent new mutation. Autosomal recessive monogenic disorder is caused by two mutated genes on two homologous chromosomes. Carriers of only one mutated gene (heterozygote) are usually asymptomatic. When both parents are carriers then there is 1 in 4 (25%) chance of having an affected child. Both sexes are equally at risk. Carriers are usually identified after birth of an affected child, family history of the condition or population screening. X linked dominant monogenic disorder is caused by single mutated gene located on X chromosome. Males and females both affected, however, male more severely affected than female and some males do not survive. Offspring of either sex have 50% chance of inheriting the disorder from affected females. The situation is different for affected males whose daughters will always inherit the gene and sons will never inherit. X linked recessive monogenic disorder is caused by single mutated gene located on X chromosome. Males are affected and females are usually carriers (asymptomatic) or mildly symptomatic. Female carriers have 50% chance of transmitting the mutated gene to their offspring. Half of the sons will be affected and half of daughters will be carriers.

Multi-factorial polygenic disorders are caused by an interaction of many genes and many exogenous (environmental) factors. The inheritance pattern is complex and risk to the relatives is less than monogenic disorders. Each of this category disease has different risk to relative and hence requirement for genetic counseling. Common examples are most birth defects, hypertension, diabetes mellitus, etc.

Chromosomal disorders are due to loss/gain (numerical) or abnormal arrangement (structural, including microdeletion/microduplication, translocation, inversion, ring, isochromosome, etc) of one or more chromosomes (whole or part), producing excessive or deficient genetic material. Normal chromosome complement in human is 46 (46, XX in female and 46, XY in male) and known as diploid state, while one set of 23 chromosomes seen in gametes (23, X in female or 23, Y and 23, X in male) is known as haploid. Error in segregation of chromosomes during cell division leads to gain or loss of chromosome and known as aneuploidy. It includes missing of a member of the pair (monosomy) or presence of more than two chromosomes in a pair (trisomy for 3 numbers, tetrasomy for 4 numbers, etc).

Errors in mitosis result in mosaicism, i.e. two types of cells originated from single cell. Rarely, admixture of 2 or more zygote results in chimerism, i.e., two or more types of cells originated from two or more zygote in early embryonic life. Some common numerical chromosomal disorders are trisomy 21 (Down syndrome), trisomy 18 (Edward syndrome), trisomy 13 (Patau syndrome), monosomy X (Turner syndrome), 47, XXY (Klinefelter syndrome), etc. Microdeletion/microduplication are characterized by small (< 5 Mb) chromosomal deletions/duplications in which one or more genes are involved. They are frequently associated with multiple congenital anomalies or neuropsychiatric disease. The phenotype is the result of haplo-insufficiency/over-expression of genes in the critical interval. The examples of microdeletion syndromes are velocardiofacial syndrome (22q11.2), Prader-Willi syndrome/Angelman syndrome (15q11-13), Williams's syndrome (7q11.23), etc. The examples of micro duplication syndromes are Charcot-Marie-tooth neuropathy 1A (17p12), Alzheimer disease (21q21.3), spino-cerebellar ataxia type 20 (11q12), Pelizaeus-Merzbacher disease (Xq21-22), and Parkinson disease (4q21), etc. In translocation a portion of chromosome is transferred to another chromosome. There are two main types of translocations, i.e. reciprocal and Robertsonian. In reciprocal translocation, segments from two (or more) different chromosomes are exchanged. In Robertsonian translocation, the long arm of one acrocentric chromosome is translocated to the long arm of another acrocentric chromosome with loss of short arms of both the chromosomes, resulting in one chromosome, derived from two chromosomes. Translocations can be balanced (no gain or loss of genetic material) or unbalanced. In inversion a portion of the chromosome breaks, flips around and rejoins, resulting in change of orientation (physical position) of the genetic material whereas in ring chromosomal ends break (near the tip) and rejoin each other to form a ring. Isochromosome is an abnormal chromosome with two identical arms, i.e. either two short (p), or two long (q) arms. It is formed by faulty chromatid separation during segregation. Other rare chromosomal abnormalities are chromosomal breakage/instability/fragility, etc. Normally, each member of the pair of chromosomes is derived from each parent. Sometimes, both members of chromosome pair (part or whole) may be derived from one parent (uniparental). Occasionally the entire set of chromosomes may derive from one parent as in molar pregnancy (complete mole where all 46 chromosomes come from father). Sometimes an individual may have an additional set of haploid (triploidy; 69) or diploid (tetraploidy; 92) chromosomes.

Mitochondrial disorders are a wide variety of genetic diseases found to be associated with dysfunction in mitochondrial DNA (mtDNA). Mitochondrial disorders can be caused by a mutation in the nuclear DNA regulating mitochondrial DNA, by a mutation in the mtDNA or by yet an unknown genetic mechanism. These diseases affect eye, brain, muscle, liver and sperms predominantly. These defects can either be inherited from mother (sperm do not contribute mitochondrial DNA at fertilization) or acquired through somatic mutation with age. Males do not transmit the disease and all offspring of affected mother will have the disease however in variable forms and difficult to predict severity in offspring until recently following development of next generation sequencing (NGS) platforms and whole mtDNA sequencing.

Genomic disorders are a group of diseases that result from genomic rearrangements, such as insertions, deletions, duplications, inversions, etc. Human genome is a highly dynamic structure. Approximately 5% (~800 genes) of the human genome is structurally variable in the normal population. Genome architecture makes the genome susceptible to rearrangements through recombination mechanisms. Non-allelic recombination between low-copy repeats (LCRs) results in loss or gain of genomic segments. LCR DNA spans 10-400 kb, shares ~97% sequence homology and provides the substrate for recombination, thus predisposing to rearrangements. The mechanisms by which rearrangements contribute to various phenotypes (such as diversity, traits, susceptibility, behavior or disease like microdeletion/duplication syndromes, schizophrenia, autism, etc) are diverse and include gene dosage alterations, gene disruption, gene fusion, position effects, mutations, etc. Rearrangements introduce variation into our genome and serve as evolutionary function in addition to diseases, including cancer.

Epigenetic is the study of changes other than DNA sequence that affect gene expression, including the methylation of DNA and modifications to DNA-binding proteins. Epigenetic mechanisms include packaging of genome and gene activation ability. Epigenetic changes can be inherited across cell divisions or across generations and can have a profound effect on an individual's phenotype including cancer.

### Reproductive Genetic Counseling

Reproductive genetic counseling is a communication process concerning the occurrence and the risks of recurrence of reproductive genetic disorders within a family. This aims to provide the patient with clear and comprehensive understanding of all the important implications and possible options of the disorder; to facilitate rational decision [5]. Decision should come from counselee and not from counselor. This preferably should be non-directive & non-biased manner. Genetic counseling aims to help the individual or family as follows:

1. Comprehend the medical facts including diagnosis, cause, probable course of disorder, available management including precision, prediction & prevention in the family
2. Appreciate the way heredity contributes to the disorder and the risk of recurrence in relatives
3. Understand the options for dealing with the risk of recurrence
4. Choose the course of action, which seems appropriate to them in view of their risk & family goals and act in accordance with that decision
5. Make the possible ways of adjustment with the disorder in an affected family member and/or to the risk of recurrence of that disorder in the family

