The Challenge of Life

Prenatal screening and diagnosis

Pontifical Academy for Life

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PICCIN

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Preface

Over recent years, there has been rapid technological growth in medicine. In the field of prenatal diagnosis. Sophisticated examinations using technological instruments and laboratory equipment have made it possible to collect information on the fetus' health, even early in the pregnancy. As with all technology, biotechnologies not only modify our way of interacting with the world, they also alter our way of understanding it. Technology emphasizes "product", processes and quality control. This is an insufficient approach when Here we witness a tendency to treat profound issues regarding the meaning of human life as problems of mere functionality. This leads to an understanding of normality that excludes imperfection and vulnerability. These features, however, are an integral part of the human condition, which has always included unexpected, unpredictable and surprising elements.

Hence, these new ways of understanding and acting on the prenatal stages of human life have profound anthropological and social implications. They lead to a radical transformation in the way of experiencing and understanding humanity and the maternal-fetal relationship. The consequences of these changes are not always fully comprehended. At times we think that technology expands the range of viable options and freedom of choice. We often forget that new technologies, by creating new choices, put an end to others. They come with a general framework for interpreting phenomena and "creating a culture". Moreover, the complexity and amount of information does not simplify the decision-making process, but rather exposes it to new emotional stress and intense socio-cultural conditioning. Finally, there are also commercial interests pushing towards the spread of technology that do not take the impact and delicacy of the management data deriving from it into consideration.

For all these reasons, it has become necessary to create an up-to-date and balanced interdisciplinary scientific framework for this complex matter. This text

provides solid scientific background and the consensus of experts from various fields to guarantee critical awareness and a constructive approach. The Pontifical Academy for Life has willingly supported and encouraged this work while being aware of the importance of providing an up-to-date and sensitive approach to profound anthropological and cultural implications, an essential premise for any ethical assessment. Therefore, I am deeply satisfied with the work that has been carried out, especially as it also includes concrete suggestions on how to support parents and families in the choices facing them, while respecting the values belonging to each individual. I'd like to highlight another unique element of this text: the positive feedback from the representatives of the Jewish, Islamic and Buddhist faiths. This text is a precious resource to provide support to all of those involved in this process—parents, young patients (the unborn or newborns), and healthcare workers and managers—so that they may protect their rights and assume their responsibilities.

Mons. Vincenzo Paglia
President of the Pontifical Academy for Life

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Introduction

PRENATAL EXAMINATIONS: CRITERIA FOR RESPONSIBLE USE

Ultrasounds and prenatal genetic diagnoses became widespread in medicine in the 1970s, and soon after established themselves in obstetrical practice. It has since become possible to diagnose pathologies during pregnancy that were previously manifest only at birth.

Innovations in biomedicine

The recent gains made in the biological sciences, which are linked to technologies, have brought about dramatic changes. The combined expertise of various fields of knowledge such as genetics, computer science, data processing and omics has produced a rapid increase in unprecedented possibilities. In medicine, there has been a profound conceptual change in the way reproduction, development and relationships are understood. It has become possible to explore previously invisible spaces of the mother's body with direct and indirect imaging and use invasive procedures for diagnostic or therapeutic purposes. The fetus is more exposed to and subjected to intervention becoming a full-fledged patient and entering more often into a public and legal domain where it was once almost absent (Boltanski L, 2013).

These biomedical, economic, and socio-cultural factors cannot be ignored when trying to understand the significance of the tests available. Procreation is not only a biological fact. It calls into play the meaning of existence and the way cultures process it. Through the attitude we have towards development and the welcoming of children, we can not only see the deep relationship of couples but also that of society as a whole with regard to life and the future. The development of biotechnologies and prenatal diagnosis examinations have brought about a profound transformation in the way the development is understood (Thiel MJ, 2018).

The latest features of the tests, which are closely examined in the following pages, can be summed up in four points: 1) expansion in the field of analysis (no longer just the karyotyping of single genes, but the entire genome); 2) simplification of procedures (reduced invasiveness, costs and exam times); 3) an increase in the range of non-genetic examinations available (pathologies, maternity, etc.); 4) more widespread use, with a progressive increase in people affected and a less distinct line between diagnoses and screening. This work provides current and scientifically authoritative information in accessible language.

Health and disease: scientific data and relational significance

The elimination of genes considered to be pathological or disadvantageous via the voluntary termination of the pregnancy is a trend that is spreading, even when their significance is unclear. This is a product of a distorted cognitive interpretation, which understands a person as reducible solely to a genetic structure (Sabatello M, Juengst E, 2019). This practice remains widespread even though a less reductive perspective has been clearly outlined in the official documents of international organizations (UNESCO, 2003). Indeed, from a scientific point of view, individual genes do not constitute the only determinants of a phenotype. There is a close and dynamic interconnection between the innate and acquired traits (between nature and nurture). They cannot be separated or contrasted, except for monogenetic or Mendelian cases.

The same notions of health and disease keep the role of the environment in mind, in varying proportions. They are not "deterministically" deducible from each individually considered gene, except in the case of some monogenetic Mendelian diseases. They are also a product of the reciprocal interactions that are established among them and within the wider system where the organism is located and where it finds (or does not find) the equilibrium that allows it to live. The environment can play an important role in the majority of cases of complex diseases. Therefore, the clinical effects of a genetic deficit (on the health of the organism) also depend on the properties of the environment, which can also be changed by medical treatment (Dupras Ch, Ravitsky V, 2016). Furthermore, in the case of monogenetic (or Mendelian) diseases, the results of the tests do not automatically declare the entire clinical severity. The predictions are even more uncertain regarding the (pathological phenotypic) effects of specific genetic variants (Richards S et al, 2015). Epigenetic developments have also contributed to the role and functioning of genes by demonstrating that lifestyle, which is modulated both by the personal and cultural choices made where the person is located, has an impact on health. These

factors not only influence the regulation of genes but also the transmission of hereditary traits to the descendent.

Moreover, the particular sensitivity of the time when prenatal examinations are performed deserves to be highlighted (Mancuso S, Benagiano G, 2021). The pregnancy is in fact a moment when unique relationships in the personal experience between mother and child are established as the woman experiences the presence of another in her body. The molecular and cellular dialogue between the fetal (as well as the immunological) self and the maternal self leads to the creation of an exchange, both on a biological and experiential level. The external reality of the other body is not recognized as an enemy to be resisted, but as a guest to be welcomed (and adopted) in a complex process. This is a dynamic of crucial importance in which prenatal clinical examination plays a significant role. especially ultrasounds. The symbolic value of this dynamic goes far beyond the relationship between mother (and more broadly, the parents) and child, influencing the formation of a social link on the level of inter-human coexistence.

Decision and information

Some examinations for screening predict a probabilistic relevance for a condition. Others for diagnosis have a higher level of certainty. In any case, both have emotional resonance. Such examinations concern situations that depend on a variety of largely unknown factors. Their administration is therefore extremely complex. The decision-making process, which leads to the choice of undergoing or not undergoing genetic testing, is equally sensitive. Such responsibility is legitimately the parents. We must be attentive to the multiple pressures to which the couple is subjected, from the setup of the healthcare system to the organization of society and its culture. We find ourselves within cultural conditions that freely give us permission to ask how much is socially induced based on economic forces (Han BC, 2016).

In a high-tech society, the logic of risk assessment and control dominates. The attempt to protect oneself from any risk exposure, and free oneself as much as possible from it, makes the uncertainty which is an integral part of human existence difficult to accept. After all, life itself in the universe and its changes imply precarity and contingency (Pievani T, 2019). In human existence, there is a general condition of frailty and vulnerability, of which disease is just one expression. We do not die because we get sick, but rather disease occurs because we are mortal. The pursuit of the elimination of every imperfection, including genetic ones, hides the fact that mutations and variability will present themselves

in new forms, as well as in their variants that despite seeming disadvantageous, could reveal themselves to be competitively advantageous in unpredictable conditions (see the reduced susceptibility of those with thalassemia to malaria or even Covid-19) (Lansiaux E et al, 2020).

Socially representing ourselves as perfect is false because the human condition itself is incomplete. The fetus is to be considered a human being whose vulnerability, frailty and dependence are limits that relate (in a certain sense) to a state of imperfection. It is necessary to take care of them so they are less of an obstacle for the fetal flourishing but these are also deficiencies and limits that define the encounter with others where relations are established. These experiences are constitutively part of the search for the meaning of existence which can show up in unexpected ways and different from the healthy projections onto the sick (Wilkinson D, Savulescu J, 2014). This does not mean giving up on diagnoses and treatments but increasing use and research within a wider horizon that favors a path to welcoming (not subordinated to pre-established conditions, even genetic ones) (Habermas J, 2002) and humanizing people. Asking why one should undertake prenatal diagnoses is part of this perspective, developing a more critical approach than is often proposed with a view of greater assumption of responsibility.

Basic examinations should be proposed to everyone, but with critical awareness of which exams to explore and which to avoid. This means wisely using the principle of proportionality of the diagnostic means and objectives. The purpose is not the eradication of difference and the reduction of variability but rather to better help people affected by the identified disease. This objective requires a change in perspective on the acceptance of disability and the support offered to the disabled and their families.

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CHAPTER 1

Why prenatal diagnosis? Risk perception and its consequences

MEANING OF THE TERM "RISK"

The Italian dictionary defines the term "risk" as the "possibility of suffering harm related to more or less predictable circumstances" (Treccani, translation of the authors). It specifies that in medicine, risk is a condition that is threatened by an elevated statistical incidence of particular pathological events. The reference to the eventualità (possibility) is more nuanced in Collins' English definition of risk as "the possibility of incurring misfortune or loss; hazard". The word in the French-speaking world takes on the meaning of "Possibility, probability of a fact, of an event considered as an evil or a damage" (Larousse, translation of the authors). The Anglo-Saxon conception of risk in prenatal diagnoses is expressed in purely probabilistic terms. It is the basis of the development of today's widely-used screening techniques that express how many fetuses in percentage terms, in a population with common variables (maternal age, smoking, etc.), suffer from a certain condition. Risk is strongly linked to the individuation of pregnant women to whom specific examinations should be proposed to diagnose or define the probability of a pathologic event.

RISK FACTORS

The risk factors that will be discussed can lead to various unfavorable outcomes in the pregnancy: abortion, fetal or neonatal death, structural deformations in the fetus, and growth or fetal development anomalies that can contribute to adverse outcomes in the short or long term. In Appendix 1, Physiopathological features of female reproductive potential and the development of the fetus and placenta), some pathophysiological principles regarding the development of the fetus and placenta are identified that can assist in understanding how risk factors taken under consideration.

Fetal risk factors can be divided into three types:
- a generic species risk;
- risks related to family history;
- risks correlated to the mother's lifestyle and the evolution of the pregnancy.

Generic species risk

Approximately 3% of newborns are affected by chromosome pathologies (about 1%), congenital defects (about 1%), or Mendelian diseases (around 1%). Three fundamental mechanisms that are shared by the general population contribute to this risk:
- new mutations;
- interaction between genomes inherited from the parents;
- the effect of environmental factors on the genome.

New mutations

New mutations closely connected with fetal medicine. Prenatal diagnoses have demonstrated that fetal diseases with chromosomal and non-chromosomal anomalies can originate from a healthy couple.

a. *Chromosomal anomalies*. The invasive techniques of prenatal diagnoses began with amniocentesis. They were at the same time as recognition of the correlation between the increase in maternal age at conception and the frequency of some chromosomal aneuploidies. In the first decades of prenatal diagnoses, maternal age was the main indicator for the analysis of the fetal karyotype.

   The chromosomal aneuploidies represent the number of anomalies in the chromosomes. Currently, no single essential and sufficient cause is identifiable for the development of abnormalities due to the number of chromosomes. However, there are known risk factors that influence the rate of chromosomal anomalies differently, as will be shown later on.

   The recognition of independent risk factors for the development of such pathologies has allowed prenatal diagnostics to diversify the screening options offered to the couple. However, one mustn't forget that the screening exams share a common aim: providing an estimate of risk, which is to be understood as the probability that the fetus will be affected by a chromosomal pathology. Meiotic nondisjunction during the maturation of the oocyte (first and second division) is recognized as the main pathogenetic mechanism of the aneuploidies. Given the timeframe between the beginning of the first meiosis and the

Table 1.1 Risk of chromosomal aneuploidy at birth in relation to maternal age			
Mother-to-be's age	**Aneuploidy's risk**	**Mother-to-be's age**	**Aneuploidy's risk**
15	1/1859	31	1/459
16	1/1789	32	1/392
17	1/1712	33	1/332
18	1/1631	34	1/278
19	1/1546	35	1/230
20	1/1456	36	1/172
21	1/1361	37	1/188
22	1/1264	38	1/153
23	1/1167	39	1/97
24	1/1070	40	1/76
25	1/972	41	1/59
26	1/876	42	1/45
27	1/784	43	1/34
28	1/695	44	1/25
29	1/611	45	1/19
30	1/532	46	1/14

end can be particularly long, even decades, one can understand how errors in segregation can take place in this phase. Other mechanisms at the basis of the aneuploidy of the gametes linked to the mother's age (like loss of cohesion between sister chromatids during the latent phase) have been studied. Therefore, the first risk factor identified as the basis of the phenomenon is constituted by the mother's age. The occurrence of the majority of trisomy is linearly (trisomy 16) or exponentially (as in the case of trisomy 21 and 18) higher with the increase of age.

The biological considerations cited here turn out to be all the more important if analyzed in the context of the clear demographic trend of postponing the pregnancy for social, familial and personal reasons.

The study of relationships between nondisjunction and maternal age conventionally sets the age of 35 as the threshold recommended for the monitoring of the pregnancy, based on an empirical risk of aneuploidy at birth estimated at around 1:230 (Table 1.1).

Other mechanisms have been studied based on the aneuploidies of gametes linked to maternal age such as the loss of cohesion between sister chromatids during the latent phase. The frequency of the majority of trisomies increases

with the linear (trisomy 16) or exponential (as is the case in trisomy 21 and 18) increase in age.

Age represents the baseline risk in the diagnosis of aneuploidies. Moreover, there areindividual and laboratory correction factors that modify the risk that a fetus can be affected by a chromosomal pathology. It is no coincidence that the first basic method of screening trisomy in the 1970s foresaw the mother's age as being the only variable, with the result that amniocentesis was proposed to all women over 37, making up 5% of pregnancies. Later, the age was lowered to 35 and today this group makes up, in industrialized countries, more than 30% of pregnancies.

The nondisjunction mechanism correlated to the mother's age has been identified in the progressive reduction of the number of chiasmas in the meiotic prophase, with a subsequent excess in errors at first division, likely in relation to the proportional elongation of the dictyotene (stop of the first prophase) with the advance in the woman's age. Therefore, it is not surprising that around 95% of trisomy 21 and XXX aneuploidies come from nondisjunction in the oogenesis, as with 90% of trisomy 18 and 85% of trisomy 13. The XXY aneuploidy comes from the mother in 55% of cases, while the X monosomy originates in oogenesis in just a third of cases.

The risk of reoccurrence of the same chromosomal pathology in the mother who has already given birth to an affected child varies according to the pathology. For trisomy 21 the risk of recurrence only slightly increases (1% up to 40 years old, based on the mother's age). In the case of Translocation Down syndrome, the risk is higher if only one of the parents is a carrier of the translocation in a balanced way. For a girl affected by Down syndrome, the risk of transmitting the disease to her children is 1 in 3.

b. *Non-chromosomal anomalies.* The findings from genomic analyses have highlighted a completely different rate of non-chromosomal mutations, with a significantly greater contribution from the father. In short, it has been calculated that, from 20 to 40 years old, the number grows on average from 40 to 90 for each paternal conception and from 10 to 20 for each maternal conception. The data indicates that parents with an age between 20 and 40 can transmit between 50 and 100 new mutations at each conception, the frequency of which is only minimally influenced by the age of the mother and significantly correlated to that of the father (Fig. 1.1).

The non-chromosomal mutations can consist both in point mutations, as in the case of autosomal dominant diseases, many of which have been shown to have a clear correlation with paternal age (e.g. achondroplasia), and in

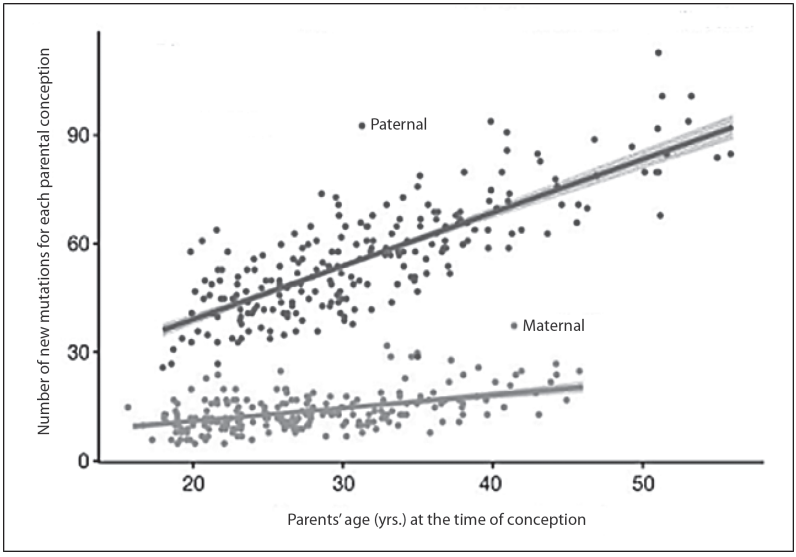

Figure 1.1. Number of new mutations per proband at conception in relation to parental age (modified from Jónsson et al, 2017).

variations within repeated sequences that are often associated with complex phenotypes (e.g. congenital defects, autism, etc.) (Toriello VH et al, 2008; Southard AE et al, 2012). The mechanisms at the basis of this increase in mutations can be traced back to the characteristics of spermatogenesis, particularly to the growing number of mitotic divisions in the spermatogonia, preceding meiosis. In fact, while the number of cellular divisions that occur starting from the embryo to puberty is estimated to be around 30, this number increases to around 380 at the age of 28 and around 540 at 35, resulting in a potential increase in errors during the duplication of the DNA.

c. *Post-zygotic mutations-mosaicism* (chromosomal and non-chromosomal). The genome inherited at conception can encounter post-zygotic mutations during embryo-fetal development and postnatal life. Such mutations give rise to mosaicisms, i.e. the coexistence in the same person of cellular populations with a different genetic pool. The symptomatic mutations may regard chromosomes or the nuclear or mitochondrial genome with very different clinical implications regarding the type of mutation and the typology and the number of cells and tissues involved (Acuna-Hidalgo et al, 2015). They are

stochastic events that are not correlated to known risk factors. The majority of post-zygotic mutations are asymptomatic or not attributable to specific clinical conditions. The extent of this phenomenon has been clarified over the last years, both on mouse models and human beings, particularly with the variations deriving from the transposition of mobile elements present in the non-coding portion of the genome. For example, it has been calculated that, even when using a conservative estimate, at least one cell in 300 in the brain possesses a single insertion, which indicates the presence in the brain of over 100 million different genomes (Erwin et al, 2014). These modifications occur starting from the first phases of embryogenesis and their implications remain completely unknown.

