

**NEW ASPECTS AND THE INTEGRATION OF THE
FIRST AND EARLY SECOND TRIMESTER
SCREENING TO THE FETO-MATERNAL MEDICINE**

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Ph.D thesis

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**University of Szeged
SZEGED, HUNGARY**

2015.

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List of publications related to the dissertation

- I. **Response to “Comment to “Nasal bone length: prenatal thickness ratio: a strong 2D ultrasound marker for Down syndrome””**
Károly Szili, Andrea Szabó, János Szabó
Prenatal Diagnosis 2015, (in press) Impact Factor: 2.514
- II. **Új módszerek a Down-szindróma második trimeszterbeli ultrahangszűrésére: az orrcsonthosszúság és a praenatalis lágyrész-vastagság mérésének statisztikai elemzése**
Szili Károly, Szabó Andrea, Vanya Melinda, Bártfai György, Szabó János
Orvosi Hetilap Orv.Hetil., 2014, 155(47), 1876–1881 (precalc. Impact Factor: :0.390)
- III. **Is it Possible to Improve 2nd Trimester Screening Efficacy with a Combined 1st and 2nd Trimester Nasal Bone Length Normogram?**
Szili K., Szabó A.Sz., Vanya M., Szabó J.
The Journal of Reproductive Medicine (In press, Accepted: Oct. 2014) Impact Factor: 0.688
- IV. **Nasal bone length:prenatal thickness ratio: a strong 2D ultrasound marker for Down syndrome**
Szabó Andrea, Szili Károly, Szabó János Tamás, Sikovanyecz János, Isaszegi Dóra, Horváth Emese, Szabó János
PRENATAL DIAGNOSIS Volume 34, Issue 12 (pages 1139–1145) Impact Factor: 2.514
- V. **Az egészséges életér—az otthoni mikrokörnyezet vizsgálati modellje**
Lipták-Váradí Julianna, Szili Károly, Vanya Melinda, Széll Márta, Szabó János, Szabó Andrea, Kató Lilla
ÉPÍTÉS ÉPÍTÉSZETTUDOMÁNY 41:(3) pp. 271-282. (2013) Impact Factor: 0
- VI. **A prenazális lágyrész vastagodás a 21-es triszómia ultrahang jele a második trimeszterben**
Szabó Andrea, Szili Károly, Szabó János Tamás, Isaszegi Dóra, Horváth Emese, Sikovanyecz János, Szabó János
MAGYAR NŐORVOSOK LAPJA 76: pp. 24-27. (2013) Impact Factor: 0
- VII. **Early embryonic heart rate and pregnancy outcome (*citable abstract*)**
Szili K., Ferencz E, Szabó A, Szabó J, Sikovanyecz J
ULTRASOUND IN OBSTETRICS & GYNECOLOGY 40:(S1) pp. 234-235. (2012) IF:3.14
- VIII. **Diagnosis and counseling of women with single umbilical artery should be confined to first-trimester (*citable abstract*)**
Szabó J, Horváth E, Szili K., Sikovanyecz J
ULTRASOUND IN OBSTETRICS & GYNECOLOGY 36:(S1) p. 119. 1 p. (2010) IF:3.14
- IX. **Effects of maternal epilepsy and antiepileptic therapy in women during pregnancy**
Melinda Vanya, Nóra Árva-Nagy, Károly Szili, Délia Szok,György Bártfai
Ideggyógyászati Szemle/Clinical Neuroscience Impact Factor:0.382

Aims and objectives of the dissertation

1. To provide a comprehensive literature review in the main scopes of fetal medicine including prevention; etiology of prenatal conditions and diseases; ultrasonography, biochemical and non-invasive prenatal screening and of course diagnostics.
2. To summarize the clinically important and published findings in the field from the prevention up to the diagnostics.
3. To find and collect the most sensitive screening methods and markers of the trisomies.
4. To create and publish the population specific normograms of ultrasonography markers of the Hungarian population
5. To find and describe the best ultrasonography screening methodology for autosomal trisomies.
6. To develop more sensitive screening methods or techniques with the combination of new and old screening markers.
7. To confirm or deny the first and only publication in our knowledge about combination of nuchal translucency and ductus venosus pulsatility index in the first trimester.
8. To observe the possible efficacy of the second trimester facial markers in the first trimester screening.
9. To observe the combination of the first and second trimester nasal bone length normogram.

1. Introduction

1.1. Health

Definition of health

Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.

1.2. Prevention

Definitions of the prevention:

To define the prevention and find the correct word on it we need to check several definitions of prevention.

(1) "Prevention in nursing care: actions directed to preventing illness and promoting health to reduce the need for secondary or tertiary health care. Prevention includes such nursing actions as assessment, including disease risk; application of prescribed measures, such as immunization; health teaching; early diagnosis and treatment; and recognition of disability limitations and rehabilitation potential. In acute care nursing many interventions are simultaneously therapeutic and preventive".(1)

(2) "The management of those factors that could lead to disease so as to prevent the occurrence of the disease." (2)

(3) "The prevention in disease control terms includes measures designed to prevent the introduction of a disease into areas where it does not already exist, and improve the resistance of the population and reduce the chances of the infection spreading, when the disease already exists in the population." (3)

(4) "Action so as to avoid, forestall, or circumvent a happening, conclusion, or phenomenon (for example, disease prevention).(4) (5) mid-15c., "action of stopping an event or practice," from Middle French *prévention* and directly from Late Latin *praeventionem* (nominative *praeventio*) "action of anticipating," noun of action from past participle stem of *praevenire* (see prevent). (5)

In contrast to the „promoting health” was found alone in a definite, and the words of „disease” and „factors” were used in a several times by other definitions.

Prevention in Fetal Medicine

During literature review the definition of prevention in fetal medicine was not found. Thither are many reasons, why it is necessary and why not recommended to create the definition preventive fetal medicine. Nevertheless, to keep the ethical aspects the definition of prevention in fetal medicine must be legalized.

To use definitions of prevention in the fetal medicine, we bear to adjust and mix their aspects. „The prevention in fetal medicine is actions directed to promoting their health before, during, and after the pregnancy, to decrease and assess risk of risk factors and to preventing the fetomaternal diseases, abnormalities and adverse outcomes of pregnancy.”

This complex definition is not fully satisfactory but could be useful to see the grandness of this thesis, and might be used by some other publication, besides.

1.3. Medical Genetics

Medical Genetics is a medical specialty made up of clinical geneticists who are physicians certified in multiple or different clinical specialties.

The aim of the medical genetics is to find, to prevent, to observe and to cure factor or the background of the single gene, polygenetic, epigenetic and complex disease in humans.

Before a genetic test, there is pre-test consultation where a genetic counselling with detailed information have been given to the subject(s) (and their relatives if necessary). The result and another detailed counselling will be given after the post-test genetic counselling.

1.4. Fetal Medicine

Definition of fetal medicine

A multidisciplinary branch of medicine that trades with the growth, development, prevent, care, and treatment of the fetus and with environmental components that may harm the fetus. The major area of fetal medicine is the major physical anomalies. Which could be observed cc. They are seen in approximately 3-6% of newborns. (6) The "major physical anomaly" means a physical anomaly that has cosmetic or functional significance, another 1-3% will have malformations (including internal, genetic - Biochemic, structural, mental, or perceptive condition) detected later in childhood or life. These congenital malformations account for about 20% of deaths in the perinatal period.

Scope of the fetal medicine

Fetal medicine has many interrelated studies such as maternal medicine, obstetrics, public health, midwifery, gynecology, birth rate, medical genetics and genomics, epigenetics, Neonatology, Perinatology, pediatrics, radiology...etc.

Aim of the fetal medicine

The aim of the fetal medicine to observe, define, prevent, evaluate factors of fetal development and find solution or prevention strategy to fetal diseases.

Many countries has been 'separated' from another branch of medicine and using as a unique field with or without maternal medicine (fetal medicine or feto-maternal medicine).

Thither was a substantial demographic change in the maternal age in the Hungarian population. A drastic decline can be observed in the proportion of women under 25, while a significant increasing tendency in the proportion of women over 30 or more. The rate of 35 year old or older pregnant increased 15.6% till 2009 with more than 7% from 2001. (see Figure 1)

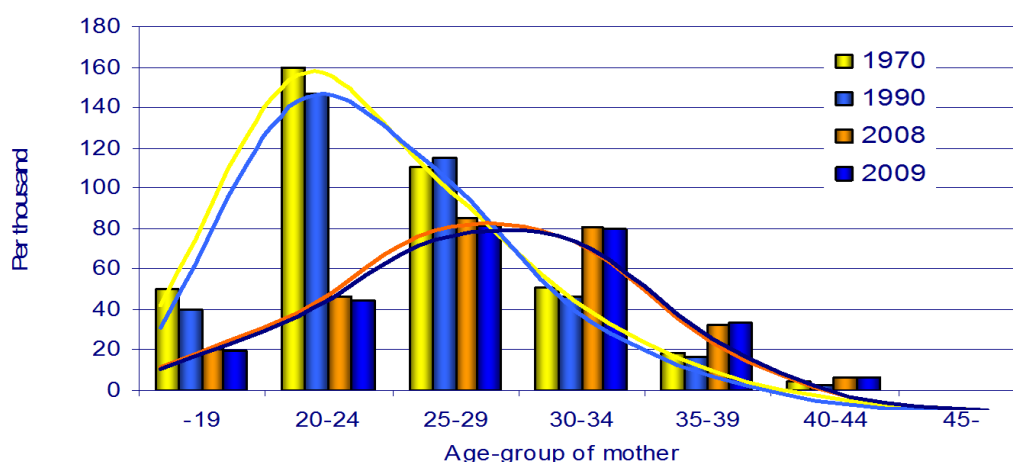


Figure 1 Live births by age-group of mother per 1000 females of corresponding age between 1970 and 2009
Source: Hungarian Congenital Abnormality Registry (HCAR) 2011

Department of Hungarian *Congenital Abnormality Registry* (HCAR or VRONY) is a good monitor of fetal defects in Hungary. Approximately 5-6% of fetuses have congenital abnormalities at birth in Hungary (HCAR National Report of Birth Defects, 2011).

The incidence of fetal anomalies has been increased during the last decades (Figure 2), however many studies suggesting that's could be the issue of the modern lifestyle or the improved health and more developed technologies.

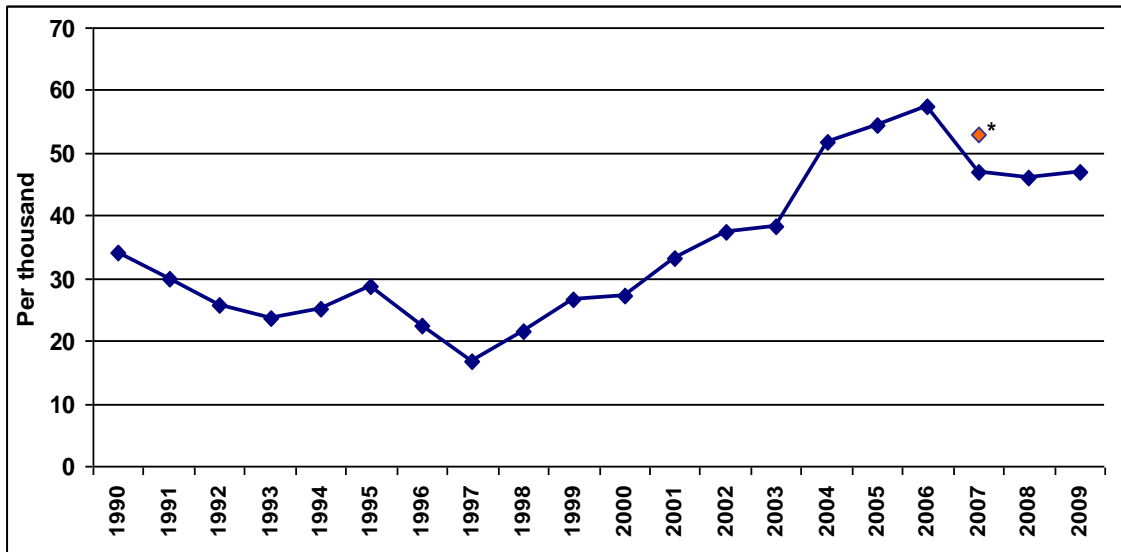


Figure 2 Birth defects (cases per thousand) between 1990 and 2009 in Hungary (Source: HCAR 2011)

(Figure 2. Orange rhomboid in 2007 is without minor anomalies: hernias, haemangioma, etc. The blue line is online notifications.)