### Genetic Counseling in Infertility Practice

#### Genetics of infertility

The infertility is defined by the absence of conception after 12 months of regular unprotected intercourse. Infertility affects approximately 10% of couples of reproductive age [7]. Genetic abnormalities are thought to account for 15–30% of male and 10-15% of female infertility, including chromosome aberrations and single gene mutations. Genetic variability and epigenetic factors affect reproduction and fertility from gametogenesis to birth. Genetic research has expanded in the last few years, and more and more genetic causes are coming up. This is going to change of previous estimates of genetic contribution of infertility. Genetics is becoming more important following the development of *in vitro* fertilization (IVF) and intra cytoplasmic sperm injection (ICSI) as these procedures lead to more genetic abnormality in offspring. The use of ICSI has raised major concerns about safety for the offspring, since it bypasses the physiological protective mechanisms related to normal fertilization. Natural selection prevents the transmission of mutations causing infertility. This protective mechanism is bypassed by using assisted reproductive technology (ART). The risk for genetic causes of infertility will increase in future generations [8]. Various tests are now available to explore the genetic causes of infertility. Identification of genetic factors in infertile couple helps in appropriate counselling thus management. There are several genetic etiological factors underlying infertility (Table 1) and these are chromosomal abnormalities, Yq microdeletion, CNVs, monogenic, multi factorial, epigenomic, mitochondrial, etc. Sperm chromosomal alterations [9] are also highly prevalent in spermatogenic impairment but not with necrozoospermia [10]. Infertile males with oligo/astheno/teratozoospermia (with normal blood karyotype) have ten-fold increase of chromosomal abnormalities in their sperms, including diploidy, disomy and nullisomy. Based on prevalence data routine karyotyping of infertile men within explained spermatogenic failure is widely recommended before ART. Sperm fluorescent in situ hybridization (FISH) is commonly used to determine the proportion of aneuploidy present in sperms of infertile men. Testicular sperm from men with non-obstructive azoospermia display higher rate of aneuploidy in spermatozoa than ejaculated sperms. Increased sperm aneuploidy increases the risk of IVF/ICSI failure and fetal aneuploidy. Indications for sperm FISH are repeated in vitro fertilization failure, oligospermia, nonobstructive azoospermia (testicular sperm), teratozoospermia, necrozoospermia, Klinefelter's syndrome (mosaic and nonmosaic), translocations, exposures to gonadotoxins, chemotherapy, pesticides exposure, etc. Various Y chromosome microdeletions/azoospermic factor (AZF) are predominantly found in non-obstructive azoospermia or severe oligospermia. Testing of AZF has a prognostic impact for sperm extraction, since no sperm can be retrieved in AZFa and AZFb, while there is a fair chance in AZFc. Copy number variations (CNVs) have not yet defined as a cause of male infertility, but that seems inevitable. In our ongoing study (unpublished) we have found CNVs in pseudo autosomal regions 1 (PAR 1) with early maturation arrest cases. Link between epigenetics and male infertility involves protamine packaging of the sperm genome. Sperm chromatin compaction is increased twenty-fold compared with somatic cells following the replacement of 90–95% of histones in the genome by the highly negatively charged and arginine-rich

nucleoproteins, protamine. Integration of protamine 1 and protamine 2 into the sperm genome during the elongation phase of spermatogenesis normally occurs in a strictly controlled 1:1 fashion. Significant deviations in the ratio have been associated with alteration in motility, morphology, and fertilization capacity as well as increased DNA fragmentation [11,12]. However, existing data do not support a consistent relationship between abnormal sperm DNA integrity and reproductive outcomes. At present, the results of sperm DNA integrity testing alone do not predict pregnancy rates achieved through natural conception or with ART. However, it is clear that homozygous mutations in key epigenetic regulators affect male fertility. In our preliminary study on epigenomic factors using Illumina 450K global methylation array in maturation arrest we have observed global hypomethylation in several cases (3 out of 12; work in progress, unpublished data). Monogenic disorders associated with infertility are Kallmann syndrome, Laurence Moon Biedl syndrome, Prader Willi syndrome, Noonan syndrome, androgen receptor mutations or CAG triplet expansion, 5  $\alpha$ -reductase deficiency, FSH-receptor mutation, LH receptor defects, mitochondrial gene defects, etc. Female specific genetic causes of infertility are fragile X premutation, FOXL2 mutations (blepharophimosis-ptosis-epicanthus inversus), galactosemia (GLAT mutations), POLG mutations (mitochondrial disease), adrenal hyperplasia and structural anomalies of the X chromosome such as terminal and interstitial deletion [13]. Two loci on Xq22-q26 and Xq27-q28 appear to be critical (DIAPH2 gene in proximal Xq22, XPNPEP2 gene in Xq25, DACH2 gene in Xq21.3 and POF1B gene in Xq21.3) for the premature ovarian failure (POF). About 10% of infertility is due to POF [14]. Fragile X mental retardation 1 (FMR1) gene premutation is defined as 50-200 repeats and may be associated with premature ovarian failure as well as low response to ovarian stimulation during IVF.

Genetic abnormality	Male Infertility	Female Infertility
Chromosomal		
Numerical	47,XXY; 46,XY/47,XXY; 46,XX male	45,X; 45,X/46,XX; 46,XY; 47,XXX
Structural	Dicentric Y	Ring X; Isodisomy Xp; Isodisomy Xq; Xq22-26 del; Xq27-28 del
Sperm chromosome	Aneuploidy (disomy, diploidy, nullisomy, etc)	Not applicable
CNVs		
Yq microdeletion	AZFa, AZFb & AZFc	Not applicable
Other CNVs	CDY (Yq); TSPY (Yp); PAR1 (Yp/Xp)	Not applicable
Monogenic		
Cystic Fibrosis gene	CFTR gene	CFTR gene
GnRH gene	Kallmann syndrome (KAL1, KAL2, etc)	Kallmann syndrome (KAL1, KAL2, etc)
Androgen receptor gene	Androgen insensitivity, androgen receptor CAG triplet expansion, etc	Androgen insensitivity
Other gene mutations	5 $\alpha$ -reductase deficiency, LH receptor defects, etc Laurence Moon Biedl syndrome, Prader Willi syndrome, Noonan syndrome, etc	FMR 1 premutation (50-200 triplet repeats), FSH-receptor, LH/hCG receptor, FOXL2 mutations, GLAT mutations, adrenal hyperplasia, WNT4 gene, DIAPH2 gene, XPNPEP2 gene, DACH2 gene, POF1B gene, etc Laurence Moon Biedl syndrome, Prader Willi syndrome, Noonan syndrome, etc
Epigenetic	Sperm protamine packaging	
Mitochondrial gene defects		POLG mutations

Table 1: Table showing genetic causes of infertility.

### Genetic Counseling in Infertility

Genetic counselling is a communication process concerning the occurrence and the risks of recurrence of genetic disorders within a family. This aims to provide the patient with clear and comprehensive understanding of all the important implications and possible options of the disorder and to facilitate rational decision [1]. In general, decision should come from counsellee and not from counsellor. This should be non-directive & non-biased manner. However, this may be challenged in special circumstances and may be more proactive. The ideal genetic counselling avoids a directive approach and concentrate on the medical, psychological and social circumstances so that couples can make decisions that are appropriate for them. The nondirective decision-making approach may sometimes prove unattainable, especially in situations where ambiguity in decision making. Shared decision-making can then provide a complementary approach when trying to balance the tensions between evidence-based guidance and the need to respect patient choice. Counselling provides help in areas of psychological assistance, technical explanations and discussing relationships. Genetic counsellor should be sensitive for infertility diagnosis as it carries social stigma, inferiority complex, familial disharmony/anxiety and ethics of various treatment options. Where genetic risks are related to the cause of infertility, genetic counselling is always required. There are several options available to couples viz., avoid having an affected child; having no children, having no genetic testing, having prenatal/preimplantation genetic diagnosis, or conception using donor gametes, or adoption. For prenatal/preimplantation genetic diagnosis (PGD) or use of donor gamete the genetic counselling should be provided by trained professionals. Counselling should begin with a thorough history, including the medical, social, reproductive and genetic histories of both partners. In situation where both partners are known to carry genetic defects (causing infertility) there can be high chance of transmitting the disease to child. In this situation, clinicians and infertility clinic personnel may feel that it is not ethical to proceed and ART should not be offered to the couple unless followed by preimplantation and/or prenatal diagnosis. In some countries, law may govern these matters but, in the absence of law, this type of conflict makes the doctor's role very difficult. In this situation the interests of future child should take precedence over the interests of a couple. In this situation pre-implantation genetic diagnosis is helpful to have a normal baby. Indications of genetic counselling are advanced age of couple, parent with known genetic disorder/carrier (chromosomal, Yq microdeletion, cystic fibrosis, etc), before offering assisted reproduction & preimplantation genetic diagnosis, gonad/gamete cryopreservation, donor gamete use, etc. Traditionally genetic counselling in infertile couple is done before offering assisted reproduction. Pre ART counselling is important in identification of risk factors, disease states & potential teratogens, and prompts elimination of teratogens as well as to discuss preventive measures through carrier screening, preimplantation screening and prenatal screening. This provides anticipatory guidance. The patient's ethnicity, medical history and genetic family history are key elements in this evaluation.

