PRENATAL DIAGNOSTIC CENTER

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PRENATAL DIAGNOSIS

The Prenatal Diagnosis is the branch of medicine in obstetrics which studies and applies techniques that reveal the normality or the presence of various types of disease in the fetus; those being hereditary or genetic disorders.

The **Prenatal Diagnostic Center of Villa Donatello** is able to provide state of the art performance in prenatal diagnosis, combining operator expertise with
the use of the latest generation of ultrasonic devices equipped with 3D / 4D probes and validated genetic screening tests.

Our center uses the **Voluson E10 GE** ultrasound machine, equipped with a volumetric electronic probe, making it the most advanced ultrasound instrument worldwide in the field of obstetrics - gynecology. Exceptional 3D and 4D volumetric reconstructions (which also highlight movements) allow the best qualitative evaluation throughout the fetal development period.

The new "transparency" application allows visualization of internal cavities and early diagnosis of neurological alterations as of the first trimester. This is the same technique used in the embryo-fetal cardiovascular field which improves the possibilities for observation and therefore diagnosis.

With regard to the field of **Ultrasound Diagnostics** and **Screening of Chromosomal Abnormalities and Diagnostic Techniques**, the following non-invasive and invasive examinations are available:

ULTRASOUND SCREENING

What is ultrasound?

Ultrasound is a non-invasive diagnostic technique that uses high frequency sound waves, that is, sound waves with a frequency higher than the one perceptible by the human ear. This technique produces visual imaging, allowing for the exploration of the internal structures of the human body and therefore also the fetus.

By placing the electrode probe on a maternal abdomen, ultrasound beams are emitted and travel through the amniotic fluid which act as a propagation medium. Once they reach the fetus, they are partially reflected and transformed into visible images on the monitor of the device.

Why is obstetric ultrasound used?

We can consider obstetric ultrasound as an integral part of the visit. It can accurately determine the gestational age of pregnancy, determine the number of fetuses, detect vital parameters, exclude the occurrence of major malformations, monitor growth, and render the fetal position in the uterus.

Ultrasounds have been used in obstetrician practice for about thirty years and no harmful, long-term effects on the fetus have been reported.

For this reason, with the procedures adopted today, the diagnostic use of ultrasound is considered to be free from risk.

With regard to the number of ultrasound examinations to be made during pregnancy, the SIEOG Guidelines (Italian Society of Obstetric and Gynecological Ultrasound) indicate three times for physiological pregnancies, which are generally distributed in the three trimesters of pregnancy, as follows:
I trimester (10-12 weeks)
II trimester (19-21 weeks)
III trimester (30-34 weeks)

In the case of risk-based pregnancies or doubts emerging during the diagnostic screening, an additional diagnostic ultrasound examination is recommended.

Diagnostic ultrasonography aims to confirm or negate any suspicion of malformations in the fetus, therefore providing the mother and/or the couple with the most accurate counseling possible on neonatal prognosis.

THREE DIMENSIONAL ULTRASOUND

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What is three dimensional ultrasound?

One of the most important advances in the field of image technologies over the last twenty years has been the development of three-dimensional (3D) and three-dimensional in time, that is, the fourth dimension (4D). The understanding of multi-planarism and the application of this concept has allowed to improve the study of the physiology and pathology of many biological systems, including the female reproductive system, the uterus and fetus. The specific anatomical features of these biological structures have shown to be in correlation with the three-dimensional characteristics, that is, allowing the possibility to sample on infinite and reproducible planes (another fundamental 3D innovation). The methodology of 3D technology uses the fine-tuning of the volumetric probe, which consists in the acquisition of numerous scans with mechanical or, more recently, electronic techniques, to produce high quality images.

Three-dimensional ultrasound has predominantly entered in the field of embryo-fetal ultrasonography and ultrasound diagnostics, demonstrating from the outset the importance of application in spatially complex anatomical structures, such as the brain and heart, but also allowing to evaluate the size of the fetus and to explore organs, biological systems and surfaces in relation to the three spatial planes.

Regarding the study of fetal anatomy, the minimum requirements are indicated by SIEOG (the Italian Society of Ultrasound in Obstetrics and Gynecology) however the present tools available are mostly volumetric probes and therefore the passage to using 3D-4D is part of the current ultrasound observation.

In obstetric applications, it is possible to use 3D volumetric apparatus for a more detailed study of fetal dysmorphologies, thanks to the more realistic visualization of the fetal face. This holds true also for the somatic characterization of chromosomal pathologies.

During screening, some functions (tools) of the system are aimed at detecting Down Syndrome in the first trimester, as well as the study of early fetal malformations.

