University of Medicine and Pharmacy CRAIOVA

DOCTORAL THESIS

ABSTRACT

The complex evaluation of the fetus at 11-14 weeks - screening efficiency of chromosomal abnormalities

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PERSONAL CONTRIBUTIONS

Introduction

Ultrasound has become an integral part of a new subspecialty, or at least recently introduced in our country (but known in the U.S. since the early 1950 to 1970), maternal-fetal medicine. It offers a new approach, involving several areas: genetics, teratology, imaging, obstetrical diagnosis, maternal-fetal physiology, endocrinology, as well as the relationship between maternal health and fetal pathology.

The purpose of this science is an integrated approach for the maternal and intauterin patient and to adopt a philosophy dominated by interest in fetal behavior – in the concept of maternal-fetal medicine, the fetus is equally important as a patient with the mother.

As innocuous technique itself, however, obstetric ultrasound has generated and generates abusive medical and medico-financial behaviors, fueled by anxious developments specific to contemporary societies. Also Roy Filly said in this regard that "ultrasonic diagnosis is used by many and understood by very few." On the other hand, diagnostic ultrasound has its own pitfalls, and should be pointed out that, although sometimes it seems certain diagnosis requires a permanent distrust of the sonographer, who must reserve the right to review before stating a diagnosis, especially when it comes to one of seriousness. "Never the reality on the screen does not represent the reality of the studied object " - it must be every sonographer hidden thought, especially one who is also the obstetrician (a clinician, and an explorer) and looks at his dual profession with responsibility and adulthood.

First trimester obstetric ultrasound may fit a patient relatively suddenly in the risk group, even when she was considered "risk free", or quantify this risk more precisely when it is already known, thus improving management of birth in selected cases .

The need to understand this fascinating area that is obstetric ultrasound, to understand the structure, and then the desire to provide these things pushed me to accomplish this thesis

State of knowledge

Chapter I-Chromosomal Abnormalities - describes the main ultrasonographic markers used in the diagnosis of chromosomal abnormalities in the first and 2nd trimesters and the frequency of their changes in different chromosomal syndromes, according to studies in the literature.

Chapter II- first trimester ultrasound screening for fetal abnormalities - the protocol of the first trimester sonographic screening detection of chromosomal abnormalities is described in this chapter. Arguments are brought in favor of this type of screening and measurement techniques of used markers are described by presenting standard sections to be obtained fora described and also those that are under evaluation.

Chapter III - invasive diagnosis maneuvers: amniocentesis and chorionic villi biopsy - the chapter contains a brief description of the two maneuvers together with their standard performing technique, indications, contraindications and risks derived from their practice. An important element described in this chapter is the counseling prior to screening.

Personal contributions

Objectives of the doctoral thesis - Internationally in recent years numerous studies have been conducted on the development of testing options to assess the risk for a pregnant woman to have a child with chromosomal abnormalities. But screening decision is a personal one that can be influenced by the presence or absence of a family history of aneuploidy or genetic diseases of obstetric or medical history of the mother, its attitude to abortion, education, religious convictions and economic concerns.

Specialists concern is to bring new screening strategies aimed mainly to the reduction of births of children with chromosomal abnormalities.

Few studies are available in our country regarding antenatal screening methods for chromosomial abnormalities, the research aiming to conduct a modal study- genetic, imaging and biochemical to permit the assesment of the performance for this screening method in the antenatal diagnosis of fetal chromosomal abnormalities.

Objective of the study was to evaluate the performance of the screening method, indications for amniocentesis or CVS which we performed followed by genetic tests to diagnose fetal chromosomal abnormalities.

Another important aspect aimed during the study was to determine the degree of association between aspects of chromosomal abnormalities and fetal ultrasound to estimate the riskiness of individual markers using likelihood estimation analysis.

Methods - the doctoral theme researched in this paper was coordinated by Prof. Univ. Dr. Nicolae Cernea and was included in the grant "MARKERS OF SCREENING AND EARLY MANAGEMENT CRITERIA IN FETAL Chromosomopathies (SONOSEROSCREEN)" PC 41-041 conducted in the Clinic of Obstetrics and Gynecology Emergency County Hospital of Craiova.

The study aimed mainly to assess the efficiency of ultrasound in the first trimester and early detection of chromosomal abnormalities and limitation of the invasive maneuvers (CVS, amniocentesis) performed for genetic purposes and was carri out between January 2008 - September 2011 in te Clinic of Obstetrics and Gynecology of the Emergency County Hospital Craiova and the Department of Obstetrics and Gynecology of the Municipal Philanthropy Hospital Craiova.

This prospective study included all patients who presented to first-trimester screening to assess the risk of chromosomal abnormalities associated with each pregnancy. A detailed anatomic evaluation protocol was applied to these pregnancies. It is shown that the study of fetal anatomy optimizes the chromosomial screening outcome - which means increased predictive value, together with the reduction of false-positive.

In this study we aimed to assess the efficiency of the screening test at 11-13 +6 weeks, evaluating the efficiency of ultrasound and biochemical markers, the major benefit of detecting anomalies in the first trimester ultrasound, amniocentesis rate evaluation, evaluation of genetic results.

First trimester prenatal screening protocol

The survey was conducted over 3 years (January 2008 - September 2011) in a prospective morphological and genetic study of first trimester pregnancies pursued in two university clinics of UMF Craiova.

The main criteria of eligibility for inclusion in this study:

• On request

• History of pregnancies with chromosomopathies

• Obvious ultrasound malformation

• Age over 37 years

• Family chromosomopathies

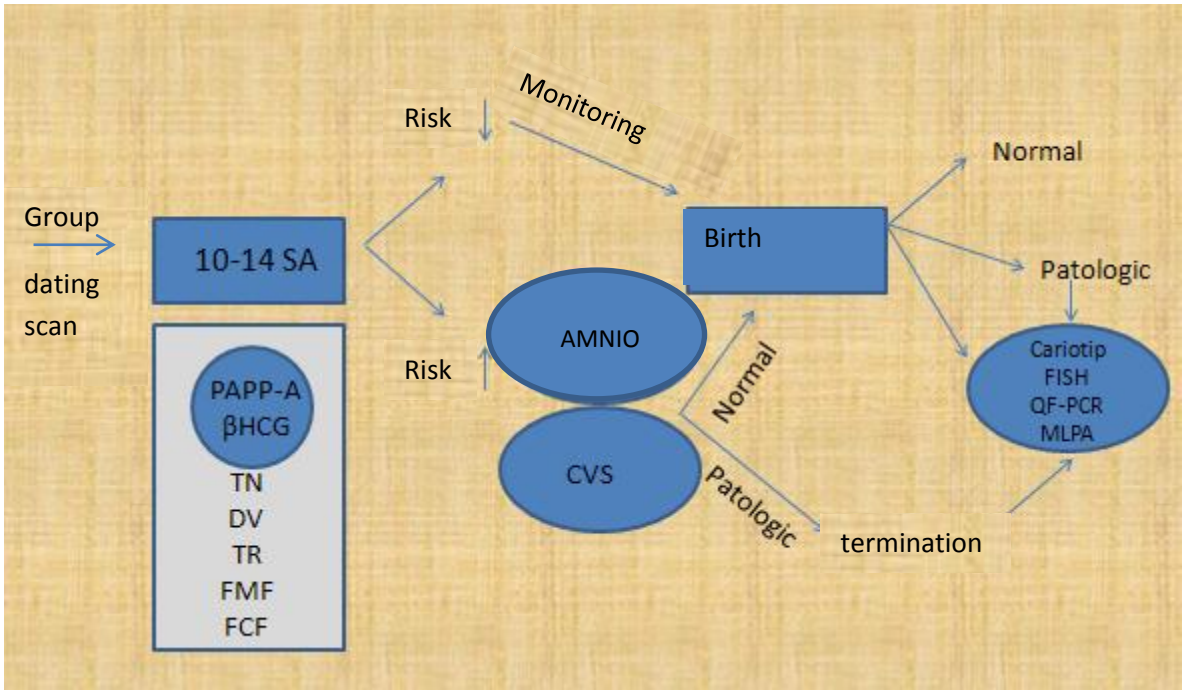


Fig. 2 The protocol of monitoring pregnancies included in the study

Among maternal history were marked the general personal (cardiac, renal, hematologic, collagen diseases, endocrine pathology, pulmonary pathology, etc..) where I have not noticed significant influence on the incidence of chromosomal abnormalities. Also the pregnant women were surveyed on personal obstetrical history (number of births, abortions, causes of abortion - spontaneous / provoked ectopic pregnancies sterility / infertility).

EVALUATION OF THE ULTRASOUND MARKERS OF CHROMOSOMOPATHIES AT 11-13^{+6} WEEKS

As an approach method, the assessment was made both by transabdominal ultrasound and transvaginal ultrasound. For data collection we used Ultrasound Voluson 730 Pro and Expert machines, GE Medical Systems Kretztechnik, OHG4871 Zipf Austria conventional trans-abdominal probe of 3.5 MHz and 7.5 MHz endovaginal. We also used GE Voluson E8 Expert The ultrasound machine. I also used 3D/4D color Doppler velocimetry.

The benefits of ultrasound examination at 11-13 weeks include, correct dating of pregnancy, first trimester misscariage diagnosis, risk assessment of Down syndrome and other chromosomal abnormalities and the presence of certain major congenital anomalies, multiple pregnancy diagnosis and correctly specifying chorionicity and amnionicity. Major advantages of the ultrasound are the precocity of diagnosis and the possibility of estimating maternal-fetal outcome from the first trimester of pregnancy (recently demonstrated that certain parameters of ultrasound 11-13 SA can be used to select patients at high risk of developing preeclampsia in the third trimester).

All patients carries a risk of having a fetus with chromosomal abnormality

• The risk depends on the a priori maternal age

• Subsequently, it is modified by ultrasound parameters and blood test values, between 11-13 weeks of gestation, and hence the risk for a specific pregnancy.

• The risk of trisomy 21 (Down syndrome) increases with maternal age and decreases with increasing gestational age, as 30% of affected fetuses die in utero, between 12-40 weeks

• The risk for T21 increases with maternal age, but because lots are uneven (there are many more young pregnant patients), most of the T21 fetuses are from mothers under 35 years

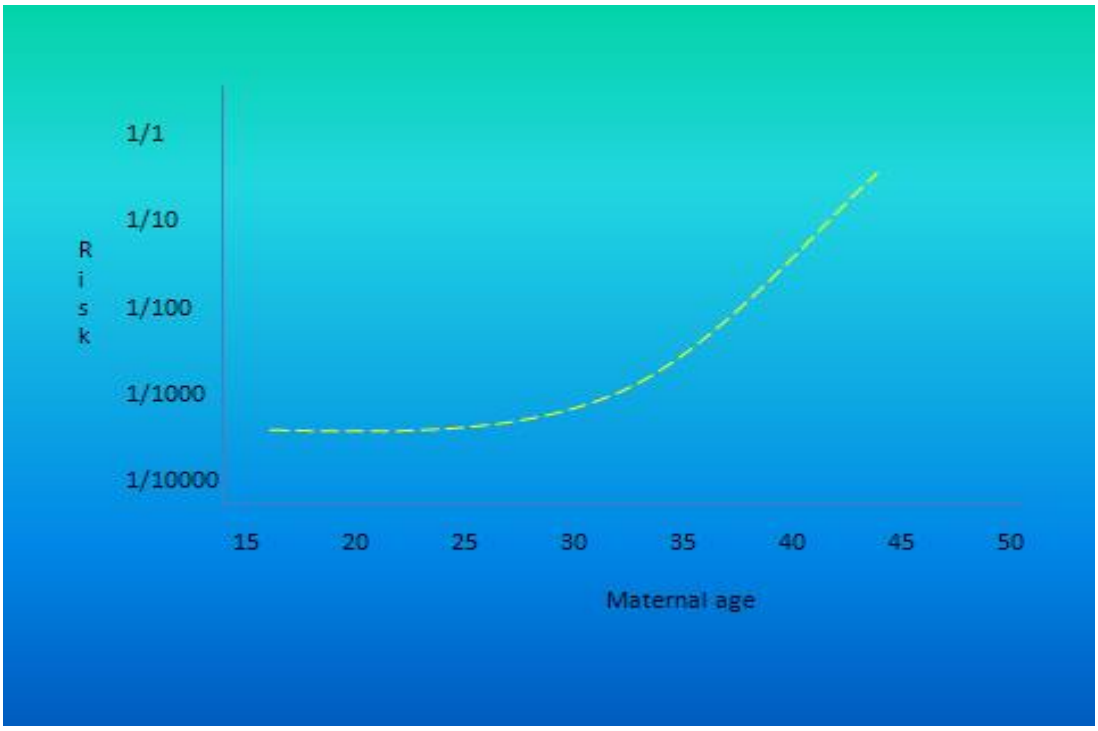


Fig. 3 Evolution of T21 risk in relation to maternal age

Results - During the period 01.01.2008-01.09.2011 combined test was performed at 2214 patients. Criteria for inclusion in the screening were:

- Age over 37 years - 320 cases
- History of pregnancy with chromosomopathies -37 cases
- Family chromosomopathies-27 cases
- On request 1787 cases
- Obvious ultrasound malformations - 43 cases

DETAILED PRENATAL SCREENING PROTOCOL FOR FIRST TRIMESTER

Combined screening protocol (biochemical and ultrasonographic evaluation profile 11-13^{+6} weeks) for the first trimester was applied in 2214 cases. To perform prenatal screening we used a cut-off risk of 1/250, which has established a group of 137 pregnancies (6.18% of the population studied) who had high risk of chromosomal abnormality and the remaining 2077 patients (93.82%) had no risk at the prenatal screening.

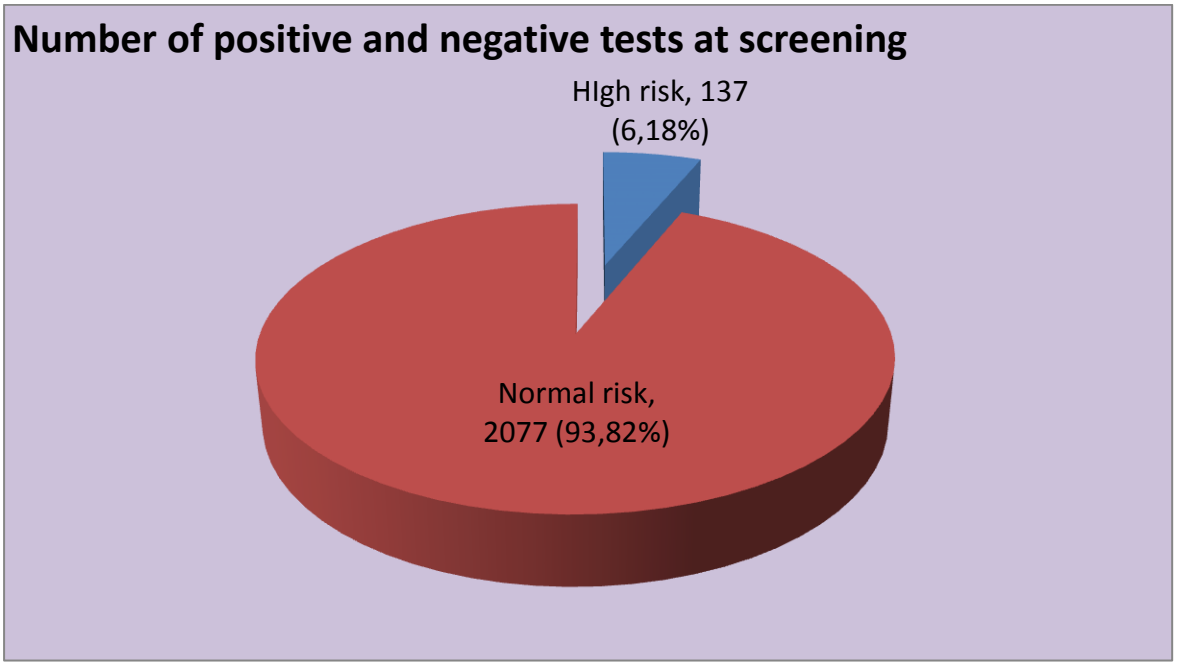
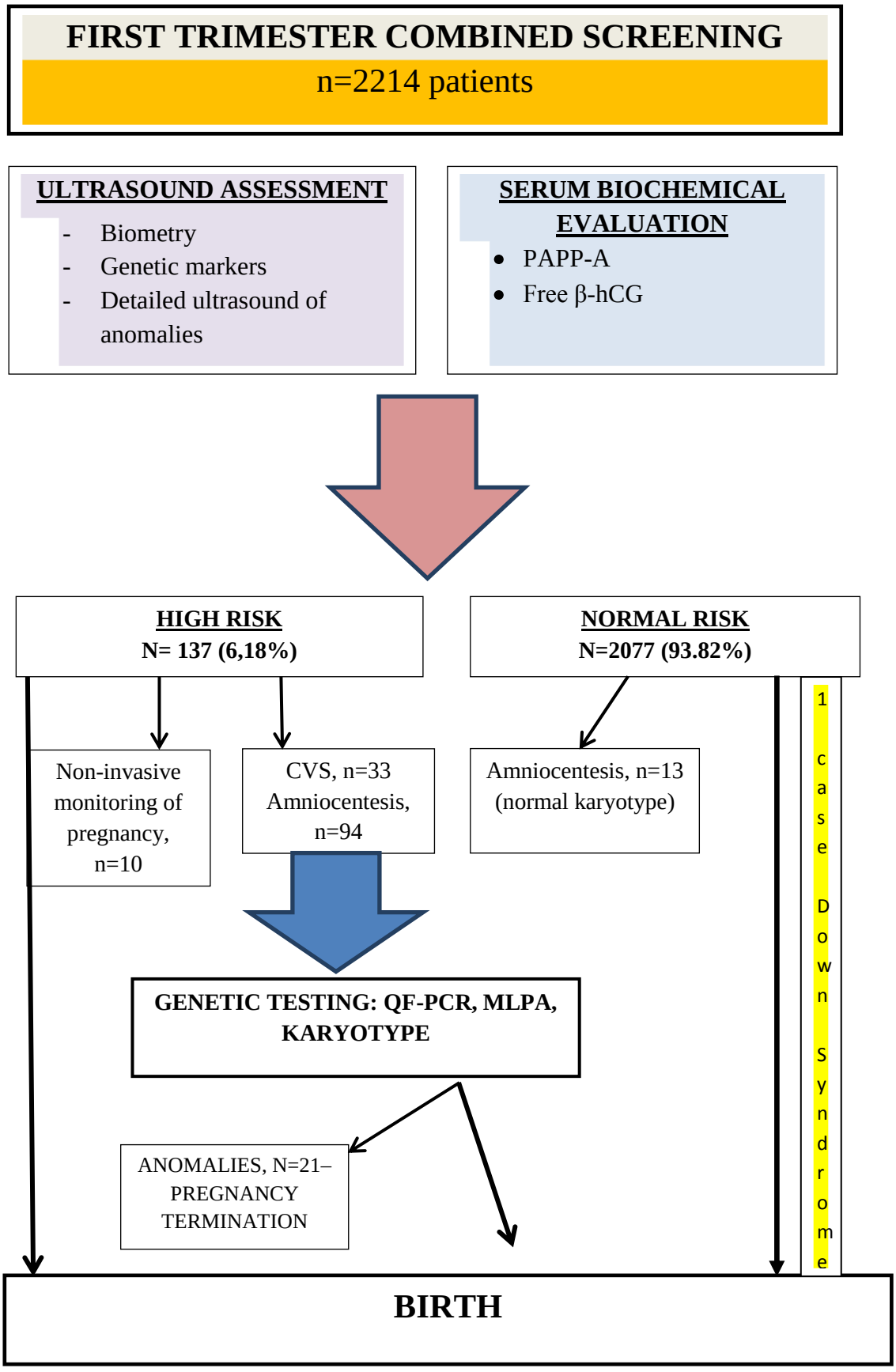