Preconception period is the optimal time to review the importance of preventive measures of transmitting genetic disorders in offspring. It also provides the opportunity to address the risks associated with environmental hazards and medications, and general risk of congenital anomaly or chromosomal abnormality associated with parental advanced age. Preconception counseling clinic is progressively becoming integral part of modern reproductive care. This should cover risk associated with advanced age, ethnicity, individual with balanced chromosomal translocations, risk of fetal malformations associated with drugs/radiation exposure, etc. Y chromosome microdeletion analysis should routinely be offered to all men with severe oligozoospermia or azoospermia. A positive test would provide a firm diagnosis of the man's problem, type of Yq deletion may assist the clinician in determining the best type of ART treatment. If infertility is secondary to AZF micro deletion then all male offsprings will inherit that micro deleted Y chromosome and will experience infertility whereas no female offsprings will have the defect. The couple can make choices here. The couple may choose for ICSI and PGD to have normal daughter. This is an appropriate use of PGD and couples should be encouraged to consider this option. If the couple elects to have an AZF microdeleted son, they will need to be aware of accumulating knowledge/interventions that may help their son preserve or optimize any future fertility options viz., sperm/gonad cryopreservation around pubertal age. In the next several years we may find out more in regards to the biology and pathophysiology of an AZFc microdeletion, perhaps even discovering therapies. The awareness of their son's AZFc microdeletion may allow the couple to employ possible therapies (including future developments in the field of stem cells) to help their son to become a biological parent. Similarly, cystic fibrosis transmembrane reductase (CFTR) mutations have implications for clinical infertility practice. When the male partner has congenital bilateral agenesis of vas deferens (CBAVD), it is important to test the female partner for CFTR mutations as well. If she is also found to be a carrier, then there must be



very careful consideration about whether the couple wishes to proceed with ICSI using the husband's sperm as the chance of a baby with cystic fibrosis will be 25% if he is heterozygous or 50% if one partner is homozygous & other is heterozygous or have PGD and/or prenatal diagnosis to avoid an affected offspring. A careful genetic counseling should also be offered to female partner carrying FMR1 permutation and candidate for ART, as premutation predisposes to further expansion of the triplet repeat in the germ line. Therefore pre-mutated women are at high risk of delivering male children with mental retardation. Here preimplantation/prenatal diagnosis for fragile X syndrome should be offered.

### Genetic risk from ART procedures

There is an increased risk of transmitting genetic defects to the offspring originated from ART. Genetic counselling should be a crucial step prior to ART procedure. There is a fourfold increase in incidence of sex chromosomal abnormalities, threefold increase in incidence of structural chromosomal defects [15] and a six fold increase in the incidence of imprinting defects [16] in babies conceived by ART/ICSI besides epigenetic effects related to *in vitro* culture, cryopreservation, handling stress, etc. This may also be due to retrieval of epigenetically immature germ cells from the testes. ICSI is used for the treatment of male infertility since 1992 or selected female infertility viz., limited quantities of oocytes, anomalies of the zona pellucida, etc. In addition, ICSI is used for treatment of polyspermy or poor fertilization in a prior IVF cycles. Here sperm (ejaculated or epididymal or testicular) is injected directly inside oocyte. However, there has been concern of chromosomal, genetic, congenital and developmental abnormalities in children born after ICSI [17-19]. Available data so far have shown that there is a small but definite increased risk of chromosomal abnormality [18], in particular sex chromosome abnormalities, major congenital malformations [20], etc. At present in many European countries ICSI is considered unethical and illegal. Counselling process should cover adverse effects of ART viz., risk of a major birth defect [21], epigenetic effects and imprinting effects [22], etc. Imprinted genes play key role in embryonic growth and behaviour [22]. Epigenetic changes affect transcriptional activity and control developmental plasticity, including cell-type-specific gene expression [23,24]. Studies on animal models have established that environmental factors, such as ovulation induction, culture medium composition and/or embryo manipulation, etc affect epigenome and impact on the conceptus, including birth weight. Animal models such as mouse and cow, commonly suffer from the so-called large-offspring syndrome [25]. This is more so with round spermatid nuclear injection (ROSNI). Genomic imprinting normally occurs during gametogenesis and this may not be completed early in the round spermatid stage. Genomic imprinting is a process through which alleles of given genes are expressed in a parent-of-origin-specific manner. Genes that are subject to imprinting often play key roles in embryonic development and behaviour. In human, several defects in imprinted genes are linked to syndromes such as Beckwith–Wiedemann, Prader–Willi, Angelman and Silver Russell. Increased prevalence of imprinting disorders related to ART has been reported. Studies suggest a possible link between ART (including *in vitro* culture) and genomic imprinting disorders [26]. Cryopreservation, a technique commonly used for gamete/embryo storage, also has effect on gene expression, telomere length, replication senescence, plasma/nuclear membranes, chromatin condensation [27] and chromosomal aneuploidy [28]. Similarly, when donors are chosen for oocyte or sperm donation, one should evaluate family history and appropriate genetic tests to prevent any transmission of genetic defect to offspring.

### Future perspective

Recent progress in stem cell research in particular induced pluripotent stem cell as well as germ line stem cell isolation and culture may provide a platform for *in vitro* gamete development [29,30] and may open a new era of gametogenesis in a dish and personalized infertility treatment in coming years. Once genomic screening technologies are used as part of predictive medicine practice, high-risk groups may be identified before development of disease and appropriate measures may be instituted before too late in near future. Cases like Klinefelter syndrome, Turner syndrome, Yq microdeletion, etc cases may benefit in future through predictive genomic medicine practice.

### Genetic Counseling in Obstetric Practice

Traditionally genetic counseling in obstetrics practice is done in early pregnancy or whenever indications arises e.g., following detection of abnormal ultrasound findings, abnormal serum screening result, placental mosaicism, etc, however, it is preferred to have a counseling session before planning for conception (pre-conception). Preconception counseling is important in identification of risk

factors, disease states & potential teratogens, and prompts elimination of teratogens as well as offers carrier and prenatal testing. This provides anticipatory guidance. The preconception visit provides opportunity for prediction, prevention and precision intervention in appropriate time. The patient's ethnicity, medical history and family history are key elements in this evaluation. Virtually every ethnic group carries some genetic burden [31]. Preconception counseling reduces anxiety considerably during pregnancy, which arises due to lack of time and also due to too late to intervene. The content of genetic counseling varies with the individual family situation, their concerns and indication for referral. The common indications of genetic counseling in obstetric practice are advanced maternal/paternal age, abnormal maternal serum screening test result, abnormal ultrasound evaluation during pregnancy including fetus, placenta and amniotic fluid, a previous fetus/child with genetic disorder or chromosomal disorder or congenital defect, parent with genetic disorder, family history of specific genetic disorder, maternal disorders associated with an increased risk of fetal congenital defect (e.g., diabetes, epilepsy, phenyl ketonuria, etc), possible carriers of specific harmful genes (eg., hemoglobinopathies), maternal exposure to teratogen (drugs, infections, radiation, toxins, etc) in periconception period, consanguinity, previous recurrent spontaneous abortion/intra uterine death/stillbirth, before as well as after offering prenatal screening in particular NIPT (noninvasive prenatal testing using next generation sequencing platforms) or prenatal diagnosis (e.g., chorionic villous sampling, amniocentesis or fetal blood sampling, etc) in particular using array comparative genomic hybridization or next generation sequencing platforms. The prerequisites of obstetric genetic counseling are confirmation of diagnosis, adequate time to the counselee, appropriate room (not in a busy OPD in front of other patients), confidentiality, spouse and other family members if possible should be involved, understandable conversation, nondirective, literature information and adequate follow up after procedure. The followings are the indications of genetic counseling in obstetrics practice:

### **Preconception and antenatal genetic counseling**

Ideal time for genetic counseling in obstetric practice should be before attempting pregnancy, however if does not take place then should be at first visit in pregnancy. Preconception counseling clinic is progressively becoming integral part of modern obstetric care. All women who are planning for pregnancy should attend the preconception-counseling clinic. It provides an opportunity to screen and discuss the conditions beforehand, which can adversely affect the pregnancy outcome. Several categories of patients are likely to benefit from such counseling. One such group is couples at risk for fetal abnormalities because of advanced maternal age or ethnic background. Examples include Jews of eastern European descent (at risk for carrying autosomal recessive gene for Tay-Sach's disease), individuals of African descent (at risk for sickle cell anemia), individuals of non-Jewish Caucasian descent (at risk for cystic fibrosis), individuals of north Indian descent (at risk for beta thalassemia), etc. In these conditions (autosomal recessive) simple & accurate carrier identification is possible. When both the couples are carriers of the gene in question, they may be counseled regarding the prognosis of the disease, risk of occurrence in their offspring and available prenatal diagnostic techniques. Counseling also should advise screening to all extended family members in reproductive age groups of both sides. Other group is individual with family history or previous history of any genetic disorder, fetal malformation or recurrent abortion. Complete pedigree up to three generations (5-6 generations if consanguineous marriage) should be drawn. Recurrence risk and available prenatal diagnosis possibilities should be discussed at this time. Third group of individuals includes maternal diseases like diabetes and phenyl ketonuria. In both, the risk of fetal anomalies is significantly increased (neural tube defect, congenital heart defect, microcephaly, etc), however appropriate metabolic control during early embryogenesis may reduce the risk of anomalies. Hence adequate control of disease before planning of pregnancy is important. Fetal malformation scanning by level II ultra sonography (USG) and fetal echocardiography is necessary in these situations. Increased risk of fetal malformations associated with drugs/radiation exposure should be discussed in preconception clinic. Drugs (except iron, multivitamins, folic acid) should be taken only when benefit outweighs side effects. Minimum dose, minimum number and safe drugs in pregnancy should only be prescribed. Role of peri-conception folic acid/multivitamins should also be explained [32]. During preconception visit one should elicit relevant medical history and geo-ethnic background to find out high-risk women and for this a trained genetic counselor is essential. High-risk women also include those who smoke and consume alcohol.



### Screening for carrier state

Most common indication for carrier screening in obstetric practice is to provide prospective parents with reproductive interventions/alternatives like preimplantation diagnosis, prenatal diagnosis, artificial insemination, adoption or deferral of childbearing. Carrier screening test should be simple, accurate & inexpensive. The disease should be common in the community and treatment or reproductive interventions/alternatives like preimplantation or prenatal diagnosis should be available for the identified individual. Some of the disorders meet the criteria for routine genetic screening in obstetric practice is Tay-Sachs disease [33], sickle cell anemia, thalassemia [34], etc. In India thalassemia is common (carrier status varies from 1 to 10%) particularly in some (Sindhi and Punjabi) ethnic groups. There is 1 in 1000 chance that two carriers will marry. If both parents are carrier then there is 25% chance of giving birth to a child with thalassemia major. It is recommended that all women in early first trimester or preconception stage should be screened for thalassemia trait in India, in particular north and west. If the women found positive then husband should be screened and if both found positive, then prenatal diagnosis should be offered.

### Screening for fetal malformations

All pregnant women with or without family history of genetic disorder or congenital malformations have a 3-4% risk of major congenital malformation in offspring. Most women planning for pregnancy are unaware of this baseline risk. Routine USG at 16-18 weeks can reduce this risk. Sensitivity of ultrasound for detecting fetal malformation screening in second trimester is very high even in low risk women [35-37]. All pregnant women should be offered USG at 16-18 weeks for fetal malformation as a component of routine antenatal care.

### Screening for chromosomal aneuploidy

Every pregnancy carries 0.6% risk of giving birth to a child with chromosomal abnormality; most common is trisomy 21 [1]. To reduce the birth of a child with chromosomal aneuploidy specially, trisomy 21, maternal serum first trimester dual test (beta human chorionic gonadotropin/ $\beta$  hCG & pregnancy-associated plasma protein A/PAPP-A) and/or second trimester triple test (unconjugated estriol/uE3, alpha fetoprotein/AFP and beta human chorionic gonadotropin/ $\beta$  hCG) should be offered to all pregnant women. Triple test can detect 59-67% of all Down syndrome fetuses [38] and can detect 66% cases of trisomy 18. Computer program is available to calculate the risk of having an affected pregnancy by using variables and information on maternal age, obesity, diabetes, stature, ethnicity, etc. Women with the risk at or above a specified cut-off (e.g., 1:250) are designated as screen positive. The positive triple test does not mean that the fetus is affected with Down syndrome/trisomy 18/trisomy 13 but it gives a risk figure. Amniocentesis has to be offered to confirm whether the fetus is affected or not. In women of more than 35 years, detection rate of triple test is higher (75-89%). Before offering the test women should be properly counseled regarding limitations, sensitivity and implications of positive/negative results. However, due to less sensitivity/specificity as well as high cut off value i.e., 1 in 250 (one positive case expected from 250 invasive procedures) researchers looking for better methods. One such method is non invasive prenatal screening or testing (NIPT/NIPS).

The NIPT/NIPS is an upcoming technology for screening/testing of fetal aneuploidies (in particular trisomy 21) from cell-free fetal DNA (cffDNA) present in blood of pregnant woman. The NIPT/NIPS is most commonly based on targeted massively parallel sequencing (t-MPS) involving selected chromosomal regions of interest, such as chromosomes 13, 18, and 21, in cell free DNA to determine the aneuploidy status [39]. Other methods like examining methylated DNA or epigenetic differences between fetal and maternal DNA or whole genome, are currently under exploration for detecting chromosomal aneuploidies [40]. The NIPT/NIPS has high sensitivity (above 95%) and high specificity [41]. The low false positive rate is the most important advantage of NIPT as this allows women to avoid unnecessary invasive procedures such as amniocentesis or chorionic villus sampling (CVS). The NIPT/NIPS can also determine paternity, fetal sex, fetal rhesus D (RhD) status, copy number variations (microdeletion/microduplication syndromes), or even single gene disorders [42,43] but not recommended by professional bodies [44].

Committee on Genetics (Society for Maternal-Fetal Medicine) of the American College of Obstetricians and Gynecologists in a recent publication provided their recommendation on various aspects of NIPT/NIPS [44]. In short these are pretest counseling (on risks, benefits and alternatives), limitations, cost effectiveness, posttest counseling (diagnostic test in case positive test result; termination of

the pregnancy should not be based on the NIPT/NIPS results of the cell-free DNA screening alone), genetic counseling and a diagnostic test in case of indeterminate/uninterruptable test result and test should be avoided in case of multiple gestations, fetal malformations on ultrasound examinations, etc. Patients should be counseled that a negative test result does not ensure an unaffected pregnancy and does not assess risk of fetal anomalies such as neural tube defects or ventral wall defects hence all women should be offered MSAFP screening or ultrasound evaluation. Patients should be counseled that NIPT/NIP Scan not replace the precision obtained with diagnostic tests, such as CVS or amniocentesis and limited in its ability to identify all chromosome abnormalities. Given the performance of conventional screening methods and limitations of cell-free DNA screening, conventional screening methods remain the most appropriate choice for first-line screening in the general obstetric population.