Not only the increasing maternal age, but there are many teratogen agents which are threatening and effecting fetal conditions.

Fortunately, there are many new promising results which could decrease the incidence of a numerous feto-maternal abnormalities.

1.4.1. Teratogen agents

In humans, congenital disorders resulted in nearly 510,000 deaths globally in 2010. Teratogenic agents cause approximately 7% of congenital malformations. The teratogens causes a higher risk of birth defects and developmental abnormalities. These factors could be biological (Rubeolla or Parvo B13 viruses), physical (such as high temperature or X-ray), or chemical (such as thalidomide, tetracyclines or high dosage of vitamin A). Teratogen registries have been sorted out into classes A, B, C, D, X where A and B show no evidence of risk and C, D, and X show evidence of danger. Many times the data arrives from retrospective and uncontrolled studies so information is usually not complete, although there are a few, which were well documented and proven.

There is no absolute teratogen agent so the outcome of the teratogen agent should observe by fetal medicine specialists and consulted by a medical genetics.

The effect of teratogen is highly dependent from the gestational age and the dosage of

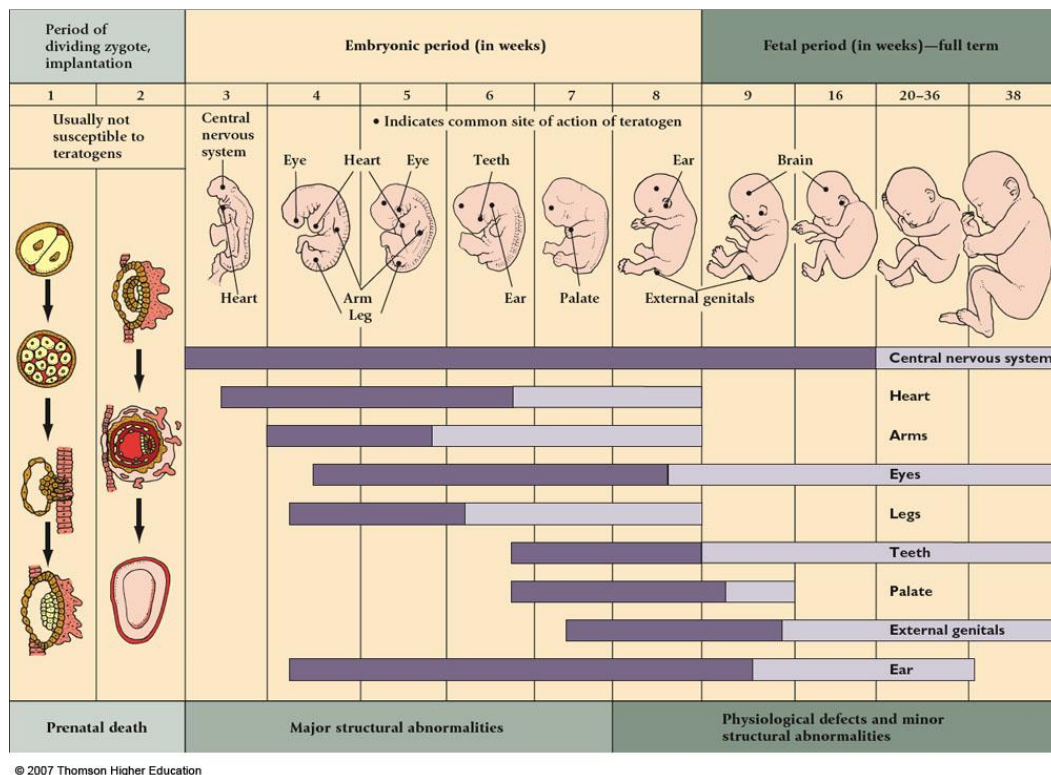


Figure 3 Sensitivity of teratogens during pregnancy (Columbia University, USA, Thomson Higher Education, 2007)

teratogen.

1.4.2. Prevention of fetomaternal diseases

The prevention of the fetal could be happened with preventive agents and with medical procedures, too. The levels of prevention must be included in the pregnancy care protocol.

Personalized pregnancy care and preventive direction should be offered to all pregnant.

1.4.3. Preventive agents

Many intrauterine and post-partum condition could be primarily prevented by different type of diets and chemical agents.

Czeizel et al. have been published about primarily prevention effect of by pre- and periconceptional folate administration in neural tube defects, limb-reduction and cardiac defects (7-9), with this findings our upcoming study preliminary results suggesting that more than 70% of neural tube defects and 30% of cardiac defects could be successfully prevented.

However, Corby reported aspirin as contraindicated agent during pregnancy in 1978. (7) One and two decades later Wallenberg et al. (8) and Beroyz et al. (12) have been published the good outcome of low-dose aspirin in the prevention of preeclampsia (PE) in a randomized trial on more than 9000 patients. Various tasks have been set up to follow the beneficial effect of platelet aggregation inhibitors during pregnancy, these subject areas highlighted the effect of Low-dose aspirin started at 16 weeks or earlier was associated with a substantial reduction in preeclampsia, preterm delivery and intrauterine growth restriction (IUGR). (9)

This effect could be extended with decreased level of protein intake and increase level of greens intake. Now aspirin is a commonly used agent to prevent preeclampsia. Before pregnancy, aspirin should be extended to adult females, who affected by anti-phospholipid syndromes (APLS).

Preliminary studies were suggested that the optimal dose of magnesium agents (such as magnesium lactate or magnesium sulforicum) in combination with vitamin B6 (pyridoxine) could lessen the danger of preterm delivery (tocolytic), fetal cardiac defects, ADHD in the childhood, maternal anxiety and tension. (10-14) In combination magnesium with Vitamin B6 and B12 have a good effect on the maternal gastrointestinal system and heart. It could also decrease the rate of negative pregnancy symptoms (such as nausea) and miscarriage. (15)

The Vitamin D prophylaxis as a prevention could decrease the deficiency of vitamin D has been linked with a greater hazard of pregnancy complications, such as preeclampsia; decrease the incidence of wheezing, asthma, rhinitis and allergic in childhood; decrease the risk of maternal gestational diabetes and a lower likelihood of a mother needing a Caesarian section. Still, the D vitamin is acting as an important agent in healthy and normal bone and immune development in utero. FDA recommendations for the daily intake is 200UI but for a pregnant or lactating woman it should be increased up to the daily limit (4.000-6.000UI/day for pregnant and 4.5-6.500UI/day for lactating women,) because the toxic dose of vitamin D is over 40.000UI/day (during the summer up to 20.000UI/day could be put out by the sun). Another positive side effect of vitamin D intake is the preconception effect because vitamin D is decreased the risk autoimmune abortion and increase the pace of fecundity in the overall population. (16-22)

Women with PCOS at least 1000UI/day intake of vitamin D had two times higher fertility rate compared to a woman with average intake (2,2UI/day in Hungary), but the clinical detail of

sentiment for the readiness of the pregnancy could be the same level as inter-pregnancy recommendations (4.000UI/day independently from the seasons). (17,18,21,23,24)

Antioxidant such as vitamin C and E could decrease the adverse pregnancy outcome (such as preterm delivery) and positive adulthood outcome (decreased incidence of age-related macular degeneration, amyotrophic lateral sclerosis, clogged arteries, scar, atopic eczema or dermatitis, heart diseases, cataract, cancer (colon, breast), dementia (Alzheimer's), Parkinson's and liver diseases). Some new randomized or cohort studies have been published on these findings till now so this will not remain level D evidence, anymore. (25-29) Vitamin E is also act as important factor in male reproduction, sufficient level of vitamin D could increase fertility. (17,18,21,23,24)

The evolution of central nervous system is very complex. Some preliminary studies proved the beneficial effects of Omega-3 acids, DHA and EPA. These factors could decrease the risk of abnormal development of central nervous system (such as schizophrenia or autism) and also could increase the level of predicted intelligence up to 20%. (21,30).

1.4.4. Methods in fetal medicine and medical genetics

1.4.5. Family planning

Family planning allows people and couples to anticipate and make their desired number of tykes and the spacing and timing of their births. It is achieved through the use of contraceptive methods and the treatment of involuntary infertility. A woman's ability to space and limit her pregnancies has a direct impact on her wellness and wellbeing as well as to the gist of each pregnancy. (WHO, 2010)

Positive family planning helps parents to hold a (healthy) baby, it is a lot more important if the parents have a familial disease.

1.4.6. Genetic counselling services

Genetic counselling should be necessary if family history and/or screening test are positive and/or maternal anxiety is higher. Each counselling should personalized to the patient(s).

1.4.7. The current status of preimplantation genetic diagnosis.

Pre-implantation genetic diagnosis (PGD) is mostly defined as the in vitro genetic (cytogenetic or molecular) testing the embryo before embryo transfer and its implantation.

1.4.8. Fetopatology and post-mortem radiology

Fetopatology and post-mortem radiology (such as CT or X-ray) is necessary to observe the cause of the fetal death.

1.4.9. Risk estimation and assessment

To evaluate the efficacy of a particular screening method and to evaluate the risk of a fetal condition, a risk estimation is necessary. Family tree should be created by the monogenic and by some polygenic disease to observe and explain the real risk. All screening tests had to have calculated sensitivity (detection rate), specificity (true negative rate), positive and negative predictive values, likely-hood ratios and cut off values (this could be chosen).

Detection rate or true positive rate: provides the proportion of actual positives which are correctly identified as true positives by the screening test. True negative rate: provides the proportion of negatives which are correctly identified as true negatives by the screening trial.

Positive predictive value (PPV): proportion of positive results that are true positives.

Negative predictive value (NPV): proportion of negative results that are true negatives.

The false negative rate is one minus the detection rate and false positive rate one minus specificity, the booth should be as low as possible or acceptable compared to the risk of diagnostic procedures.

Likely-hood ratios give a good chance to the practitioner to estimate the risk of the multifactorial hazard. The positive likely-hood ratio could increase the risk of a condition by its multiple if the marker was positive and/or higher than cut-off. Negative likely-hood ratio could decrease the risk of a fetal condition if the marker was negative or lower than the cut-off. The sum total of multiple markers' likely-hood ratio is depending on their linkage or correlation. The clinical introduction of these ratios is easy: Example: Background risk of a disease 1:1 =1, Marker one: Positive and its positive likely-hood ratio is 6x (increase the risk six times) Marker two: Negative and its negative likely-hood ratio is 0.5x (decrease the risk to half)

$$LR(overall) = BR \times LR(marker1) \times LR(marker2)$$

$$3 = 1 \times 6 \times 0.5$$

The result is three times higher than the overall risk of the condition or compared population's background risk, the likelihood ratio of the disease could be much easier explained than other methods.

The higher risk of a condition does not mean that the condition is existing because there are no absolute screening marker. Diagnostic test or analysis of other screening factor is even necessary.

Cut-off value is a term at what level and limit of risk is considered to be screen positive. Cut-off values could be based-on manual or personal selection, or study proven results (for example FPR 5% limit) or in the most case 90th-95th-or-97th-or-99th percentile were used in the literature. To this study 97th and 99th percentiles were used.

Invasive diagnostics should be offered if the cut-off risk reaching the cut-off limit or multiple marker of a condition is over cut-off value.

1.4.10. Prenatal screening

Prenatal screening for fetal malformations means to detect embryos or fetuses with normal or abnormal features during their intrauterine life.

1.4.11. Ultrasound screening

The ultrasound screening is the one of the oldest way to follow-up the pregnancy. In the last few decades, the technical development and science were reached the new era prenatal screening. These innovations help us to observe the fetus on high-definition live images or volumes. Ultrasound screening is able to detect developmental, chromosomal and structural abnormalities.

The ultrasound screening needs normal ranges, these called reference ranges, normograms, or normograms in the literature. These reference intervals are necessary to calculate the real gestational age and follow the fetal development.

To create normal ranges an advanced statistical knowledge is necessary. The complexity and requirements of the normogram creation was published by our group in Hungarian Medical Journal (Orvosi Hetilap).(31) Another publication of our research group proved usefulness of combined normograms was published.

1.4.12. Biochemical screening or maternal serum screening

Biochemical screening is introduced with AFP and HCG measurement in the middle of 80es, its overall screening performance was not higher than 40% sensitivity at 20-40% false positive rate. In the last decade multiple markers (such as PAPP-A, estradiol, PlGF) were presented in the fetal medicine.

