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New Aneuploidy Ultrasound Markers in First Trimester of Pregnancy

Nové ultrazvukové markery aneuploidií v prvém trimestru gravidity

Doctoral Dissertation

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Abstrakt

Prenatální diagnostika se ubírá několika směry - vizualizací plodů a laboratorní biochemickou, cytogenetickou a molekulárně genetickou diagnostikou.

Zatímco vizualizace neznamená a priori pro těhotenství přímé riziko, nezpůsobí zvýšení počtu komplikací, u laboratorních vyšetření tomu tak vždy není. Známá jsou rizika, která jsou spojena s invazivními metodami prenatální diagnostiky.

Množství potenciálních nechtěných těhotenských komplikací a ztrát, technická a také ekonomická náročnost invazivní prenatální diagnostiky vedou ke snaze vyhledávat potenciálně afektované jedince metodami skríningu a tím minimalizovat nežádoucí dopad invazivní diagnostiky na těhotnenskou populaci. Čím přesnější vyhledávací kriteria jsou nalezena, tím menší bude počet těhotných exponovaných invazivními výkony.

Další možností, jak snížit počet nechtěnných komplikací v souvislosti s invazivními výkony, je zjednodušení a zlepšení techniky odběrů fetálních vzorků v průběhu gravidity.

V práci jsme se prioritně zabývali dvěma oblastmi: zjištění vztahu mezi frakčním zkrácením levé a pravé komory a chromozomální výbavou plodu a zjištěním spolehlivosti nové metody odběru vody plodové a biopsie choria pomocí vakuových zkumavek.

Prokázali jsme, že vyšetření funkčních parametrů fetálního srdce již na konci I. trimestru je nejen proveditelné, ale že je možné tímto vyšetřením odlišit plody aneuploidní od plodů s normálním karyotypem. Nalezli jsme rozdíl v hodnotách frakčního zkrácení u plodů euploidních a aneuplidních. Naše měření dále naznačují, že pravděpodobnou etiologií výskytu trikuspidální regurgitace v prvém trimestru bude zvětšení pravé komory.

Ve druhé části práce jsme prokázali, že námi navržená metoda odběru vody plodové a biopsie choria pomocí vakuových zkumavek je spohlehlivá a bezpečná.

Klíčová slova: prenatální diagnostika, ultrazvuk, frakční zkrácení, aneuploidie, invazivní diagnostika, amniocentéza, biopsie choria

Abstract

Prenatal diagnostics is headed in several directions - towards visualization of fetuses and biochemical, cytogenetic and molecular genetic diagnostics in laboratories.

Whereas visualization of fetuses does not a priori represent any direct risk for pregnancy and does not increase the number of potential pregnancy complications, this is not always the case with the laboratory testing. There are known risks connected with invasive methods of prenatal diagnostics.

The number of potential unintentional pregnancy complications and losses as well as the technical and economic aspects of invasive prenatal diagnostics lead to attempts of identifying ways of detecting any potentially affected individuals by screening methods, thus minimizing the undesirable impact of invasive diagnostics on the pregnant population. The more precise the selective criteria, the lesser the number of pregnant women exposed to invasive exams.

Another way of decreasing the number of unintentional complications in relation to invasive diagnostics is to simplify and improve the fetal samples harvesting methods during pregnancy.

The work primarily focused on two areas: Determination of the relation between fraction shortening of the left and right ventricles and a fetal chromosomal complement, and verification of reliability of a new method of amniotic fluid and chorion villus sampling using new vacuum tubes.

We have confirmed that it is possible to routinely measure functional parameters of the fetal heart as early as towards the end of the first trimester of pregnancy and that the measuring results may be used to distinguish between the aneuploid fetuses and the fetuses with normal karyotype. We have identified differences in fraction shortening values in euploid and aneuploid fetuses. Our measuring further suggests that potential etiology of tricuspid regurgitation in the first trimester of pregnancy is an enlarged right ventricle.

In the second part of the work, we have proved that the method of harvesting samples of amniotic fluid and performing chorion villus sampling, using vacuum tubes developed by us, is reliable and safe.

Key Words: Prenatal Diagnostics, Ultrasound, Fraction Shortening, Aneuploidy, Invasive Diagnostics, Amniocentesis, Chorionic Villus Sampling

Content:

Addenda

1) CALDA, P., **BRESTAK M**. Amniovacucentesis vs standard syringe technique for amniocentesis: experience with 1219 cases. *American Journal of Obstetrics and Gynecology*. 2009, vol. 201, issue 6, 593.e1-593.e3. DOI: 10.1016/j.ajog.2009.06.023.