The HD Zoom, in particular, is an important feature for the study of the Nuchal Translucency (NT), since it allows magnification of the image without loss of resolution, as well as an increased frame rate. Moreover, the recent introduction of a semi-automatic measurement software for NT allows for more accurate precision, as even the smallest variations can have a heavy impact on the end result. The SonoNT tool applies automation to NT measurements and helps to avoid inter and intra variability linked to manual measurements, as well as over-underestimating the risk.

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The 3D system provides the basis for innovation in the calculation of Nuchal Translucency (NT) with automation technologies such as SonoNT: a Semi-automatic software, dedicated to measuring NT according to the guidelines of the Fetal Medicine Foundation (FMF) in London.

The particularity of 3D is also distinguished by the ability to record and store the ultrasound reading in a life-like manner, without having to resort to extrapolation techniques. This allows the ability to analyze the data acquired during and/or after the exam itself.

By using a particular correlation algorithm, a real-time acquisition of 4D allows you to obtain, within just a few seconds, the dynamic anatomical volume of the fetal heart during a complete heart-beat cycle. The anatomical volume can then be explored in multi-planar, that is by projecting the acquired anatomical life-like "reality" on three orthogonal planes. Such detailed visualization gives the operator a more complete and useful information for diagnosis.

Ultimately, the innovation of three-dimensional rendering (3D) is to obtain and offer perfectly reproducible visual planes for early diagnosis by experienced operators, without any discomfort on the part of the woman.

3D IMAGE, HDLIVE and TRASPARENCE GALLERY

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Non-Invasive Prenatal Diagnosis
Obstetric Ultrasound Scan
Combined Test
Fetal DNA Study
Invasive Prenatal Diagnosis
Chorionic villus sampling
Amniocentesis

The exams listed above will be offered within a personally customized exam itinerary, focusing to meet the most specific needs and requirements. One example is the **Advanced Combined Test**, which includes the sequential introduction of ultrasound and hormonal marker identification, useful to complete the study of particular fetal features, as well as identifying possible maternal diseases, such as hypertension, which may occur in the course of pregnancy.

In addition, the Prenatal Diagnostic Center of Villa Donatello is able to offer **genetic counseling** in case of doubt or the need to resolve complex issues.

Obstetric Ultrasound Scan 10-12 weeks

Although the recommended period for an ultrasound during the 1st trimester is between the 10th and 12th week of pregnancy, it is now possible to can carry out the first ultrasound check from the 6th week using the transvaginal probes. This ultrasound allows to identify the intrauterine position of pregnancy, the number of embryos and their vitality, as well as any associated uterine and / or anterior pathologies.

The important endpoint of the first trimester examination is dating the gestational age. By measuring the length of the fetus, it is possible to determine if fetal development corresponds to the time of pregnancy calculated on the basis of the last period.

The identification of the gestational age can have important repercussions as it can indicate the correct time for conducting particular examinations such as the chorionic villus sampling, amniocentesis, and combined test. It also has impact on the evaluation of fetal growth in later stages, as well as correct estimate of pregnancy term.

During the first trimester exam, it is possible to evaluate the number of fetuses and detect pulsatile activity of the heart. Note however that the ultrasound performed in the first trimester of pregnancy does not have the purpose to diagnose any malformations of the fetus. Instead, the screening for nuchal translucency has fueled an increasing interest in fetal anatomy evaluation in the first trimester, however the fetal anatomy evaluation standard remains in the second trimester's ultrasound examination.

The first trimester evaluation has its limits, those being the ability to only determine the gestational age and the lack of experienced operators. However, if an ultrasound image renders suspicion for malformation and / or a translucency above the 99 percentile, further diagnostic observations are requested (SIEOG Guidelines 2015).

Obstetric Ultrasound scan 19-21 weeks

The second exam at 19-21 weeks is performed mainly to study the anatomy of the fetus and exclude the presence of major malformations. The evaluation of the biometry of the fetus through the execution of some standard measurements is also performed (biparietal diameter, cranial circumference, abdomeninal circumference, femur length, etc.)

Is it possible to identify all fetal malformations in the uterus?

The ability to diagnose a malformation depends on the type and natural history of malformation involved, its size, the position of the fetus in the uterus, the amount of amniotic fluid, the thickness of the maternal abdominal wall and at what stage of pregnancy the examination is carried out. It is therefore possible, for the intrinsic limitations of the method used, that certain abnormalities, even serious ones, can go undetected during the ultrasound examination. In addition, it should be known that some malformations only occur at late stages of the pregnancy.