Interactions between genomes inherited from the parents

The additive effect of parental genomes can result in an increase in the risk of pathology through two main mechanisms: a Mendelian and a multifactorial one (Dallapiccola, Novelli, 2012).

a. The *recessive autosomal diseases* originate from homozygous pathogenetic mutations from heterozygous parents. It is difficult to define a specific risk from these pathologies, as the parents are asymptomatic as a rule. However, consanguinity can represent a risk factor for these diseases, proportional to the degree of endogamy (Table 1.2). Such a risk exists because the entire population shares pathogenic mutations in a significant number of genes-disease (probably at least 5) that get expressed in a recessive autosomal way. Therefore, in parents with links to consanguinity through common ancestral

Table 1.2 Reproductive risk in relation to the degree of consanguinity

Grade of consanguinity and percentage of shared genes	Risk of recessive diseases
First grade degree: 50% (parents, children, brothers/sisters)	30-50%
Second grade degree: 25% (aunts and uncles, nephews and nieces, grandparents)	7%
Third grade degree: 12.5% (first cousins, great nephews and great nieces, great-aunts and great-uncles, great-grandparents)	3%
Fourth grade degree: 6.25% (second great nephews and great nieces, first cousins one and a half, great-great-grandparents)	1%
Fifth grade degree: 3.125% (second cousins, first cousins two and a half)	<1%

descent, the probability of sharing the same mutation increases. Analogously, the increased frequency of a recessive autosomal disease in a population (as is the case of thalassemia and cystic fibrosis in Italy) increases the probability that the two partners will share a mutation in the same gene. This highlights the usefulness of screening capable of identifying, before conception, potential conditions of increased risk.

A particular feature in the interaction between the parental genomes is represented by the *recessive mutations linked to the X chromosome,* which as a rule, except for some rare mechanisms (Dallapiccola B, Novelli G, 2012), does not get expressed in the heterozygous females (healthy carriers), but only in hemizygous males in which the presence of the Y, as a second sexual chromosome, does not mask the effect of the mutation present on the X.

b. *Multifactorial diseases.* The more complex and misunderstood aspect concerns multifactorial diseases, which include various congenital defects (e.g., cardiopathy, cleft lip and palate, neural tube defects, etc.) and various common postnatal diseases. These conditions are considered secondary to the synergistic effect of environmental factors and genetic variation, which are capable of adding susceptibility. The hereditary component of this mechanism (so-called heritability) can be defined based on the reoccurrence of the disease between blood relatives and the identification of common variations (polymorphisms) that are significantly associated with the phenotype. Genomic studies have documented hundreds of complex diseases/traits, an association with thousands of polymorphisms (GWAS Catalog EMBL-EBI). However, the data available at the moment shows, on average, less than 15% hereditability for this phenotype; the predictive power of single polymorphisms is low, with an average additional risk of 1.1-1.5.Important differences are present in the distribution of such polymorphisms among populations, which limits the possibility of using them unless their predictive power has been previously confirmed in the relevant population. Therefore, genetic counseling on the risk of recurrence of congenital defects, traits and complex diseases remains problematic and is largely based on empirical risks rather than molecularly defined ones.

Risk related to family history

Study of the family medical history can in certain cases offer clues for increased reproductive risk, even if such risks are often present in asymptomatic parents, who are therefore unaware of them.

a. *Balanced chromosomal aberrations.* Balanced chromosomal aberrations like translocations concern over one person in 1,000 and mis-segregation, gives rise to unbalanced gametes with duplications or deficiencies. In some families, recurrent miscarriages or the presence of individuals with mental disabilities suggests segregation in the family of such aberrations. In the same way, some chromosomal inversions in asymptomatic people constitute risk factors for the production of unbalanced gametes.
b. *Genomic pathologies*. In the case of some genomic pathologies from deletion or duplication of variable portions of the genome to the order of hundreds of kb, it is always necessary to verify that the imbalance shown in the affected individual is not segregated by an asymptomatic parent (Zhao R et al, 2008). In fact, some of these abnormalities result in defect penetration and therefore can be shown at a clinical level or only be minimally symptomatic (e.g., deletion 3q29).
c. *Dominant autosomal diseases*. Some Mendelian mutations are correlated to diseases from the parents. The transmission frequency of dominant autosomal Mendelian diseases from an affected parent is proportional to the biological suitability or fitness of the mutation, i.e., to the reproductive success of the genotype. For example, the majority of adult-onset diseases have normal or nearly normal fitness, and this is why the probability is quite high that the mutation will segregate from a heterozygous parent before becoming symptomatic (e.g. Huntington's chorea). This fitness is lower for the mutations that cause physical defects in the individual, such asachondroplasia, where fitness is estimated at 20%. This implies that all the mutations originate de novo at conception, in the case of very serious (e.g., fibrodysplasia ossificans progressiva) or lethal diseases (e.g. Thanatophoric dysplasia).
d. *Recessive autosomal diseases.* Risk in a couple can be defined based on family history (e.g. the presence of spinal muscular dystrophy in a blood relation) and the later verification of the heterozygous condition for the gene disease. It should be highlighted that, given the relatively low frequency in the population of heterozygotes for pathogenetic mutations responsible for rare recessive autosomal diseases, risk must be taken into consideration only in cases where the disease has a higher frequency in the population where the partners come from (in general, a disease frequency greater than 1:10,000, which implies a frequency of heterozygotes greater than 1:50) (Table 1.3). For lower frequencies, the presence of a condition of heterozygosis in a partner does not justify the monitoring of the other partner.

Table 1.3 Genic frequency and frequency of heterozygotes calculated based on the basis of the occurrence frequency		
Disease occurrence frequency (q^2)	**Genic frequency (q)**	**Frequency of heterozygotes (2pq)**
1/1.000	1/32	1/16
1/2.000	1/45	1/23
1/5.000	1/71	1/36
1/10.000	1/100	1/50
1/50.000	1/224	1/112
1/100.000	1/316	1/158

e. *Recessive diseases linked to the X chromosome.* The case of recessive diseases linked to the X chromosome is different. Even in the case of rare or very rare conditions, the presence of a hemizygous (affected) individual in the family justifies recourse to genetic tests to exclude the mutation in all potentially at-risk females.
f. *Mitochondrial diseases.* Mitochondrial diseases concern a genome that, by definition, is inherited with the cytoplasm of the egg. The mitochondrial diseases get expressed when the level of the heteroplasmy, i.e., the percentage of mutated mitochondria, surpasses a threshold. Therefore, in the presence of an affected individual, it is necessary to exclude a subclinical level of heteroplasmy in the asymptomatic mother, which would imply an increase in reproductive risk.

Risks correlated to the mother's lifestyle and health

It has been established that the environment, understood in a broad sense, modulates the levels and the nature of epigenetic signals (Kappil et al., 2015). Therefore, the prenatal environment is also critical for the functioning of the genome. The increase in imprinting diseases in pregnancies initiated with artificial insemination techniques might fall within this scenario (Hiura H et al, 2014).

1. *Chronic maternal pathologies diagnosed and treated before the start of the pregnancy or diagnosed for the first time during pregnancy.* The enormous progress in medicine and surgery over the last decades has allowed women who are afflicted with chronic pathologies to live a long life and has allowed them to reproduce. It is very important that before dealing with a pregnancy, or as soon as possible during the pregnancy, these women have multidisciplinary counseling. The specific clinical care for these pregnancies should be

based on some basic principles and provided by a multidisciplinary team in which a gynecologist specializing in maternal-fetal medicine is the responsible physician for the woman/couple. The gynecologist should interact with various specialists and surgeons who themselves must have knowledge and experience of the treatment of the respective pathologies during pregnancy. It should always be kept in mind that the pregnancy can have a short-term or long-term effect on the progression of the disease. The disease, or its treatment, can interfere with the outcomes of the pregnancy. The pathology can result in problems for the development of the fetuses in various ways.

a. *Increased risk of malformation.* The presence of a maternal malformation (e.g., in the cardiovascular or central nervous system) can increase the risk of malformation in the fetus in the same system. For example, pregnant women afflicted with cardiopathy have a 1-4% risk of having an afflicted child versus around .8% in the general population. Even some chronic maternal diseases can increase the risk of malformation in the fetus: preexisting diabetes increases the risk of malformations by 10, in particular cardiovascular ones. and caudal regression syndrome by two hundred (https://www.orpha.net). This risk is reduced if the diabetes is well-monitored from the moment of conceptionbut still remains higher than the risk in the general population (https://www.orpha.net). For other pathologies, such as epilepsy, it is too difficult to clarify the role of the disease and the role of the medication used in the treatment of the disease (see the paragraph Medications below).
b. *Risk of anomaly of excessive fetal growth.* Diabetes, especially if it is not well managed, carries a risk of fetal dysmetabolism and accelerated growth. Accelerated growth can cause dystocia during childbirth, with often serious consequences like brachial palsy and hypoxal/anoxic fetal distress. Secondly, dysmetabolism makes the fetus more susceptible to hypoxia during labor and less prepared to adapt to the extrauterine life with a consequent risk for brain damage.
c. *Risk of fetal growth restriction.* Many maternal pathologies can interfere with the growth of the fetus, either because they directly entail a direct reduction in the oxygen intake and/or nutrients by the fetus, or because they contribute to the development of diseases in the pregnancy, pre-eclampsia in particular. Today there are no therapies available to treat this in-utero pathology f progressive hypoxia that leads to irreversible brain damage and eventually to the death of the fetus. Currently, prenatal di-

agnosis—via ultrasound to evaluate the growth of the fetus and Doppler flowmeter and cardiotocography (CTG) to indirectly evaluate the oxygenation conditions of the fetus—helps in choosing the moment in which to deliver the fetus to minimize the risks of death and outcomes resulting from very early or very late birth.

   d. *Risk of preterm birth.* With serious maternal pathologies, an iatrogenic preterm birth could become necessary, both to protect the health of the mother and the fetus. In these cases, it becomes critical to monitor the mother and the fetus to identify the best moment for the delivery. On the other hand, there are many maternal conditions, like infections, that represent a significant risk factor for preterm birth.

2. *Medications.* The intake of medication during pregnancy can have a teratogenic effect (in the first trimester of the pregnancy); can alter the growth and the functional development of the feus; and have toxic effects on the fetal tissue (second or third trimester) or harm the newborn (near childbirth) (Appendix 2. Principles of teratogenesis). Unfortunately, randomized studies are not possible, for obvious reasons, and existing studies are encumbered by bias. Databases like TOXNET (TOXLINE in https://pubmed.nbci.nim.nih.gov) are very useful for being guided in the choice of the safest medication when it is indispensable for the treatment of the pathology. In any event, the pathology must always be adequately treated because the risk of a negative impact on the pregnancy is still greater than the risk of taking an appropriately chosen medication (TOXLINE in PubMed).
3. *Lifestyles*
   a. *Smoking.* Elevated levels of active and passive smoking during pregnancy areassociated with an increased risk of maternal complications and adverse outcomes, not only for the fetuses during the current pregnancy but potentially also future ones (Rauschert S et al, 2019). Smoking reduces the fertility of women and also the fertility of men whose mothers smoked during pregnancy (Practice Committee of the American Society of Reproductive Medicine, 2018). It has been documented that smoke increases the risk of abortion and preterm birth (Kelkay B et al, 2019), and reduces fetal growth (Terzioglu F et al, 2019). Quitting smoking before the start of the pregnancy, or in the first weeks, has a positive effect with regard to at least some of these complications (Brand JS et al 2019; Sonej S et al 2019; Kondracki AJ et al, 2019). There are no studies at the moment that document a modification of risk with the use of electronic cigarettes.

Several strategies should be proposed and discussed with the woman to encourage her to stop smoking given the pregnancy.

b. *Alcohol*. Elevated levels of alcohol consumption in pregnancy result in a delay in the physical and neurobehavioral development of the child and dysmorphic facial features (fetal alcohol syndrome). The risks that low to moderate consumption causes are less clear, as well as the threshold below which the consumption of alcohol during pregnancy can be considered safe. In some studies, complications like low birth weight, fetal growth restriction, preterm birth and malformations are reported. Some studies correlate the adverse outcomes of alcohol consumption to the third trimester, while others the first trimester (Nykjaer C, 2014). In the face of these uncertainties, as a precautionary principle, all guidelines advise against the consumption of alcohol for pregnant women or those who are planning for a pregnancy (World Health Organization, 2014; World Health Organization, 2016).
c. *Drugs*. The use of drugs and narcotics (amphetamines, opioids, benzodiazepine, cannabis, cocaine, heroin) are associated with adverse events in pregnancy: placental abruption, rupture of the uterus and withdrawal syndrome in the newborn. The data concerning cognitive deficits in children of mothers who take cocaine are contrasting (Terplan M, 2015) and there are few reports concerning a link with structural malformations (David AL et al, 2014). Interventions during or before the pregnancy to reduce the use of narcotics are strongly recommended, even if there is no strong evidence of their efficacy (World Health Organization, 2014; Terplan M, 2015).
d. *Nutrition*. An adequate intake of proteins, lipids, carbohydrates, vitamins and minerals is very important for the health of the mother and the development of the fetus. Maternal malnutrition is associated with an increase in fetal mortality and low birth weight. In specific circumstances recommendations consist of an increase in proteins in the diet and/or supplementation with iron and folic acid and/or supplementation with calcium and/or supplementation with vitamin A (World Health Organization, 2016). Supplementation with folic acid (0.4 mg/die) is recommended for all women, independently from the diet, for the prevention of neural tube defects in the fetus. Obesity, which is clearly linked to unhealthy dietary habits, is also spreading to disadvantaged populations and represents a risk factor forpregnancy. Obese women have an increased risk of hyper-

tension in pregnancy, gestational diabetes, induced labor, emergency caesarean sections, fetal macrosomia and a large fetus for the gestational age. The risk is even greater the greater the pre-pregnancy body mass index (Masturzo B et al, 2019; Doi L et al, 2020).

4. *Working conditions.* The work environment and working conditions of the mother, and often also those of the father, can negatively influence the outcome of the pregnancy, causing infertility, abortion, preterm birth, and developmental defects and malformations in the fetus. Chemical substances, gas and radiation can have a teratogenic effect (Appendix 2) and/or development of the fetus. Physical exertion, strenuous work shifts, and stressful jobs can induce preterm birth or fetal growth restriction. The data in the scientific literature is not unambiguous and there are many risk factors with various mechanisms of action. The general recommendation is to consider the work anamnesis at the start of the pregnancy or before the start of the pregnancy and evaluate the measures to implement to avoid risks correlated to specific factors.
5. *Environmental conditions.* In experimental studies, the many pollutants known as teratogens, which have been demonstrated to have adverse effects on pregnancy (infertility, abortion, preterm birth, fetal growth restriction), are prevalent in the atmosphere. Additionally, considerable attention has been placed on the so-called endocrine disrupting chemicals, which can cause genetic/epigenetic alterations in the normal development of the fetus. However, there have not yet been epidemiological studies able to establish a causal link with certainty between life in specific environments (incinerators, landfills, factories, mines) and fetal malformations (Baldacci S et al, 2018).
6. *Ionizing Radiation.* The hypothesis that ionizing radiation can cause nondisjunction in cells in active meiosis or mitosis goes back to the 1970s. The experimental context of these studies has considered the great catastrophes of the last century (Chernobyl, nuclear experimentation areas) and targeted clinical settings (use of radiation for diagnostic ends). A significantly higher risk of nondisjunction was found both in mothers and fathers who were previously exposed to ionizing radiation during the pause in meiosis I (in the case of women) and meiosis II. The genetic damage from stochastic ionizing radiation is obviously called into question in the pathogenesis of non-chromosomal pathologies. Therefore, even though the pathogenic role of these agents has been scaled back over the last years, when taking charge of a woman in her fertile age, one cannot avoid taking the principles of prevention into account concerning the diagnostic exam guidelines for the emission of ionizing radiation.

7. *Vertically transmitted infections.* Various viruses, bacteria and protozoa can infect the mother and, via the placenta, the fetus; these infections can be asymptomatic or have a benign progression in the mother but cause serious damage to the fetus. The agents that are known today are viruses (rubella virus, varicella-zoster virus, cytomegalovirus, parvovirus B19, HBV, HCV, HPV, HSV, HIV), bacteria (Treponema pallidum) and protozoa (Toxoplasma gondii). The risks vary depending on the pathogen and its capacity to pass through the placenta and the moment of the pregnancy in which the infection is contracted. For example, the probability that the rubella virus causes fetal malformation in the first trimester of the pregnancy is more than 90%; later, the risk is lower, and after 17 weeks of gestation there is no longer a risk of malformation. The infection from toxoplasma causes serious fetal damage during the first trimester; the seriousness of the damage is lower in the second trimester and even lower in the third trimester. However, the probability that the Toxoplasma gondii passes through the placenta and infects the fetus is very low in the first trimester (< 1%), increases in the second, and is very high in the third. Varicella contracted in the second trimester results in a low risk of fetal malformation (1% - 2%); if contracted near childbirth, it carries a risk of neonatal death due to the infection of the newborn who is not yet able to produce antibodies (and no longer receives the mother's). This is the case in the majority of viral infections (e.g. the pertussis virus).
   Some infections are susceptible to primary prevention (Table 1.4) with the administering of the vaccine in receptive women (e.g., rubella) or the vaccine in the newborn of positive mothers (HBV); for others, a vaccine is not available (e.g. cytomegalovirus, toxoplasma, treponema), but primary prevention is possible by adopting behavioral and hygienic standards. Some are amenable to secondary prevention through therapies that reduce the transmission risk of the disease from the mother to the fetus (e.g., toxoplasmosis, treponema, HIV). For others, tertiary prevention is possible (e.g., fetal transfusions to correct anemia in the case of hydrops from the Parvovirus).
   There is also the possibility for pregnant women to be vaccinated both for influenza (in the II and III trimesters) and, even more significantly between the 27th and 35th week, against pertussis, diphtheria, and tetanus. The purified antigen vaccine against pertussis is neither dangerous for the pregnant woman nor the fetus. It is needed to protect the child in the time that passes from birth to the first vaccine (the 61st day of life). During this period, the

Table 1.4. Indications for screening and prevention/treatment of main infections				
Pathology	**Effect**	**Screening**	**Prevention I**	**Prevention II**
Citomegalovirus	Neurosensorial impairment	No	Yes, healthy behavior	No
Hepatitis B	Chronic hepatitis, cirrhosis, hepatocellular carcinoma	Yes	No	Yes, vaccine in newborn
HIV	Neonatal infection	Yes	No	Yes, antiretroviral treatment at the moment of birth
Rubella	Malformations CNS, heart, Intrauterine growth restriction	Yes	Yes, maternal vaccination before pregnancy	No
Beta-hemolytic streptococci	Early neonatal infection	Yes	No	Yes, antibiotic treatment in labor
Syphilis	CNS impairment, kidney and liver damage	Yes	Yes, healthy behavior	Yes, medication during pregnancy
Toxoplasmosis	CNS impairment liver and spleen damage	Yes	Yes, healthy behavior	Yes, medication during pregnancy
Varicella	CNS, skin, limb impairment		Yes, maternal vaccination before pregnancy	No

newborn is susceptible to this dangerous infection for its complications and high mortality rate (7 out of 10 children who die from pertussis are younger than 2 months).