1.4.13. Combined Screening

The combination of the ultrasound and biochemical markers has improved the efficacy of the screening. The ultrasound dating by using CRL is successfully solved the vulnerabilities of the biochemical screening. The correct dating is allowed to use them as MoMs values (Multiple of Medians) which are age, habitual and gestational age specific values. These values are really useful in biochemical screening but not as much as during the ultrasound screening.

1.4.14. Non-invasive prenatal testing

Non-invasive prenatal testing (NIPT) is based-on fetal cell free DNA in maternal plasma. This method is could be easily used in the clinical practice, but actually basically big companies earn a huge profit. Olive Kagan and et al. has been published (in press in UOG) a QUALY based study comparing the new and old methods for screening for trisomies, this publication was suggested that NIPT is more expensive and not wide specturumed as combined or ultrasound screening.

This method is brand new. Till this time, there is no restrictions, no ethical observation or no further control of the samples is exits. This will be a very big and serious deal, because two (mother and fetus) plus a half (father) patients genetic data could be observed without a warning or acceptance. Private life and health insurance companies could use these data in future to find risk gene of complex disease (such as cancer, cardiovascular diseases...etc). The parents and fetus does not known about this risk.

Another possible field of usage is the Y detection to determine the sex of the fetus, which could be easily lead to demographic problems such as it happened before in the Far East regions (like China) during the introduction of CVS in 1980s.

1.4.15. Prenatal diagnostics

Prenatal diagnostics means to detect genetic diseases in the fetus using invasive procedures and genetic techniques.

1.4.16. Invasive procedure

The aim of the invasive procedure is to get a sample from a fetal cell culture for a genetic test. Because of the needle puncture for sampling the *invasive prenatal method* is the other term used. Ultrasound controlled sampling could be performed in the first trimester (Chorionic

Villus Sampling - CVS), or in the second trimester (Amniocentesis - AC) or later in the pregnancy (cordocentesis - CC). The major problem of the sampling procedures is the risk of abortion, while the advantage is the certainty of the result which is above 99.8 per cent.

Reviewing the literature, from end of 80s up today, a huge decrease of the invasive procedure associated risk could be observed. Commonly known, AC and CVS risk were about 1-2%, however the education, trainings, and experience proves that there is no significant difference if the procedure technique was appropriate and the protocols were followed. The latest publication in this field suggesting that the procedure of miscarriage

The decision has been made by the parents, which based-on the estimated risk of aneuploidies in contrast to the risk of miscarriage associated with invasive procedures.

Recent Guideline of the Hungarian College of Clinical Geneticist and of Obstetrics and Gynaecology (2010) recommends that all pregnant women of 37 years of age or over should be offered invasive testing to obtain a definitive diagnosis of fetal karyotype. However, from an ethical point of view the couples are left to have an autonomous decision if they want to have an invasive test or not. At genetic counselling the patient is advised to the possibility that they can skip the expensive screening and can go straight for invasive testing.

A huge series of studies were presented on the risk of post procedure miscarriage. The significant discordance between the number should be observed and should be implicated to the genetic counselling. (32-37)

Table 1 Risk of miscarriage with and without invasive procedures (literature review)

Invasive Procedure	Risk of PPM or SM	Optimal GA	Sens. / Rep
Chorionic Villus Sampling	0.17-2.7%	10-12wks	98% / 2.3%
Spontaneous miscarriage	cc. 1%	10-12wks	N/A
Amniocentesis	0.06-2.3%	14-20wks	99% / 1.3%
Spontaneous miscarriage	less than 0.5%	14-20wks	N/A
Cordocentesis	0.3-3%	From 18-20wks	98% / 2.6%
Spontaneous miscarriage	less than 0.1%	From 18-20wks	N/A
PPM: Post Procedure Misscarriage; SM: Spontaneous miscarriage. Optimal GA: Optimal gestational age of procedure, Sens.: Sensitivity (incl. polluted sample or unsuccessful procedures); Rep.: Repetition of procedure is necessary. wks: weeks			

However, the invasive techniques have been become a routine and the likelihood of fetal loss is decreased to cc. 1.1 in the last decades. During the pre-test genetic counselling their complications and risk of iatrogenic fetal abnormalities should be present to the patients.

2. Fetal loss and aneuploidies

More than 75% of the pregnancies has been ended with spontaneous abortion until the 6 weeks. Most of these abortions are affected with some kind of numeric abnormalities (see Table 1.). The demographic trends have shown the increasing maternal age at the first and second pregnancy, which increase the risk of genetic abnormalities.

The trisomy is the major factor in the background of the early fetal loss. (See Table 3.) The prevalence of all types of chromosomal abnormalities at birth is 1.88% (including asymptotic and balanced cases).

2.1. Human karyotype

The diploid (2N euploid) human karyotype contains 46 chromosomes. Two sex and forty-four autosomal chromosome are each of them paired.

Human karyotype could be investigated cytogenetically from different types of samples (such as blood, or other tissue). For fetal karyotyping an invasive procedure is required.

2.2. Aneuploidies

Aneuploidy is the one of the major categories of chromosome mutations in which chromosome number is abnormal. An aneuploid is an individual whose chromosome number differs from the 46 by part of a chromosome set. Generally, the aneuploid chromosome set differs from 46 by only one or a small number of chromosomes. Aneuploids can have a chromosome number either greater or smaller than that 46. Aneuploid nomenclature is based on the number of copies of the specific chromosome in the aneuploid state. For example, the aneuploid condition $2n - 1$ is called monosomic (meaning “one chromosome”) because only one copy of some specific chromosome is present instead of the usual two found in its diploid progenitor. The aneuploid $2n+1$ is called trisomic, $2n-2$ is nullisomic, and $n+1$ is disomic.

The frequency of all chromosomal abnormalities has been analyzed in Table 3, using data of United States Environmental Protection Agency (Texas). A significant difference was observed between under pregnancy (Sp. AB and fetal death) and newborn samples.

The features, symptoms, outcome and prognosis of the most common aneuploidies (Down Syndrome, Edwards syndrome, Patau syndrome, Tuner syndrome, trisomy 16 and polyploids have been summarized in Table 2.

2.2.1. The Down syndrome

In 1886, Dr. John Langdon Down was described firstly Down syndrome as the part of the “Observations on an Ethnic Classification of Idiots” (38). Nowadays, the Down syndrome is the most frequent numerical chromosomal abnormality. Down syndrome is also known as trisomy 21, but the old terminology, mongoloid idiotism must not be used in the clinical practice.

The trisomy of 21 chromosomes causes several but a wide range of developmental errors, physical and mental handicaps, most of them could be observed already in the fetal life. No effective cure of Down syndrome has been developed until now. Fetus affect with Down syndrome showed promising results with fetal programing, but this is only some approaches to decrease the symptoms. Nowadays, the prevention by early diagnosis and therapeutic abortion could be the possible choice.

The prevalence of registered Down syndrome cases has been increased since the middle of the 80es’. The birth prevalence of Down syndrome has not increased significantly, which means the efficacy of screening for trisomies is not optimal or the rate of Down syndrome affected fetus is increased. (See Figure 5) Prevalence of Down syndrome between 2001 and 2009 was 1,78 ‰ (1:563 total births) in Hungary.

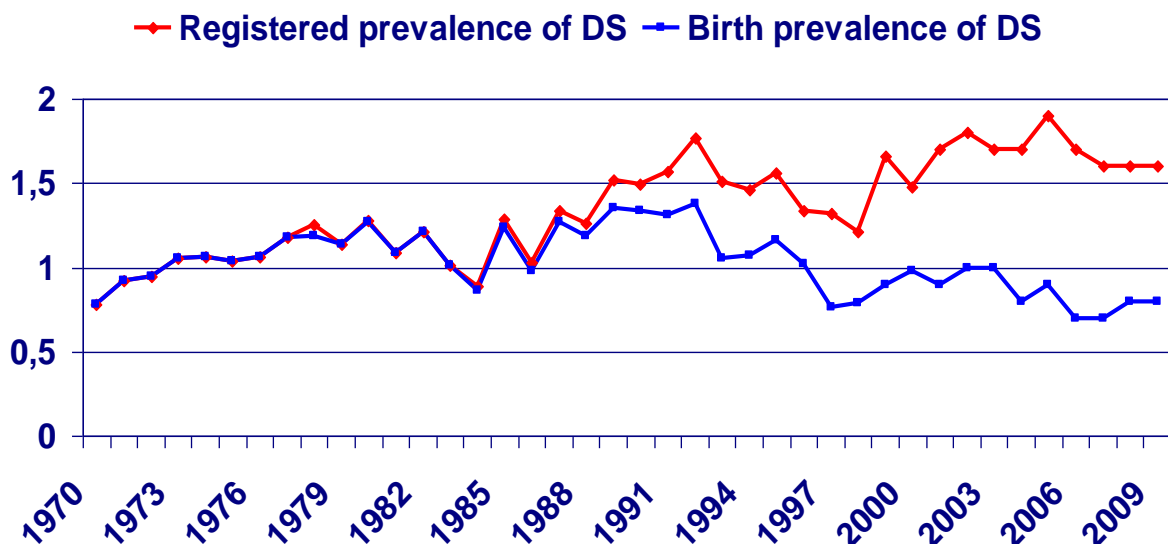


Figure 4 The prevalence of Down Syndrome (cases/thousand) between 1970 and 2009.

The correlation between the new screening method of Down syndrome and registered prevalence of Down syndrome could be observed from the middle of 80es. However, the rate of case should not be changed.

2.2.2. Edwards syndrome

Trisomy 18 was independently described by Edwards (31) et al and Smith (32) et al in 1960. Among liveborn children, trisomy 18 is the second most common autosomal trisomy after trisomy 21. Trisomy 18 is characterized by severe psychomotor and growth retardation, microcephaly, microphthalmia, malformed ears, micrognathia or retrognathia, microstomia, distinctively clenched fingers, and other congenital malformations.

2.2.3. Patau syndrome

Trisomy 13 or Patau syndrome is a trisomy disorder. It is caused by the presence of a whole extra copy (or occasionally partial extra copy) of chromosome 13. Patau syndrome, in 75%-90% of cases, is the result of the presence of a whole extra (third) copy of chromosome 13, hence its alternative name of trisomy 13 (or simple trisomy 13). Patau syndrome is caused by a chromosomal translocation in 5%-10% of cases, and to mosaicism (whereby only some cells have the extra copy of chromosome 13) in 5%. In occasional cases, it is only a part of chromosome 13 that is extra (partial trisomy 13). Occurrence in live births is about 1 in 9,500 (in the absence of any prenatal detection program), and rises with increasing maternal age.

Trisomy 13 is associated with a high rate of spontaneous loss of pregnancy (64% loss rate from the 2nd trimester onwards) and very poor chances of survival in neonates (median survival is 10 days).

2.2.4. Aneuploidy of the Sex Chromosomes

However, sex chromosomes are rarely affected by aneuploidies, their screening and diagnosis is still not solved. There are numerous syndrome known with additional X or Y chromosomes (ei. XXX, XXY, XYY... etc.), or with the lack of one sex chromosome (X0).

Tuner-syndrome (XO) could be observed as the most common aneuploidy during fetal life. However, the high rate of spontaneous abortion is decreasing its birth prevalence. Other sex aneuploidy are not commonly affected by spontaneous abortion like Tuner but XXX or Superwoman syndrome has shown a higher rate than the others, where significant difference between lost fetuses and newborns could not be observed.