Preimplantation genetic screening/diagnosis (PGS/PGD) is highly technical approach for detecting genetic/mitochondrial/chromosomal abnormalities in early-stage embryos using genetic or molecular cytogenetic methods. Single/few cell genomic methods based on DNA microarrays or next generation sequencing (NGS) have been used for PGS/PGD [45]. It is an alternative to prenatal diagnosis for the detection of genetic disorders in couples at risk of transmitting a genetic condition to their offspring. Preimplantation genetic screening using DNA microarray/NGS improves the effectiveness of in vitro fertilization by selecting normal embryo for implantation [46]. However, before preimplantation genetic diagnosis, genetic counseling by a certified genetic counselor/geneticist must be provided to ensure that patients fully understand the risk of having an affected child, the impact of the disease on an affected child, and the benefits and limitations of all available options for preimplantation as well as prenatal screening/diagnosis. Couples should be informed that preimplantation genetic screening/diagnosis can reduce the risk of conceiving a child with a genetic abnormality. Invasive prenatal or postnatal testing to confirm the results of preimplantation genetic diagnosis should be encouraged because preimplantation genetic screening/diagnosis has technical limitations.

### **Prenatal diagnosis**

The couple at risk should be informed about all prenatal diagnosis options. Risk of pregnancy loss associated with the prenatal diagnostic procedures viz., chorionic villous sampling (CVS), amniocentesis & fetal blood sampling are of 1-2%, 0.5% & 2-5% respectively [47-49] and this fact should be explained to the couple before procedure. Limitation and implication of test result (normal/carrier/affected) should be discussed. Reproductive options like termination of pregnancy should be discussed before offering the test. Written informed consent should be taken before hand. Before attempting prenatal diagnosis, precise defect or mutation must be demonstrated in the proband or affected relatives in case of monogenic disorders. The common indications of prenatal diagnosis requiring genetic counseling are advanced maternal age, positive triple test, USG detected fetal malformations or abnormality in amniotic fluid volume, previous pregnancy or child with chromosomal abnormality, carrier parent for a chromosomal translocation or rearrangement, carrier (both parents) of autosomal recessive disease, increased risk for X linked recessive disease for which molecular diagnosis by mutation analysis or linkage is available, etc.

### **Advanced parental age**

Advanced maternal age is an important factor affecting reproduction. Advanced maternal age is the most common indication for prenatal diagnosis. The risk of having a child with chromosomal abnormality increases with the maternal age [50], most common of which is trisomy 21. Trisomy 21, 18 and 13 are most common autosomal aneuploidy and increase with maternal age. Sex chromosomal abnormalities, that increase with maternal age are 47, XXY (Klinefelter syndrome) and 47, XXX. The risk of spontaneous abortion also increases with advancing maternal age. The risk of abortion in mother of twenties is about 15% and this frequency increases gradually to 40% or more by age of 40 years. Advanced paternal age does not seem to carry any increased risk of aneuploidy as seen with increased maternal age, however, the collective risk for autosomal dominant diseases, e.g., Marfan syndrome, neurofibromatosis, achondroplasia and Apert syndrome, are increased. It is likely that autosomal dominant diseases are increased in offspring with increased paternal age. There is also an increased risk of X linked recessive diseases such as hemophilia and Duchenne muscular dystrophy in maternal grandsons when the grandfather was older ( $\geq 55$  years old) when his daughter was conceived. Maternal age of 35 years is taken as cut-off age for invasive prenatal diagnosis because the statistical chance of having a chromosomal defect in the fetus (1:178)

at maternal age of 35 years is equal or greater than the risk of amniocentesis (1:200). Previously it was thought that amniocentesis should be offered to all pregnant women more than 35 years and triple test (hCG, MSAFP, uE3) should be limited to the women less than 35 years. When the results of triple test screening were compared with the strategy of offering triple test to all pregnant women above 35 years and amniocentesis only if triple test screen was positive (cutoff 1:250 or less), same number of Down syndrome could be detected by triple test screening. This strategy reduces the number of amniocentesis to half and hence saving unnecessary procedure related to pregnancy loss. Moreover, detection rate of triple test is higher in women > 35 yrs age. So all pregnant women > 35 yrs should be offered triple test as initial steps and not directly invasive procedure. Presently following introduction of non-invasive prenatal test (NIPT) using free DNA of maternal blood the frequency of invasive test for trisomy 21 and 18 has reduced drastically. Although NIPS/NIPT is very specific and sensitive for trisomy 21 and/or 18 detection, patients should be informed that cell-free fetal DNA screening does not replace confirmatory tests, such as chorionic villus sampling or amniocentesis and limited in its ability to identify all chromosome abnormalities. Before as well as after offering these tests (triple test or NIPT or CVS/AC) women should be properly counseled about the sensitivity, limitations, and interpretation of positive and negative results.

### Previous childbirth with neural tube defect

Neural tube defect (NTD) is the commonest congenital malformation of the central nervous system. Worldwide prevalence is one per thousand live births [51] and higher prevalence (as high as 11 per thousand live births) in northern India [52-54]. After birth of a child with NTD couple should be counseled about prevention in subsequent pregnancies. Recurrence risk after one affected (isolated NTD) child is 3-5%, which is 10 times higher than the general population [55]. It increases to about 10% after two affected children and to about 25% after three such births. Peri-conception folic acid (4 mg per day for at least one month prior to conception and three-month post conception) reduces the risk of NTD by 70% [32]. Affected child (photograph if unavailable) should be examined (preferably by a geneticist) for correct diagnosis. This is important for correct counseling as several monogenic syndromes and chromosomal disorders [56-59] are associated with NTD. These carry different recurrence risk and may not be amenable to prevention by peri-conception folic acid treatment. Besides genetic factors, environmental factor like maternal diabetes or maternal intake of carbamazepine or valproic acid during pregnancy should be excluded. Maternal serum alpha feto-protein (MSAFP) and ultrasound examination at 16-18 wks should be advised in subsequent pregnancy. MSAFP (taking cut-off > 2.5 MOM using BPD for estimating gestation age) has a detection rate of 82-85% at 16-17 weeks with 1.4% false positive rate [60]. In skilled hand and in high-risk women ultrasound has a detection rate of 92-98% with false positive rate of 0.6-0.8%.

### Previous child with chromosomal abnormalities

Family history of chromosomal abnormalities may increase risk for an individual up to third degree relatives. For a couple with prior trisomic fetus, the recurrence risk is about 1%. Down syndrome can be due to pure trisomy 21 (95%) or mosaic trisomy 21 (1%) or robertsonian translocations (4%). Recurrence risk in translocation and trisomy is different. Therefore karyotype of Down syndrome child should be advised before counseling. In trisomy 21, maternal age at conception is important [61]. The recurrence risk is approximately 1% (greater than the maternal age specific risk). This represents a significant increase over the maternal age specific risk if maternal age is less than 35 years. If translocation Down syndrome case (13;21, 14;21, 15;21 or 21;21, 21;22) is detected in the affected child, parental karyotype should be advised. In 7-10% cases translocation is inherited from one of the parents and most commonly (90%) from mother [62]. If mother is carrier then recurrence risk is about 15% (except for 21;21 where risk is 100%) and if father is found to be carrier risk is 5%. If karyotype of both the parents is normal then translocation is de novo and unlikely to recur, but a recurrence risk of 2-3% is given [63]. Previous practice in this situation was amniocentesis at 16-18 weeks or CVS at 11-12 weeks followed by cytogenetic study (conventional and/or molecular). However, in present practice if the couple does not opt for amniocentesis then NIPT can be offered with adequate pre and post-test counseling.