8. *Twin births.* The frequency of twin birth varies throughout various populations, with the highest rate in Central Africa, specifically in Nigeria, and the lowest in Southeast Asia and South America (Smits J, Monden C, 2011). In the Occidental world, it is on the risewith a rate that varies from 1% to 1.6% in its various countries (Pison G et al, 2005). The factors that influence the rate of twin births are correlated to genetics, nutrition, maternal age, and the use of artificial insemination. Twin births can be dizygotic (two eggs fecundated by two spermatozoa) or monozygotic, deriving from an abnormal subdivision of a single zygote. The dizygotic twins are always dichorionic-diamniotic; the monozygotic twins can be dichorionic-diamniotic, monochorionic-diamniotic ormonochorionic-monoamniotic (very rare). Overall, less than 20% of twin births are monochorionic. Independently of chorionicity, twin pregnancy carries an increased risk both for the mother and the fetuses: pathologies

in pregnancy (hyperemesis, diabetes, hypertension, anemia, cholestasis of pregnancy, genito-urinary infections), postpartum hemorrhage, fetal underdevelopment, and both spontaneous and iatrogenic preterm birth (correlated to the abovementioned pathologies) are more frequent (Cheong-See F et al, 2016; Santana DS et al, 2018). These risks are greater in the case of multiple pregnancies. Monochorionic pregnancies carry additional risks for the fetuses: twin-twin transfusion (TTTS), twin anemia-polycythemia sequence (TAPS) and the rarer twin reversed arterial perfusion (TRAPS). Structural malformations, in particular cardiac ones, are more frequent and can concern both fetuses or, more frequently, only one of the twins. Cases of conjoined twins are rare (1:50.000). Finally, it should be remembered that some of the abovementioned risks, like multiple pregnancies, are more frequent in pregnancies achieved with artificial insemination techniques.

9. *Maternal age.* A certain reproductive risk is present in the entire fertile periodbut tends to increase towards the curve ends. Both a lower (< 20 years old) and higher (> 40 years old) maternal age carry risks for the pregnancy, the mother, and the fetus/newborn. A low maternal age is associated with an increased risk of eclampsia, puerperal endometritis and infections, preterm birth, low birth weight and stillbirth (Ganchimeg T et al, 2014). Advanced maternal age is associated with an increase in the risk of perinatal mortality, preterm and extreme preterm birth, low birth weight, macrosomia, neonatal intensive care, and caesarean section (Kenny L et al 2013; Laopaiboon M et al, 2014). As far as maternal outcomes are concerned, deaths, "near misses", and serious pathologies are more frequent (Laopaiboon M et al, 2014). As far as congenital defects are concerned, structural ones do not correlate with maternal age except for a slight increase in the defects of the closure of the abdominal wall in younger women.

   Instead, there is a known (Table 1.1) correlation between maternal age chromosomal aneuploidies due to nondisjunction (Sauer MV, 2015; Goetzinger KR et al, 2017; Odibo AO et al., 2006; Miller DA, 2005; Raitio A et al, 2019; Durfee SM et al, 2013)

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CHAPTER 2

Screening and Diagnoses

The term "screening" refers to an examination strategy within a specific group, e.g., pregnant women, used to identify those with a greater risk of a disease that represents a significant problem in terms of (higher) prevalence and (serious) clinical symptoms. The clinical impact of screening tests has been to reduce the use of examinations via invasive techniques e.g., amniocentesis and the sampling of chorionic villi with their associated risks.

Sensitivity (SENS), specificity (SPEC), positive predictive value (PPV) and negative predictive value (NPV) are indicators of the validity of a screening test. An ideal screening test should show a SENS and a SPEC of 100%. However, these values are technically unreachable and screenings always exhibit some degree of error.

NON INVASIVE, BIOCHEMICAL, AND BIOPHYSICAL TECHNIQUES

Screening tests for the monitoring of the so-called "low risk" physiological pregnancy were developed at the same time as invasive prenatal testing. Currently, screening testing use ultrasounds and the analysis of the maternal blood (Figure 6-8).

1. *Ultrasounds carried out at the 11^{+0} – 13^{+6} week* measure the dimensions of the fetus and nuchal translucency (NT), a lymphatic space between the spinal column and the posterior region of the fetus' neck. An increase in NT shows an increase in the fetal subcutaneous edema, which usually resolves itself before the second trimester. This anomaly could be accompanied by chromosomal aneuploidies, some genetic diseases, maternal infectious diseases, and fetal heart defects (Senat MV et al, 2002; Bakker et al, 2014). The test can supply false positive results, but its sensitivity increases when it is associated with a blood sample with some serological markers.

2. The *Combined Test* has been implemented since the 1990s in the screening of trisomy 21. Carried out between the 11+0 and 13+6 weeks of gestation, this test uses a combination of maternal age, the thickness of NT, the blood sample on the maternal plasma of the free fraction of the beta chorionic gonadotropin (free β-hCG) and of the elevated molecular weight of the glycoprotein, the "Pregnancy Associated Plasma Protein A" (PAPP-A). An algorithm allows for the attainment of a "personalized" risk value (e.g. 1/2000; 1/350; 1/50; etc.), or one that is specific to each pregnancy. It has a sensitivity of around 80-90% and a false positive rate (FPR) between 4-5% and 7% (Snijders RJ, 1998; Nicolaides KH, 2005; Park SY, 2016; Santorum M, 2017). Alongside trisomy 21, the combined test also allows for the early detection of trisomy 18 and 13, respectively the second and third most frequent chromosomal anomalies with a sensitivity of 92-95%, while considering a percentage of false positives between 1% and 4% (Park SY, 2016; Santorum M, 2017). At a gestational age of 11+0 and 13+6 weeks, the relative prevalence of trisomy 18 and 13, compared to 21, is 1:3 and 1:7 (Snijders RJ, 1994; Snijders RJ, 1995; Snijders RJ, 1999). All three trisomies are associated with an increase in maternal age, the relevance of the NT and a reduction in the concentration in maternal plasm of PAPP-A, but in trisomy 21 the concentration of free β-hCG is higher, while in trisomy 18 and 13 it is lower (Snijders RJ, 1994; Snijders RJ, 1995; Snijders RJ, 1998; Snijders RJ, 1999; Tul N, 1999; Spencer K, 2000; Nicolaides KH, 2000; Wright D, 2008).
   Studies carried out over the last ten years have shown that it is possible to obtain improved results in the screening of the first trimester:
   – by carrying out biochemical tests at 9-10 weeks and the ultrasound at a gestational age of 12 weeks. However, the organizational complexity and the fact that there would be blood samples of women whose pregnancies are no longer evolving should be evaluated (4-6%);
   – by including additional parameters in the ultrasound assessment (like the nasal bone, the evaluation of ductus venosus and tricuspid valve blood flow, etc.) that require a higher-level ultrasound, ultrasound technicians trained to carry out detailed examinations and more time requirements inconsistent with a screening test.

   In clinical practice, the most widely used operational diagram includes the carrying out of biochemical tests and ultrasounds at the same time between the 11+0 and 13+6 weeks (Bindra R, 2002; Spencer K, 2003; Spencer K, 2000).

3. *The Tri-test* is carried out at a gestational age between 15 and 18 weeks, following the ultrasound dating of the pregnancy. It consists of a sample of maternal serum with some products of the feto-placental metabolism (alpha-fetoprotein, free estriol and total chorionic gonadotropin: AFP, uE3 and hCG). An algorithm based on maternal age and the results of the above-mentioned blood sample allows for obtaining a risk value. This "late" test is less accurate in the assessment of risk and provides a lower predictive performance (sensitivity of 70-80%) but can be proposed to women who have not had access to the I trimester test.
4. *The quadruple test* is the integrated tri-test with the addition of another biochemical marker, inhibin A, that is not included in the most commonly used software in many countries, including Italy. This marker does not significantly increase sensitivity and specificity and is expensive.
5. *The integrated test* is carried out in two moments: (1) at 11+0-13+6 weeks there is the ultrasound with NT measurement (as in the combined test) and the PAPP-A blood sample and (2) at 15-17 weeks there is a blood sample of the maternal blood of Estriol, alpha-fetoprotein, and chorionic gonadotropin. The results and the mother's age are "integrated" and an algorithm provides the risk value. The combination of this data increases the accuracy of the prediction and also allows for the risk evaluation of malformation in the spinal column (e.g., spina bifida)

   It is not easy to introduce and explain the concept of "risk". It must be made clear what the result means in practice: for example, a risk of 1 in 100 is higher than 1 in 1000. A risk of 1 in 100 means that only one fetus in 100 with the same result is affected, while 99 in 100 are healthy. The only way to know definitively if it is affected is to carry out a diagnostic test that, in the case of trisomy 21, 18 and 13, is the study of the fetal karyotype on chorionic tissue (obtained from the chorionic villi sample – see appendix) or on amniotic fluid (obtained with amniocentesis – see appendix).

   If the test result shows a risk of 1 in 1000 it means that only 1 fetus in 1000 is affected, while 999 in 1000 are healthy. The only way to know if the fetus is affected is to carry out a diagnostic test (chorionic villi sample or amniocentesis) which involves an additional risk of abortion (obtained with amniocentesis—see appendix). Comparing the two risks (risk of having a sick child and the risk of aborting a healthy child due to complications linked to an invasive procedure) can often help in decision-making. even if the two negative events have different meanings and significance for each woman. The question for

Table 2.1. Different screening tests and the relative markers

	1° trimester			2° trimester			
Maternal age +	NT	PAPP-A	fβ-hCG	fβ-hCG/ hCG	AFP	uE3	Inhibin-A (optional for the integrated test)
Combined test							
Tri-test							
Quadruple test							
Integrated test							
Biochemical integrated test (or serologic)							

the woman/couple is: are you worried about having a sick child or having an abortion due to the possible consequences of an invasive exam? In an individual case, it is often difficult, if not impossible, to clearly attribute the abortion to the invasive procedure, so it is important that the woman be motivated in her decision and refer to centers/operators with proven experience, knowing that there is additional risk. Tables 2.1, 2.2 and 2.3 present the screening tests available in the I and II trimesters, their sensitivity considering a fixed FPR

Table 2.2. Sensitivity of the various tests considering a fixed FPR rate of 5% according to the SURUSS and FASTER studies

Maternal age +	Period	SURUSS	FASTER
nNuchal translucency	1° trim.	60%	64-70%
Combined test	1°trim.	83%	82-87%
Tri-test	2° trim.	77%	69%
Quadruple test	2° trim.	90%	81%
Biochemical Integrated Test	1° + 2°	85 (89)%	(85-88)%
Integrated Test without Inhibin A	1° + 2°	92%	---
Integrated Test with Inhibin A	1° + 2°	93%	94-96%

(from: Wald NJ et al. 2003; Malone FD et al. 2003)

Table 2.3. Evaluation of safeness of different diagnostic tests for Down syndrome (from SNLG, Sistema Nazionale per le Linee Guida, Ministero della Salute Italiano, Istituto Superiore di Sanità. Aggiornamento 2011).

Test	False positive rate	Numbers of not afflicted fetus loss every 100.000 women	Numbers of fetus diagnosed with Down syndrome due to fetus loss correlated to the procedure
Combined	6.1%	44	3.9
Double	13.1%	94	1.8
Triple	9.3%	67	2.6
Quadruple	6.2%	45	3.8
Integreated serum	2.7%	19	9.1
Integrated	1.2%	9	19.2

rate of 5% and the calculated rate of fetal losses for fetuses not affected by each test.

Other options are:

6. *the sequential test*. A first report is produced in the 1st trimester (after NT or after the combined test) and a final report in the second trimester. In this way, there are two results that can be inconsistent.
7. *The contingent test* involves a test in the first trimester (NT or combined test) and then, it is decided whether to continue with further tests or stop. Based on the use of two results the pregnancy is divided into three categories:
   - high risk, which is offered with a diagnostic test (villi sampling or amniocentesis) and to which various cut-offs have been proposed (1 in 10, 1 in 50, 1 in 100);
   - low risk, which is recommended to not be carried out with other tests because the result is considered to be satisfactory (1 in 1,000, 1 in 2,000, 1 in 2,500);
   - intermediate risk, with a risk between high and low, which is offered with additional tests.

   The risk cut-offs proposed in the literature and used in clinical practice are different (see above) with different results in terms of sensitivity and specificity and depend on various factors:
   - the economic resources available;
   - the objectives of the screening program: do you wish to reduce the number of invasive procedures or identify the highest possible number of af-

flictions? In the first case, the cut-off increases for high-risk (e.g., 1 in 10) in the second case the cut-off is lower (e.g. 1 in 2,500).

During the pre- and post-test consultation, the pregnant women must be informed of the various options, including the possibility of invasive prenatal diagnoses.

The additional tests in the contingent tests in the intermediate risk group can be:

a. *ultrasound*, the assessment of the nasal bone, the ductus venosus and the tricuspid valve, which allows for the identification of a higher number of afflictions. Each of the additional ultrasound markers can be evaluated in a single pregnant woman with a subsequent increase in sensitivity up to 93-96% and a contemporary reduction of the FPR to 2.5% (Kagan KO, 2009; Maiz N, 2009; Nicolaides KH, 2005; Cicero S, 2001; Cicero S, 2006; Matias A, 1998; Huggon JC, 2003; Faiola S, 2005; Zvanca M, 2011).
b. *fetal DNA Test or NIPT* (Non-Invasive Prenatal Testing), which has greater sensitivity (around 99%) and specificity compared to traditional tests.

8. *NIPT (Non-Invasive Prenatal Test)*: starting from the first trimester of pregnancy there is cell-free fetal DNA or cffDNA in the maternal bloodstream, which can be non-invasively sampled and used for the study of some fetal pathologies. Starting from the 10a week, there is enough cffDNA to carry out screening tests. The percentage varies between 0 and 25% with an average at 12 weeks of 12%. This technique is currently used in some countries to make diagnoses on fetal sex in pregnancies at risk of diseases connected to the X chromosome, like Duchenne muscular dystrophy, focusing invasive diagnoses and molecular tests only on pregnancies with male fetuses. The current techniques analyze the cffDNA fragment without differentiating between the fragments from the mother and those of the fetus. These tests are called NIPT (Non-Invasive Prenatal Test) and are considered "non-diagnostic" because they are based on a mixture of maternal and placental DNA. The specificity of the tests on cffDNA, in the studies with over 10,000 samples, reveals a <1/1,000 percentage of false positives, in confirmation of the percentage of feto-placental discrepancies that emerged from the chromosomal analyses carried out on the cytotrophoblast.
   The percentage of false negatives in the population relevant to the test is <1/100 (sensitivity 99%). Some international guidelines set objectives, choices and prescription methods and propose informed consent modules for the

patients. NIPT is mostly used to determine the risk of chromosomopathy, specifically aneuploidies 13, 18 and 21 and sexual chromosomes which constitute only a part, albeit the most significant one (50%-70%), of the chromosomal aberrations present in the fetus. It is currently considered the best screening test for identifying trisomy 21 on account of the results reported in the screening of the first trimester. The specificity of NIPT, relative to the screening of the aneuploidies and sexual chromosomes, is lower compared to the one obtained for the autosomes.

The scientific literature does not agree with the results reported by some authors near 100%. This is attributable to different mechanisms, even on a biological basis, and to constitutional mosaicisms confined to the placenta in the mother, which have been detected in a high percentage of cases (8,6%). In some pathologies like DiGeorge syndrome, the detection rate varies between 44.2% to more than 80% (Martin et al, 2017; Helgeson et al, 2015). At the moment there are no studies available concerning the general population; therefore, it is not yet possible to establish the validity and clinical utility of this screening when it is used on all pregnancies. At the moment the ccfDNA tests aimed at deletion syndromes are not recommended by the leading professional scientific associations, given the limited number of prospective clinical studies available (Committee on Practice Bulletin, 2016). It is worth remembering that some studies have shown that the weight and/or body mass index (BMI) of the pregnant woman can impact the concentration of cffDNA in her plasma. For example, obesity is associated with a reduction in fetal fractions (Ashoor et al, 2013; Wang et al, 2013). This data could be correlated with the increase of volume in the maternal circulation, which translates to a greater dilution of the fetal DNA (Canick et al, 2013). Alternatively, the reduced fetal fraction may be caused by an increase in apoptotic events or necrosis of the stromovascular fraction and the adipose tissue in obese women during pregnancy (Haghiac et al, 2012).

Comparison between NIPT and "traditional" screening methods

The results available indicate that NIPT is the prenatal screening test with the greatest sensitivity and specificity for the screening of trisomy 21, with a prediction accuracy of 99.9%. The accuracy is lower for trisomy 18 and 13 (around 98%) and the aneuploidies of sexual chromosomes (>96%). In all cases of high-risk NIPT, it is best to proceed to confirmation through a diagnostic test (amniocentesis or chorionic villi sampling). In around 2% of cases, a result is not

supplied due to the inadequacy of the sample correlated to a low concentration of the cffDNA in the maternal plasma. Used as contingent screening after the combined test, it offers the best model in terms of cost/benefits. Screening must be offered as part of a consultation with specialists in medical genetics and/or obstetrics and gynecology and a rigorous process of informed consent. The applications of screening to genomic pathologies (microduplications and microdeletions) and Mendelian diseases are not validated at the moment for their clinical use but are carried out in the context of research programs (for a general review see Consiglio Superiore di Sanità: Screening del DNA fetale non invasivo (NIPT) in sanità pubblica 2021, https://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua =italiano&id=3097).

Obstetric ultrasound

The ultrasound is the first method that offered the possibility of directly observing the fetus in its natural environment of the uterus, and following its development from the first weeks of pregnancy to the end. Its widespread use has substantially changed the assistance of pregnancy. The scientific data published over the years have defined its potentialities and limits in screening, diagnoses and monitoring of many fetal pathologies. Additionally, the use of the ultrasound as support is by now widespread and irreplaceable for obstetric checkups in detecting the presence of the gestational sac and/or of the fetus in the uterus, the fetal heartbeat, fetal movements, the number of fetuses, the lie of the fetus, and the amount of amniotic fluid. This is defined as an "office ultrasound" and is an integral part of the obstetric checkup. Results are reported in the obstetric file with the other parameters to be detected at each pregnancy checkup, with no specific clinical review requested.