Table 1 Clinical feature of aneuploidy, pregnancy outcome and prognosis

Clinical features of the aneuploidy		
Trisomy	Clinical Features	Outcome (without iatrogenic abortion)
21	Cardiac defects, duodenal atresia, mild-to-moderate mental retardation, thyroid problems, hearing loss, increased risk of leukemia, seizures	Only 1:37 fetuses survive the intrauterine life and 80% of children could reach 60 yr of age or older
18	Cardiac defects, renal anomalies, severe mental retardation, intrauterine growth restriction, omphalocele, central nervous system defects, breathing and feeding difficulties, "rocker-bottom" feet, micrognathia, low-set ears	Only 1 from 149 fetuses survives the intrauterine life and more than 90% of children die before or shortly after birth; <10% reach 1 yr of age
13	Cardiac defects; renal anomalies; cleft lip, palate, or both; holoprosencephaly; microcephaly; omphalocele; severe mental retardation; deafness; seizures	Only 1 from 86 fetuses survives the intrauterine life and most children die in the first days or weeks of life; <10% reach 1 yr of age.
16	Cardiac defects; renal anomalies; multiplex CNS abnormalities	The most fetuses (99.9%) and remaining children die in the first days.
XO	Smaller height, lack or less developed primary and secondary genitals, anovulation, infertility, recurring miscarriage	Only 1:1800 fetuses survive the intrauterine life and 90% of children could reach 50 yr of age or older
XXX	Various phenotypes are known, rate of infertility, gyn. cancer (cervix, breast, uterus)	Only 1:5 fetuses survive the intrauterine life and 80% of children could reach 65 yr of age or older
XXY	Various male phenotypes are known, rate of infertility, higher rate of breast cc.	Only 1 from 2 fetuses survives the intrauterine life and 80% of children could reach 60 yr of age or older
Triploid and tetraploid	Multiplex abnormalities and fetal hydrops could be observed.	The most fetuses (99.9%) and remaining children die in the first days in intensive care.

Table 1 Summarize the prevalence of genetic syndromes in the intrauterine life and by birth.

Table. 2. Prevalence of aneuploidy		
Affected chromosome	Sp. Ab or fetal death*	Newborn**
1	0.00%	0.00%
2	2.12%	0.00%
3	0.71%	0.00%
4	1.27%	0.00%
5	0.00%	0.00%
6–12	7.48%	0.00%
13	1.71%	0.02%
14	3.67%	0.00%
15	4.24%	0.00%
16	16.39%	0.00%
17	0.13%	0.00%
18	2.97%	0.02%
19–20	0.69%	0.00%
21	4.67%	0.13%
22	5.65%	0.00%
Subtotal (trisomy):	51.69%	0.17%
Trisomy or monosomy of sex chromosomes		
XXX	0.05%	0.05%
XXY	0.05%	0.05%
XO	18.00%	0.01%
XXX	0.28%	0.05%
Subtotal (sex chr):	18.39%	0.17%
Translocations		
Balanced	0.19%	0.19%
Unbalanced	3.00%	0.06%
Subtotal translocation	3.19%	0.25%
Polyploid		
Triploid	17.00%	0.00%
Tetraploid	6.00%	0.06%
Other (such as mosacism)	3.73%	0.65%
Subtotal polyploids and other	26.73%	1.29%
Total:	100.00%***	1.88%****
*Data based on 7500 spontaneous abortions with genetic analysis.		
**Data based on 85.000 births with genetic analysis.		
***Spontaneous abortion or fetal death had been associated with genetic syndromes in cc. 50% of all abortion cases.		
****Birth population had been karyotyped.		
Source: Data calculated from United States Environmental Protection Agency, Texas 1998		

3. Efficacy of prenatal screening and diagnostics for trisomies

Hungarian Congenital Abnormality Registry is monitoring the efficiency of the screening *in* Hungary. Comparing to European results overall Hungarian detection is a bit lower than the average, but it could not reach the 65% since the beginning. The rate increase has been slowed down after 2006 and there is a need to find new techniques and finding the weaknesses of the screening policy.

Most common weaknesses are the lack of the population specific normograms, lack of the quality control and the lack of pregnancy wide 'to do' protocol.

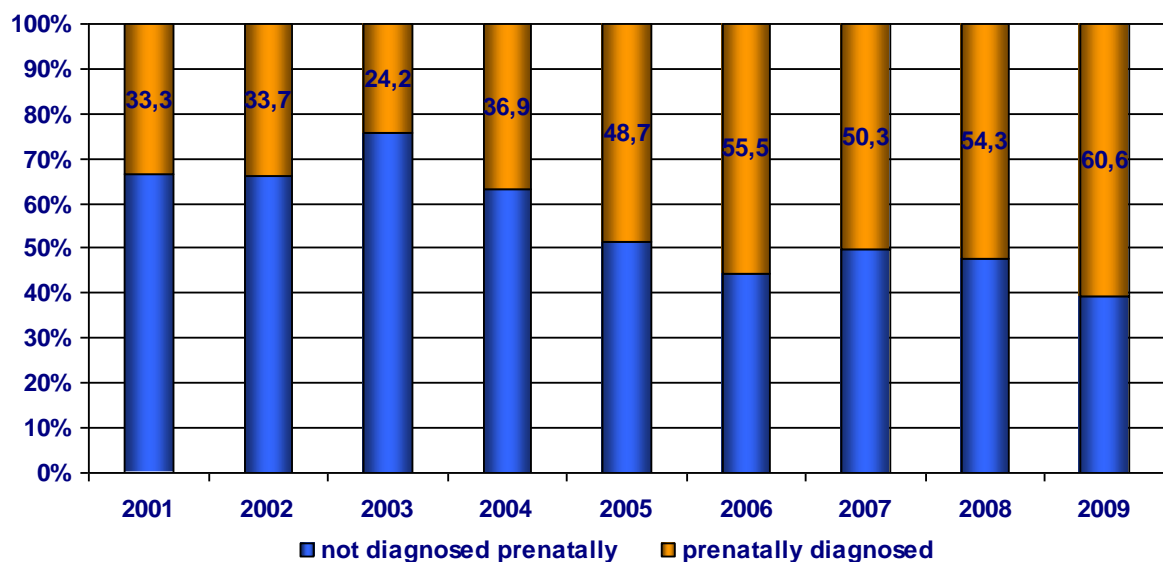


Figure 5 Efficacy of the prenatal screening in Hungary 2001-2009 by HCAR

4. Basics of the ultrasound screening

The importance of the fetal biometry reference normograms during the screening for trisomy is well-known (39,40)(39,40), and some preliminary studies have been highlighted the importance of local normal curves and charts.(41)To our knowledge, there was not any study to establish the Central European normograms.

Appropriate methodology has been published fetal biometry charts and equations for various populations using the correct methodology are now available in the international literature. (39,42-45) Although there were many previous publications of the measurement and normal

ranges of the human fetal biometric parameters, none of all had specific data on the Central European Region.

The first trimester fetal biometric characteristics have been observed, analyzed and published many times by the Fetal Medicine Foundation (FMF) London and its co-operators, but many papers highlighted the racial growth chart differences. (41,46-50) Our study was established based on these findings.

The new sonography era of the screening for Down Syndrome (DS) has been started by Szabó and Gellén with their breakthrough publication about nuchal translucency thickness in 1990. (51) Following their paper many studies have been proved its clinical importance of their hypothesis and results. (52-56)

From the beginning of the 20th Century, facial markers introduced to the trisomy screening. In the last decade, the importance of facial marker in the first(57-60) and second trimester (61-68) was published several year ago and our good preliminary results also proved the high efficacy of the fetal profile ratios in the second trimester.(69) These results were suggested that to introduce them and to create normograms to observe these markers and ratios in the first trimester, too.

The aim of the study was to establish the local fetal growth charts and normograms of fetal biparietal diameter, femur and humeral length, nuchal translucency, prenasal thickness, nasal bone length, ductus venosus PI, and PT-to-NBL and NBL-to-PT ratio from about 10 weeks and fetal heart rate and CRL from the 37 days of gestation to the midtrimester.

The secondary aim was to improve efficacy of screening for chromosomal abnormalities at the first trimester ultrasound screening.

5. Material and Methods

5.1. Materials:

This prospective observational study has been designed to measure, and describe the normal biometric parameters. All included 4321 cases scans have been performed from January, 2008 to February, 2014 in the MEDISONO Fetal and Maternal Health Research Centre and the Department of Medical Genetics, University of Szeged, Szeged, Hungary.

This study contains for (both low- and high) mixed-risk obstetric populations, and ethnically over 99.8% of pregnancies were a Caucasian population of Hungary. The study protocol was approved by the Regional Ethics Committee of the University of Szeged and all procedures were in full accordance with the Helsinki Declarations.

5.2. Measurements

All measurements were performed by one experienced sonographer using transabdominal ultrasound (GE Voluson E8 Expert, GE Healthcare Cpf, Austria). This sonographer was a holder of The Fetal Medicine Foundation's (FMF) Certificate of Competence for first-trimester scanning. All measurements were repeated for 3 times and the best one were selected.

Measurements of fetal biometry such as CRL were followed the INTERGROWTH-21st measurement of fetal crown rump length and standardization of ultra-sonographers (2010). CRL was measured in the mid-sagittal section, a neutral horizontal position, using the optimal magnification with the correct calliper position. The intersection of the callipers were placed on the outer borders of the skin over the head and rump.

NT and DVPI measurements were fully followed the FMF criteria. DVPI-to-NT and NT-to-DVPI were established by the division NT and DVPI. Measurements of BPD were obtained from a transverse axial plane of the fetal head showing a central midline echo broken in the anterior third by the cavum septi pellucidi, if already present. BPD was measured from the outer border of the skull.

The femur length (FL) was measured from the greater trochanter to the lateral condyle if it was exiting and shown, as if on the two ossification border of the bone.

To measure the FHR, M-Mode was used in acquiring volume with automatic calculation.

Facial profile (NBL and PT)

On the basis of technical descriptions of NBL (70,71) measurements and our experience, both measurements could be obtained in the same image if the face of the transducer was positioned parallel to the nasal bone. The insonation angle should be close to 45 degrees. The following image settings were used: low gain, medium dynamic contrast, and maximum magnification so that the fetal head occupied the entire screen. Images were adjusted to ensure the correct midsagittal plane and sharp margins of the skin and the nasal bone. The diencephalon, nasal bone, lips, maxilla, and mandible were used as reference points for the correct measurements of NBL in the midsagittal plane (72,73). The following image settings were used: low gain, medium dynamic contrast, and maximum magnification so that the fetal head occupied the entire screen. Images were adjusted to ensure the correct midsagittal plane. (58,73) Briefly, PT was measured as the shortest distance from the lower margin of the frontal bone to the outer surface of the overlying skin. The margins of the nasal bone are the

proximal and the distal ends of the white ossification line. The NBL(70,71) and PT(73) were measured using the same view. If it was possible NT, NBL and PT were measured on the same image. PT-to-NBL and NBL-to-PT were established by the division of NBL and PT. Additionally, between April 2008 and December 2013, 2549 women were included into another study and followed-up in the first and second trimester to improve the second trimester screening efficacy. First and second trimester measurements were combined in a normogram and compared to second trimester Down syndrome cases.

5.3. Inclusion and exclusion criteria:

All healthy singleton pregnancies have been included were not have been confirmed any abnormalities by the fetus and mother.

Exclusion criteria were: IUGR, any fetal (including fetal and neonatal mortality) or maternal disease/ disorder, IVF or induced pregnancies, rejecting the participation of study.

Absent nasal bone was an exclusion criteria for the facial profile ratio group. PT and NBL measurement were accepted if both could be observed and measured on the same image.

The main exclusion criteria were when the difference between the three repeated measurements were higher than 10% of the measured value. (Only the measurement of the marker was excluded in this case, not the patient case.)

5.4. Statistical analysis and data collection

All measurement data and volumes have been sent to astraia software (astraia GMBH, Munich, Germany) via DICOM. Data analysis performed by a single medical bioinformatics specialist with Microsoft Office 2013 (Microsoft, Redmond, Virginia) and SYSTAT (SyStat Software Inc., San Jose, CA, USA) Woman was referred by LMP but all data were based on the CRL measurement in this study, no correction have been made by other parameters. GA was calculated from CRL measurement at 10th week.

Statistical analysis was performed using SigmaStat. Regression analysis was used to determine the percentiles (mean and 5th, 95th percentile) and regression analysis was used to estimate the relation between CRL and other parameters. Euploid and trisomy group were compared with independent sample t-test ($p \geq 0.001$).

No reproducibility analysis was taken, but previous studies have been confirmed a non-significant difference. This study not concerning about newborn or child characteristics, and no other maternal, neonatal or fetal biometrics were not observed.

6. Results

From the 4321 women, 3356 accepted the consent form (77.67%) and participated in our study. Thirty seven women were excluded because of missed abortion at the first scan. One Edward's syndrome case and five Down syndrome cases were diagnosed during the ultrasound measurements.

Furthermore 103 cases were excluded because of maternal (26), fetal (74) or neonatal (8) disorders. The Edwards syndrome case had spontaneous abortion a few days after the CVS at 11th week. Down syndrome cases were cytogenetically proved by AC during the early midtrimester.