### Recurrent abortion

Approximately 0.8 to 1% couples experiences three or more pregnancy losses [64]. In about 5% of couples with recurrent abortions the cause is a balanced translocation in one of the parent as compared to 0.55% in general population [65]. Therefore, after ruling out non-genetic causes (endocrinological, immunological, uterine and infection) karyotype of couple should be advised. If a translocation in one of the couple is detected then careful genetic counseling should be offered. If the translocation is reciprocal then the risk for

abnormal offspring is similar regardless of whether father or mother carries the translocation, however, if the translocation is Robertsonian then the risk is greater if mother carries the translocation. The unbalanced karyotype that can result in the zygote is associated with increase pregnancy loss rate and an increased risk of live born fetus with chromosomal abnormality. Hence, prenatal diagnosis in future pregnancy should be advised.

### Stillbirth

Chromosomal studies in stillbirth fetuses have shown that the incidence of chromosomal abnormality is about 10 times (5.6%) as compared to live births (0.6%). It is more in macerated fetus (11.9%) as compared to non-macerated fetus (4.18%) [4]. Chromosomal analysis may be considered in all such cases. It provides the family a more definitive cause of the loss and more definitive recurrence risk for future pregnancy [66].

### Polyhydramnios and Oligohydramnios

Polyhydramnios is associated with structural abnormalities or chromosomal abnormalities in 10-20% cases. Severity appears to be directly related to increased incidence of abnormal findings. Brady, *et al.* [67] found 3.2% incidence of chromosomal abnormality in fetus with polyhydramnios and most authors recommend genetic studies should be offered when any fetal structural anomaly (even subtle) is seen in association with polyhydramnios. Isolated moderate to severe polyhydramnios is often (1.4%) associated with chromosomal abnormalities and many authors recommend offering chromosomal study. Similarly, oligohydramnios is associated with an euploidy and fetal malformation like renal agenesis, renal dysplasia, posterior urethral valve, etc.

### Early onset intrauterine growth retardation

High prevalence of fetal chromosomal abnormalities is associated with early fetal intrauterine growth retardation/IUGR (61%). In high-risk pregnancy approximately 5% small infants may have chromosomal abnormality. About 25-35% of fetus with chromosomal abnormality will have no structural anomaly on USG except IUGR. Presence of early onset IUGR itself is an indication for chromosomal studies.

### Ultrasound detected fetal malformations

Antenatal screening of all pregnant women at 16-18 weeks permits detection of major structural malformations [37,67]. Detection rate in experienced hands vary from 50-75% [68]. USG detection of fetal malformation is a common event these days. Very often couple faces dilemma to continue the pregnancy. In this situation couple should be carefully counseled regarding diagnosis, prognosis and future implications. The prognosis of the fetus depends upon the type & severity of abnormality, associated malformations and chromosomal/genetic anomalies. In this regards fetal autopsy is very important as this contributes extensively to establishing a definitive diagnosis and thus impacted on genetic counseling [69]. Autopsy may leads to refinement of the risk of recurrence in as many as 36% of cases [70]. Utility of fetal autopsy in reproductive genetic counseling is significant. Approximately 35% of all fetuses with structural malformation have chromosomal disorder [71,72]. Detailed ultrasound for associated malformations and fetal echocardiography [73] should be advised in this situation. If malformations are known to be commonly associated with chromosomal abnormalities (Table 2) and couple decides to continue the pregnancy, fetal karyotyping by amniocentesis should be advised before giving definitive counseling. In case of termination fetal autopsy should be carried out for not only confirmation of diagnosis but also for genetic counseling including future prevention.

### TORCH (Toxoplasma, rubella, cytomegalo, herpes & other) infections in pregnancy

TORCH infections in pregnancy are associated with increased risk for fetal malformations and other sequel. Risk of infection to the fetus vary with the type of infection, gestational age and whether primary or secondary infection (Table 3). Prenatal screening for TORCH infections (except rubella) is not suggested in population with low prevalence. In India routine screening for TORCH in pregnancy or in cases of recurrent spontaneous abortions are not indicated as most population have exposure due to high prevalence rate, hence hard immunity before reproductive age is expected. Serologic screening for TORCH in pregnancy will complicate the situation and produces unnecessary anxiety to parents as well as doctors as interpretation of a serology test without a pre-pregnancy titer remains virtually impossible. Moreover millions of obstetric patient would have to undergo pre-pregnancy titer, which again are

not justified keeping financial constraints in mind. There is no standard reference laboratory where positive results can be confirmed (except isolating organism through culture or DNA diagnosis) and critical decision regarding fetal therapy or pregnancy termination can be made. So unnecessary TORCH testing should be avoided. In pregnancy this can be limited to symptomatic patients, those with suspicious ultrasound findings and possibly to those who are immuno-suppressed e.g., post transplant or HIV infected group [74]. Rubella vaccine should not be advised in pregnancy. If acute infection is detected in pregnancy, risk of fetal infection should be explained. Option of prenatal diagnosis of fetal infection should be offered by combination of ultrasound examination (findings like hydrops, ascites, ventriculomegaly, polyhydramnios, cardiac anomalies, limb abnormalities, microcephaly, IUGR etc) [75], microbial culture (from amniotic fluid or cord blood) [76], immunoglobulins level (IgM/IgA in cord blood) or DNA diagnosis (by polymerase chain reaction of specific DNA sequence of the microbes from amniotic fluid or cord blood [77,78] before making decision for termination of pregnancy as in most cases fetus are unaffected.

USG detected fetal malformations	Risk of chromosomal abnormality
Structural heart defect	32-48%
Septate nuchal membrane > 3 mm <15 wks > 5 mm 15-20 wks	56-60%
Simple nuchal membrane > 3 mm at < 15 wks or > 5 mm at 15-20 wks	9-33%
Duodenal atresia	30-40%
Congenital diaphragmatic hernia	2-20%
Omphalocele	30-40%
Choroid plexus cyst, short femur length Intracardiac echogenic foci, mild pyelectasia	Risk not significantly increased

**Table 2:** Table showing risk of chromosomal abnormality with ultrasound detected fetal malformations [80-85].

Type of infection	Risk of fetal infection	Sequels
Toxoplasmosis: 1 <sup>st</sup> trimester 2 <sup>nd</sup> trimester 3 <sup>rd</sup> trimester	10-25% 40-50% 60%	abortion, still birth, IUGR, chorioretinitis, hydrocephalus, intracranial calcification, hepatosplenomegaly, etc
Rubella: < 12 weeks > 12 weeks	60% 5%	IUGR, hearing loss, eye defects, heart defects, microcephaly, intracranial calcification, thrombocytopenia, hepatosplenomagaly, etc
CMV	30-40%	Chorioretinitis, microphthalmia, MR, intracranial calcification, microcephaly, deafness, thrombocytopenia, hepatosplenomegaly, etc
Genital herpes Vaginal delivery Recurrent infection	40-60% < 5%	Abortion, skin lesions, scar, microcephaly, microphthalmia, encephalitis, chorioretinitis, etc
Varicella zoster < 13 week 13-20 week around birth	< 0.4% 2-4% 25-50%	Cortical atrophy, MR, skin scarring, hypoplasia of extremities, etc Neonatal varicella

**Table 3:** Table is showing risk of fetus with maternal TORCH infection [86,87].

### Mitochondrial disorders in family

Diseases due to mitochondrial DNA (mtDNA) defects show some specific characteristics, which make it very challenging to estimate recurrence risks correctly and to predict whether a future child will be affected. One of the reasons is heteroplasmy (mixture of normal and mutant mtDNA as well as differences among tissues). Clinical features are manifested when mutant load (ratio of mutant to normal mtDNA) exceeds a specific threshold. However, the exact threshold to disease expression is not yet known. This makes it complex to predict test result in relation to development of disease symptoms. Further, extreme shifts in mutant load can be observed between mother and child and also between siblings thus makes counseling very difficult. Although a scientific and ethical debate about the possible reproductive options for carriers of mtDNA mutations is developing, however not much information exists regarding reproductive genetic counseling [79]. We are facing now women at risk of transmitting mtDNA mutation and asking for options to avoid the birth of another affected child. Until a consensus comes, women with mtDNA disorder may be offered to undergo assisted reproduction with donor oocyte to prevent the disorder in the offspring.