The ultrasound plays a central role in prenatal diagnosis, particularly in the diagnosis of malformations. It allows for the accurate assessment of the age of the fetus (when carried out in the first half of the pregnancy); the following of its physiological growth and the detection of abnormalities in the fetal organs, systems, the placenta, the amniotic fluid; and deviations from the norm. Moreover, it is an integral part of all the biochemical screening methods and NIPT (see above) and an indispensable support instrument in invasive methods, from the simplest (see amniocentesis and sampling the chorionic villi) to the most complex ones (see surgical therapies).

The ultrasound is widely used as a screening and diagnostic test of structural malformations in the fetus. Much research has been carried out to determine

which strategies offer the best results in terms of efficacy, the accuracy in identifying malformations, and efficiency, the clinical implications of the diagnosis. There are three clinical implications of prenatal diagnosis:

- monitoring the evolution of the malformation in utero, which allows optimizing the timing, place and the method of birth to ;
- in utero therapy (see surgical therapies);
- termination of the pregnancy.

Currently, the most widely adopted screening policy in the world is to offer an ultrasound to every woman around a gestational age of 20 weeks (between 18-19 and 21-22). The specificity of the screening test in recognizing malformations in this period is very high (99%). On the other hand, the sensitivity varies according to the organ(s) afflicted and type of malformation at a maximum of 68% - 90% for nervous system malformations and a minimum of 6% - 30% for cardio-circulatory system malformations. The average for the total malformations reported in various studies is 30% - 60%. The sensitivity also varies based on the competence of the operators carrying out the exam, the instrumentation used and the organization of the screening. This explains the differences observed in the studies coming from different countries: from 70% to 10% considering malformations as a whole.

To optimize and homogenize the results, scientific associations have given instructions about the methodology with which to conduct the screening test so the dimension of the fetus, its movements, the anatomy of its organs and systems, amniotic fluid and the placenta are systematically checked according to standardized methods. The objective of this test is to verify that the anatomic-functional context is normal. If deviations from the norm exist regarding any aspect of the test (dimension of the fetus, anatomical structures, etc.), the woman must be referred for a diagnostic test. It is still under discussion whether there is enough evidence to carry out a screening for structural malformations in the fetus already at the end of the first trimester (12 – 14 weeks) when the sensitivity of the method is lower and there is a greater frequency of false positives. The usefulness of carrying out an ultrasound in the third trimester is discussed in a similar way, with the same view of diagnosing malformations, which could improve sensitivity by detecting the presence of late-developing malformations.

The diagnostic ultrasound aims to identify or exclude a specific fetal pathology and is carried out on a population of pregnant women who have a greater risk of a fetal pathology than the general population. The risk can be represented by a suspicion established at the screening ultrasound or by factors present before

the pregnancy (e.g., maternal diabetes, family history of malformations, intake of known teratogenic medication, etc.) or that appear during pregnancy, such as infections (see chapter 1). The test must be carried out in a focused way to recognize the abnormalities for which there is risk; indeed, it is also called a "focused ultrasound". It must be carried out with high-quality instruments, equipped with Doppler, color Doppler, and the possibility of visualizing in 3D and 4D. The person carrying out the exam, in addition to being competent in the use of the instrument, must have specific knowledge of malformation pathologies, their anatomic and functional characteristics, and the natural development of malformations of various organs and systems. For the definition of the diagnosis and its prognosis, it can be useful to make use of techniques (MRI, invasive diagnosis, etc.) and complementary experts (geneticists, pediatricians specialized in various disciplines, etc.). The exam should be carried out in a reference center or expert center that not only offers a diagnosis but also counseling for the woman/couple and the establishment of the diagnostic and therapeutic process.

It is necessary that ultrasound, both screening and diagnostic is preceded by information on its aims and utility, and limits, to support truly informed consent. For any type of ultrasound, it is essential:

- to use a correct working methodology as explained in the guidelines;
- draft an adequate report on the type of ultrasound carried out.

It is especially important to dedicate time, at the end of the exam, to explain what was detected, especially in terms of normality (which can require a short amount of time, especially if the reason for having the exam and its aims have already been previously explained) or pathology (which can potentially require further consultations).

INVASIVE DIAGNOSTIC TECHNIQUES

The analyses of the fetal karyotype and the search for specific genetic mutations are based on cytogenetic and molecular investigations carried out on fetal cells or those of the trophoblast. The sampling of these tissues/cells takes place via the introduction of a needle under ultrasound guidance. Such minimally invasive techniques are carried out in the firsttrimester (biopsy of the trophoblast or villocentesis or sampling of the chorionic villi) or in the second trimester of pregnancy (sampling the amniotic fluid or amniocentesis). The accuracy of the diagnoses carried out through these two techniques is considered to be greater than 99%.

Randomized studies carried out on large populations of pregnant women have highlighted that the risk of fetal loss linked to such methods currently

stands at 1:300-500 for amniocentesis and slightly higher for villocentesis, even if more recent studies seem to demonstrate a substantial overlapping in the risk of the two methods.

Genetic analyses

For many years prenatal genetic diagnosis has solely used amniocyte cultures that have been sampled via transabdominal amniocentesis in the second trimester, more precisely in the 16ª-18ª week of amenorrhea. The genetic tests use desquamation cells of the skin, mucus, digestive tract, respiratory tract, urogenital tract and the amniotic sac either cultivated *in situ* and analyzed on clones to optimize the identification of mosaicisms and maternal contamination or cultivated in jugs to increase cellular growth at the expense of clonal individuality. For genetic diagnoses, the fetal leucocytes are examined from the eighteenth week of amenorrhea are used (but only rarely) to settle interpretative doubts of mosaicisms identified on the amniocytes.

Since the 1980s diagnosis in the first trimester has become available around the 11ª-12ª week of amenorrhea, based on villocentesis and the acquisition of the cells of the trophoblast, chorionic villi, which can be analyzed with direct techniques for spontaneous mitosis of the cytotrophoblast. Diagnosis is available on average within 48-72 after the sampling, or on short-term cultures after the treatment of villi with proteolytic enzymes capable of disintegrating cytotrophoblast and the syncytiotrophoblast. Given the various advantages and disadvantages of the two methods, their combined use increases diagnostic accuracy since the cells analyzed through the two techniques have different embryonal origins. This is particularly important in cytogenetic diagnoses since around 2% of samples show a mosaicism (constitutional, confined to the placenta from maternal contamination). The laboratory that carries out cytogenetic analyses must be able to settle the interpretative doubts between first-level mosaicisms (single-cell pseudomosaicism), second-level mosaicisms (multiple-cell pseudomosaicism), third-level mosaicisms (true mosaicism) and contamination.

The principal indications for prenatal cytogenetic diagnoses concern:

- advanced maternal age, (empirically equal to or over 35;
- the presence of a balanced chromosomal anomaly (translocation, inversion), a homogeneous aneuploidy or a mosaicism (small supernumerary chromosome, autosomal trisomy or a sexual chromosome);
- previous pregnancy with an aneuploid fetus;
- an alteration of the combined screening test of the I trimester of pregnancy

(biochemical alteration in the mother suggesting an increased risk of aneuploidy and/or an ultrasound alteration suggesting a chromosomal pathology (e.g., increased thickness of the nuchal fold;
– the necessity of confirming the "diagnosis" of the aneuploidy at the cffDNA exam of the isolated sample from the maternal blood.

Traditional cytogenetic analyses are accompanied by and/or integrated with molecular cytogenetic techniques, in particular array-based Comparative Genomic Hybridization or SNP-array (*Single Nucleotide Polymorphisms*). Such an examination allows for the identification of variations in the number of copies, such as deletions or duplications/amplifications. Deletions are losses of a portion of the genome, while duplications/amplifications are defined as an excessive number of copies of inferior dimensions to those of the chromosomal resolution, which highlights imbalances of dimensions over 10 Mb (millions of base pairs). It is estimated that at least one in every 300 newborns is affected by one of the genomic pathologies. It is good practice to sample, at the same time as the fetal sample, the blood of the parents, which is indispensable for correctly interpreting the potential imbalance revealed in the fetus.

The fetal samples can also be used for biochemical examinations aimed at identifying metabolic diseases or at obtaining DNA to use for the molecular analysis of Mendelian diseases.

The study of the fetal karyotype (cytogenetic analyses) and the search for genetic defects (molecular analyses), in case of the confirmation of one or more congenital malformations, constitute an indispensable instrument for prenatal consultations and the elaboration of specific pre and postnatal care strategies Moreover, the genetic consultation based on the results of such investigations allows for the clarification of the couple's and other family member's future reproductive risk.

FROM TRADITIONAL TECHNIQUES TO "OMICS" ANALYSES

Depending on the timing and clinical indications, prenatal genetic diagnoses use a series of diverse examinations, including karyotype, arrays and molecular analyses. Their appropriateness is evaluated based on the utility of the result in the management of the pregnancy and not only their diagnostic capacity. In this sense, in pregnancies that do not carry a specific risk, indiscriminate application of predictive genetic examinations should be discouraged.

Next generation sequencing (NGS), and in particular the analyses of the exome or whole exome sequencing (WES), are changing the diagnostic approach

to genetic diseases. Considering that the primary objective of prenatal genetic analyses is to provide information fetal pathologies and their clinical management, unambiguous results must emerge from genomic analyses.It is important that the analyses are carried out on the trio (fetus + parents) and include the most complete clinical data possible (ultrasounds, family history, potential autopsy findings in the case of the termination of the pregnancy). The limitation of the objective data of the fetus and the lack of knowledge of the prenatal phenotype of rare diseases, which are mostly diagnosed after birth, often make the interpretation of the results from genomic analyses difficult. That's why on the report there must only be the variants classified as pathogenetic or likely pathogenetic, indicating the variants of uncertain or unknown significance (Variants of Uncertain Significance - VUS) only when they are associated with a known clinical background. Consequently, there must be a pre- and post-test consultation and management of the entire diagnostic process at accredited laboratories with professionals who are in full possession of the specialized clinic and laboratory expertise.

Time is critical in providing prenatal diagnosis. WES requires relatively long times, including those necessary for confirming the potentially pathogenic variants identified with alternative methods. With optimal organization it is possible to obtain the results within about ten days. WES, analogously to other genetic examinations, has numerous limits, as it does not identify the Copy Number Variants (CNV), small intragenic rearrangements and the mutations from triplet repeat expansion.

There are still few scientific studies on the applications of WES in prenatal diagnoses. They have a limited focus particularly on the diagnostic yield and lack follow-up data and r cost-effectiveness analyses. Best et al. (2018) have carried out a meta-analysis of 31 studies with a normal karyotype and CGH-array and have reported diagnostic success between 6.2 and 80% of cases. The highest diagnostic yields were obtained when the examinations were carried out on the trio (fetus and parents) or on individual fetuses that showed multiple defects or ultrasound pictures traceable to distinct groups of diseases (e.g., skeletal dysplasia). The ability of WES to increase the diagnostic yield during pregnancy has also been shown by a study by Xue et al. (2020), which analyzed a series of fetuses that had presented an increase in nuchal translucency with a negative CGH-array. Other studies, like those by Lord et al. (Lord et al, 2019), while confirming the potential of WES to increase the diagnostic yield, have highlighted the organizational and ethical issues in the use of this analysis in the prenatal field; the impor-

tance of resorting to WES only in select cases:the necessity of having the expertise to interpret the results; and the difficulties of the pre- and post-test consultation.

In summary, the studies available today are varied for the number of cases analyzed, the inclusion criteria, the gestational period monitored and the criteria used in the interpretation of the results. The clinical information is often limited to ultrasound findings, and only a limited number of studies have had an autopsy or postnatal follow-up. The International Society for Prenatal Diagnosis (SPD, 2018) has drawn up some recommendations aimed at laboratories that offer prenatal genomic analyses. In summary:

1. Multigenic panels, WES and WGS (analyses of the entire genome) can be used in pregnancies with malformed fetuses, ones negative to Chromosomal Microarray Analysis (CMA) or when there is a suspicion of a pathology associated with the single nucleotide variation (SNV), or in cases of a family history of non-diagnosed anomalies.
2. The introduction of "omics" techniques in routine prenatal diagnosis is not recommended, except in specific cases or for research purposes.
3. Analyses should only be carried out at accredited laboratories that meet defined quality standards, with experience in the execution and management of "omics" analyses, and within a process that includes a multidisciplinary pre- and post-test consultation.

Therefore, the literature and scientific organizations agree that the use of genomic analyses during the prenatal period can increase the diagnostic yield. However, at the moment, they should be reserved to select cases, such as defining the risk of reoccurrence of a pathology while considering the organizational and ethical criticalities and the limited knowledge at the time. Additionally, beyond the WES's technical limits, (e.g., the incomplete coverage of some genomic regions), its use in prenatal diagnosis is made even more complex by the limited development of the fetal phenotype, which evolves over time, and from the incomplete knowledge of critical genes during development (Feldkamp ML et al, 2017).

Table 2.4. Sensibility of the ultrasound screening of fetal malformations in Europe (EUROCAT, 2015)

Registry	Total number of cases	Number prenatally diagnosed	Percentage of all cases (95% CI)
French West Indies (France	608	419	68.9 (65.1-72.5)
Wessex (UK)	2200	1413	64.2 (62.2-66.2)
Paris (France)	3211	1799	55.5 (53.7-57.2)
East Midlands & South Yorkshire (UK)	6269	3023	48.2 (47.0-49.5)
Northern England (UK)	3008	1445	48.0 (46.3-49.8)
Isle de la Reunion (France)	1735	818	17.1 (11.8-19.5)
Thames Valley (UK)	2484	1141	45.9 (44.0-47.9)
Vaud (Switzerland)	1116	475	42.6 (39.7-43.3)
Auvergne (France)	1966	818	41.6 (39.4-43.8)
Hainaut (Belgium)	1179	487	41.3 (38.5-44.1)
Wales (UK)	5157	1929	37.4 (36.1-38.7)
Basque Country (Spain)	1797	672	37.4 (35.2-39.7)
Odense (Denmark)	380	208	35.9 (32.1-39.8)
Tuscany (Italy)	2579	913	35.4 (33.6-37.3)
S Portugal	778	271	31.8 (31.6-38.2)
N Netherlands	1876	611	32.6 (30.5-34.7)
Ukraine	6624	1135	31.3 (29.8-32.8)
Styria (Austria)	1105	336	30.1 (27.8-33.2)
Emilia Romagna (Italy)	3972	1080	27.2 (25.8-28.6)
SE Ireland	448	112	25.0 (21.2-29.2)
Valencia Region (Spain)	4950	1126	22.7 (21.6-23.9)
Saxony-Anhalt (Germany)	2419	547	22.6 (21.0-24.3)
Malta	416	92	22.1 (18.4-26.3)
Zagreb (Croazia)	686	146	21.5 (18.4-24.5)
Mainz (Germany)	632	132	20.9 (17.9-24.2)
Norway	7094	1429	20.1 (19.2-21.1)
Cork and Kerry (Ireland)	1044	181	17.3 (15.2-19.8)
Hungary	16101	1747	10.9 (10.4-11.3)
Total	**79067**	**24505**	**31.0 (30.7-31.3)**

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CHAPTER 3

Fetal therapies and quality of life

The goal of prenatal medicine is to modify the results of defects and ameliorate the natural history of pathologies diagnosed in utero through the study of pregnancy and fetal development. Once the prenatal diagnosis is established, the results and the implications of the detected pathology must be communicated to the parents. This piece of information is essential in offering the possibilities offered by pre and postnatal interventions must orient the approach to the prenatal diagnosis. The couple must be given an account of these possibilities in a timely manner, while also considering the limits of the therapies and the possibilities of improving the quality of life of the unborn or newborn. It is necessary to consider the concept of quality of life held by all parties before having pre-natal diagnosis.

FETAL THERAPIES

Treatable defects which are diagnosed in utero include fetal anemia, diaphragmatic hernia, cystic lung lesions, congenital heart diseases, obstructive uropathy, defects from the closure of the neural tube, sacrococcygeal teratoma, and some conditions linked to the development of the amniotic bands.

There are many causes of serious fetal anemia ranging from maternal-fetal infections, feto-placental hemorrhages, alloimmunization resulting from maternal-fetal incompatibility, and transfusions between twins.

Correction of serious fetal anemia is made possible through *fetal transfusion* which can be carried out using two techniques that require, respectively, the common cord access (IVT – Intravascular transfusion) or peritoneal access (IPT – Intraperitoneal transfusion).

The main purpose of fetal transfusion therapy is the prevention of hydrops due to the accumulation of liquids in the subcutis, lungs, abdomen, orpericardi-

um, with possible development of fetal heart failure. Intrauterinetransfusion can prevent prematurity and allow for a delay in the decision to give birth

The rate of complications, less than 10% for these procedures if carried out in a qualified center limits the use of the procedure. There have been reports of cases of fetal distress, with the need to carry out an urgent cesarean section, premature rupture of the membranes (pPROM), intense abdominal pain, unusual vaginal bleeding, increased infection risk and fetal damages.

Complicated monochorionic twin pregnancies represent around 20% of spontaneous twin pregnancies and can encounter complications like twin-to-twin transfusion syndrome (TTTS), TAPS (twin anemia polycythemia sequence), selective intrauterine growth restriction (selective IUGR), and twin reverse arterial perfusion sequence (TRAP). The diagnosis of a monochorionic pregnancy and the earliest possible identification of complications are essential in offering proper consultation and possible in utero therapy shown itself to be effective in reducing adverse fetal-neonatal outcomes.

The presence of a single placenta with vascular anastomosis between the two fetuses can cause serious complications. These complications are defined as twin-to-twin transfusion syndrome (TTTS), which is characterized by polyhydramnios and polyuria in the recipient twin and oligohydramnios and oliguria in the donor twin. The prognosis is unfavorable if effective measures are not taken: 90% of non-treated cases can lead to perinatal death. Selective fetoscopic laser coagulation via placental anastomosis is the only effective therapy for TTTS. A randomized European clinical experiment demonstrated the advantage of the laser technique over other treatments. The advantage for the twins that receive the therapy has been confirmed six months after the birth, with a significantly higher survival rate ($P = 0.002$) and a lower rate of neurological complications (31% vs 52%). The procedure lasts seventy minutes on average and a longer operation time correlates to a greater rate of adverse effects. As with all fetal surgical procedures, the learning curve is important, and the cases must be sent to experienced centers specialized in the treatment of TTTS. The complications linked to the procedure are the persistence and reoccurrence of anastomosis, fetal hemorrhaging and fetal death.

Even the twin anemia-polycythemia sequence can be the result of the persistence of residual anastomotic groups. The premature rupture of the membranes occurs in about 27% of cases after the procedure. The formation of amniotic bands capable of causing ischemia of the limbs in 1-2% of cases has been described as a consequence of laser therapy.

Fetal surgery is still hampered by significant rates of obstetric complications, even serious prematurity and mortality. Recommendations for this type of therapeutic intervention must be extremely selective and assessed in the context of the risk induced by the intervention itself versus the intrinsic risk of the pathology. This creates an ethical debate on the use of the proposed procedures with the fetus taking on a central role as the patient. Delicate ethical and legal questions arise regarding the responsibility of the mother for therapeutic interventions benefitting the unborn.