2) CALDA, P., **BRESTAK, M**. Chorionic villus vacu-sampling in 377 consecutive cases. *Prenatal Diagnosis*. 2009, vol. 29, issue 11, s. 1075-1077. DOI: 10.1002/pd.2345.

3) CALDA, P., **BRESTAK, M**., TOMEK, V., OSTADAL, B., SONEK, J. Left ventricle shortening fraction: a comparison between euploid and trisomy 21 fetuses in the first trimester. *Prenatal Diagnosis*. 2010, 30(4), s. 368-71. DOI: 10.1002/pd.2500.

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5) **BRESTAK, M**., CALDA, P., MCKENNA , D.,SONEK, J. Comparison of right ventricular measurements and SFRV in fetuses with and without tricuspid regurgitation at 11 0 and 13 6 weeks' gestation. *Journal of Maternal-Fetal and Neonatal Medicine*. 2013, s. 1-4. DOI: http://dx.doi.org/10.3109/14767058.2013.863867.

1. General Part

1.1 Introduction

Advancements of the ultrasound diagnostics throughout the last 15 years, predominantly in the area of the ultrasound machine resolution, allowed for improved visualization of the fetus already towards the end of the first trimester. The most important development was that of transvaginal ultrasonography, using high-frequency probes, supporting realistic portrayal first of the developing embryo (sonoembryology) and later of the fetus.

Knowledge of formation of the individual embryonic structures and their ultrasound depiction represents the basic prerequisite for evaluation of a normal, and at the same time abnormal, embryonic development. Embryo undergoes natural developmental stages, divided into individual stadia. The generally followed system of the individual stadia of embryonic development - “Carnegie stages” - divides the first eight post-ovulation weeks into 23 stadiums (5). While the Carnegie stages are based on precise determination of the stages based on outer morphology and histological structure of particular organs, Jirásek divided pre-natal development from conception to delivery into 10 stadia (J’ stadia), based on external markers without the need for histological processing (Tab 1). Embryonic and fetus development, as described by embryologists, has been proved by sono-embryologic studies (2, 3, 4). It can thus be suggested that healthy embryos of the same age are in a particular developmental interval of the same size and in terms of development resemble one another. This expectation is conditioned by normal ovulation, conception and nidation.

The pre-embryonal period ends at implantation, which occurs on day 7 post-conception. During the pre-embryonal period, the conceptus is transported from the ovary, though the oviduct, into the uterine cavity. The pre-embryonic period cannot be monitored ultrasonographically. This option is only available from the start of the embryonal period.

Embryonal period begins with blastogenesis. Fetal period starting with post-conception week 9, or gestational week 11. The fetus is characterized by fused eyelids and distinct human somatic characteristics. Therefore, it is appropriate to respect the timeframe between the embryonal and fetal periods and not to refer to a fetus of 10 weeks and older as an embryo and vice versa.

1.2 Aneuploidy

Aneuploidy is a condition in which the number of chromosome (the chromosome number) in the nuclei varies from that considered normal for the given animal species. There is not an exact multiple of a complete set of chromosomes - i.e. diploid, respectively haploid amount. This state is the so called numeric chromosomal aberration (deviation in the overall number of chromosomes). While with plants, aneuploidy is usually not a problem - either being a fatal flaw or without any serious consequences in terms of life, growth and reproduction, for the majority of animal species aneuploidy results in severe impairment for the individual.

1.2.1 The Etiology of Aneuploidy

Aneuploidy is caused by separation spindle or centromere malfunctioning resulting in nondisjunction of homologue chromosomes in the first meiotic division or chromatids in the second meiotic division, causing that the created gametes contain a chromosome in excess or lack certain chromosomes. A chromosome may also be delayed during anaphase and thus not included in the daughter nuclei. This mechanism causes origination of a completely, uniformly trisomic or monosomic organism. If this malfunctioning occurs after zygote has been formed a mosaic is created, i.e. a state when an individual has a proportionate distribution of two different clones of cells with different karyotypes.

Thanks to studies of polymorphous molecular markers it is know that with trisomy of the 21stchromosome the non-disjunction most frequently occurs during the first meiotic division. Majority, but not all, trisomies 21 (up to 90%) are of maternal origin. However, for example, lethal trisomy of the 16th chromosome, often present in material from spontaneous aborts, is caused by non-disjunction during the first meiotic division and is always maternal.