Figure 1. The incidence of positive tests for screening

In this prospective study, we sought to investigate the applicability of the new ultrasound markers in the first trimester screening protocol of chromosomal abnormalities (double test). The validation of these new markers would enable the detection of a significant proportion of fetal anomalies, which could lead to non- invasive early detection of a higher number of chromosomopathies. Another advantage would be that investigating viewable fetal

morphology from 11 to 13^{+6} weeks of gestation with the confirmation of normality of structures would lead to reclassification of pregnancies with increased or intermediate genetic risk into the reduced risk group for chromosopaties. Recent software for the calculation of genetic risk have considered many early fetal morphological parameters (The First trimester Screening Programme Fetal Medicine Foundation and Astraea).


FIRST TRIMESTER COMBINED SCREENING
n=2214 patients
ULTRASOUND ASSESSMENT
- Biometry
- Genetic markers
- Detailed ultrasound of anomalies
SERUM BIOCHEMICAL EVALUATION
• PAPP-A
• Free β-hCG
HIGH RISK
N= 137 (6,18%)
NORMAL RISK
N=2077 (93.82%)
Non-invasive monitoring of pregnancy, n=10
CVS, n=33
Amniocentesis, n=94
Amniocentesis, n=13 (normal karyotype)
1 case Down Syndrome
GENETIC TESTING: QF-PCR, MLPA, KARYOTYPE
ANOMALIES, N=21– PREGNANCY TERMINATION
BIRTH

Gestational age was based on last menstrual period or on the measurement of CRL. In case of patients who did not know the exact date of the last menstrual period, gestational age determination was based solely on ultrasound parameters. Patients whose pregnancy was obtained through IVF, gestational age determination was calculated based on the date of embryo transfer.

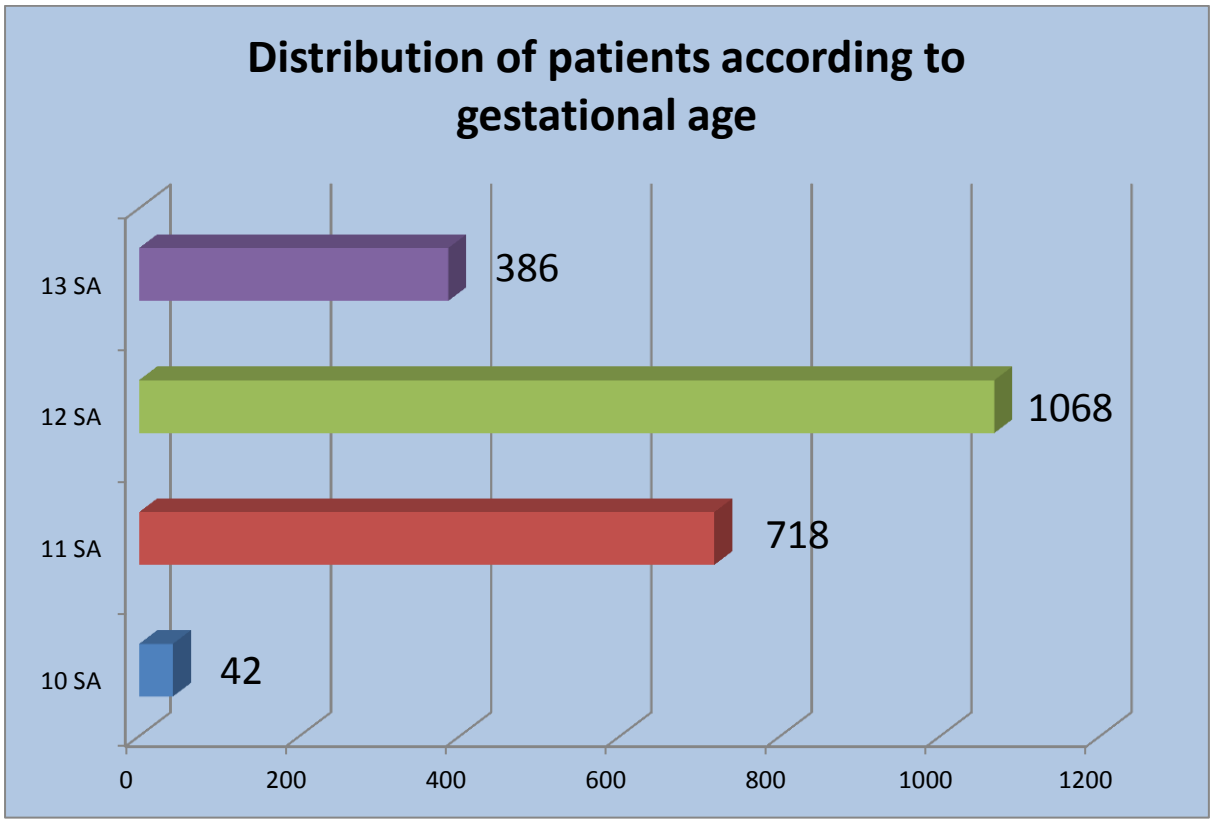


Chart 3. Distribution of patients according to gestational age on which the screening was performed

IVF pregnancy was obtained in a total of 24 pregnant women (1.08%), the other 2190 (98.92%) obtaining spontaneous pregnancy. Twin pregnancies were found in 22 cases (0.99%), of which two were monochorial-monoamniotic, 4 monochoriale-biamniotic and 16 bicoriale, biamniotice. Of the 2214 pregnant women 453 were smokers and 1761 nonsmokers. There were 8 patients suffering from diabetes insulin dependent, and 2 patients were treated with anticonvulsants. Measurement of nuchal edema, nasal bone, tricuspid valve and and ductus venous flow, FMF angle and heart rate was performed between 11 and 13^{+6} weeks.