Some fetal pathologies result in neonatal mortality. Seeing that these procedures are being carried out earlier and earlier, one should consider their reduced effectiveness due to extreme prematurity. It is necessary to examine the risk and benefits of the possible therapeutic interventions, both for the mother and the fetus, for an adequate ethical assessment.

Some of the conditions for which a surgical approach has been developed include:

1. *Congenital diaphragmatic hernia (CDH)* is the incomplete formation of the diaphragm during embryogenesis which results in herniation of the abdominal viscera in the chest. This leads to pulmonary hypoplasia and pulmonary hypertension that can result in cardiorespiratory insufficiency in the neonatal period. There is a wide spectrum of severity in the disease. Progress in neonatal care and the introduction of extracorporeal membrane oxygenation (ECMO) have improved outcomes in many cases but the most severe defects are still associated with morbidity and mortality. Various techniques have been developed over the last 30 years to treat congenital diaphragmatic hernia in utero. Fetoscopic endotracheal occlusion (FETO) is the option that has by far shown the best result and has lower maternal morbidity and shorter surgery times.
2. *Pulmonary pathologies* (CCAM and BPS) Congenital cystic adenomatoid malformation (CCAM) is a rare condition characterized by an altered development of the last portion of the bronchioles (terminal bronchioles) that increases abnormally until the creation of cystic formations. Bronchopulmonary sequestration (BPS) is characterized by the presence of islands of pulmonary tissue provided by the circulatory system and not by the pulmonary one. The two conditions are not exclusive and a certain level of overlap between the two pathologies exists (in this regard alternative nomenclature has been suggested). Their prognosis is generally good, with a notable spontaneous rate of repair (15%-65% in the case of cystic adenomatoid disease and

68% in the case of pulmonary sequestration). The surgical treatment of embolization, ablation with radiofrequency, interstitial laser, and open surgery is generally reserved for preterm newborns (< 32 weeks) who have already developed fetal hydrops. Inducing the maturation of the fetal lung with steroids remains of vital importance as it constitutes first-line treatment for fetal lung lesions.

3. *Congenital heart disease*. Aortic stenosis and pulmonary atresia represent congenital heart diseases for which various in utero treatments have been developed. Fetal aortic valvuloplasty has been made possible percutaneously or via fetoscopy. The primary objective of this surgery is the improvement of postnatal outcomes and the prevention of ventricular remodeling. The expansion of the pulmonary valve in utero is aimed at correcting the atresia even in fetuses that have already developed hydrops.
   Through a percutaneous or laparoscopic opening, with the aid of the ultrasound, one accesses the valve and expands it using a balloon. Another potentially treatable pathology in utero is hypoplasia of the left heart. There have been reports of techniques with percutaneous pacemakers in cases of fetal bradycardia that are not responsive to medical therapy.
4. *Neural tube defect*. Spina bifida is a congenital malformation of the central nervous system caused by an incomplete closure of the neural tube in the first 28 days of gestation. The global incidence of spina bifida is around 4.63 for every 10,000 births.
   Spina bifida occulta includes a group of spinal malformations with intact overlying skin that are not associated with hydrocephalus. The neurological consequences of these forms are generally milder. The neurological effects of spina bifida worsen during gestation. These observations have developed the "two-hit" hypothesis, according to which the neurological deficit derives from two factors: the failure of the formation of the neural tube and the traumatic and chemical lesion caused by the amniotic fluid. The corollary of this theory is that early repair in utero should improve the prognosis of the affected fetus. In utero repair of the defect is associated with more favorable neurological outcomes even if it increases the risk of preterm birth and premature rupture of the membrane.
5. *Lower urinary tract obstruction* (LUTO). The creation of a vesicular-amniotic shunt is the main intervention carried out in the case of lower urinary tract infection. Other fetal interventions include ablation of the valves that obstruct the urinary tracts via fetoscopy, fetal vesicostomy and fetal ureterostomy. The main objective of these procedures is not to preserve renal function, but rath-

er to obtain enough liquid amniotic fluid to prevent pulmonary hypoplasia and increase the possibilities for the fetus to adapt to postnatal life. The main recommendations for prenatal surgery are ultrasound identification of the obstruction (hydronephrosis, fetal megacystis or renal alterations), single pregnancy, the exclusion of concomitant malformations, oligohydramnios, anhydramnios and normal karyotype. In female fetuses, the shunt is not usually proposed, because the most severe forms of urinary obstruction are present (obstruction or urethral atresia). The urethral valves can be corrected with the resection of the valve itself, while urethral atresia requires a more complex surgical intervention.

It is possible to obtain a sample of fetal urine through a vesicocentesis to study renal function and the severity of the case. Good renal functionality before the intervention is considered to be a favorable prognostic factor, even though the risk remains of the newborn developing renal failure later on.

The creation of a vesicular-amniotic shunt is an invasive procedure with specific complications like the retraction or occlusion of the shunt (up to 34% of cases) and the development of fetal ascites. Other possible complications are hernias of the abdominal wall, vesical lesions, the preterm premature rupture of the membrane (pPROM), preterm labor and stillbirth. The overall rate of post-procedural complications is around 40%. Because the prognoses for fetuses with LUTO can be poor, the choice of treatment should be made by carefully evaluating the costs and benefits.

6. *Sacrococcygeal teratoma.* The prognoses for fetuses with sacrococcygeal teratoma are generally good. Some serious complications can develop in utero such as heart failure, hemorrhage and stillbirth. For fetuses who develop hydrops, the mortality in utero draws closer to 100%. Severe maternal complications have been described, such as "mirror syndrome", a severe form of preeclampsia. If the mother is in good condition, various surgical procedures are available: open fetal surgery, ablation with radiofrequency or laser and sclerotization with alcohol. The survival rate reported after open fetal procedures ranges from 33% to 75%. In the majority of less-severe cases, it is possible to delay the treatment until after birth.

Fetal surgery is in rapid developmentbut there are few conditions today that are treatable with prenatal surgical interventions. Some of the interventions described are still marked by a high rate of maternal-fetal complications (preterm birth, pPROM, stillbirth, placental abruption, uterine lesions and increased rate of utero rupture in later pregnancies). The complexity of the subject contributes

to the difficulty of clinical trials. The bioethical issues are still under discussion (see the declarations of the American College of Obstetricians and Gynecologists in this regard). The study of these techniques should be implemented in specialized centers and monitored by competent authorities to develop.

QUALITY OF LIFE

The traditional duty of a doctor is to act in the best interests of the patient. In pregnancy, this includes the mother and the fetus. TWe encounter competing values in this perspective. Therefore, clarification is required.

The concept of the quality of life (QoL) is relevant in assessing medical benefits and risks. The notion of QoL is not in conflict with the dignity and inviolability of human life, as they are complementary from the point of view of health and wellbeing. For some QoL is the main criterion for medical interventions. The assumption underlying this perspective is that a greater or lesser degree of QoL changes the value of human life itself. This "anthropological" jump is no small matter as it an understanding of life not as an ontic good but a useful good, whose value is subordinate to its "quality". TIt should be placed as a goal through the care and solidarity of the health system and the whole of society. For a correct understanding of QoL, education is necessary.

Interpretation of the quality of life

Because of the inherent dignity of each human being, there are no essential differences in value between different human lives. Dignity is an inherent, constitutive characteristic of human beings in such a way to make them "priceless". It is neither measurable nor modulable based on a quantitative gradation. Healthcare decisions cannot take it as being the definitive criterion.

In our pluralist society, QoL is interpreted in different ways so we find confusion in clinical and ethical discussions. One interpretation concerns a subjective assessment by the person. Such an assessment is an essential component of the person's values and preferences. It is the basis of freedom of choice for individual's looking for medical care to obtain pain relief and functional improvement. Improving quality of life through diagnostic and therapeutic advances and support and care, guided by the patient, constitutes a fundamental aim of medicine. This is not possible in the case of the fetus.

Another perspective, however, interprets QoL as an evaluation of an observer who looks at others unable to express preferences in therapeutic choices. This is

the case of the fetus/newborn or a person incapable of giving consent. This meaning of QoL causes many problems in the field of prenatal diagnosis, particularly for pathologies with serious prognoses. The perspectives that the doctor presents to the parents implicitly refer to some standards of QoL, based on what is held to be acceptable or unacceptable in the social and healthcare context in which it takes place. For a fetus/newborn affected by spina bifida and myelomeningocele, the QoL may be held to be lower and marked by severe dysfunctions, to the point of being unacceptable, with death being preferable to the continuation of life as in the Netherlands Groningen Protocol. Neurosurgeons with experience in this field could present the parents with the perspective of surgical intervention that allows for an acceptable QoL. Therefore, it is necessary to carry out an overall assessment of the risks and benefits of the available treatments in concrete circumstances concerning all the subjects involved. In this assessment, the criterion of QoL cannot be considered exclusively but should be integrated into the overall assessment.

There is an essential interdependence of fetus and mother and child and family. In the case of the fetus and newborn, the impact of its disease on the life of the parents and the entire family unit should be considered. TIt is necessary to avoid healthcare decisions that hurt the child or increase the burdens on the family or society.

The understanding of a good life in each culture is relevant. When one says, for example, that the newborn will have a learning disability and therefore poor QoL, one is expressing the value system of a society that emphasizes intelligence and productivity. In countries in which the healthcare network is more comprehensive and provides social and rehabilitation services and special education (Thiel MJ, 2019), the acceptance of disabled children occurs more easily and comfortably compared to countries in which healthcare policies penalize these services.

Difficulty in recognizing and welcoming forms of difference that are perceived as "other" and that do not correspond to the tacitly shared ethos exerts a strong influence on the welcoming of disabled people. The guiding criterion of clinical decisions should always be the one that is in the greatest interest of the child, even if it enters into conflict with the interests of the family. These obstacles can be overcome if the healthcare team can adequately tend to the communication and plan a care path, trying to find a solution and favoring the integration of the immediate emotional impact into a broader, long-term framework. Due to the complexity of factors in play including health, professional, social and

cultural dimensions all the parties must assume their individual and collective responsibilities.

Quality of life and post-diagnostic perspectives

During the prenatal consultation, the expression of a disease being "compatible or incompatible with life" is often used. Given the prognostic difficulties and the wide variety of perspectives on the notion of QoL , there needs to be a broad vision when responding to the questions the families are facing. The communication of the diagnosis represents the crucial moment in which, in addition to the type of pathology and its clinical characteristics, one must indicate which life prospects the fetus/newborn will have. In the case of Down's syndrome, for example, the couple must be supplied with the necessary information to understand the specific clinical condition. It is necessary to make sure to provide the couple with a framework that includes alternative solutions to the termination of the pregnancy. The same thing happened for children with Down syndrome: from the moment in which they began to undergo corrective surgical interventions, their lifespans increased and the relative importance of the genetic syndrome in the assessment of treatments to be used is now considered differently, being a standard procedure to treat them like all other children who do not have genetic syndromes.

For example, both Edwards syndrome (3/10,000 live births) and Patau syndrome (2/10,000 live births) are rarer than Down's syndrome but have a decidedly greater impact on cognitive and motor skills.

It is important to distinguish between the various aneuploidies distinguishing trisomy 13 and 18 from trisomy 21. Cases of mosaicism have been reported for trisomy 18 and 13, but these are rare. The natural development of these syndromes is changing. The causes of newborn death with trisomy 18 are mainly respiratory, central apnea and obstruction of the respiratory tracts caused by craniofacial malformations, cardiac or infectious. With the advances in *neonatal intensive therapies*, especially assisted ventilation, and invasive and non-invasive techniques , the causes of death have been progressively reduced, increasing the life expectancy of these newborns. The treatment of heart failure due to congenital heart diseases and palliative surgery has reduced the mortality from cardiac causes as well. The certainty that children with trisomy 18 will encounter an early death or a low QoL is changing thanks to early interventions that can increase their lifespans and improve quality. This is highly variable and based on the capacity of parents and families.

Families of children with handicaps often meet in associations for peer support sharing of the experiences and the "progress" made by their children. The literature now demonstrates that children subjected to surgical correction/palliation for congenital heart disease, in carefully selected cases, have a greater lifespan than those who are not treated. so, treatment of heart disease is no longer considered "futile" or "out of proportion".

From quality of life to quality of care

Many life-limiting conditions are diagnosed before birth as a result of progress made in fetal assessment. Facing the prenatal diagnosis of a pathology with an unfavorable prognosis or marked by severe malformation or threat to life, it is necessary to change perspective focus on the quality of care that the child should receive.

An important contribution to changing attitudes towards these children affected by pathologies has come from the philosophy of palliative care and perinatal hospices. They are not yet widespread but have shown the ability to welcome and accompany a child and family affected by a prenatal pathology as an alternative to the termination of the pregnancy. Perinatal hospice contains specialized hospital multidisciplinary team consisting of specialized medical, nursing, psychological pastoral and bioethical professionals, among others, whose aim is to welcome, support and accompany families who find themselves facing prenatal diagnoses with serious pathologies.

For the families that choose to bring the pregnancy to term, it is important to establish a personalized healthcare plan for each child after the birth. When the diagnosis of a serious pathology is certain and the prognosis unfavorable in the short term, the decision should be aimed at providing a care plan limited to guaranteeing therapy for the pain and comfort of the child.

The congenital conditions that limit life can be defined according to two categories: (1) a condition that limits life with a foreseeable early death within a few hours or days, with or without intensive care and (2) a condition that limits life, but with longer foreseeable survival. In the second case, intensive therapy can prolong life for days or weeks, but the burdens could be judged to be greater than the overall benefits that one can reasonably expect. In this second situation, the assessment of the elements in play is highly delicate and demanding. or malformations, which are often incompatible with life, while providing comfort care.

In conclusion, QoL cannot be the defining criterion of the dignity of the person and, therefore, cannot be the criterion that justifies interventions aimed

at selecting, through the termination of the pregnancy, who has a right to life. Rather, QoL has the role of recognizing and pursuing the "global" good of the fetus/newborn. It is, therefore, necessary to consider the clinical condition of the affected subject and the ability of the person to pursue the objectives and goals of life, understood as values that transcend the physical life. Lastly, decisions based on the concept of a "dignified" life or "quality" life are dangerous if made by an outside observer. The subtle difference between intrinsic dignity and perceived dignity must be clear to all who make decisions for those who cannot personally do so. The extreme complexity of the situation should be considered seeing how the person in question cannot express an opinion.

Discussing choices of QoL with the parents regarding the fetus/newborn does not imply an obligation on the part of the doctors to go against their moral conviction based on the desires of others, but the freedom of the doctor, as well as the woman/couple, to decide should be considered equally based on the ethical, moral, social conditions in which they live and the possibility of care they can provide for the child. The doctor/researcher is also concerned with helping truth and life come together, a task that cannot be carried out with ascetic neutrality, even when one must properly respect another's freedom. Scientific truth, the values of human life, freedom and responsibility and the conscience of the parents and doctor are the most important concerns when making ethical medical decisions.

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CHAPTER 4

Information, quality, and importance of communication

Prenatal diagnostic technology, particularly fetal ultrasound imaging, has allowed for unprecedented insight into fetal health and development. Understanding the accuracy and purpose of specific diagnostic interventions has important implications for communication and decisions for optimal maternal lifestyle and prenatal care.

COMMUNICATION AS PART OF THE MEDICAL ACT

The ultrasound is an instrument that helps create an emotional relationship with the unborn who. becomes more real from the moment in which the embryo and, later, the fetus is visualized. The experience of fetal movements on the part of the mother in the second trimester of the pregnancy strengthens the mother-child relationship. Its physical presence and "personality" becoming another important step in the psychological transition towards parenthood.

Ideally, pregnancy is a positive, peaceful and fulfilling experience culminating with the birth of a healthy child. For the majority of couples, the ultrasound represents a step to be confronted without anxiety or worry and is appreciated, especially when it satisfies the expectation of the parents.

However, the prenatal diagnosis can also bring disquieting news on the health and development of the unborn causing a collapse of expectations and cause genuine ailments for the mother and couple.

The safety of the fetus plays a significant role as well. If for some parents the ultrasound is one of the most emotional moments and full of positive expectations, it is also true that many parents feel anxious and worried about what the exam might reveal. The prenatal diagnosis of a congenital anomaly in the fetus is often a devastating event for families. Following such an event, the family members must come to terms with the loss of the idea of having the "perfect child"

and the sudden evaporation of life plans. They are often faced with the choice of terminating or continuing with the pregnancy. Not infrequently, however, the diagnosis and especially the prognosis of the defect is uncertain, representing a further complication in acceptance and decision-making.

Non-invasive prenatal screening methods such as the combined test, cffDNA or NIPT, and invasive villocentesis and amniocentesis create even more worry and fear of pathological outcomes because they are often offered, especially with invasive techniques, as second-line tests in case of increased risk or the diagnosis of a verified or suspected, fetal anomaly.

When the first trimester screening, the ultrasound or the genetic analysis, results in an unexpected outcome, such as a soft-marker, the suspicion of a congenital malformation or the diagnosis of a chromosomal anomaly, it is necessary to carry our precise care strategies aimed at accompanying and supporting the parents in managing their anxiety and stress and in making decisions that inevitably they will be faced with. A key point in these strategies is without a doubt information.

INFORMATION TO THE COUPLE

The communication of an unfavorable diagnosis to the couple, whether it is a congenital malformation or a genetic anomaly, is a particularly delicate moment in the experience of the pregnancy as it produces a sharp and unexpected interruption in the realization of the life "plan" that the parents had fetus of and imagined. The moment of the first diagnosis and the following meeting are particularly significant and complex because everything that gets said and explained at that moment, how it is said, the doctor's attitude or tone of voice and the surrounding environment, can decisively condition the rest of the communication process and, as a consequence, influence the parents' attitude and choices. It is as if an emotional rather than rational imprinting takes place. This is particularly true for an ultrasound diagnosis of a congenital malformation, which is more often than not a completely unanticipated event.

Despite significant technological and medical-scientific progress, with a refinement in diagnostic capabilities and progressively earlier prenatal diagnosis, today's most common scenario for congenital anomalies is an ultrasound screening in the third trimester or a "morphological" ultrasound. It consists of an ultrasound test for the general population that must be carried out in the fifth month of pregnancy (in Italy between 18 and 21 gestation weeks) and that includes, among other things, the study of the fetal anatomy with an aim to excluding the

main congenital malformations of some organs and systems. The organizational model for pregnancy care in some healthcare systems in industrialized countries requires that the screen test (such as the "morphological" ultrasound) act as a first-level "filter" aimed at the identification of high-risk cases of pathology or with pathologies already present, in addition to reassuring the couple of the fetus' wellbeing and the absence of pathologies. The high-risk or pathological cases are then referred to second-level structures that are tasked with confirming the diagnosis, prenatal consultation and the clinical care of the pregnancy and the pathology in the postnatal period.