Descriptive data analysis of the results

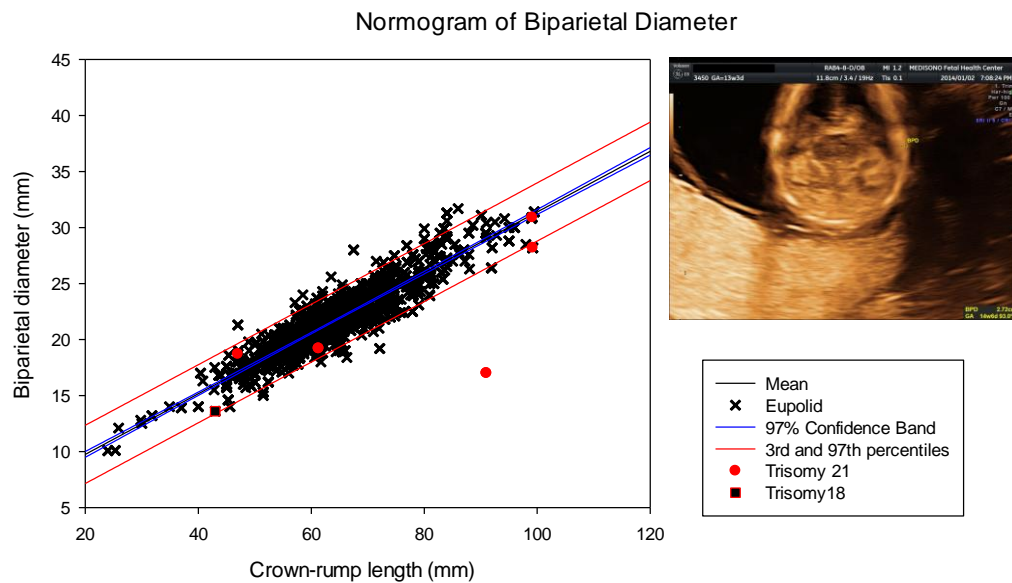
The mean maternal age in euploid and trisomy cases was 33.83 years (16.6–47.1 years) and 35.83 years (26.1–41.3 years), respectively. The mean gestational age was 77.58 days (26.0–120 days) for euploids. The population specific maternal age, CRL, FHR, FL, BPD, NT, NBL and PT descriptive statistics of euploid fetuses (mean, standard deviation, maximum, 75%, median, 25% and minimum values) of the euploid fetuses were summarized in Table 3.

Table3.	Mean	Std Dev	Std. Error	Max	Median	Min
GA by LMP (days)	77.579	14.033	0.249	120	73	26
NT (mm)	1.845	0.632	0.0323	6.9	1.8	0.6
FL (mm)	8.942	2.319	0.1	16.8	8.7	3.9
BPD (mm)	21.977	3.536	0.0786	40.9	21.5	10.08
NBL (mm)	2.381	0.415	0.0213	4.2	2.3	1
PT (mm)	1.412	0.369	0.019	3.2	1.4	0.1
FHR (bpm)	162.542	15.468	0.279	215	164	68
Ductus Venosus PI	1.099	0.165	0.00843	1.7	1.1	0.65

The following figure series represents the results of the study and the created normograms with control cases.

Biparietal diameter (BPD)

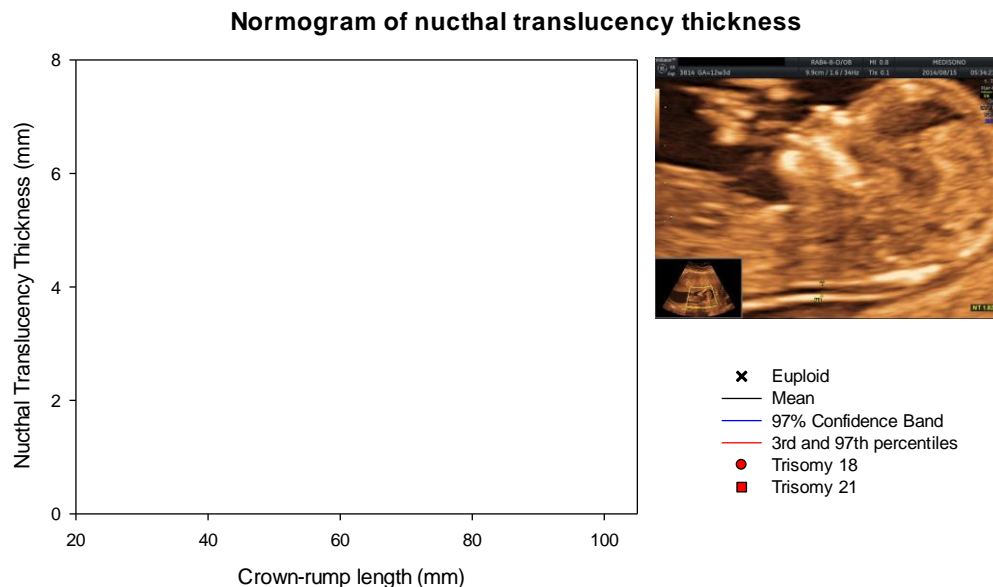
All biparietal diameter measurements and distribution were summarized in Figure 6.



Where the black crosses were the measurements, black/gray line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles. Red rectangles were the Down syndrome cases. The red dots were Edwards's syndrome case.

Nuchal translucency (NT)

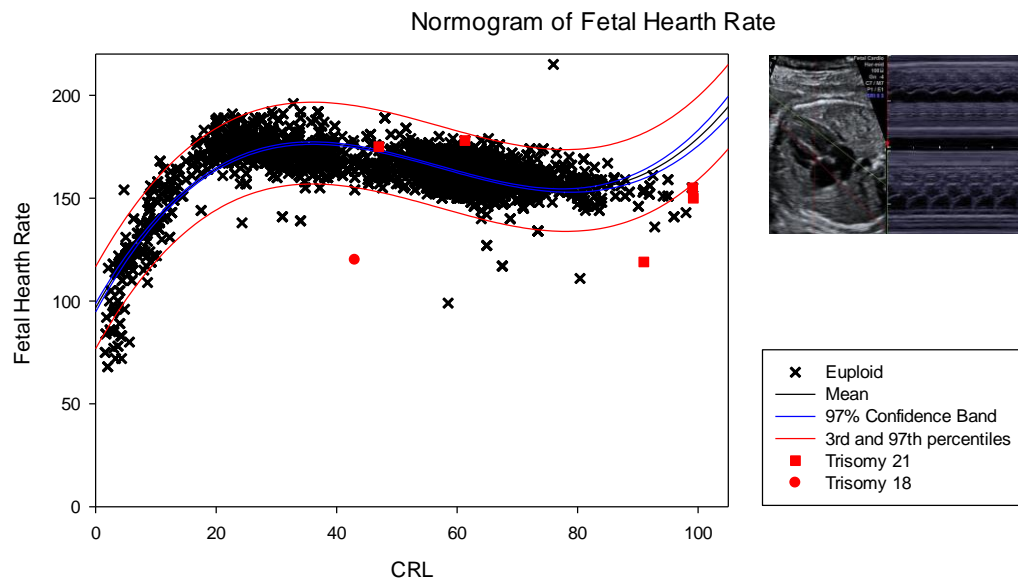
All nuchal translucency measurements and distribution were summarized in Figure 7.



Where the black crosses were the measurements, black/gray line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles. Red rectangles were the Down syndrome cases. The red dots were Edwards's syndrome case.

Embryonic or fetal heart rate (EHR /FHR)

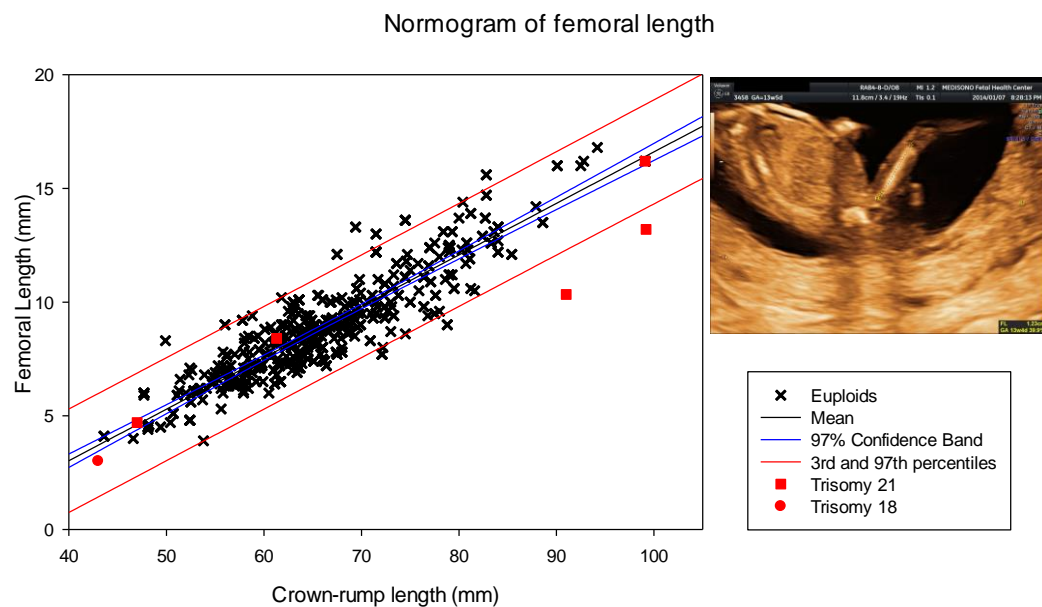
All fetal heart rate measurements and distribution were summarized in Figure 8.



Where the black crosses were the measurements, black/gray line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles. Red dots were the Down syndrome cases. The red rectangles were Edwards's syndrome case.

Femur length

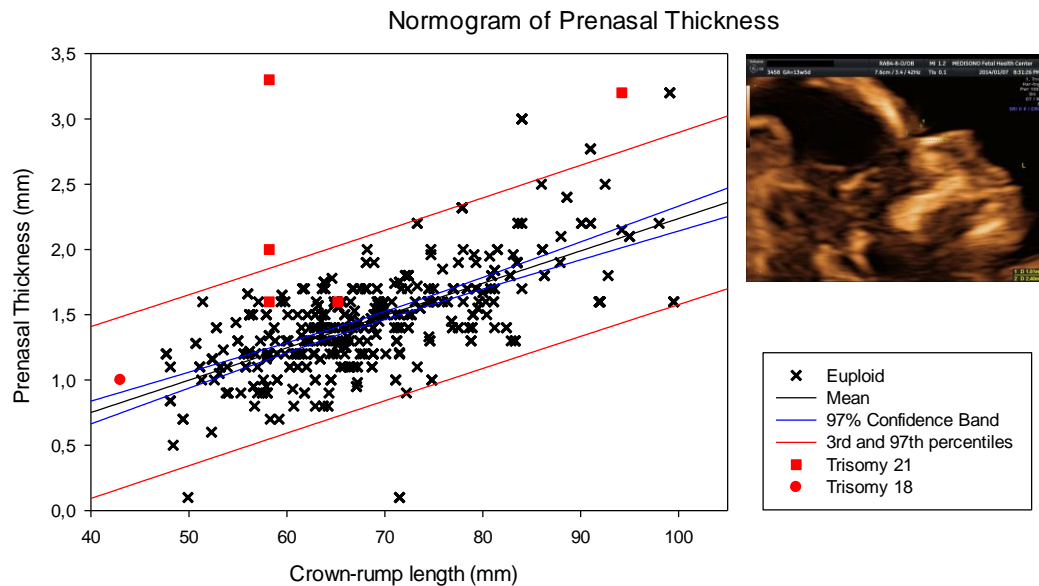
All femoral length measurements and distribution were summarized in Figure 12.



Where the black crosses were the measurements, black/gray line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles. Red rectangles were the Down syndrome cases. The red dots were Edwards's syndrome case.