Most of pregnant women with positive risk were within the age group 35 to 40 years (72 patients), followed by the age group 30-35 years (21 patients). In the age group over 40

test was positive in 19 patients, in the age group 25-30 years the test being positive in 15 patients, the remaining 10 patients were in the age group 20-25 years.

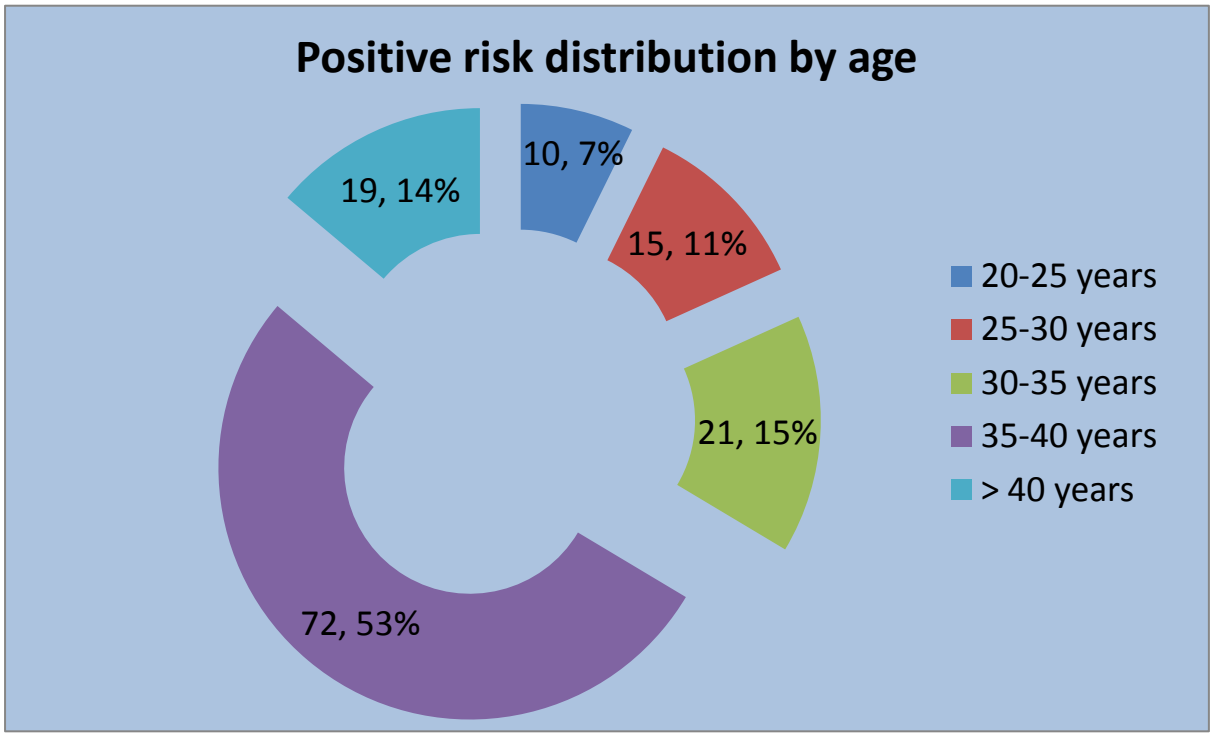


Figure 4. Risk distribution by age

Of the 137 cases with positive tests, four patients obtained pregnancy through IVF, and the remaining 133 pregnant pregnancy weere obtained spontaneously. Twin pregnancies were found in 4 cases out of 137 with positive risk, one being monochorial-monoamniotic, 2 bichorial-biamniotic and one monochorial-biamniotic. Smoking and positive tests were 47, the remaining 90 patients were non-smokers. Insulin-dependent diabetes mellitus was present in 3 patients with positive tests. Of the 137 cases with positive tests, ultrasound visible malformations were found at 11 patients (3 cases with cardiac malformations, cystic hygroma, 4 cases, 2 cases, the fetus was with multiple malformations, 1 case - diaphragmatic hernia, 1 case-hydrocephalus).

No	Chromosomal abnormalities detected by molecular methods and classical karyotyping	Abnormal morphological and functional markers	Serum biochemical results
1	trisomy 21	• absent NB	PAPP-A 0.62 Corr.MoM hCG 1.87 Corr.MoM
2	trisomy 21	• NT 3.4 mm • Exomphalos	PAPP-A 0.71 Corr.MoM hCG 1.8 Corr.MoM
3	trisomy 21	• NT 3.7 mm, • Enlarged FMF, • Renal pyelectasis, • hyperechoic bowel.	PAPP-A 0.52 Corr.MoM hCG 3.41 Corr.MoM
4	trisomy 21	• NT 3.7, • absent NB, • "a" wave reversed at the level of DV.	PAPP-A 1.31 Corr.MoM hCG 2.65 Corr.MoM
5	trisomy 21	• NT 4.1, • atrioventricular septal defect, • Tricuspid Regurgitation (TR).	PAPP-A 0.74 Corr.MoM hCG 2.38 Corr.MoM
6	trisomy 21	• NT 3.9 mm, • absent NB, • Tricuspid Regurgitation, • reversed "a" wave at the level of DV.	PAPP-A 1.51 Corr.MoM hCG 5.13 Corr.MoM