In fact, the diagnostic sensitivity of ultrasonography screening, that is the ability to detect the malformations present at birth, is quite low in various scientific studies that has examined this aspect. The experience available so far suggests that routine ultrasound examinations allow to identify 30 to 70% of the major malformations, with percentage variations depending on the device being used.

Obstetric Ultrasound scan 30-34 weeks

The third exam (usually performed at 30-34 weeks) is performed primarily to evaluate fetal growth and identify the conditions showing slowed growth which can result in a higher risk of perinatal mortality.Other important elements to consider during the third trimester examination which may have an important impact on the obstetric practice are the controls for late-onset fetal malformations (ex. malformations in the urinary tract, the digestive tract, central nervous system, etc.), the placental insertion site, the evaluation of the quantity of amniotic fluid, and finally the definition of the fetal position in the uterus.

First Trimester Combined Test

The combined test is a screening test consisting of a blood test in order to analyse placental hormones free-betaHCG and PAPP-A, together with an ultrasound examination aimed at measuring the nuchal translucency thickness (NT). It is performed in the first trimester, between the 11th and 13th week + 6gg and is used to calculate the risk that the fetus may be susceptible for chromosomal abnormalities, such as trisomy 21, 18 and 13. In addition, the ultrasound examination may detect some fetal congenital malformations at an early age.
As mentioned, the combined test is a screening test and has no diagnostic. Instead it acts as a sort of filter used by obstetricians to identify pregnancies at risk for chromosomal anomalies. The data results from the ultrasound examination combined with the values of maternal hormones, free-betaHCG and PAPP-A, provide an individual risk assessment for each woman.

How reliable is the combined test?

Published international literature indicates that the combined test identifies 85-90% of fetuses with Down syndrome, with only a false positive of 5%. The measurement of nuchal translucency is detected at 70-80%.
It is very important for the test to be carried out by experienced operators who follow protocols, are subject to rigorous quality controls, and who can provide accurate clarification and information on the test results.

Combined Test: from two-dimensional to three-dimensional

By introducing the measurement the nuchal translucency during the screening for Down's Syndrome, we can say that the 1st quarter ultrasound "forces" the practitioners to a more accurate study of fetal morphological features.

The Combined Test Steps

- Pre-test counseling and hormone blood test (9-12 weeks).
- Combined test + additional markers: nasal bone, venous duct, tricuspid.
- Uterus arteries in order to evaluate the risk of intrauterine growth
- Post-test counseling

PRENATALSAFE®

What does PrenatalSafe evaluate?

PrenatalSAFE® is a non-invasive prenatal test that, analyzing circulating cell-free fetal DNA isolated from a sample of maternal blood; from the most common to the rarest, structural alterations in all fetus chromosomes and the presence of mutations related to severe genetic pathologies.

PrenatalSAFE 5

It screens for Trisomy 21, Trisomy 18, Trisomy 13, sex chromosomes (X and Y) aneuploidies and includes fetal gender (optional).

PrenatalSAFE Karyo

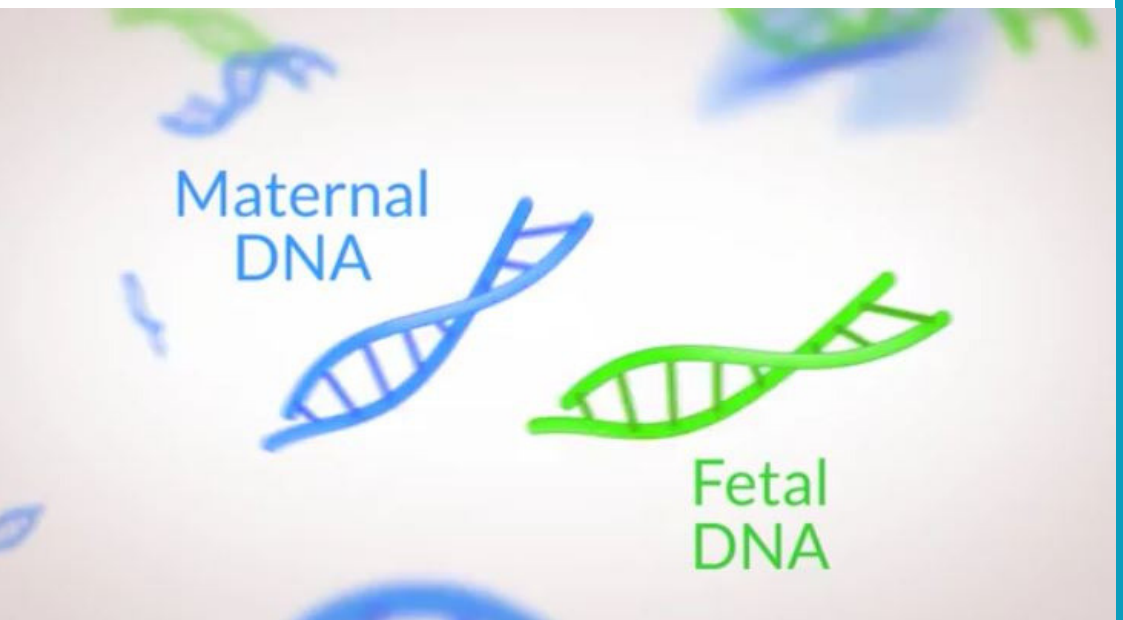
It screens for aneuploidies and structural chromosomal abnormalities (deletions or segmental duplications) across the fetal genome.