The first communication of the diagnosis to the couple inevitably takes place at the moment of screening. This takes place in technically "ideal" conditions by a medical sonographer with proven ability and experience with equipment whose technical specifications fully satisfy the needs of the case. However, the process of communication is almost invariably inadequate for a response that is appropriate to the needs of an unfavorable diagnosis.

The post-diagnostic prenatal consultation

The emotional impact of "bad news", comprehension of all the information related to the clinical conditions, and the level of acceptance of the care paths proposed are strongly conditioned by the ways they are communicated. The prenatal consultation is aimed at communication and on developing an empathic, trusting relationship with the mother or the parents, which is necessary for assuming one's professional responsibilities.

Every aspect of communication must be taken under proper consideration. Particular attention should be paid to the terminology utilized. Terms like malformation, disability and impairment can give rise to ideas and convictions related to the fetal prognosis. The term "disability" can be used to refer to the loss of physiological and/or psychological functions. It is important to understand the couple's beliefs about the impact of disability will be on the QoL of the unborn.

– The purposes of communication after the diagnosis of a fetal pathology include:
  1. description of the type of anomaly shown;
  2. possibility of an in-depth examination of the diagnosis (when necessary);
  3. definition of fetal and postnatal prognoses (results);
  4. possibility of in utero treatment (maternofetal risks);
  5. assessment of maternal risk correlated to fetal pathology;
  6. necessity of maternofetal monitoring and a postnatal follow-up;

7. possibility of postnatal treatment (neonatal risks and later results);
8. choice of location, method and timing of the delivery;
9. the prospect of supporting the fetus with a pathology incompatible with life;
10. clinical ethics consultation and the shared ethical-care document, where possible;
11. the prospect of perinatal hospice.

– Before the meeting:
  - *Establish who is to participate*: usually the mother, the couple and any people indicated by the parents like relatives or within this setting.
  - *Decide which professionals should be present*: based on the pathology in question, the gestational period, the objective of the meeting and the psychological state of the couple. The number of professional figures involved at the same time should not be excessive: the gynecologist, the pathology specialist, and the neonatologist are the key figures along with the perinatal psychologist. They have the task of introducing the couple, with their history and past and present experiences (potentially gathered in a preliminary interview), to the team of specialists and "orchestrating" the consultation; interpreting the parents' worries and anxieties; and adjusting communication, in content and manners, according to the objective. The presence of trainees is only possible with prior consent from the parents.
  - *The space*: should be a different place and time than those used for the ultrasound. The room should be private, quiet and exclusively dedicated to the activity of the consultation with seating for everyone present. It is advisable to turn off the ringtones for both landlines and mobile phones to avoid interruptions and a consequent loss in attention.
  - *Agree upon the content of the meeting*: it is important to agree upon the contents of the meeting with the various professionals, depending on whether it is the first meeting or a follow-up, and based on the information provided by the psychologist with regard to the couple. The pertinence, accuracy, consistency and clarity of the information received in the various meetings eases comprehension on the part of the parents and reduces the gap between the contents shared and processed. To this end, it would be appropriate to limit the number of topics addressed in each meeting by as much as possible to avoid overloading the couple with what is often complex and difficult information to absorb and remember. Moreover, it is recommended to use outlines and diagrams that are pre-printed or made directly by the professionals, as they are useful for

improving the understanding of the anatomical characteristics of the pathology and clarifying the results of the genetic analyses.

– During the meeting:
  A feeling of acceptance from the couple and a positive willingness to listen from the professional figures depends on the creation of a "comfortable" and reassuring atmosphere.
  - At the beginning of the meeting, approach the family and clearly indicate where everyone can sit down within the room. All the members of the medical team must introduce themselves, clearly mentioning their names and roles.
  - Address the parents and other potential family members with their names.
  - Ask the parents if they have already chosen the name of the boy or girl and if they wish to use it to refer to him or her.
  - Get ready to listen and be open to those present:
    - invite the couple to express their outlook on the fetus' pathology, even with a reconstruction of the various stages of the process carried out until that moment;
    - always use direct and easily understandable questions;
    - accept possible moments of silence connected to emotionally "difficult" moments;
    - use a gentle and friendly tone of voice;
    - maintain eye contact with those present;
    - avoid interruptions;
    - do not impede on emotions, welcome various points of view or various ethical and religious approaches;
    - give due consideration to non-verbal aspects of communication (looking away, sighing, crying, hesitating, etc.).
  - Provide the couple with the necessary information to face the stage they are going through.
  - Provide information with honesty, ensuring the parents that they will always be provided with clear information on the conditions of the mother and the fetus; do not hesitate to communicate uncertainties or lack of knowledge, always starting from the best prospects (e.g., speaking of survival rather than mortality).
  - Proceed according to a "path" with stages (e.g., describing the situation, necessity of diagnostic/monitoring tests, therapeutic or care paths/possibilities), giving them the necessary time to assimilate the information

and checking their understanding through targeted questions. Adjust the information according to the necessities of the couple.

– After the meeting:
  - At the end of the meeting, the couple is often disoriented, having just been forced to redefine and reprocess the image they had; consequently, they are prey to various emotions that are difficult to put together and express. Therefore, it is important to recognize, value and make sense of their worries, fears, confusion and, sometimes, even apparent indifference from one or, rarely, both of the partners.
  - Briefly go over what was said during the meeting, once again looking for unanswered questions (what the main worries of each parent are, what news has yet to be provided or has not been clearly understood, what can be truly useful in that particular moment).
  - Make oneself available to once again discuss the most complex issues and the most delicate subjects.
  - Suggest writing down questions that might come up after the meeting and that could be asked in a future meeting.
  - Provide a written report that contains a comprehensible synthesis of the meetings' contents (highlighting the parts one wishes to reinforce), the information regarding the next steps in the care path, general information and contact details (phone and/or email) of the professionals who took part in the meeting.
  - Take leave by planning, when necessary, the next meeting, which can be established not only based on objective clinical concerns but also on the psychological state of the couple to mitigate and manage the anxiety and worry during the time between the various tests.
  - The mother/couple must know who will be taking care of their child/get to know the space where the child will be hospitalized and cared for; if possible, the mother and the father should be able to visit the Neonatal Intensive Therapy specialized department before the birth.
  - The psychological specialist who meets with the parents in the prenatal phase should be the same one that works within the departments of the medical-nursing team.

Description of the type of anomaly shown

For further understanding of the pathology shown in the prenatal diagnoses and the various pre and postnatal scenarios that they entail, it is appropriate that

some salient and easily interpretable images are used, i.e., images taken from reference books, illustrative anatomical tables or simple drawings made by the same doctor leading the meeting. This information can be integrated with an adequate explanation of the genetic tests and their meanings.

Possibility of in-depth examination of diagnosis

All of the possible instruments that will be potentially useful for diagnostic precision or investigation must be identified with their clear risks and benefits.

Definition of the fetal or neonatal prognoses (results)

The possibility that one's child could die before being born or immediately afterward has a strong psychological impact on the future parents. However, distress from the possible suffering and the "disability" that can derive from a serious congenital anomaly can weigh even more heavily than the fear of loss. The possibilities offered by medical science today have progressively reduced the rate of fetal loss and neonatal death; however, neonatal and pediatric morbidity have not experienced a parallel reduction. The materno-fetal medical team's task is to present, within the limits of scientific and medical technology, every possible scenario from the perspective of survival/mortality, complications, and long-term results.

Possibility of in utero treatment (materno-fetal risks)

Over the last decade, within maternofetal medicine, the highly specialized sector of in utero "surgical" therapy has progressively developed. The rationale for treatment of the fetus affected by a congenital malformation consists of the possibility of modifying the natural development of the anomaly early on in an attempt to improve its outcomes. The potential benefit of this approach must inevitably be counterbalanced by a rigorous assessment of the maternofetal risks.

Today it is correct to state that in utero surgical therapy is only feasible if:

- the prenatal diagnosis is reliable and documented in detail;
- the physiopathology of the congenital anomaly is well known;
- there is reasonable certainty of a negative prognosis in the absence of treatment;
- the surgical technique under consideration has been widely tested and validated;
- maternal wellbeing is not at risk of being compromised.

Necessity of materno-fetal monitoring and postnatal follow-up

The concept of a care path, the continuity of care and the possibility of sensing the presence of a well-structured and organized medical-nursing and social-health support network is of fundamental importance in the creation of a trusting relationship between the parents and the medical-nursing team.

It is necessary to put forward as precise and foreseeable a program as possible, even allowing the couples who travel from afar to visit the fetal medical center to organize their work schedules and manage family needs based on the pre- and postnatal test schedule.

Possibility of postnatal treatment (neonatal risks)

A fundamental role in the prenatal consultation is carried out by an expert specialist of the postnatal medical-surgical treatment (neonatologist, otolaryngologist, general surgeon, neurosurgery, maxillofacial surgeon, plastic surgeon, expert in metabolic diseases, etc.). This specialist's task is to present the treatment possibilities by outlining their characteristics, through simple and comprehensible terminology, aims, timeframes (even the potential necessity of repeated interventions) and, most of all, risks.

Choice of location, method, and timing of the delivery

The detection of a congenital pathology of the fetus often requires the reorganization of the care program, especially with regard to the location, timing and often the delivery method.

Based on the type of anomaly diagnosed, it will be necessary to direct the pregnant woman towards a leading center for "at risk" delivery and intensive neonatal care, or it will be possible to allow the delivery to take place in the originally chosen location with the woman's gynecologist.

In the first case, the contacts between the couple and the medical-nursing staff of the "new" birth location will be established early on to quickly create a solid and effective relationship of trust that can take the place of the previous one, allowing for a reassuring balance. In the second case, it would be appropriate to set up an active collaboration between the gynecological and neonatology teams responsible for the delivery and the neonatal care and fetal medicine center specialists, also to evaluate the need to transfer the newborn to the childbirth center at the hospital specialized in the medical-surgical postnatal care.

The prospect of accompaniment the fetus with a pathology incompatible with life

The guiding criterion must always be the care and accompainement of the fetus in a continuative and integrated approach. The possibile conflict with the opinion of the parents can be circumvented by adequately providing the information, the support that can be provided and managing the emotional impact that this information could create, favoring the assumption of specific responsibilities by all the parties involved.

The clinical ethics consultation and the shared ethical-care document, where possible

The clinical ethics consultation, where it is possible carried out by a single consultant, a group or a committee, can support the pre and postnatal consultation. The objective is to make ethical decisions that emerge in the specific situation of the diagnosis of a serious fetal pathology easier. This consultation can take the form of a shared ethical-care document as an expression of a plan of interventions to undertake, sharing care objectives and values that take all the parties involved into consideration (parents, gynecologists, obstetricians, neonatologists, nurses, psychologists, etc.).

This document is a tool to personalize the decisions related to a specific case in which the interdisciplinarity helps to identify the greatest good for the fetus and mother, ensuring that the clinical and contextual aspects cause one to rethink the theoretical aspects as well, which get illuminated and enhanced by the specific case.

The prospect of perinatal hospice

Perinatal hospice is a healthcare, scientific and ethical response to pathological prenatal diagnosis based on fetal medicine and prenatal palliative care. It focuses on the fetus as a patient even in the most extreme pathological conditions. It assumes an interdisciplinary specialist approach that, in the face of prenatal diagnoses of serious pathologies and malformations often incompatible with extrauterine life or with life-limiting conditions, aims to achieve, through a relationship model, "shared care". The objective is to provide "integrated" specialist care that is not only founded on medical-scientific expertise or state-of-the-art diagnostic-therapeutic techniques but also on the family's experience and testimonials to support the couple in welcoming, caring for and attending to their child.

Cultural-societal contextualization

Couples counseling related to prenatal diagnosis, especially when communicating a congenital fetal condition, may require the presence and intervention of a neonatologist/pediatrician who will care for the newborn. In more complex cases, such as rare or ultra-rare diseases, the contribution of a specialist pediatrician in the diagnosed condition is also useful. This specialist should outline the care program to the couple, which includes, when necessary, further diagnostic investigation. If possible, they should suggest the most appropriate and effective treatment, taking into account ongoing scientific advancements.

However, if providing up-to-date medical-scientific information is necessary for an informed decision by the couple, it is equally important to illustrate the various aspects of the experiences of patients with the identified prenatal diagnoses, including future quality of life considerations, while considering the healthcare and social context in which the child/adult will live.

a. When it comes to medical-scientific information concerning established fetal pathology, advancements in biomedical research have led to the availability of highly precise and reliable diagnostic technologies. Notable examples include next-generation sequencing (NGS) for genetic-molecular investigations and the enhanced resolution of prenatal imaging equipment in detecting birth defects. However, even in cases where accurate prenatal diagnosis of such defects (such as malformations, inherited metabolic diseases, genetic disorders, etc.) has been made, general geneticists, gynecologists, or non-specialists may not possess the latest knowledge regarding the specific pathology. In such instances, the involvement of a specialist becomes extremely useful, if not indispensable, for that particular condition. The information provided to the couple enables them to make informed decisions regarding reproductive choices. It is worth considering the potential for innovative treatments that the unborn child could undergo after birth, particularly if initiated at an early stage or if the condition manifests after a few months/years of life rather than being congenital. Certain innovative therapeutic approaches are revolutionizing the prognosis of highly debilitating or potentially fatal diseases.

   Gene therapies are already available that can replace the defective gene with a corrected copy of the gene or, in the near future, with the technique of genome editing, which can correct in the defective gene the site with the incorrect nucleotide sequence, realizing an example of true precision/personalized therapy. Additionally, ongoing development in drug therapies has shown sig-

nificant results, particularly in inherited metabolic diseases, as well as certain neurological or neuromuscular conditions like spinal muscular atrophy.
While this information provides an objective overview of the new treatments available for newborns, it is important to address the high costs associated with these treatments, raising concerns about equitable distribution and economic sustainability within national healthcare systems.
On the other side of the coin, considering quality of life is crucial when communicating a diagnosis. It requires a shared assessment with the couple of the family and social context in which the infant, with varying degrees of disability, will live, and the presumed impact on their quality of life.
In addition to the objective description of the detected pathology and its related therapeutic interventions, the person who provides the couple with a consultation should help them to better understand the organization of the healthcare networks available in the community to welcome, care for, and rehabilitate a child with problems, and to guarantee his or her inclusion in the education system. For example, the welcoming of children with Down syndrome is better socially fostered in Germany than in France and notably, Denmark, which hopes to become "a society without Down syndrome". This framework should help parents, who have already been disappointed in their expectations for the birth of a child with problems, to have a clearer perception of the quality of life of the unborn and implement their choices with greater awareness. In this regard it is good to remember that the information on diseases, which the public easily arrives at on often unreliable websites, predominantly describes the clinical cases at the most severe end of the phenotypic spectrum, causing the worries that push them to lean towards terminating the pregnancy.
Quality of life, summarizing what already presented, is the other side of the coin in the communication of the diagnosis. It is represented by a shared assessment between the couple and the family and social context in which the newborn, with varying degrees of disability, will have to live.
The danger that pregnancy terminations for pathological conditions of varied seriousness create discrimination and stigmatization for those with congenital or acquired disabilities is highlighted more and more in the bioethical literature. With regard to these themes, there are international initiatives created by associations of patients and their family members. Some examples can be seen in the documents of the Nuffield Council on Bioethics (2006) and in scientific and print-media articles that illustrate the associations' posi-

tions. For example, the conclusion of a recent review in the NEJM on Down syndrome claims: "People with Down syndrome and their families generally have a positive attitude and express their desire for a higher quality of life based on the strengths and abilities of the child or the affected adult. The published guidelines provide recommendations and standards to allow people with Down syndrome to reach their full potential".

For this reason, the condemnation expressed by Pope Francis on the "culture of waste", it is necessary that the medical-scientific community also respect the evaluations of people with disability and their families on the actual quality of their lives, engaging institutions to promote the rights of people with disabilities to receive adequate care through public services.

b. It is likewise crucial to improve the public's sensitivity towards so-called "differently abled" people. It is a task that not only involves individual areas of society, although the healthcare field is of particular relevance, but also society's overall mentality and culture. In fact, the predominant culture in countries in which prenatal diagnosis is most practiced, and being progressively spread throughout the world, is characterized by criteria of efficiency and personal and work performance standards. Given the power of technology, such criteria tend to become references for the assessment of every situation, even when the lives of people are in play. Based on this, one starts to evaluate the suffering and the disability only in relation to advantages and utility. From here, a systematic opposition to disability is derived with a healthy dose of prejudice. It is seen as being a condition automatically linked to suffering, to be suppressed (by any means) in favor of the greatest aggregate wellbeing within reach—and that here happiness lies.

However, one immediately must ask if this equation between health/efficiency and happiness is sustainable and if happiness is in total opposition to suffering. Must one excludes the other as if there were an inverse relationship between them? This is a rather challenging question. However, we can at least call back to the common observation that not only does suffering belong fundamentally and constitutively to life but also that there are very large subjective variations in how limitation and disability are experienced. We know that a subject with a disability is not necessarily unhappy, just as a person in a good state of health and without a handicap is not automatically happy.

Here a further important element emerges: good physical health is not definable abstractly and does not coincide with ideals of perfection that are practically unrealistic and non-existent. Coming to terms with one's frailty is part

of the human condition, with exposure to vulnerabilities up to genuine impairments. These aspects should be recognized as part of every existence and not only attributable to those considered fragile. Therefore, one can see how problematic it is to exclude those who do not correspond to some functional parameters based on a definition of normality dictated by those in positions of power, who are consequently capable of "discarding" others not corresponding to those standards. Without giving any undue complacency to pain ("dolorismo") or inert resignation in enduring it, one must recognize it as a transition through which it is possible to access a more profound sense of life. It can progressively give rise to a new and more profound understanding of the human being. Disability challenges us to overcome the difficulties we experience in making sense of our limits and frailty, urging us to assume greater awareness of the resources present in reciprocal solidarity to allow for a more authentic coexistence of the human family against the backdrop of common exposure. From this point of view, the exclusion and erasure (eugenically achieved) of those who find themselves in a more fragile state of life leads to a genuine loss in our common humanity. This is the context one should refer to in distinguishing between the choices to make in concrete circumstances, in the light of history and the effective characteristics of the people directly involved. It is important to promote an inclusive "sense of community" capable of welcoming and helping each new life so that couples and families are supported by the sympathy of everyone towards those weaker and more disadvantaged in this world.

Training courses: setup and core areas

Education and further training of healthcare workers are fundamental for offering a comprehensive and proper diagnostic-care pathway to the woman/couple. All the healthcare workers involved must have received adequate training which is aimed at the specific role that the worker has in the care of the pregnant woman, the delivery and the newborn.

The training courses can be carried out in various ways, based on the social and economic situation of the various circumstances.

- Distance-learning courses that use tools like USB drives or the internet with the possibility of interactions between the teachers and participants in group discussions.
- Residential courses in small or large groups. It can include cascade training where those who have participated in the course become the trainers in their fields with the use of slides that have already been discussed and shared in the

residential course.