		• Enalrged FMF angle, • Exomphalos that contains liver, • Single umbilical artery (SUA).	
7	trisomy 21	• NT 4.2 mm, • absent NB, • Tricuspid Regurgitation, • reversed "a" wave at the level of DV, • megacystis, • atrioventricular septal defect.	PAPP-A 0.83 Corr.MoM hCG 4.24 Corr.MoM
8	trisomy 21	• absent NB	PAPP-A 0.72 Corr. MoM hCG 1.97 Corr. MoM
9	trisomy 21	• NT- 3.7 mm • Absent NB • Enlarged FMF	PAPP-A 0,82 Corr. MoM hCG 1.94 Corr. MoM
10	trisomy 21	• NT 4.1 mm • Absent NB	PAPP-A 0.52 Corr. MoM hCG 3.2 Corr. MoM
11	trisomy 21	• Absent NB • Tricuspid Regurgitation	PAPP-A 1.2 Corr. MoM hCG 2.83 Corr. MoM
12	trisomy 21	• Absent NB • reversed "a" wave at the	PAPP-A 0.84 Corr MoM

		level of DV • enlarged FMF	hCG 2.42 Corr. MoM
13	trisomy 21	• NT 4.1 mm • hyperechoic bowel	PAPP-A 1.71 Corr. MoM hCG- 3.8 Corr MoM
14	trisomy 21	• NT- 3.9 mm • Absent NB • reversed "a" wave at the level of DV	PAPP-A 0.73 Corr. MoM hCG 4.1 Corr. MoM
15	trisomy 21	• NT 3.4, • Enlarged FMF, • Multiple choroid plexus cysts localized bilateral, • Diaphragmatic hernia, • Single umbilical artery, • unilateral lesion of the forearm (radius hypoplasia) • Hands fixed in flexion position.	PAPP-A 0.34 Corr.MoM hCG 0.93 Corr.MoM
16	trisomy 18	• NT 3.5, • absent NB, • Exomphalos that contains liver	PAPP-A 0.68 Corr.MoM hCG 0.56 Corr.MoM
17	Trisomy 18	• NT 4.2 • reversed "a" wave at the level of DV • tricuspid regurgitation • enlarged FMF • Atrioventricular septal defect	PAPP-A 0.76 Corr MoM hCG 0.72 Corr MoM

		• vicious hands position (contracture) • Early growth restriction • horseshoe kidney	
18	trisomy 13	• Laparoschizis, • holoprosencephaly, • Early growth restriction • Abnormal heart, • low heart rate.	PAPP-A 0.32 Corr.MoM hCG 0.92 Corr.MoM
19	Monosomy X	• Severe edema of the head, neck, thorax and abdomen; • omphalocele, • Umbilical cord cyst, • reduced left ventricle, aortic arch hypoplasia.	PAPP-A 0.41 Corr.MoM hCG 1.38 Corr.MoM
20	Deletion of chromosome 10 short arm	• TN 3.7 • venous duct agenesis • 2nd: abnormal facial profile, short femur, short humerus, vicious position of the hands	PAPP-A 0.62 Corr MoM hCG 1.2 Corr MoM
21	triploidy	• TN 3.8 • common arterial trunk • Hydrocephaly	PAPP-A 0.66 Corr MoM hCG 2.4 Corr MoM

Table No V. sonographic and biochemical characteristics of pregnancies with fetuses with chromosomal abnormalities