PrenatalSAFE Complete
is the most technologically advanced non-invasive prenatal test currently available. Through the analysis of circulating cell-free fetal DNA (cfDNA) in maternal blood, it screens for fetal karyotype and severe genetic disorders in the fetus.

Who is PrenatalSafe recommended for?

PrenatalSAFE® is suggested in the following cases:

- Patients wishing to avoid an invasive diagnostic procedure;
- Positive results on maternal serum screening;
- Advanced maternal age (women that are >35 years old);
- Advanced paternal age (men that are >40 years old);
- The ultrasound scan shows fetal anomalies, suggesting aneuploidy.
- Personal/family anamnesis of chromosomal abnormalities
- Partner(s) carrier of balanced Robertsonian translocation of chromosomes 13 or 21.
- Low risk pregnancies.



GENESAFE®

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- Low risk pregnancies.

How does it work?

During pregnancy, some DNA fragments of the fetus circulate in maternal blood.
Circulating cell-free fetal DNA (cfDNA) in maternal blood, derives from placental trophoblasts and is present in the plasma in variable quantity, depending on the gestation week. This DNA is detectable starting from the 5th week of gestation; its concentration increase in the following weeks and disappear right after childbirth. The quantity of cfDNA from 10th weeks gestation is sufficient to ensure the elevated sensitiveness and specificity of the test.

GeneSafe™ test is performed through the cfDNA study, by carrying out a blood sample from the pregnant woman with a gestational age of at least 10 weeks.
Through a complex laboratory analysis, the cfDNA is isolated from the plasmatic component of the maternal blood.
Successively we proceed to the bioinformatics analysis to detect mutations that cause a specific genetic disease, thanks to an innovative technology named Next Generation Sequencing (NGS).

GeneSafe™ includes three levels of investigation depth to make the prenatal screening closer to the needs of each pregnant woman:

Genesafe inherited

allows detecting inherited genetic diseases in the fetus
GeneSafe™ Inherited test allows detecting mutations on 4 genes responsible for the genetic diseases most frequently found in the Italian population, such as Cystic Fibrosis, Thalassemia-Beta,
Sickle cell anemia, Deafness autosomal recessive (both type 1A and 1B).

Genesafe de novo

allows the screening of severe genetic diseases not inherited from parents (de novo)
GeneSafe™ DE NOVO, allows detecting mutations on 25 genes in relation to 44 monogenic diseases. The mutations detected by GeneSafe™ DE NOVO test can occur randomly in the fetus. These mutations, named de novo, are not detectable with pre-conception screening performed on parents as non-hereditary. The presence of de novo mutations in one of the investigated genes can cause skeletal dysplasia, congenital heart defects, multiple congenital malformation syndromes, and/or neurodevelopmental disorders.

GeneSafe Complete

allows detecting in the fetus for both inherited and de novo single-gene disorders
If GeneSafe™ COMPLETE test if performed together with genome-wide cfDNA screening PrenatalSAFE™ Karyo test, it provides the most comprehensive information available from a non-invasive prenatal test to date.

INVASIVE TECHNIQUES: CHORIONIC VILLUS SAMPLING and AMNOCENTESIS

These two exams can identify a disease or defect, that being either from a chromosomal or genetic disorder. The advantage of these tests is that they have a definite answer, while the disadvantages are the risks associated with the invasiveness of the procedure.

The guidelines SIEOG 2015 and ISUOG 2016 offer the following recommendations:

- preliminary counseling for the couple to provide information on:
- general exam information
- techniques and execution period
- response times
- side effects
- risk of miscarriage
- test limits

Furthermore, it should be noted that:

• There is no scientific evidence for a particular preparation before examination;

• The use of antibiotics or miolytics has not been proven to have any benefit;

• There is no need to schedule down time after the examination, other than that needed for possible psychological recovery;

• The clinical operator must have completed an adequate training period;

• In the case of multiple pregnancies, the clinical operator must have adequate experience in the specific field.