The drafting of documents can be a common tool for both possibilities, allowing for a discussion and a later possibility of interacting between trainers and participants.

The contents can and must be adequate to the training target:

- a general part must be the cultural heritage of everyone (like the possibility of screening and diagnostic tests and options for pregnancy and birth);
- a more specific part dedicated to the healthcare workers involved in the specific activity (ultrasound, screening test, prenatal diagnoses, labor management, neonatal care).

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Conclusion

The key reference point that has guided the reflections of this document is the respect and acceptance of human life from its beginnings while being aware of the unique relationship established between mother and child during pregnancy and, more broadly, of the parental relationship. The prenatal diagnoses considered concern a development phase of the fetus in which its human quality is almost universally recognized, save for some minority opinions.

The attempt has been to provide scientifically up-to-date and comprehensive information for this important subject matter, despite not being a specialist paper. In fact, it concerns a topic that has profound cultural implications, given that it puts our understanding of health and sickness into question and pertains to the acceptance of those who do not immediately correspond to conventionally accepted standards. Our difficulty in understanding vulnerability as universal and the normal condition of human life emerges in this context. It brings us to recognize reciprocal care as the fundamental paradigm of human relationships and social coexistence.

With this in mind, the questions of diagnostic techniques, which over recent years have made great progress and continue to proceed at a rapid pace, have been looked at. However, this growing body of knowledge does not eliminate a margin of uncertainty, not only due to the characteristics of the techniques but also due to the context in which they are used. Indeed, genetic and biological data are not the only determining factors in the clinical framework, which also depends on the interaction of the environment, caretaking possibilities and resources that the healthcare system and social services can make available. The support and acceptance provided by the various surroundings and cultural sensibilities play an important role as well.

Awareness of cultural context is necessary to provide appropriate informa-

tion regarding how to interpret the data, diagnosis, and perceived quality of the fetus's life. The prognosis and the methods used for life cannot be considered irrespective of specific and concrete situations because of the many contextual variables involved in the clinical situation of the individual.

It seems useful to present some summarized recommendations that express what has been developed and argued throughout this document:

1. Scientific development of both the biotechnologies and therapeutic possibilities and continual training is indispensable for health and well-being. It is of the highest importance to only use validated tests, make careful critical analyses of the commercial pressures exercised in the field of prenatal diagnosis, and avoid carrying out useless or exaggerated interventions from a medical point of view.
2. Information: communicating adequate information to the couple is a task that takes great responsibility and tact. The information must be up-to-date, honestly correspond to the state of knowledge and the medical situation detected and aim at helping the couple make the best choice in a non-directive way, without undue interference.
3. Genetic consultation: it is important that, in its connection with the prenatal diagnosis, it be accompanied by a written and, when indicated, multidisciplinary report according to how practicable it is in the various healthcare systems. It should always be presented in its connection with the prospective cure
4. The choices of the mother/couple on these topics are often very demanding and delicate, so adequate guidance and support are necessary. This guidance is, as much as possible, an integral part of the comprehension and assumption of the many values that are always in play in these situations.
5. Not only do healthcare institutions play a fundamental role in putting these indications into practice, but they also support the comprehension of their implications for the entire community on a cultural and a social level.

Comment from the Buddhist Religion

The document sent from the Pontifical Academy "For Life" proposes an interreligious discussion on some prenatal diagnostic techniques and their dissemination, social impact and, most importantly, their ethical implications. The Italian Buddhist Institute Soka Gakkai has only recently approached, and with great caution, bioethical questions, trying to avoid the simplification of extremely complex problems that risk creating a negative impact on the life of individuals and their environment. Therefore, if on the one hand it is difficult to offer answers regarding the "completeness and balance of the paper" for the lack of specific scientific expertise in the content, on the other hand it is possible to outline the general principles on which the ancient wisdom of Buddhism operates, in particular on the teachings of the Buddha Nichiren Daishonin. From its beginnings, Buddhism was born as a search to transform the suffering of birth, disease, old age and death: questions that involve all human beings without exception. The development of medical techniques and research have allowed for the achievement of significant success in the cure of diseases but have at the same time opened new frontiers and new reflections in relation to scientific innovations. This process has given rise to bioethics and the constant need to look for new responses to the rapid development of technoscience in relation to human beings. Buddhism entered into today's bioethical debate with the presumption of the supreme dignity (or sacredness) of life that becomes—also through the principles of responsibility, compassion and care—a profound form of practical wisdom. This brief contribution to the themes presented by the Academy "For Life" is based on the ideas of Daisaku Ikeda, president of the Soka Gakkai International, and Master for all the members of the global organization. Throughout his life, Daisaku Ikeda has spread the teachings of the Buddha Nichiren Daishonin in 192 countries and territories of the world, giving birth to and developing a global

movement aimed at achieving world peace and transforming the suffering of life. Generally, with regard to the theme of the development of technology and human science, Ikeda constantly highlights the necessity of the parallel development of a cosmic, or symbiotic, humanism that is capable of guiding each scientific advancement "towards the good". The term "symbiotic" also defines the role of care and protection that the human species must carry out towards all sentient and non-sentient beings that inhabit our common home: Mother Earth. In Ikeda's opinion, it is critical to find a new ethic that is compatible with the changes taking place in medicine and capable of guiding them. A new ethic that sets itself up as a frame of reference in a historical moment where two great threats loom: the development of nuclear technologies and the development of audacious biotechnologies (G. Bourgeault, D. Ikeda, R. Simard "On Being Human"). In Italy, in the field of bioethics, religious culture (a predominantly Catholic one) and secular culture oppose one another: the former is based on the "sacredness of life" and the latter on the "quality of life". Within this dialectic, Buddhism, staying away from dogmatic positions, upholds the fundamental importance of dialogue by trying to express a synthesis between the two opposites of sacredness and quality. Moreover, the Buddhism of Soka Gakkai does not provide "exact" responses at the moment to every problem brought on by bioethics but invites every person to assume responsibility for their decisions based on their knowledge of, responsibility towards and relationship with all forms of life, which are inextricably interrelated to one another. Generically speaking, the Buddhist position is defined as passive or relativist. But nothing is further from the Buddhism of Nichiren Daishonin than these adjectives: his resolve is not doctrinarian or based on precepts, as much as it is a resolve "directed" towards Life. The second President of the Soka Gakkai Josei Toda, in the cell where he was kept by Japanese nationalists, was enlightened by the fact that "the Buddha is life!". Starting from this key point, Soka Buddhism takes a position on every religious, political and ethical question. Nichiren Daishonin writes that "Life is the foremost of all treasures" and: "it is expounded that even the treasures of the entire major world system cannot equal the value of one's body and life" (The Gift of Rice, A Collection of Writings of Nichiren Daishonin, Istituto Buddista Italiano Soka Gakkai, Firenze, 2008, vol.1, pg .997). In his dialogue with the historian Toynbee Ikeda, Daishonin also states "As a religious man, I am deeply conscious of the paramount, irreplaceable value of human life; I deeply believe that all human actions must be based on an awareness of the greatness of life. On the other hand, birth control—the lessening to some extent of the possibility of giving birth—is by no means trampling on the dignity of life. On the contrary, if such measures can

alleviate the chronic starvation of the developing nations, they are a practical way to demonstrate even greater respect for life. As long as it effectively promotes the continued survival and expansion of mankind, birth control deserves our support (…) The attitude that man must breed to the limit must be changed, for if it is allowed to go unaltered it will further aggravate the population crisis and will effectively destroy respect for life." (A. Toynbee, D. Ikeda, Dialogue, Man Himself Must Choose, Kodansha International, 1976 pg. 110/111). Carefully reading the numerous books of Ikeda's dialogues written with scientists, philosophers and peace scholars of each religion, it is clear how he always firmly includes great attention to the particularity of the case, always going back to the fundamental approach of the supreme dignity of life. For example, Toynbee was favorable of euthanasia and Ikeda responded: "While recognizing that freedom to assist another to escape unbearable pain by taking his life or to seek death for oneself is a logical conclusion of humanistic thought, I am afraid that, should this idea be regarded with less than maximum caution, it could degenerate into the kind of undervaluing of life that I have often condemned. (…) I believe that whatever means are available ought to be applied in attempts to lessen suffering. Maximum efforts must be made to this end. But human agencies must not be allowed to affect the inherent right of life itself to survive. Pleasure and pain have no intrinsic dignity, whereas life has a dignity for which there is no equivalent. Consequently, no pleasure and no pain can weigh as much in the scales of judgment as the dignity of life." (Dialogue, Man Himself Must Choose, pg. 161). In the Buddhist vision, human life has no price as the nature of the Buddha is intrinsic in each person and assisted death can deprive someone of the possibility of manifesting it. That's why Buddhism is against euthanasia. The Buddhist approach pursues health by reinforcing the intrinsic "dynamic harmony" in existence and goes tirelessly in search of what is called "quality of life", a fundamental concept in modern bioethics. Each individual has great potential for self-realization; when one is conscious of this, it is necessary to be wise to see it in others as in ourselves. "Compassion" means acting to overcome the sufferings of life together with others while respecting their human dignity. That is why Ikeda advocates for the birth of a new ethic that is capable of constantly guiding the new medical-scientific discoveries. With regard to the document "For Life", we are convinced that the general principles that have been outlined can offer a sufficient basis for the religious vision of our institute. In general, the Buddhist writings hold that existence begins at the moment of conception: " Modern prenatal physiology clearly reveals conditions in the mother's womb that were impossible to

determine in ancient times. To deal with the issues of in vitro fertilization and artificial insemination, we must re-examine our interpretation of the nature of life based on scientific and medical findings. Buddhism teaches that birth, like death, is a process. (…) Recent technological progress has been made at a dizzying speed. It is not surprising that technology, which makes artificial manipulation of human life possible, is generating new and serious ethical problems. The possibility of prenatal examinations has added new aspects to the issue of artificially induced abortions. Prenatal tests like amniocentesis, ultrasounds and chorionic villi sampling (CVS) allow us to monitor the very early stages of fetal development and identify the growing number of congenital and hereditary disorders" (D. Ikeda, R. Simard, G. Bourgeault, On Being Human, Middleway Press, 2003, pg. 130/132). Normally, in the case of a discovery of a congenital malformation, the decision to allow the fetus to live until the birth is left to the mother. The burden of "abnormality" in a child falls onto the shoulders of the woman, while it should fall to society as a whole. "Providing equipment for prenatal testing is important—writes Ikeda—but we must also create the kind of social system that can support and advise women trapped in untenable situations" (ibidem, pg. 132). At this point fundamental bioethical questions: what is meant by genetic anomaly? What does the vulnerability or the predisposition to this or that disease consist of? What is "abnormality"? What is a handicap? When can we judge when these disorders are serious enough to justify abortion? "Two questions among the many—writes Professor Bourgeault—capture the attention. First is the definition of the boundary between normal and abnormal (or pathological). To establish this boundary is to define the quality of human life. The second question, underlying the first, deals with so-called soft eugenics" (ibidem, pg. 133). In Master Ikeda's thinking, to define quality of life one must not draw boundaries, relegating anything beyond them as abnormal: "Instead we must do everything in our power to build the kind of broadminded society in which people living with disabilities do not have to consider themselves "handicapped" and can manifest their full potential" (ibidem, pg. 133). Going back to the crucial theme of abortion, it is closely connected with the attitude held with regard to the fetus by the mother, both parents, family and society as a whole. "The issue requires serious, considered debate. Buddhist compassion extends to respect for fetal life. It prefers avoiding abortion in favor of other methods of contraception. Of course, other considerations play a role. When birth endangers the mother's life or when pregnancy is the result of rape or other violent sexual relations, the will of the parents, especially the mother, must be respected

in making a decision. If genetic therapy progresses far enough in the future and provides solutions for certain problems, it should be considered as an option, but first it must be carefully and seriously examined to determine the best way to use it. Of course, all possible precautions must be taken to prevent therapy from degeneration in the manipulation of people for nontherapeutic ends." (ibidem, pg. 136). Buddhism is founded on tolerance. Just as the Buddhist precept against murder is extended to the child, the spirit of tolerance recognizes the dignity of every existence by adopting a society that attributes full value to people and allows them to live in complete self-respect. Modern society as a whole must abandon the logic of exclusion in favor of inclusion, making harmonious existence a possibility. Along these lines, "dialogue" takes on a central role in Buddhism as an instrument that redefines the space of possible choices, reconciling practical wisdom and theoretical knowledge. Indeed, respect for life somehow imposes the predominance of practical wisdom over theoretical wisdom. The superficial view that makes Buddhism seem like it lacks precise instructions, in reality, does not consider the practical wisdom of Buddhism, which refuses aprioristic and rigid rules for human behavior. Ultimately, all scientific discoveries do nothing more than push human beings on the complex path of exploring the meaning of our human community. Let's conclude by reaffirming that we agree with the For Life paper when it affirms the need to "promote an inclusive "sense of community" capable of welcoming and helping each new life so that couples and families are supported by the sympathy of everyone towards those weaker and more disadvantaged in this world". The biggest challenge that the Soka Gakkai Buddhists face and will continue to face in the future is making sure that the awareness of being part of a "human community" and living together on Mother Earth prevails in the heart of everyone on this planet.

Comment from the Jewish Religion

Under close reading, the text ***The Challenge of Life*** offers an accurate examination of the possibilities offered by prenatal diagnosis with neutrality and scientific accuracy. We share the conviction that prenatal diagnoses should promote the development of, in the case of malformations, in utero and neonatal surgical techniques. At the same time, we share a commitment to a more inclusive society that promotes the rights of people with disabilities. We respect the document's bioethical approach; however, with regard to post-diagnostic consultation, we would like to point out that the rabbinic Hebrew outlook presents different ways and foundations of reasoning, which can consequently lead to different directions and choices.

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Comment from the Islamic Religion

The document ***The Challenge Of Life*** is a precise scientific analysis of a shared bioethical structure aimed at protecting the mother, giving the utmost consideration to the human embryo and the interactive cellular and molecular "dialogue".

Prenatal diagnosis, i.e., the standards for a diagnostic assessment of the health of the embryo and the fetus during the progression of the human pregnancy, contributes to the development of the conception of the "fetus as a patient" and opens to the development of other possible therapies. Despite the persistence of a significant gap between the diagnostic and therapeutic capabilities in the prenatal field, the prospects for the treatment of pathological states (congenital or acquired) in the fetus open a complexity of issues connected with the assessment of the relationship between risk and benefit in the interests of the subjects involved.

The underlying aim of the paper is agreed upon. While considering the fetus as a patient, it does not propose the elimination of those who are different, but rather looks to accept people with disabilities to create a society that is more inclusive and attentive to the rights of all.

Dr. Massimo Abdellah Cozzolino
Secretary General of the Confederazione Islamica Italiana

Glossary

Abortion
Spontaneous or procured interruption of the pregnancy with the expulsion or removal of the fetus (or embryo) from the uterus.

Achondroplasia
Achondroplasia is a hereditary, fully penetrant autosomal dominant disorder. The most common is characterized by rhizomelia (upper and lower limbs grow less compared to the rest of the body), lumbar lordosis, brachydactyly, macrocephaly with bulging forehead and midfacial hypoplasia.

Alloimmunization
Anomaly of the maternal immune system that produces antibodies against foreign erythrocyte antigens and can be caused by fetal-neonatal hemolytic disease.

Amniocytes
Cells derived from the skin and other fetal tissues that are normally found shed in the amniotic fluid. In the case of invasive diagnostic exams (amniocentesis) they are utilized for they study of the fetus' chromosomal and genetic set.

Aneuploidies
Numeric anomalies of chromosomes: they are characterized by a greater or lesser number of chromosomes compared to the standard amount.

Autosomal (diseases- dominant- recessive)
It concerns genes hosted by autosomes (non-sexual chromosomes). In autosomal dominant disease, a normal copy of the gene is present while the other homologous copy is mutated. The term "dominant" means that the alteration of just one of the two copies of the genes is sufficient to cause the disease. The

altered gene "dominates" the other normal one. Consequently, a parent that carries a mutated copy of one of the genes is ill, and each pregnancy has a 50% risk (one possibility in two) of having a sick child, independently of the sex of the unborn child.

Autosomal recessive diseases are only found in people who have inherited two altered (mutated) copies of a gene. Both the copy inherited from the mother and the father are mutated. The term "recessive" means that the alteration of just one of the two copies of the genes is sufficient to cause the disease. The parents are carriers of just one copy of the altered gene (the other copy is normal) and therefore are not ill: they are healthy carriers. Two healthy carriers that want to have children have, with each pregnancy, a 25% chance (one in four) of having a sick child. The probability is independent of the sex of the unborn child.

Bias

Tendency to deviate from the average statistical value, and therefore a distortion of the sample. Selection bias describes the impossibility of selecting samples without being in some way influenced by prejudices and distortions that end up altering statistics.

Cardiotocography

Exam used in pregnancy (after the 28th week) that evaluates the fetal heart rate and the contractile activity of the uterus, in addition to correlations between the two variables under consideration. It is currently the most utilized method for having information on fetal homeostasis in real time.

Chiasma

A normal chromosome in which its locus is replaced by that of the homologous copy, encapsulating the concept of two chromosomes that, once overlapped, exchange part of their set.

Chorionic (mono-di)

The term "dichorionic" refers to the presence of two placentas and therefore two distinct gestational sacs (most frequent scenario). In the "monochorionic" pregnancy a single placenta is present from which two or more umbilical cords come out.

Chromatids

Two genetically identical subunits where the chromosomes are made during the process of mitotic and meiotic cell division.

Chromosomal aberrations

Anomaly in the number and structure of the chromosomes generally deriving from errors that take place during the cellular division after mitosis and meiosis. They can be recognized in deletions, duplications, translocations, conversions, ring formation.

DNA

The nucleic acids called deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) contain the information that gets transmitted from one generation to another. In the majority of cases the DNA constitutes the genetic makeup that identifies each person. Only in some organisms, such as certain viruses, is this information contained in the RNA.

Doppler flowmeter

Ultrasound exam that, by using the Doppler effect, allows for the evaluation of the peripheral resistances present in the arterial and venous blood vessels examined. It was introduced in prenatal medicine at the end of the 1980s, opening a completely new chapter in the comprehension of fetal coping mechanisms and fetal distress.

Dysmorphisms (facial)

Cranial-facial dysmorphism is characterized by malformation due to incorrect development during intrauterine life. The anomalies can concern the cranium (craniosynostosis: premature closure of the cranial sutures), the face (dysostosis: anomalous development of the facial bones), or both.

Early neonatal death

Death in the first 7 days of life over the total of living births.

ECMO

Extra Corporeal Membrane Oxygenation (ECMO) is an extracorporeal circulation procedure which is used as a support in subjects with heart or respiratory failure. Thanks to ECMO the heart and lung functions are temporarily carried out by an external machine and it is possible to intervene with a medical treatment on the patient.