Prenasal Thickness (PT)

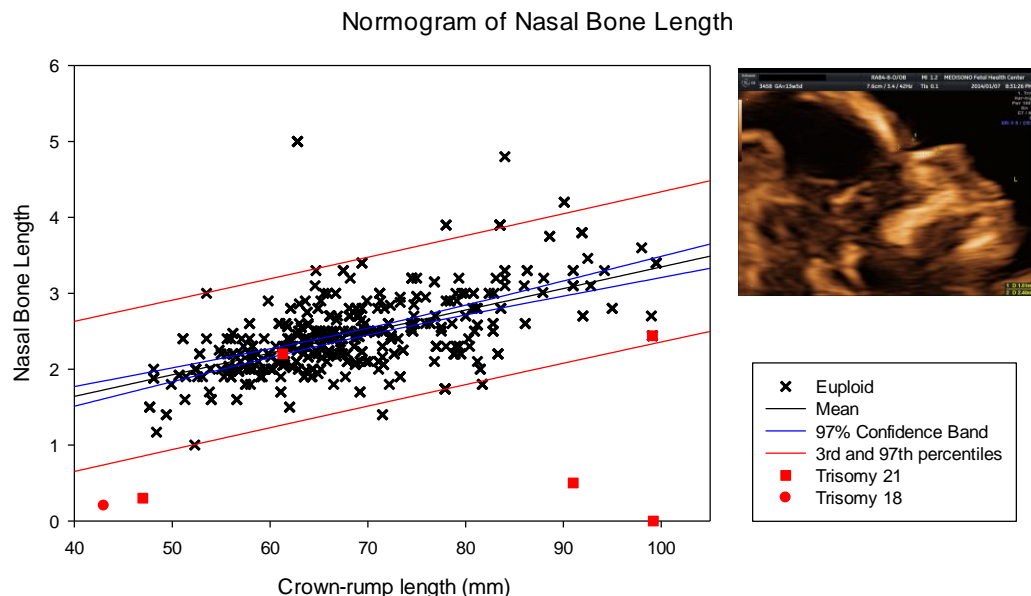
All prenasal thickness measurements and distribution were summarized in Figure 10.



Where the black crosses were the measurements, black/gray line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles. Red rectangles were the Down syndrome cases. The red dots were Edwards's syndrome case.

Nasal bone length (NBL)

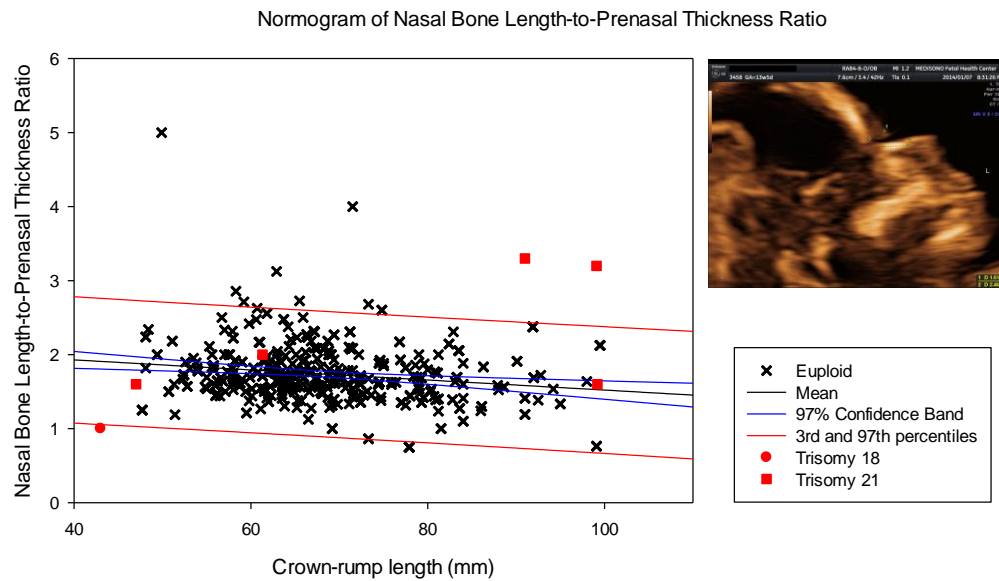
All nasal bone length measurements and distribution were summarized in Figure 11.



Where the black crosses were the measurements, black/gray line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles. Red rectangles were the Down syndrome cases. The red dots were Edwards's syndrome case.

Nasal bone length-to-prenasal thickness ratio (NBL:PT)

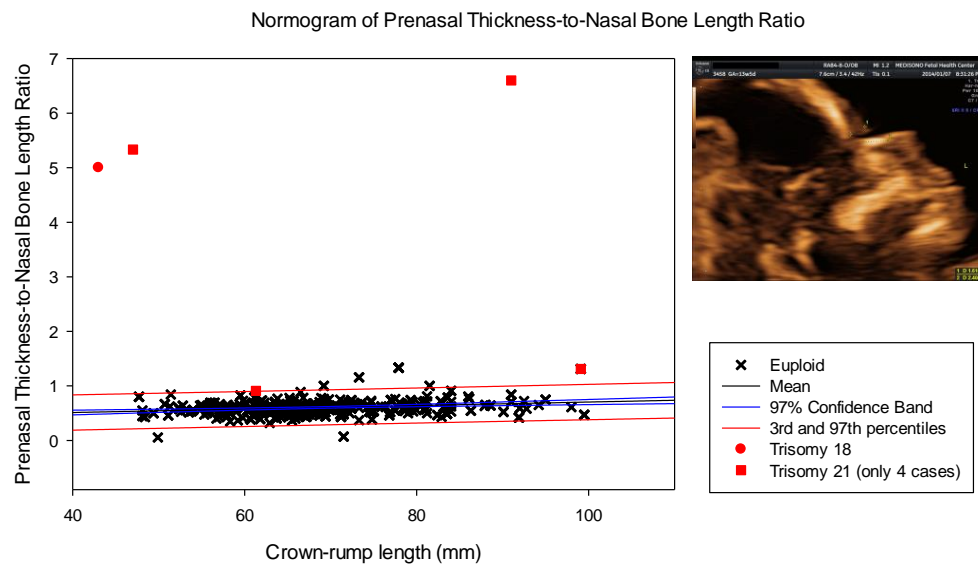
All nasal bone length-to-prenasal thickness ratio distribution were summarized in Figure 12.



Where the black crosses were the measurements, black/grey line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles. Red rectangles were the Down syndrome cases. The red dots were Edwards's syndrome case.

Prenasal thickness-to-nasal bone length ratio (PT:NBL)

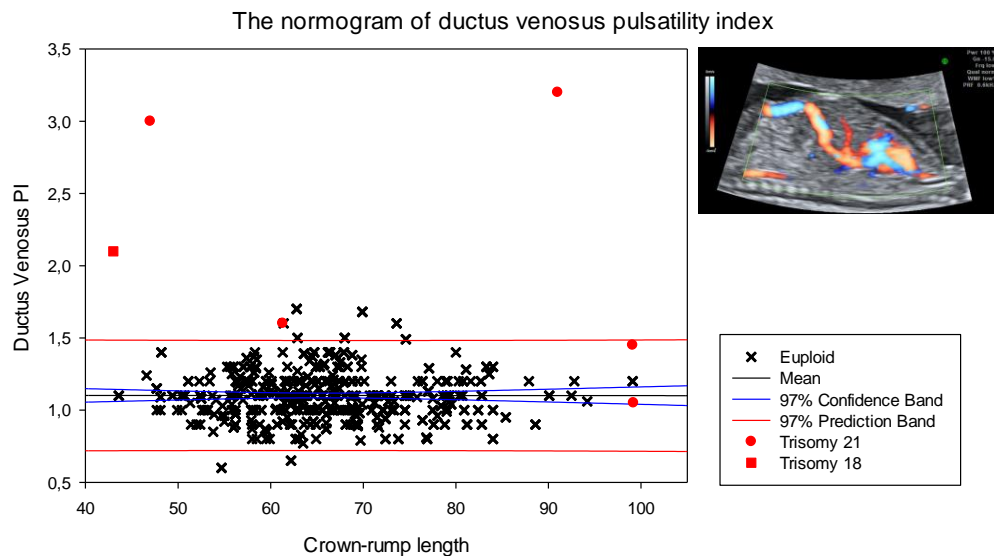
All prenasal thickness-to-nasal bone length ratio distribution were summarized in Figure 13.



Where the black crosses were the measurements, black/grey line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles. Red rectangles were the Down syndrome cases. The red dots were Edwards's syndrome case. One case with absent nasal bone was excluded because of the zero division.

Ductus venosus flow pulsatility index (DVPI)

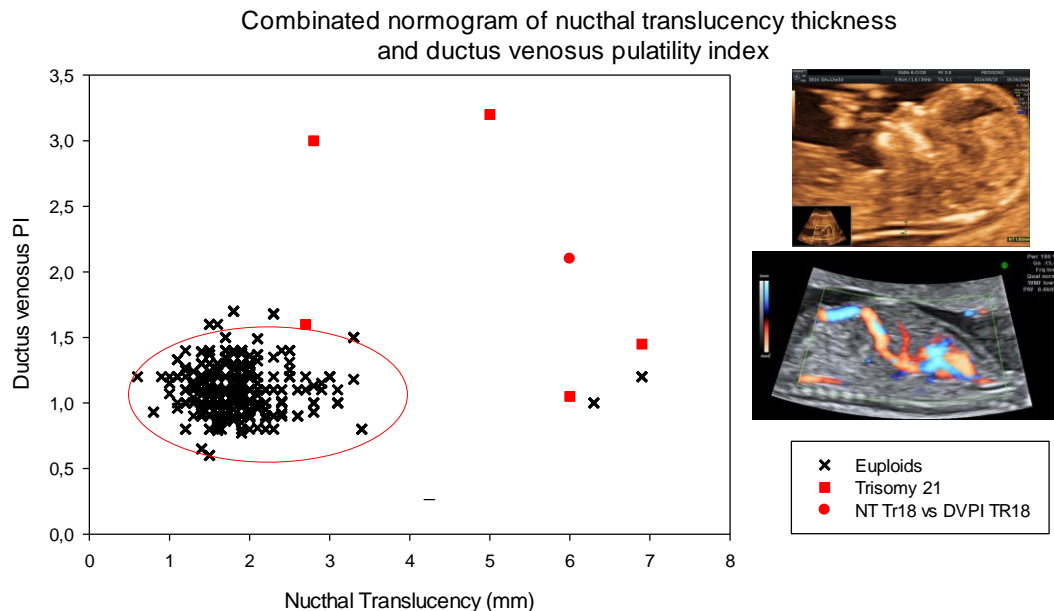
All ductus venosus pulsatility index distributions were summarized in Figure 14.



Where the black crosses were the measurements, black/grey line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles. Red rectangles were the Down syndrome cases. The red dots were Edwards's syndrome case.

Nuchal translucency versus ductus venosus flow pulsatility index (NT/DVPI)

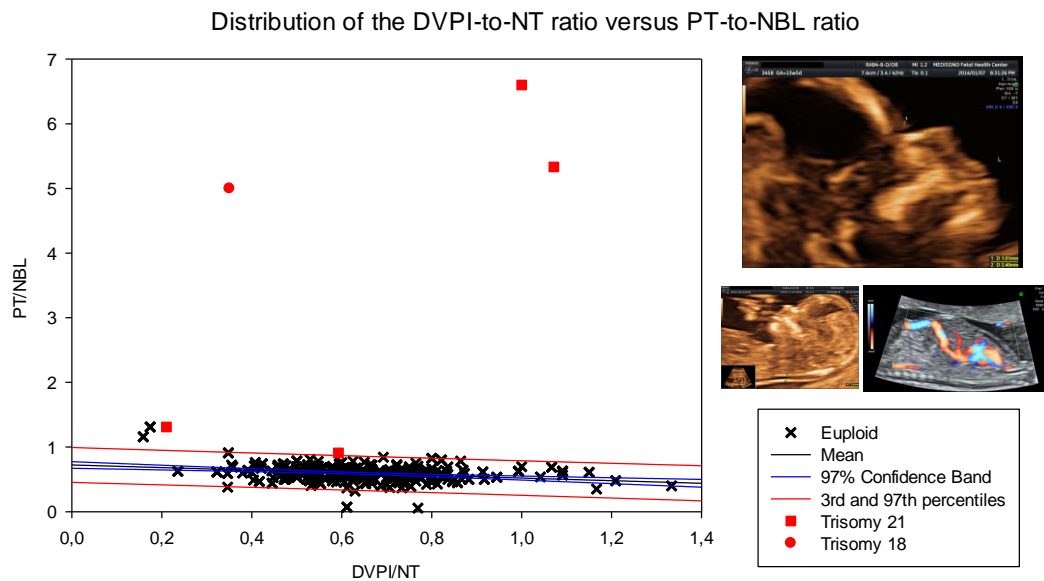
All combined nuchal translucency and ductus venosus pulsatility index distribution were summarized in Figure 15.