Chorionic Villus Sampling (CVS)

What is it?

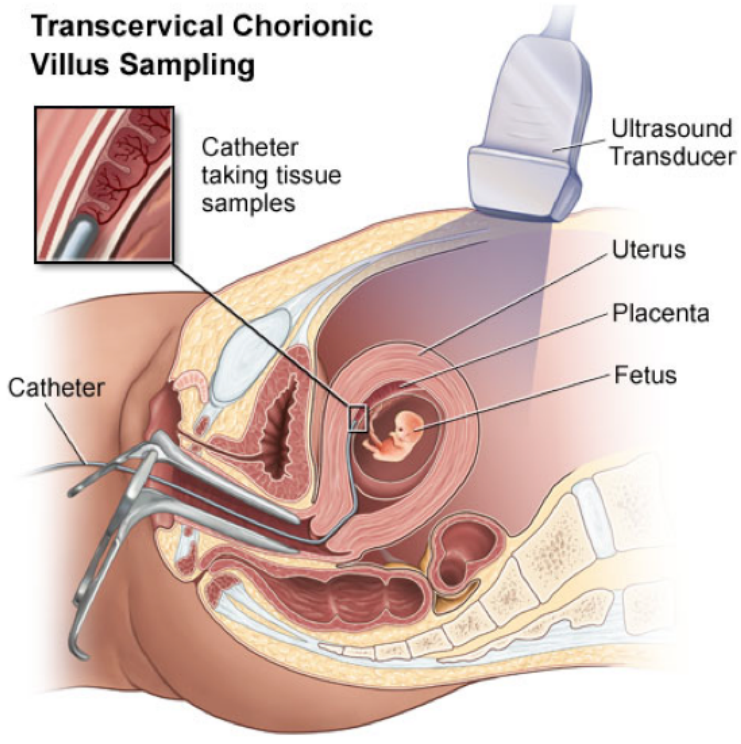
CVS is a form of prenatal diagnosis to detect disorders in the fetus. It entails sampling and testing of the placenta (chorionic villus) since it contains the same chromosomes as in the baby's cells.

When is it performed?

The test can be performed from the 10th week until the end of pregnancy. Note that performance before the 10th week may cause abnormalities in the fingers of the fetus, therefore it is preferable NOT to perform the test before the 11th week for safety reasons. In Italy, in order to have the possibility to interrupt the pregnancy, the test is made between the 11th and 12th week. Rarely is the test performed after the first quarter.

How is it performed?

The test is performed trans-abdominal by inserting a needle through the abdomen, uterus and into the placenta. Ultrasound is used to help guide the needle, and a small amount of tissue is drawn into the syringe. Local anesthesia will be used and in most cases it is done in less than a minute with a very small discomfort for the woman.



What happens after the test?

In the following 24-48 hours, abdominal discomfort may occur which can be managed with over the counter painkillers, if needed. In rare cases vaginal bleeding may occur, however, it is not an indication of a miscarriage. If the abdominal pain continues with an increase of temperature and/or there is a continued loss of blood, your medical doctor should be contacted.

When will the test results be ready?

For Down Syndrome and some other major chromosomal diseases, the results are available within three days, but often they can be ready the day after the examination. For rare defects, two weeks may be necessary. For non-conclusive results, the examination is repeated in less than 1% of the cases.

What are the risks associated with the exam?

The risk of miscarriage is less than 1% and is statistically reduced about one week after the exam.

Amniocentesis

What is amniocentesis?

Amniocentesis is an invasive technique used in prenatal diagnosis to examine amniotic fluid cells of fetal origin for chromosomal abnormalities.

How is it performed?

With the guidance of an ultrasound, a thin needle is placed in the uterus to take a small amount of amniotic fluid, containing fetal tissue. The procedure usually does not take more than a minute and does not require local anesthesia, nor rest in the days following the exam.

What are the side effects?

Abdominal cramps may occur in the following 24-48 hours. If the abdominal pain increases with vaginal bleeding, loss of amniotic fluid, and an increase of temperature, contact your doctor.

When will the test results be ready?

Within 72 hours the results of the major chromosomal diseases will be available, although they can be reported within 24 hours. Note that for some rare defects it may take two weeks.

What are the risks associated with amniocentesis?

The risk of miscarriage is about 1% (the same as for Chorionic Villus Sampling) and statistically diminishes after 5-7 days. It should be mentioned that the risk is also tied in part to the operator's experience. In addition, to avoid a minimal risk of developing alterations to the fetal lower extremities, the exam is normally performed no earlier than the 16th week.