### Bibliography

1. Milunsky JM and Milunsky A. "Genetic counseling in perinatal medicine". *Obstetrics and Gynecology Clinics of North America* 24.1 (1977): 1-17.
2. Plachot M., et al. "Chromosome analysis of oocytes and embryos". *Human Reproduction* 3.1 (1998): 125-127.
3. Boue J., et al. "Retrospective and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions". *Teratolgy* 12.1 (1975): 11-26.
4. Alberman ED and Creasy MR. "Frequency of chromosomal abnormalities in miscarriage and perinatal deaths". *Journal of Medical Genetics* 14.5 (1977): 313-315.
5. Milunsky A. "Genetic disorders and the fetus: diagnosis, prevention & treatment". *The American Journal of Human Genetics* 77.5 (1992): 896-897.
6. Simpson JL and Elias S. "Prenatal diagnosis of genetic disorders". In Creasy RK, Resnik R [eds.]: *Maternal fetal medicine: Principle & practice*. WB Saunders, Philadelphia(1994): 61.
7. De Kretser DM. "Male infertility". *Lancet* 349.9054 (1997): 787-790.
8. Faddy M., et al. "Intra-cytoplasmic sperm Injection and Infertility". *Nature Genetics* 29 (2001): 131.
9. Halder A and Tutschek B. "Analysis of meiotic segregation in human nondecondensed interphase spermatozoa by multicolor rapid direct FISH". *Indian Journal of Medical Research* 107(1998): 94-97.
10. Halder A., et al. "Absence of sperm meiotic segregation error of chromosomes 1, 9, 12, 13, 16, 18, 21, X and Y in a case of 100% necrozoospermia". *Asian Journal of Andrology* 5.2 (2003): 163-166.
11. Carrell DT, et al. "Altered protamine expression and diminished spermatogenesis: what is the link?" *Human Reproduction Update* 13.3 (2007): 313-327.
12. Oliva R. "Protamines and male infertility". *Human Reproduction Update* 12.4 (2006): 417-435.
13. Toniolo D. "X-linked premature ovarian failure: a complex disease". *Current Opinion in Genetics and Development* 16.3 (2006): 293-300.
14. Coulam CB., et al. "Incidence of premature ovarian failure". *Obstetrics & Gynecology* 67.4 (1986): 604-606.
15. Fragouli E and Wells D. "Aneuploidy in the human blastocyst". *Cytogenetic and Genome Research* 133.2-4 (2011): 149-159.
16. Gutierrez-Mateo C., et al. "Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos". *Fertility and Sterility* 95.3 (2011): 953-958.
17. Golombok S. "Parenting and the psychological development of the child in ART families" in Vayena E., et al (edn): *Current Practices and Controversies in Assisted Reproduction, Report of a WHO Meeting*. Geneva: WHO (2001): 287-302.
18. Braude P and Rowell P. "Assisted conception II-*in vitro* fertilization and intracytoplasmic sperm injection". *BMJ* 327.7419 (2003): 852- 855.
19. Bondulle M., et al. "Medical follow-up study of 5-year-old ICSI children". *Reproductive BioMedicine Online* 9.1 (2004): 91- 101.



20. Bonduelle M., et al. "A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, *in vitro* fertilization and natural conception". *Human Reproduction* 20.2 (2005): 413– 419.
21. Hansen M., et al. "The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization". *The New England Journal of Medicine* 346 .10 (2002): 725–730.
22. De Rycke M., et al. "Epigenetic risks related to assisted reproductive technologies. Risk analysis and epigenetic inheritance". *Human Reproduction* 17.10 (2002): 2487– 2494.
23. Jablonka E and Raz G. "Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution". *The Quarterly Review of Biology* 84.2 (2009): 131–176.
24. van Montfort AP, et al. "Assisted reproduction treatment and epigenetic inheritance". *Human Reproduction Update* 18.2 (2012): 171–197.
25. Velker BA., et al. "Embryo culture and epigenetics". *Methods in molecular biology* 912 (2012): 399–421.
26. Marques CJ, et al. "Genomic imprinting in disruptive spermatogenesis". *Lancet* 363.9422 (2004): 1700– 1702.
27. Winston RM and Hardy K. "Are we ignoring potential dangers of *in vitro* fertilization and related treatments?" *Nature Reviews Molecular Cell Biology* 8 (2002): S14– S18.
28. Iwarsson E., et al. "A high degree of aneuploidy in frozen – thawed human preimplantation embryos". *Human Genetics* 104.5 (1999): 376–382.
29. Anand S., et al. "Chemoablated mouse seminiferous tubular cells enriched for very small embryonic-like stem cells undergo spontaneous spermatogenesis *in vitro*". *Reproductive Biology and Endocrinology* 18 (2015): 13-33.
30. Sriraman K., et al. "Mouse Ovarian Very Small Embryonic-Like Stem Cells Resist Chemotherapy and Retain Ability to Initiate Oocyte-Specific Differentiation". *Reproductive Sciences* 22.7 (2015): 884-903.
31. Milunsky A. "Choices, not Chances: An essential guide to your heredity and health". Boston, Little, Brown, 1989.
32. MRC vitamin study research group. "Prevention of neural tube defects: results of the Medical Research Council vitamin study". *Lancet* 338 (1991): 131-137.
33. American College of Obstetricians and Gynecologists (ACOG). "Screening for Tay Sachs disease". Committee opinion no. 162. Washington DC: ACOG.
34. American College of Obstetricians and Gynecologists (ACOG). "Hemoglobinopathies in pregnancy". Technical bulletin no. 220. Washington DC. ACOG.
35. Chitty LS., et al. "Effectiveness of routine ultrasonography in detecting fetal structural anomalies in low risk population". *BMJ* 303 (1991): 1165-1169.
36. Shirley IM., et al. "Routine radiographic screening for fetal malformations by ultrasound in unselected low risk women". *British Journal of Radiology* 65 (1992): 564-569.
37. Gonclave LF, et al. "The accuracy of perinatal ultrasonography in detecting congenital anomalies". *American Journal of Obstetrics and Gynecology* 171.6 (1994): 1606-1612.
38. Wald NJ., et al. "Antenatal maternal serum screening for Down's Syndrome: results of a demonstration project". *BMJ* 305.6850 (1992): 391-394.
39. Zimmermann B., et al. "Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci". *Prenatal Diagnosis* 32.13 (2012):1233-1241.
40. Papageorgiou EA., et al. "The Epigenome View: An Effort towards Non-Invasive Prenatal Diagnosis". *Genes (Basel)* 5.2 (2014): 310-329.
41. Palomaki GE., et al. "DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study". *Genetics in Medicine* 14.3(2012):296-305.
42. Helgeson J., et al. "Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing". *Prenatal Diagnosis* 35.10 (2015): 999-1004.
43. Chitty LS and Lo YM. "Noninvasive Prenatal Screening for Genetic Diseases Using Massively Parallel Sequencing of Maternal Plasma DNA". *Cold Spring Harbour Perspectives in Medicine* 15.9 (2015): a023085.