Where the black crosses were the measurements, red lines were the 1st and 99th percentiles limit circle. Red rectangles were the Down syndrome cases. The red dots were Edwards's syndrome case.

DVPI/NT-to-PT/NBL ratio

All combined ductus venosus pulsatility index-to-nuchal translucency ratio versus prenatal thickness-to-nasal bone length ratio distribution were summarized in Figure 16.



Where the black crosses were the measurements, black/grey line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles. Red rectangles were the Down syndrome cases. The red dots were Edwards's syndrome case.

Statistical Performance of the markers

Table 4 summarized the statistical performance of the marker of Down syndrome.

Table 4. Statistical performance of the markers	DR	Spec	LR+	LR-
Fetal Heart Rate (FHR)	50.00 %	99.02 %	68.07	0.34
Femur Length (FL)	40.00 %	99.07 %	43.04	0.61
Biparietal Diameter (BPD)	40.00 %	98.79 %	33.10	0.61
Nasal Bone Length (NBL)	66.67 %	99.17 %	80.67	0.34
Prenasal Thickness (PT)	50.00 %	99.36 %	78.50	0.50
NBL/PT ratio	33.33 %	98.28 %	19.42	0.68
*PT/NBL ratio	80.00 %	98.93 %	74.56	0.20
Nuchal Translucency (NT)	83.33 %	99.63 %	225.83	0.17
Ductus Venosus PI	66.67 %	98.89 %	60.22	0.34
DVPI+NT	100 %	98.51 %	67.25	<0.00
DVPI+NT+PT/NBL ratio (3D diagram)	100 %	99.17 %	120.25	<0.00
NT/DVPI-to-PT/NBL ratios	100 %	99.20 %	125.67	<0.00
DVPI/NT-to-PT/NBL ratios	100 %	99.47 %	188.50	<0.00
DR: detection rate or sentivity; Spec: Specificity (FPR=1(00%)-Spec.); LR+: Positive likelihood ratio; LR-: Negative likelihood ratio				

Significant differences were observed between euploid and trisomy group from the aspect of nuchal translucency, fetal heart rate, nasal bone length, prenasal thickness, ductus venosus PI, prenasal thickness-to-nasal bone length and ductus venous-to-nuchal translucency to prenasal thickness-to-nasal bone length ratios. ($p > 0.001$)

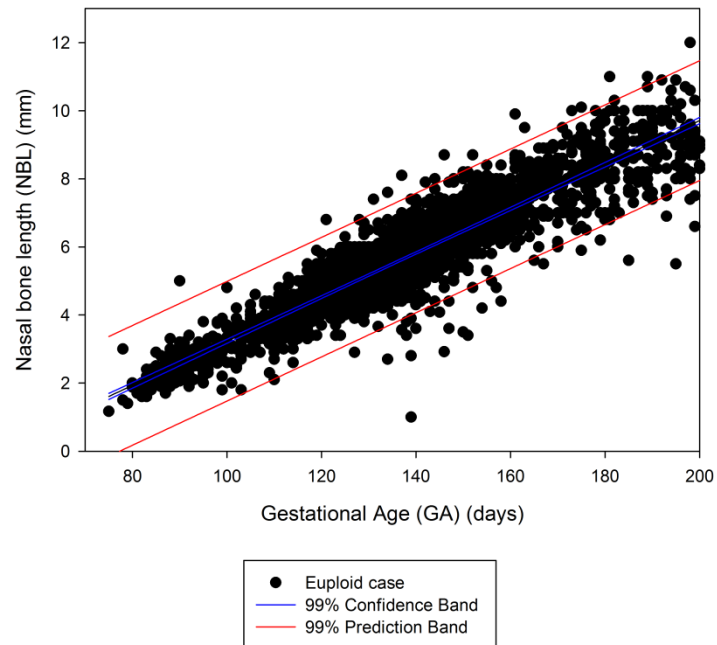
IMPORTANT NOTES:

- three times repeated measurements of NBL caused almost no measurements marked on 40-55mm of CRL region because difference of the measurements where higher than study limit (10% difference which was higher than the callipers internal space).
- Where NBL was zero, there was a zero division in the results by PT/NBL ratio. These cases were excluded.

Second trimester results on combined first and second trimester normograms

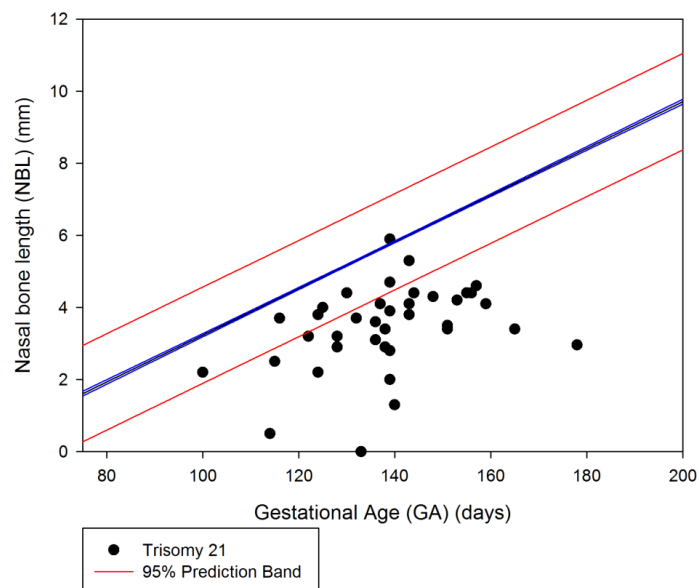
Forty-one (1.6%) of 2549 were affected by trisomy 21. Maternal age ranged from 16 to 47 years (median 29.5) and the gestational age from 14 to 27 weeks (median 19.57 weeks). The nasal bone length ranged from absence (0.00 mm) to 12 mm and volume capture duration ranged from 1 to 49 minutes (median 7 minutes).

Measurements and normal ranges of nasal bone length



Where the black crosses were the euploid measurements, black/grey line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles in Figure 17

Trisomy 21 cases with the normal ranges of nasal bone length



Where the black crosses were the trisomy 21 measurements, black/grey line was the median, blue lines were the confidence lower and upper lines, and red lines were the 3rd and 97th percentiles in Figure 18.

Down syndrome cases were plotted on normogram (Figure 2). Cut-off value was setup to the 5th percentiles line. Forty-one cases of trisomy 21 were identified (cytogenetically) and all of them were detected between 14th and 28th weeks. In 33 cases the measured NBL values were lower than the 5th percentile and 8 cases of trisomy 21 fetuses were higher than 5th percentile, respectively. These results showed 80.49% sensitivity with 98.17% specificity. (See in Table 3.). Positive and negative likelihood ratio for trisomy 21 fetuses were 43.98 and 0.2, respectively. There was significant difference between the nasal bone length of euploid and trisomy 21 fetuses ($P \leq 0.001$).

7. Discussion

7.1. The explanation of the results

This study represented the high sensitivity ultrasound screening methods and reference charts of the fetal biometric parameters of the Caucasian population.

NT was the first and the most sensitive screening marker of Down syndrome. This study proved its strength in first trimester screening.

DVPI was found one of the most sensitive marker of trisomies during the first trimester. It has high sensitivity and a medium-high specificity on trisomies. This finding was also confirmed by our study.

The most sensitive marker was the combined DVPI and NT plot, but the best result was reached when NT and DVPI were combined with the facial markers.

Our preliminary results were proved the efficacy of PT, NBL and their ratios in the second trimester. Slightly, these markers were fitted to the first trimester scan. They could be measured easily on the same image with NT. The common measurement, possibility could decrease the necessary time of observation and extremely increase efficacy of screening. In contrast with previous result PT-to-NBL is overwhelming in the first trimester.

The FL, BPD and HL should act as an important screening marker of the early IUGR and not for the trisomies. These markers may help to identify the bi- and unilateral cranial and limb anomalies during the first trimester.

The clinical aspects of these findings were the introduction of the facial profile ratio to the first trimester screening, using DVPI-to-NT and PT-to-NBL ratios in the first trimester as new markers of trisomies.

The ductus venosus-to-nuchal translucency to prenasal thickness-to-nasal bone length ratio reached an impressive 100% detection rate of 0.6% false positive rate. The risk of chromosomal defects is very high and the first line of management of such pregnancies should be the offer of NIPT or chorionic villus sampling (CVS) for fetal karyotyping.

The latest screening strategy for first and second trimester was introduced by Nicolaides et al. in a congress. (Advances in Fetal Medicine Dec 2013 London). Their opinion was to focus on the neck on first and focus on the face (facial profile) in the second trimester. These was a summary of the long development and research of the screening for trisomy, booth direction were introduced and published from several groups in last two and the half decades. (51,53,60,65,69,70,74-83)

CRL, BPD, FHR and FL were measured from the beginning of the obstetric ultrasound era. These markers were easily measured with the low-resolution devices, but provided much useful information about fetal development to the examiner. Our study had been set the normograms of these markers and tried to use them in the screening for trisomy. Excluding FHR, these markers have efficacy in the developmental and well-being scans, and they should not be used for trisomy screening. FHR proved a really high sensitivity and a fair specificity to detect fetal defects in the early pregnancy. Our previous observation also proved its importance during the early first trimester scans to find the pregnancy outcome or the early and late fetal loss from the 6 weeks.(84)

NT were the real first marker of autosomal trisomies (51) but several study proved its usefulness in different conditions and diseases (See Appendix Table 1).(75) Current paper used NT after the first trimester and proved a really good efficacy on trisomy.

Nasal bone length was proved high screening efficacy during the pregnancy. However, in the first trimester its repeatability is very low and there is no linear increase till 60mm of CRL, but the production lines were padded to the border of the plot and it was useful to screen out cases in the early pregnancy.

Prenasal thickness (PT) was published a several years later by Maymon et. al. PT(65) was improved the second trimester screening efficacy for trimsomies. Current study as a preliminary studies(85) before used PT as a first trimester marker –successfully. Szabó et

al.(69) published facial profile based ratios and its inverse counterpart and proposed that they could be utilized as well in the first trimester. This study confirms the usefulness of these markers in the first trimester. However, during the second trimester NBL-to-PT was better than the PT-to-NBL, although PT-to-NBL was better in the first trimester. The problem with this ratio is the zero division so if there is no nasal bone, it is unable to use for risk estimation.

7.2. The limitations of the study

The limitations were the local population and strict inclusion policy of the healthy euploid cases and the number of the overall cases and patient number of trisomy group.

Another limitation of the study is the nasal bone development; margins of the nasal should be identified.

7.3. Combined normograms

Table 5. Fetal nasal bone length normogram and trisomy 21 : review of the literature

Authors	Population	Method	Case	Tr21	DR	FPR	LR+
Bromley et al. 2002(3)	<i>Mixed</i>	2D	239	16	69	5	11
Cicero et al. 2003 (4)	<i>Mixed</i>	2D	1016	34	60	1	50.50x 132x*
Bunduki et al. 2003 (5)	<i>Mixed</i>	2D	1631	22	59.1	5.1	11.6x
Chen et al. 2004 (9)	<i>Chinese</i>	2D	198	NI	N/A	N/A	N/A
Benoit et al. 2005 (6)	<i>Mixed</i>	3D	38	20	75	8	N/A
Sutthibenjakul et al. 2009 (12)	<i>Thai & mixed</i>	2D	295	18	77.7	0.7	N/A
Geipel et al. 2010 (17)	<i>mixed</i>	3D	870	37	65	5.8	14x
Szabó et al. 2014 (16)	<i>Caucasian</i>	2D	1330	33	75.8	1.88	41,32
This study in 2014	<i>Caucasian</i>	2D	2590	41	80.5	1.83	43.98x

Population : population of the study; Method : 2D or 3D ultrasound were performed.; cases: number of euploid cases; Tr21 : number of trisomy 21 cases; DR : detection rate; FPR : false positive rate; LR+ : likelihood ratio;

*by Caucasians

*N/A not applicable or not available; NI : not included

Our data demonstrate that hypoplastic nasal bone between 14 and 28th gestational weeks was found in 1.83% of euploid and 80.49% of trisomy 21 fetuses, respectively. These findings are showed a better screening performance than to previous studies with 2D ultrasound. (8, 16) The nasal bone length was used as an isolated marker in our study. However, other studies used nasal bone length in combination bi-parietal diameter (BPD), femur length (FL) and moreover they used multiple of medians (MoMs) values instead of simple measurement value in millimetres. Our analysis showed that using MoMs in a statistical evaluation is misleading. Since the millimetre measurements day-by-day are more reliable and much easier to use in practice than a more complex summarized and corrigated values.