Distribution of ultrasound markers for high risk combined test

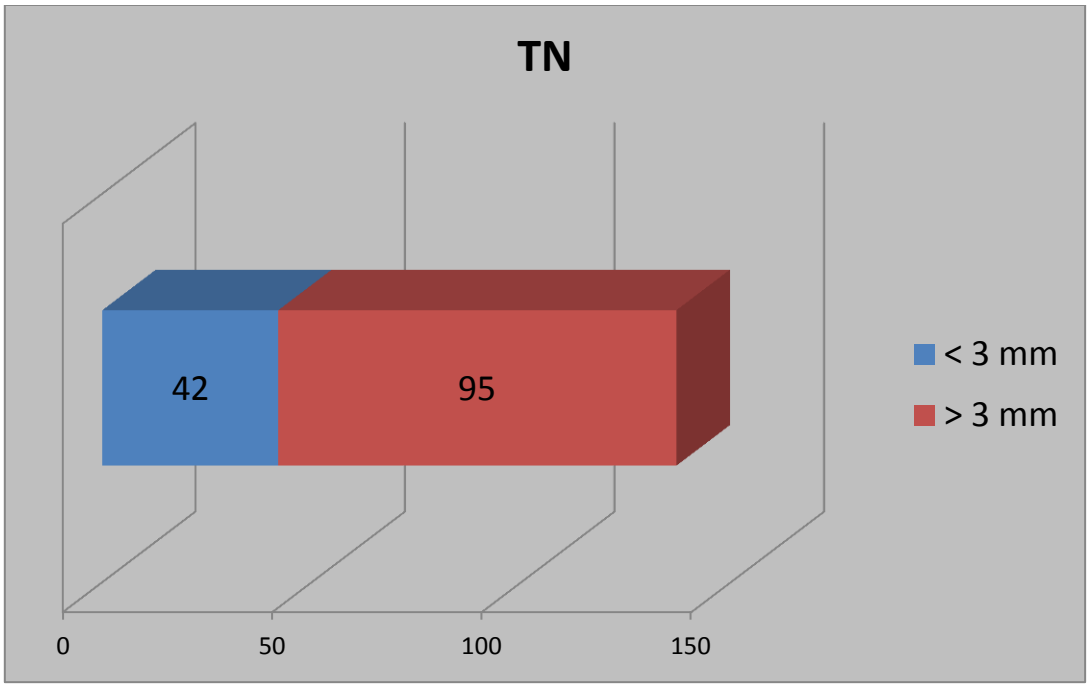


Chart 5. nuchal translucency

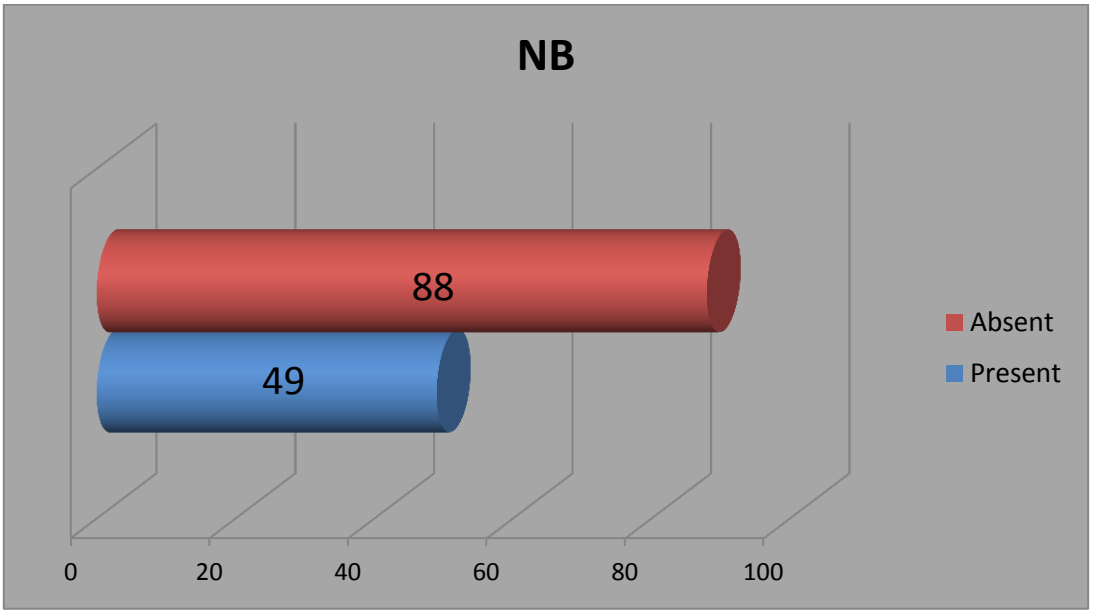


Chart 6. nasal bone

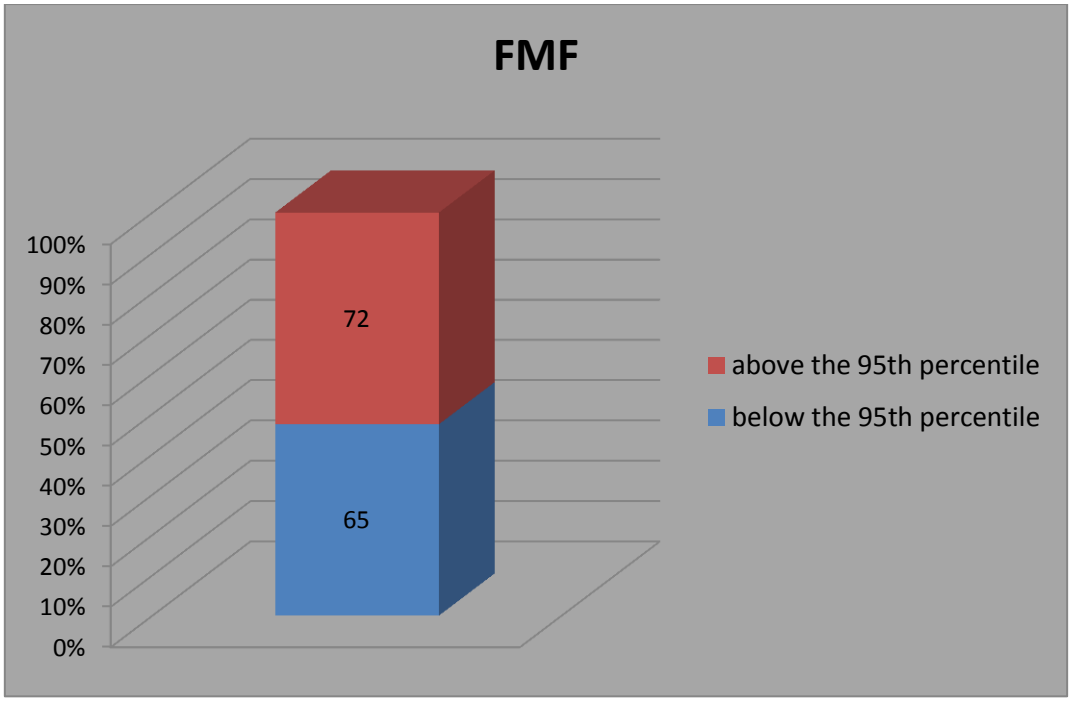


Chart 7. Fronto maxillo facial angle

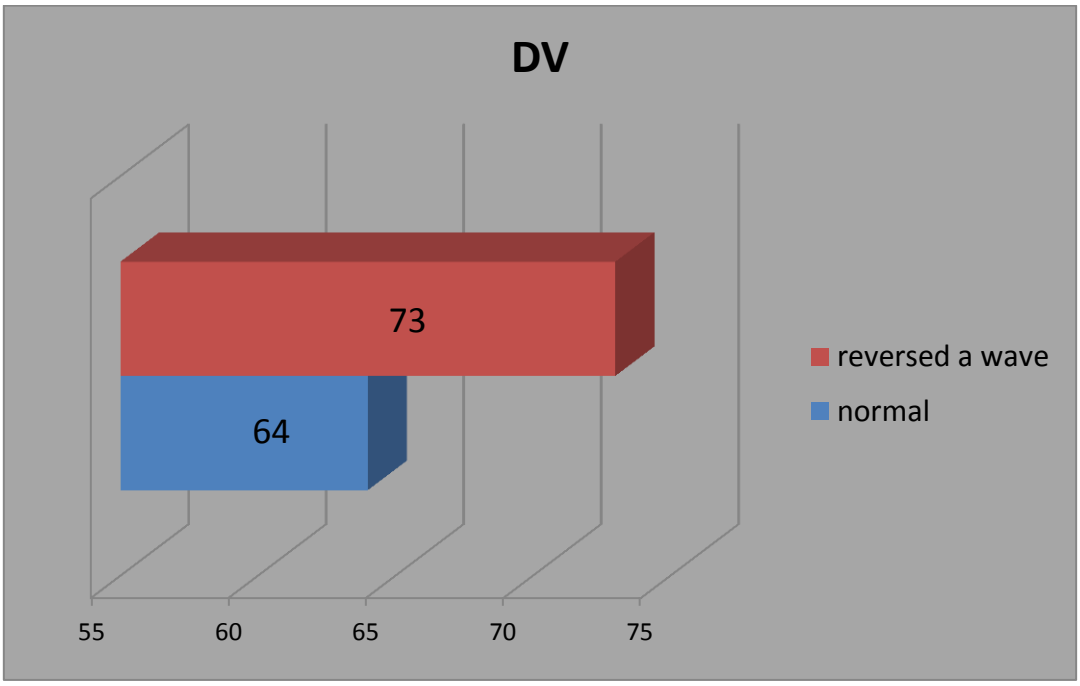


Chart 8. ductus venous

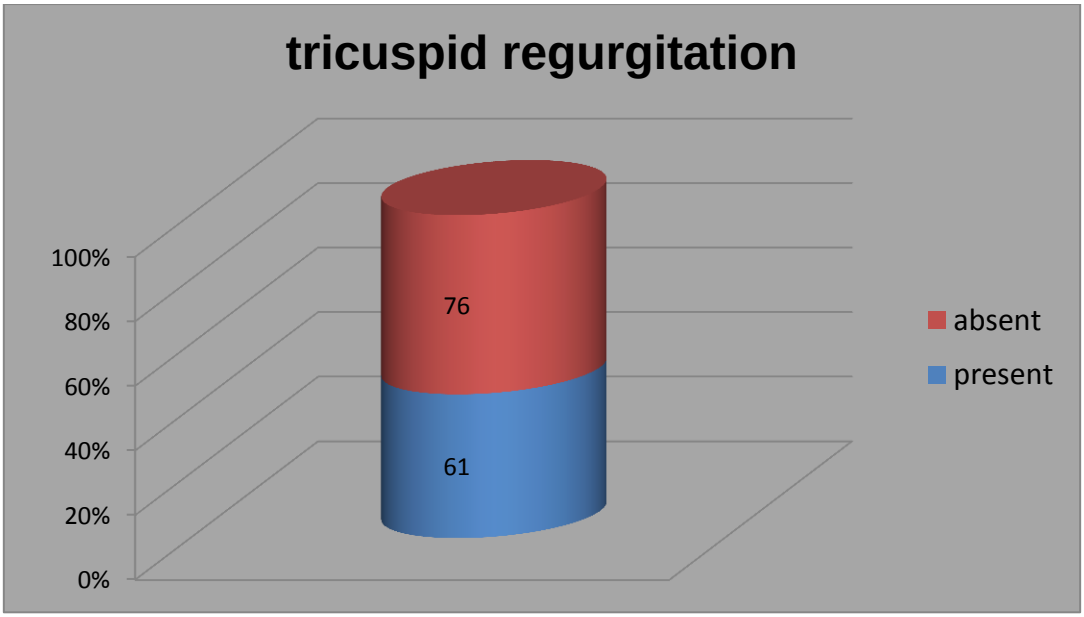


Chart 9. tricuspid regurgitation

After genetic counseling 68.61 % of patients have given consent for the invasive genetic investigation. Of the 137 cases with positive tests , amniocentesis was performed in 94 cases, chorionic villi biopsy in 33 cases, exposing the 21 chromosomal abnormalities. The 10 patients who refused amniocentesis or CVS, they were contacted by phone after the probable date of birth and 9 confirmed the birth of children with normal phenotype, in one case a child was born with Down phenotype confirmed by karyotype. Besides the 94 patient with positive test who have been investigated through genetic invasive maneouvers, amniocentesis was carried out on request in 13 patients with a negative test, resulting normal karyotype. In patients with combined test positive, the risk of chromosomal abnormalities was statistically significant. The distribution by age of the 22 pregnant women who were confirmed by amniocentesis or by phone: nine were over 40 years old, two 39 year old, two 38 year old, one 37 year old, four were aged 35 years, two were 23 and two 20 years. Major indications for performing screening were advanced maternal age, abnormal ultrasound markers in first-trimester screening, or screening ultrasound abnormalities.

Conclusions - Despite decades of research dedicated to prenatal screening, strategies regarding the best method for detection of prenatal abnormalities are in constant improvement.

Following the implementation of detailed screening protocol in the first trimester - which included biochemical testing (free β-hCG and PAPP-A), detailed assessment of

functional markers and ultrasonographic fetal morphological ones - a higher percentage of detection of fetal anomalies could be revealed. The combination of morphological markers resulted in a more accurate assessment of fetal anomalies detection with a percentage only slightly lower than the CVS, thus proving invaluable as markers in first-trimester prenatal diagnosis. Given those presented in this thesis, the need to include morphological markers of fetal anatomic screening of the first trimester has been shown.