44. Committee Opinion No. 640. "Cell-Free DNA Screening For Fetal Aneuploidy". *Obstetrics and Gynecology* (2015).
45. Smeets HJ, et al. "Preventing the transmission of mitochondrial DNA disorders using prenatal or preimplantation genetic diagnosis". *Annals of the New York Academy of Sciences* 1350.1 (2015): 29-36.
46. Dahdouh EM, et al. "Technical Update: Preimplantation Genetic Diagnosis and Screening". *Journal of Obstetrics Gynecology Canada* 37.5 (2015): 451-463.
47. Thien A, et al. "An assessment of the use of FISH with chromosome specific probes as an alternative to cytogenetics in prenatal diagnosis". *Prenatal diagnosis* 20.4 (2000): 275-280.
48. Maxwell DJ, et al. "Fetal blood sampling and pregnancy loss in relation to indication". *British Journal of Obstetric Gynecology* 98.9 (1993): 892-897.
49. Antsaklis A, et al. "Fetal blood sampling: indication related losses". *Prenatal Diagnosis* 18.9 (1998): 934-940.
50. Hook EB. "Down syndrome epidemiology and biochemical screening". In screening for Down syndrome. Grudzinkas JG, Card T, Chapman M and Cukle H (eds.): Cambridge University press, Cambridge (1994): 1-18.
51. Lemire RJ. "Neural tube defect". *JAMA* 259.5 (1988): 558-562.
52. Verma IC. "High incidence of neural tube defects in North India". *Lancet* 1 (1978): 879.
53. Baird PA. "Neural tube defects in Sikhs". *American Journal of Medicinal Genetics* 16.1 (1993): 49-51.
54. Sharma AK, et al. "Incidence of neural tube defects at Lucknow over a period of 10 years from 1982-1991". *Indian Journal of Medicinal Research* 99 (1994): 223-226.
55. Tolmie J. "Neural tube defects and other congenital malformations of central nervous system". In: Principles and practice of medical genetics 3<sup>rd</sup> edition. Eds. Rimoin DL, Connor JM, Pyeritz RE, New York, Churchill Livingstone (1996).
56. Kennedy D, et al. "Prenatally diagnosed NTD: Ultrasound, chromosomes and autopsy or postnatal findings in 212 cases". *American journal of Medicinal Genetics* 77.4 (1998): 317-321.
57. Halder A, et al. "Iniencephaly: a report of 19 cases". *Indian Pediatrics* 35.9 (1998): 891-896.
58. Halder A, et al. "Iniencephaly and Chromosome Mosaicism: A Report of Two Cases". *Congenital Anomalies* 45.3 (2005): 102-105.
59. Halder A. "Lethal Developmental Defects: An Overview". *Open Journal of Obstetrics and Gynecology* 4 (2014): 1006-1036.
60. Wald J and Kennard A. "Prenatal screening of NTD & Down syndrome". In Emery 3<sup>rd</sup> edition (1997): 547.
61. Hook EB. "Chromosomal abnormalities: prevalence, risks and recurrence". In Brock DH, Rodeck CH, Ferguson-Smith MA eds. Prenatal diagnosis and screening. Churchill Livingstone, Edinburgh (1992): 351.
62. Hook EB. "Unbalanced robertsonian translocation associated with Down syndrome or Patau's syndrome: chromosome study, proportion inherited, mutation rate and sex ratio". *Human Geneicst* 59 (1981): 235-239.
63. Stienberg C, et al. "Recurrence rate for de novo translocation Down syndrome: a study of 112 families". *American Journal of Medical Genetics* 17 (1984): 523-530.
64. Durgan A, et al. "Prenatal genetic diagnosis following recurrent early pregnancy loss". *Obstetrics & Gynecology* 75 (1990): 381-384.
65. Fryns JP, et al. "Cytogenetic survey in couples with recurrent fetal wastage". *Human Geneicst* 65 (1984): 336-354.
66. Gerson SL, et al. "Prenatal Cytogenetics" *Humana press* (1999): 267.
67. Brady K, et al. "Risk of chromosomal abnormalities in patients with idiopathic polyhydramnios". *Obstetrics & Gynecology* 79 (1992): 234-238.
68. Paladini D, et al. "Prenatal diagnosis of CHD & fetal karyotype". *Obstetrics & Gynecology* 81 (1983): 679-682.
69. Pahi J, et al. "Does autopsy of antenatally diagnosed malformed fetuses aid genetic counseling"? *The National Medical Journal of India* 11(1998): 169-170.
70. Nayak SS, et al. "Clinical utility of fetal autopsy and its impact on genetic counselling". *Prenatal Diagnosis* 35 (2015): 685-691.
71. Pielt LD, et al. "Role of amniocentesis in ultrasound detected fetal malformations". *Obstetrics & Gynecology* 68 (1986):153.
72. Williamson RA, et al. "Abnormal pregnancy sonogram: selective indication for fetal karyotype". *Obstetrics & Gynecology* 69 (1987): 15.

73. Copel JA, et al. "The frequency of aneuploidy in prenatally diagnosed congenital heart disease: an indication of fetal karyotyping". *American Journal of Obstetrics & Gynecology* 158 (1988): 409-412.
74. Beazly DM and Egerman RS. "Toxoplasmosis". *Seminars in perinatology* 22 (1998): 332-338.
75. Watt-Morse ML, et al. "The natural history of fetal cytomegalovirus infection as assessed by serial ultrasound and fetal blood sampling: a case report". *Prenatal diagnosis* 15 (1995): 567.
76. Lynch L, et al. "Prenatal diagnosis of fetal cytomegalovirus infection". *American Journal of Obstetrics and Gynecology* 165.3 (1991): 714-718.
77. Hohlfeld P, et al. "Prenatal diagnosis of congenital toxoplasmosis with PCR on amniotic fluid". *New England Journal of Medicine* 331 (1994): 695-699.
78. Rovello MG, et al. "Polymerase chain reaction for prenatal diagnosis of CMV". *Journal of Medical Virology* 47.4 (1995): 462.
79. Bredenoord AL, et al. "Reproductive decision-making in the context of mitochondrial DNA disorders: views and experiences of professionals". *Clinical Genetics* 77.1 (2010):10-17.
80. Beazly OM and Egennan AS. "Toxoplasmosis". *Seminars in Perinatology* 22 (1998): 332-338.
81. Land W JB, et al. "Abnormal nuchal findings on screening ultra sonography; aneuploidy stratification on the basis of ultra sonographic anomaly and gestational age at detection". *American Journal of Obstetrics and Gynaecology* 85 (1996): 103-106.
82. Gilbert MW and Nicolaides KH. "Fetal omphalocele: associated malformations and chromosomal defects". *Obstetrics and Gynaecology* 70.4 (1987): 633-635.
83. Nyberg DA, et al. "Chromosomal abnormality in fetus with omphalocele: The significance of omphalocele content". *Journal of Ultrasound Medicine* 8.6 (1989): 299.
84. Thompe-Beeston JG, et al. "Choroid plexus cysts and chromosomal defects". *Br J Obst Gynaecol* 63 (1990): 783-786.
85. Wickstrom EA, et al. "A perspective study of association between isolated pylectasia and chromosomal abnormality". *Obstetrics and Gynaecology* 88.3 (1996): 379-382.
86. Stagno S, et al. "Primary cytomegalovirus infection in pregnancy: Incidence, transmission to fetus and clinical outcome". *JAMA* 256.14 (1986): 1904-1908.
87. Brown ZA, et al. "Genital herpes simplex virus infection complicating pregnancy: natural history and prepartum management". *Journal of Reproductive Medicine* 31 (1986): 420.

**Volume 2 Issue 1 October 2015**

**© All rights are reserved by Ashutosh Halder.**