Fifty-one (1.83%) out of 2549 euploid fetuses fell under 5th percentile While 8 (19.51%) trisomy 21 fetuses had nasal bone length higher than 5th percentile. The nasal bone was present but hypoplastic in 33 (80.49%) out of 41 trisomy 21 cases.

7.4. Practical and clinical aspects

To use these normograms some basic recommendation is still necessary.

Repeated measurements are strongly recommended to exclude the medical, technical, measurement and visual errors. If the visualization is not enough good, then the examination, it should be repeated in another time/day/. Ultrasound scans and biochemical screening are still required during the trimesters. The screening for trisomy should be more ultrasonographic oriented and extended with NBL, PT. These results lead up to 90% sensitivity, if biochemical screening is preformed than it could reach 95% of sensitivity. Higher-risk patients should be sent to the genetics department for a counselling of the diagnostics ways of the invasive procedures which will be required for the diagnosis and performed only after genetic counselling. This study could improved the efficacy of screening.

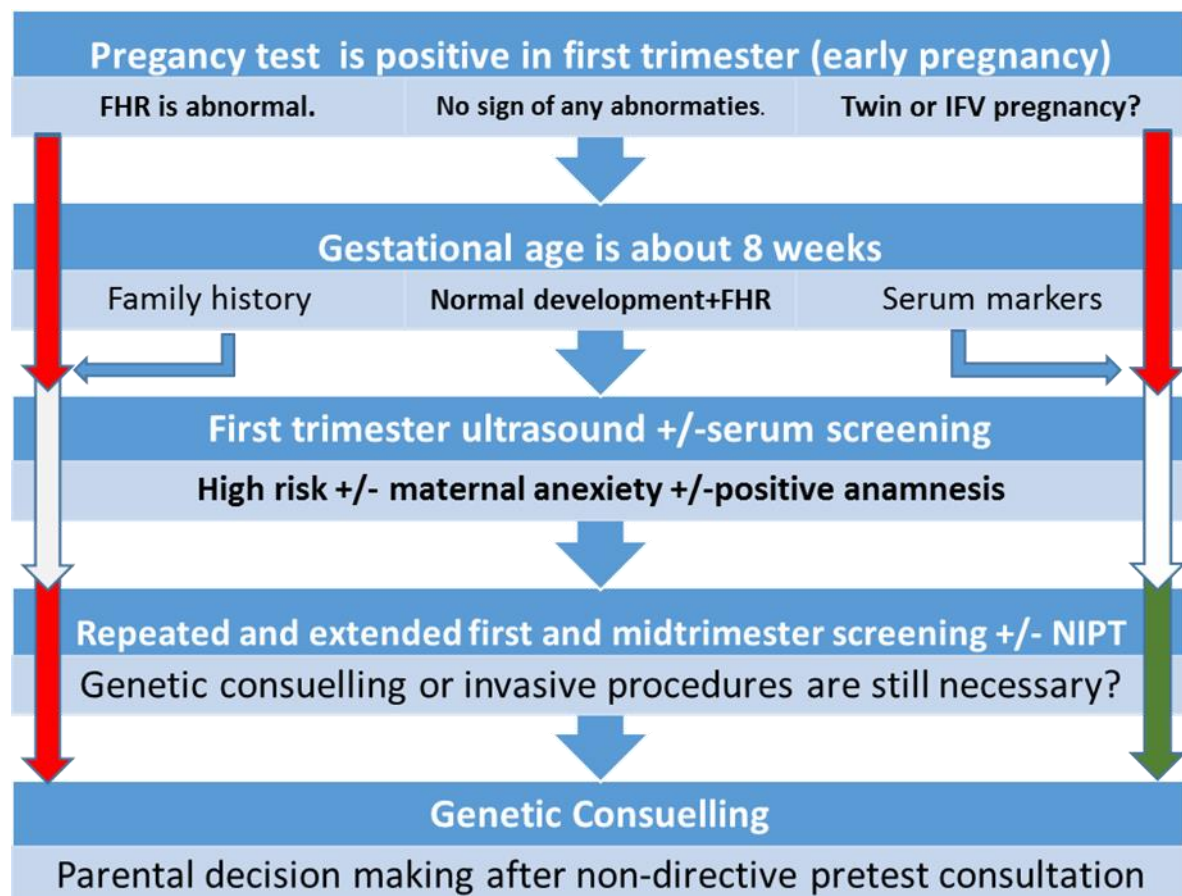
If the risk was low, but fetal growth is lower the normograms than IUGR is possible, but the measurements and dating must be rechecked to exclude the possible errors. In several cases, using the LMP to set GA leads to the wrong dating of pregnancy, so use CRL results to GA or changing markers when there is no automatized data transfer. In patients with a family history or maternal (and/or parental) anxiety of genetic syndromes that can be diagnosed by DNA analysis, the NIPT by some syndromes or the CVS sample can also be tested for these syndromes.

Place of the noninvasive prenatal tests in screening for trisomy

First of all, noninvasive prenatal tests (NIPT) are more widely offered in the wealthy and developed countries and its efficacy in screening for trisomies. On the other hand, NIPT overall screening (not diagnostic) performance is 99,5% of 1% of FPR which is impressive for a bit less than 1000EUR. However, ultrasound scans are still necessary to observe the fetal biometry for screening of other fetal structural and developmental disorder for a less than 70EUR. So it is more than dangerous, if the extended (anomaly) scans have been spent for a NIPT only.

It must not be forget that NIPT always remains a screening test, when invasive procedure offers

Figure 17 Show the developed screening and consultation methodology of trisomy.



This methodology provides the optimal prenatal care also in low-income countries where the full spectrum of biochemical screening and NIPT is not widely available for average citizens.

Conclusions

Local normograms and the most sensitive ultrasound screening model for trisomy 21, and 18 were introduced. Using these ratios could be comparable with NIPT. These measurements and methods should be incorporated into first trimester screening for trisomy. Further investigation will be necessary to observe these findings on different population and also in the second and third trimester.

8. Clinical conclusions of the study

- The increasing prevalence of DS can be primarily attributed to the increasing ratio of advanced age of mothers and changes of the environment especially the diet
- Prenatal screening could show a significant improvement year by year if our normogram and protocol could be introduced.
- The prevalence of Down syndrome and the efficiency of prenatal screening was slightly lower than the values observed in other European countries, but new references and protocol could lead to a better result such as it happened in Denmark.
- The increasing geographical inequalities in screening effectiveness demonstrated the existence of non-exploited opportunities in certain (non-properly managed) areas of Hungary by HCAR but 2D ultrasound is commonly used in the obstetrics and these markers could be easily measured in 5-15 minutes.
- Some clinical and practical training should be offered to involve the physicians to the screening.
- The combination of different ratio might be working in the second trimester screening, too.
- It is possible to improve screening efficacy if first and second trimester measurement of NBL is combined on a normogram.
- Combining different ratios could be beneficially by other markers, too.

9. New findings and newly developed methods in the thesis

- We first described the definition of “prevention in fetal medicine”
- We first described the national normograms of the fetal biometry parameters of the Hungarian population.
- We first described a new practical-based, easy-to-use and cost-effective two-dimensional measurement techniques of the NBL and PT in the first trimester.
- We first described a high risk pregnancy management protocol for low income countries
- We first described that the NIPT how to be placed into the screening system in Hungary.
- We first described that the combined the first and second trimester normogram to enhance the efficacy of the second trimester screening of nasal bone length.
- We first described in the international literature who introduced NBL and PT with their ratios in the first trimester.
- We first described that the combination of different ratios could increase the screening efficiency in the first trimester.
- We first described that highest sensitivity and specificity could be reached for the bedside in the first trimester without any biochemical or DNA test.
- We first described that development of a statistical method to a practical method of the ultrasound screening, which has comparable screening performance to NIPT but much cheaper and could be widely used also in the low income countries.

10. New observations during my fellowship

- 1) In the major trisomy markers of euploid and trisomy fetuses a significant difference was observed.
- 2) We elaborated the method how the fetal nasal bone length (NBL) and prenasal thickness (PT) can be obtained and measured in a single volume acquisition (image) during the first trimester anatomy scan.
- 3) Validated normograms have been created for the Hungarian population for the first and second trimester.
- 4) We first demonstrated the combination of nasal bone length (NBL) and prenasal thickness (PT) as a ratio could be used in the first trimester as a screening marker of aneuploidies.
- 5) These data have been supported previous observations which highlighted the importance of nuchal translucency and ductus venosus flow pulsatility index as the most effective screening co-maker of autosomal trisomy.
- 6) We first demonstrated the combination of nuchal translucency and ductus venosus flow pulsatility index as a ratio versus the combination of nasal bone length (NBL) and prenasal thickness (PT) as a ratio, could be used in the first trimester as a more sensitive ultrasonography screening marker of aneuploidies.
- 7) We first described *in the international literature* that the combination of first and second trimester NBL measurement on a mixed normogram could increase the efficacy of screening in the second trimester.
- 8) We first described *in the international literature* that the PT: NBL and NBL: PT ratio in the first trimester.
- 9) We first published *in the international literature* that the ultrasound measurements of these new markers can successfully be incorporated into the first and second trimester fetal anatomy scan.
- 10) We first described *in the Hungarian literature* that how to create and validate obstetrical normograms.

11. Acknowledgement

- This to acknowledge with thanks to my supervisor and mentor, Professor Dr. János Szabó, MD, DSc, allocating me this fantastic research topic and supporting me to realize it. I am grateful for his devoted supervisor activity and for helping me to perform the practical and theoretical part of my study.
- I would like to thank Professor Dr. habil. Márta Széll, PhD, DSc, head of the Department of Medical Genetics for her kind support in the last period of my fellowship.
- Special thanks to Dr. Emese Horváth MD, PhD for her practical recommendations.
- Special thanks to my PhD-fellows, Andrea Szabó, MD, PhD and Melinda Vanya MD for their dedicated work in preparation of the manuscripts.
- Special thanks to Roland Denk Cs, MSc, CEO and team of extra software GMBH, for their technical support and, for the invitation and sponsorship of my congress participations.
- Special thanks to my all co-authors, who helped and support my researches, especially to Szabó Andrea MD, PhD, Vanya Melinda MD, Prof. Bártfai György MD, PhD, DSc, Dr. János Tamás Szabó MD, Dr. János Sikovanyecz Md, PhD, Emese Horváth MD, PhD, Dóra Isaszegi MSc, Dr. Lipták-Váradi Julianna MSc, PhD, Prof Széll Márta MSc PhD DSc, Dr. Lilla Kató MD., Dr. Edit Ferencz MD, Roland Denk Cs, MSc, Prof. Dr. Hajnalka Orvos MD, PhD DSc, Prof. Dr- Attila Pál MD, PhD DSc, and of course to Prof. Dr. habil. János Szabó MD PhD DSc.
- Thanks for the members of the Department of Medical Genetics and MEDISONO Fetal and Maternal Health Research Centre.
- Thanks for the proofreading and spell checking for my friends and colleagues, especially Julianna Lipták-Váradi and Cindita Belencita Keto and to Ginger Proofreading Software.
- I would like to thank Dr. habil. Edit Paulik, MD. PhD, head of the Department of Public Health for her kind support.
- Last but not at least, I am extremely grateful to my parents (Judit Jákó and Károly Szili) and my sweetheart (Csilla Dézsi) and all of my family who supported me and guaranteed me the steady and quiet background.

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Appendix

Appendix table 1. Increased NT thickness associated fetal disorders
Venous congestion localized to the head and neck
Cardiac defects / dysfunction
Fetal anemia
Fetal hypoproteinemia /nephrosis
Fetal infection (ei. Parvo B13)
Altered composition of extracellular matrix
Failure of lymphatic drainage

Appendix table 2. First trimester major and minor markers of Down Syndrome
Nuchal thickening
Hyperechoic bowel
Absent nasal bone
Short humerus
Short femur
EIF
Pyelectasis
Mouth/cleft disorder

Publications of the thesis

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