Screening positive rate is directly related to the number of invasive tests and, therefore, it is important to be kept as low as possible in order to avoid unnecessary losses of fetuses. It is obvious that various new strategies for screening are promising in terms of reducing the rate of positive screening. It is really important to investigate and improve screening methods using various strategies, given that fetal medicine centers around the world differ in how the service is configured in terms of resources available. As screening for trisomy 21 is becoming more common also in less developed countries, with a limited number of sonographer and possible logistical challenges in collecting blood samples it becomes relevant the need to improve and develop various potential screening programs for the purpose of optimizing the use of existing resources.

It might be argued that screening for T18 and T13 in the first trimester is not necessary because most fetuses either die in utero or they will be detected when performing ultrasound in the second trimester. Detection rates for T18 and T13 at ultrasound in the second trimester were reported 80-86% for T18 and 90-100% for T13. However, no ultrasound screening algorithm in the second trimester is available therefore screening positive rate is unknown. The overall rate of positive screening increases only slightly (0.1%) when screening algorithm for T18 and T13 is included in the first trimester screening for T21, and it is considered acceptable to include screening for T18 and T13 in the program screening.

Most women prefer screening to be performed early in pregnancy if the fetus is diagnosed with T18 or T13, so parents can opt for a safe termination in the first trimester of pregnancy.

In the last 30-40 years the screening for chromosomal abnormalities changed from using maternal age as the only variable to using multiple markers and risk complicated algorithms that can provide specific individual risk assessment in the first 3 months of pregnancy. It became available to offer in many countries as a major advantage for many pregnant women and their fetuses because screening reduces the number of fetal losses due to unnecessary invasive procedures. In the future it may become more common also screening

for other complications during pregnancy, such as preeclampsia and premature birth by the concept developed at the chromosomal abnormalities screening. However it should be noted that also in the future, screening for pregnancy complications and for chromosomal abnormalities must always be based on an informed choice where women based on their knowledge and values make decisions individually and are free to accept or refuse screening in pregnancy.

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