Endocrine disruptors

Heterogeneous group of compounds that are potentially able to cause severe damage to one's health. The number of them and their differences make the study of their effects on humans complex.

Endogamy
In biology, a type of biological breeding in which the process of conjugation takes place between individuals of the same bloodline.

Epigenetics
A branch of genetics that studies the changes that influence the phenotype without altering the genotype—chemical modifications that do not involve changes in the sequence of the nucleotides but have a significant impact on the expression of the genome. These changes regulate the access of transcription factors to their binding sites on the DNA, establishing the functional activation of the genes.

Exome
A part of the genome made up of exons, which is the coding part of our DNA. Despite being only 1% of all our genetic material, it is responsible for all (or almost all) of the construction of our organism.

False negative and false positive
False positive: a healthy subject who comes out positive in a test. False negative: an unhealthy subject who comes out negative in a test.

Fetal mortality
Stillbirths (from a gestational age of 24 weeks and 0 days or from 28 weeks and 0 days) over total births.

Fetoscopy
Invasive exam that allows for the visualization of the fetus and the carrying out of potential surgical therapies through the introduction of an optical scope inside the uterus.

Gametes (balanced-unbalanced)
Cells that combine during sexual reproduction to form a new cell, the zygote, from which the embryo will develop. The male gametes are called sperm, while the female ones are called oocytes, ovum or egg cells.

Genome (parental)
All the genetic material that characterizes each living organism. The genetic information resides in the DNA sequence (see), which comes from the linear disposition of the four different molecules (nucleobases).

Genotype
Genetic makeup of an individual (or a group of individuals), "written" in the DNA contained in the nucleus of all the cells.

Heterozygosis
The opposite of homozygosis, it identifies a person that possesses an allele made up of two different forms of the same gene. Additionally, it indicates a carrier of a chromosomal aberration in one or two of the long chromosomes.

Ionizing radiation
Radiation capable of, directly or indirectly, causing the ionization of the atoms and molecules of the materials it passes through.

Late neonatal death
Death between 7 and 28 days of life over the total of living births.

Low birth weight
Currently, a weight that is lower than 2,500 grams is considered low (very low: lower than 1,500 grams; extremely low: lower than 1,000 grams).

Meiosis (phases)
Process of cell division through which the chromosomal inheritance is reduced by half and that leads to the formation of germ cells (gametes).

Mendelian (disease)
Disease due to genes that follow the monogenic hereditary method, i.e. due to the mutations of a single gene.

Mosaicisms
True fetal mosaicism confined placental mosaicism (CPM) and pseudomosaicism in the amniotic fluid cultures have been classified in the literature based on situations observed in laboratory practice. CPM, pseudomosaicism and true mosaicism can come from an early postzygotic mitotic error in a normal diploid embryo or from a reconstruction phenomenon of the disomy from a trisomy rescue. They represent one of the principal diagnostic problems in the determination of the fetal karyotype both from chorionic villi and amniotic fluid.

Near miss
Any event, correlated to work, that could have caused an injury or damage to health (disease) or death that, only by pure chance, did not happen: an event that in itself has the potential to produce injury.

Omics (sciences)
Disciplines that concern the study of genes in their entirety and their (genomic) relations, transcripts (transcriptomic), proteins (proteomic) and metabolites (metabolomic) that are expressed by a cell, differently from how traditional biological sciences do, which instead are concerned with studying biological processes singularly.

Perinatal mortality
Fetal deaths (from 24 weeks + 0 days or from 28 weeks + 0 days) + deaths in the first 7 days of life over total births (living and dead).

Phenotype
The set of characteristics that an individual manifests: it depends on the genotype (defined above), the interactions between genes and also external factors; consequently, it can vary also in the presence of an identical genotype.

Physiological duration of the pregnancy – gestational age
The duration of gestation is measured starting from the first day of the last normal menstruation (World Health Organization- WHO). The gestational age is expressed in completed days or completed weeks. The gestational age is not measured at the moment of conception (conceptional age) that instead is presumed to be about a further two weeks. Therefore, to calculate the due date, 280 days are counted (40 weeks) from the first day of the last menstruation, with a margin of more or less 15 days. In summary, 37+0 – 42+0 weeks, 280 days, around 10 lunar months.

Polyhydramnios
Excess of amniotic fluid that is often associated with maternal and fetal complications. The diagnosis takes place with an ultrasound measurement of the amniotic fluid.

Polymorphism
Contemporary presence in the same species of many hereditary forms (subspecies, races, variants), each of which does not make up less than one percent.

Postzygotic mutations
A postzygotic mutation produces a "mosaicism" (see) with two (or more) genetically distinct cell lines. The mosaicism can affect somatic or germinal tissues. If it hits the germinal lines, a mutation to a causative gene for a dominant or X- LINKED disease can be inherited.

Preterm birth

Birth before 37 gestational weeks (see) is considered preterm. Premature newborns are also classified as "Extremely premature" < 28 weeks, "Very Premature" from 28 to 31 weeks, "Moderately Premature" from 32 to 33 weeks, "Late Premature" from 34 to < 36 weeks.

Screening (test)

Test that allows for the identification, in an at-risk population for a specific disease, of those subjects who have a greater possibility of suffering from it. They are then directed to specific diagnostic exams that in the case of positivity allows for the adoption of, generally effective or even preventative, early therapeutic strategies.

Sensitivity

Sensitivity and specificity are criteria used to evaluate the ability of a test to identify, within a population, those fitting the sought-after "characteristic" and those without it. If a test has higher sensitivity, it will reduce false negatives. If it has higher specificity, it will reduce false positives.

Specificity

See sensitivity.

Syncytiotrophoblast

Cellular mass outside the blastocysts which are formed in the first part of the process of embryogenesis. It gives rise to the placenta and embryonic tissues.

Teratogenesis

Abnormal development of some fetal organs during the pregnancy, resulting in the birth of a child with congenital defects (Appendix 2).

Translocation (balanced)

Unusual change in the form of the chromosomes with the movement of a piece of a chromosome to another. If a parent is a carrier of a balanced translocation, it is possible that the fetus can inherit an unbalanced translation (extra segment in a chromosome and a missing one in another).

Trisomy

Chromosomal anomaly consisting in the presence of an extra chromosome retained by a determined chromosomal couple.

Weight in relation to gestational age

Appropriate for Gestational Age (AGA) is between the 10th and 90th percen-

tile for the gestational age; Small for Gestational Age (SGA) is lower than the 10th percentile; Large for Gestational Age (LGA) is a calculated weight above the 90th percentile. There are also dynamic weight assessments that do not only calculate the absolute value but also the variation compared to the expected one based on the estimated curve obtained from the last ultrasound: Intrauterine Growth Restriction (IUGR).

Zygosity (mono-di) sensitivity

Fusion of the pronuclei of the female and male gametes during fecundation.

Appendix 1
Physiopathological features of female reproductive potential and the development of the fetus and placenta

Female reproductive cells (oocytes), in contrast with male ones (spermatozoa), are produced far before birth during the development of the genital organs. Throughout life, the **"reserve"** is progressively reduced monthly until it completely ends (menopause) without the possibility of being regenerated. The female follicular population decreases from 6-7 million at 20 weeks of pregnancy to 1-2 million at the moment of birth; at puberty, there are around 500,000, which decreases to 25,0000 after 35 (1). Only 500 follicles encounter ovulation during the fertile life.

After 35 years of age, there is a decline in reproductive possibilities both for spontaneous and medically assisted procreation (1,2). This decline in fertility, registered both in Italy as well as other European countries, is associated with a consistent increase in the average age of the first birth. Istat data indicates that in Italy from 2008-2016 the average age of birth rose from 31.7 to 32.4. This trend has been documented in all European countries since the early 1980s when the average age of the first birth was around 25 (2).

Alongside the reduction in the number of gametes, there has been a progressive reduction in their quality. After 35 years of age, the rate of aneuploidies in embryos and miscarriages significantly increases (3, 4). The probability of obtaining embryos in an advanced development phase with an euploidy chromosomal population decreases year by year.

Fertilization takes place within 2-3 days from the ovulation that usually occurs between the 12th and 14th day of the menstrual cycle—i.e. after 12-14 days from the start of the last menstruation. The zygote that is formed contains 46 chromosomes, 23 deriving from the father and 23 deriving from the mother. In the first days after fertilization, very rapid cellular multiplication takes place that initially gives rise to a small undifferentiated mass of cells (morula) and a struc-

ture (blastocysts) in which trophoblast cells are identified, creating the placenta and the cells of the embryonic pole. These processes take place while the morula, and later the blastocysts, are moved from the tube to the uterine cavity, where implantation takes place in the decidua (i.e. the endometrium that covers the uterine wall after the implantation) around 7 days after conception, or 21 days after the start of the last menstruation.

During the first weeks of gestation, very rapid multiplication of cells takes place with contemporary differentiation and migration of cells that gives rise to various organs and tissues.

In the first half of the pregnancy (up to a gestational age of 20 weeks) the phenomenon of hyperplasia predominates (i.e. rapid cellular multiplication), while in the second half (20-40 weeks) hypertrophy predominates (i.e. increase in the volume of the cells and organs with a rapid weight increase).

Ultrasounds have allowed for the establishment of how the fetus grows physiologically during intrauterine life.

Concurrently, the placenta develops for the proliferation and differentiation of the cells of the trophoblast: the placental and maternal vascular network is formed to allow for an adequate exchange of gas (oxygen and carbon dioxide), nutrients, metabolic products, hormones, etc. The fetus receives all the substances which are essential to its development and growth from the mother through the placenta. It eliminates the products of its catabolism, but can also receive molecules or harmful agents (e.g. viruses, harmful gases, medicine, etc.).

The placenta is very active both in physiological and pathological conditions, producing many molecules, hormones and cytokines. In physiological conditions, the substances produced flooding into the maternal blood induce indispensable modifications in the maternal organism (an increase in cardiac output, modification in blood coagulation, etc.) to the normal development of the pregnancy. If the placenta does not adequately develop, the fetus does not receive the necessary oxygenation and supply of nutrients, and the mother can develop pathologies linked to the production of harmful molecules from the placenta. Therefore, the mother's and the fetus' health are closely correlated and, in this context, the placenta plays a crucial role.

In addition to substances produced by the placental cells, trophoblast cells also flow into the maternal blood, whose lysis, as a result of some cytokines, frees DNA fragments, which can be used for the prenatal screening of genetic diseases (see Chapter 2). This DNA is called cell- free fetal DNA (cffDNA), even if it is really trophoblast DNA and not fetal DNA.

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Appendix 2
Wilson's principles of Teratology

Susceptibility to teratogenesis depends on the genotype of the conceptus and how it interacts with the environment.

Susceptibility to a teratogenic agent varies with the developmental stage at which the exposure occurs.

Teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate abnormal embryogenesis.

The final manifestations of abnormal development are death, malformation, growth retardation, and functional disorder. Generally, the first two outcomes are correlated to early exposure and the others to late exposure.

Access of an adverse environmental agent to developing tissues depends on the nature of the agent (influences).

The manifestations of deviant development increase in degree as dosage increases from the no-effect to the lethal level.

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Appendix 3
Amniocentesis

Amniocentesis is carried out after the completion of the 15th gestational week (15+0), as at this moment there is a greater chance of a successful sampling and lower risk compared to a sampling taken at an earlier phase: early amniocentesis (10+0-14+6) does indeed present greater technical difficulties—a greater risk of fetal loss, loss of amniotic fluid, anomalies in the lower fetal limbs (clubfoot) and unsuccessful cellular cultures.

The sampling takes place transabdominally with a single 0.7-0.9mm needle (20-22 gauge) of adequate length (circa 12 mm). Before the sampling, an ultrasound is done to assess the number, vitality and position of the fetus/fetuses, the position of the placenta, and the measurement of the fetus's dimensions to choose the best-suited area for the introduction of the needle. It is a "free hand" sampling made under the continual guidance of the ultrasound. The amount of liquid sampled must not exceed 20 ml.

In twin pregnancies, it is necessary to evaluate the number of placentas and amniotic sacs before going through with the sampling (Level of evidence A). In dichorionic pregnancies, the most commonly used and least risky technique consists of taking two separate and consecutive samples, sequentially introducing two needles under ultrasound guidance. The introduction of the second needle must be carried out at the furthest point possible from the first one. In monochorionic pregnancies, one proceeds to the sampling from a single amniotic sac, after having verified the monochorionic diagnosis, in the absence of morphological anomalies of one or both the fetuses and when their growth is discordant. In the case of ultrasound anomalies in one or both fetuses, one proceeds to the sampling of both sacs to exclude potential mosaicisms. With regard to the risk of fetal loss and preterm birth, there is no evidence of a significant increase in the risk of

the procedure in multiple pregnancies compared to the base risk (Level C).

After amniocentesis, there is an ultrasound check of the fetus and the fetal heart rate (FHR) and placenta (Level C).

In pregnant women with a negative Rh factor and with a positive Rh partner, it is necessary to carry out, after amniocentesis, anti-D immunoglobulin prophylaxis to reduce the risk of Rh isoimmunization, a pathology that can also have severe consequences for the fetus/newborn (Level B).

There are no instructions for carrying out a pre-amniocentesis infectiology screening. There is no data in favor of antibiotic prophylaxis or tocolytic therapy (aimed at countering uterine contractions) to be routinely used in the preparation of amniocentesis. However, if there is HIV seropositivity, it is necessary to wait for the viral load to be undetectable. On the other hand, with regard to potential seropositivity for HCV and HBV, there are currently no contra indications.

Amniocentesis is an outpatient technique and as such does not require the hospitalization of the pregnant woman

Amniocentesis carries an additional risk of fetal loss (i.e., greater risk than that of any pregnancy) of 1-3/1000. Some data in the literature report a risk equal to 1%[1] while others have it between 0.1 and 1%[2], similar to what is reported for the biopsy of the chorial villi (Evidence I-c). This percentage can significantly increase in the presence of risk factors like a history of previous abortions (up to 7%), the presence of hemorrhaging in the I trimester (up to 6%), the presence of blood in the amniotic fluid (up to 15%) and an alfa-fetoprotein serum above 2 MoM (up to 20%) (Evidence II-c).

After amniocentesis, a risk of a rupture of the amniochorionic membrane of around 1/1000 instead of 1-2% has been reported (Evidence II-C).

The risk of fetal lesions by the needle is negligible, as the sampling must take place under the constant guidance of the ultrasound (Evidence III). An increase in the risk of preterm birth has been reported (Evidence II-C).

The risk of Rh isoimmunization after amniocentesis is not significantly higher (Evidence II-c). At the moment, there is no relevant data concerning the risk of "vertical" transmission of viral infections linked to amniocentesis.

Around 0.1% of samples can result in an uninformative test for a lack of growth in the amniocyte cell culture (Ghidini A, 2019; Windsor E, 1999).

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[1]Royal College of Obstetricians and Gynecologists, 2010.
[2]ISUOG Practice Guidelines, 2016.

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Appendix 4
Villocentesis

The villocentesis or sampling of the chorionic villi takes place starting from the 10^{+0} week of gestation (transcervically until the 13^{+0} week) (Level A). The samples taken before 10^{+0} weeks can increase the risk of abortion (Evidence II-b) and transversal defects of the limbs (Evidence II-a).

Before carrying out the procedure, an ultrasound test is given to determine the number, the vitality of the fetus/fetuses, the biometric evaluation (at least the craniocaudal length), to locate the position of the chorion frondosum (at the base of the placenta) and choose the most suitable area for the introduction of the needle (Level A).

The sampling takes place transabdominally with a single 0.7-0.9mm needle (20-22 gauge) of adequate length (around 12mm) or, less frequently, with a double needle (20 gauge inserted into a "guide"18-gauge needle) (Level A). The use of a higher gauge needle increases the success of the sample, but also the risk of complications linked to the procedure (Evidence II-b).

Alternatively, the sample can be taken transcervically by using a polyethylene catheter with an aluminum spindle (Level A) or rigid biopsy forceps (Level B). The use of this technique carries an increased risk of abortion compared to the transabdominal technique (Evidence I-b) and increases the risk of infectious complications (Evidence III).

The biopsies must be carried out under the constant guidance of the ultrasound, both to direct the instrument towards the chorion frondosum and during the aspiration of the material (Level A).

In pregnant women with a negative Rh factor and with a positive Rh partner, it is necessary to carry out, after amniocentesis, anti-D immunoglobulin prophylaxis to reduce the risk of Rh isoimmunization, a pathology that can also have se-

vere consequences for the fetus/newborn (Level B). In women who have already been immunized, the biopsy of the chorionic villi is contraindicated (Level A). There are no instructions for carrying out an infectiology screening before the villocentesis. In pregnant women with hepatitis B or C, the sample can be taken as there is no evidence of vertical transmission of the virus after the procedure (Evidence I-C and II-C). However, the data in the literature is not exhaustive and mainly refers to a few case studies. In HIV-positive pregnant women, there is no proof that the invasive prenatal diagnosis increases the risk of vertical transmission, especially if the sample is carried out at the same time that antiretroviral medication is taken and if the maternal viral load is particularly low.

It is however advisable to obtain specific informed consent, aimed at communicating that the risk of transmission is unknown at the moment (Evidence III).

There is no data in favor of antibiotic prophylaxis or tocolytic therapy (aimed at countering uterine contractions) to be routinely used in the preparation of biopsy of the chorionic villi.

The sampling of the chorionic villi is an outpatient technique and as such does not require the hospitalization of the pregnant woman (Evidence III. Level A).

The sampling of the chorionic villi carries an additional risk of abortion (i.e. greater risk than that of any pregnancy) of 1-3/1000 (Evidence I-b). The risk of abortion after the sampling of the chorionic villi does not significantly differ from the one registered after amniocentesis (Evidence I-b). Furthermore, in some scientific works, the risk reaches a value between o.2 and 2% (Saperi Doc, 2021) The risk of abortion following the chorionic villi sampling is directly correlated to various factors, like "advanced" maternal age, the number of sampling attempts, cytogenetic characteristics of the trophoblast (potential presence of mosaicisms) and inversely correlated to gestational age when the procedure is made and, especially, to the experience of the operator (Evidence I-b).

Chorionic villi sampling carried out before the 10^{a+0} week of gestation can increase the risk of a transversal defect of the fetal limbs (Evidence II-a).

Chorionic villi sampling in multiple pregnancies carries a global risk of fetal loss of 2-4%; compared to the natural abortion rate in single and multiple pregnancies, this risk does not appear to be significantly higher (Evidence III). The rate of "contamination" between twins (meaning the possibility that the material of the same time is sampled twice) is lower with the greater experience of the operator. However, the probability of repeating the sample for an unclear result has been calculated at around 2-3% (Level C).

Unusual vaginal bleeding is relatively common after transcervical sampling but rare after the transabdominal sample and therefore does not modify the final outcome (Evidence III).

Infectious complications are rare and more frequent after transcervical samples that require more than one attempt (Evidence III).

The risk of a rupture of the amniochorionic membrane caused by the sampling is a rare occurrence (around 1%).

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