Characteristic	**Stage**	**Length (mm)**	**Anatomic age (days)** from conception	Gestational age (weeks)*
Unicellular	1	0.2	0-2	
Blastomeric (16-20 blastomeres)	2	0.2	2-4	2-3
Blastodermic	3	0.4	4-6	
Bilaminar embryo stage				
Bilaminar plate	4-1	0.1	6-14	
Primary yolk sac	4-2			4
Secondary yolk sac	4-3	0.2-0.4		
Trilaminar embryo stage				
With primitive streak	5-1	0.4-1.0	15-17	
With notochordal process	5-2	1.0-2.0	17-20	
Early somite stage				5
Completely open neural groove	6-1	1.5-2.0	20-21	
Neural tube closing, both ends open	6-2	1.5-4.0	21-26	6
One or both neurospores closed	6-3	3-5	26-30	
Stage of limb development				
Bud of proximal extremity	7-1	4-6	28-32	
Buds of proximal and distal extremities	7-2	5-8	31-35	7
Proximal extremity two segments	7-3	7-10	35-38	
Proximal and distal extremity two segments	7-4	8-12	37-42	
Digital rays, foot plates	7-5	10-14	42-44	
Digital tubercles	7-6	13-21	44-51	
Digits, toe tubercles	7-7	19-24	51-53	
Late embryonal stage				

Differentiated extremities	8-1	22-23	52-56	8
Fusing eyelids	8-2	27-35	56-60	10
Fetal period	9	31-200	60-182+	11-26
Perinatal period	10	201-450	180-266+	27-40

*Tab. 1 - Jirásek J'stage (lit), *Age from 1st day of the last menstrual period*

1.2.2 Epidemiology

Prenatal diagnostics focuses predominantly on three numeric aberrations which are more or less compatible with life. The most common aberrations are trisomy of the 21st chromosome (Down syndrome - DS), trisomy of the 18th chromosome (Edwards syndrome - T18), trisomy of the 13th chromosome (Patau syndrome - T13), monosomy X (Turner syndrome), additional X chromosome in male (Klinefelter syndrome), trisomy of the X chromosome (previously also called super female syndrome), additional Y chromosome (previously also referred to as super male syndrome) and triploidia.

Trisomy of the 21st chromosome is the most common congenital defect from the above listed which is at the same time the most common congenital cause of mental retardation. Incidence of DS in population is quoted at 1 to 500 - 1000 liveborn. Quality of life and life expectancy of individuals with DS also depends on any connected anomalies, predominantly cardiac insufficiencies. The average life span of DS individuals is given between 49 and 61 years (5,6).

Trisomy of the 18th chromosome's incidence is about 1 to 5 - 6 thousand liveborn. Majority of fetuses with T18 is spontaneously aborted; average life span is quoted at 48 days with known case reports having survived till the second decennium. 5 - 10% of affected infants live longer than one year. (7, 8, 9)

Trisomy of the 13th chromosome's incidence is about 1 to 1000 liveborn. Their life span is at maximum several days, rarely months. Majority of thus affected fetuses is spontaneously aborted. About 50% of liveborn infants survive one week, 5 - 10% of affected infants live longer than one year. (7, 8, 9)

Incidence of T21 in population depends on the mother's age. The older the mother the higher the incidence. Unlike monosomy X which is age independent.

1.3 Down Syndrome as "Model" Deficiency

DS is the best described and the most population significant of the numerical chromosomal deficiencies. It is usually quoted as model deficiency due to the incidence and known, statistically confirmed, dependencies of its occurrence on the mother's age and additional parameters (biochemical, sonomorphological). Therefore, the aneuploidy screening during pregnancy is simplistically referred to as the DS screening.

1.3.1 History

The first clinical case report of phenotype in individuals with Down syndrome is attributed to Dr. John Langdon Down, who the syndrome is named after (J Langdon Down (1887): On some of the mental affections of childhood and youth. J & A Churchill). The anticipated chromosomal nature was proved by a French pediatric and genetic specialist, Jérôme Lejeun, and the aberration labelled as trisomy of the 21st chromosome - T21 (10).

1.3.2 Diagnostics

T21 diagnostics is always based on a proof of existence of an additional 21st chromosome in the cells of the examined individual. This proof may result from a DNA analysis or from the fetus karyotype examination. To perform the laboratory examination, it is necessary to obtain material from the product of conception, from the embryonic egg. In praxis, this material is a chorionic tissue sample or amniotic fluid (rarely fetal blood). This material can be obtained by chorion biopsy, amniocentesis or umbilical cord centesis. The listed procedures are connected with about 0.5 - 1% risk of complications, including a non-intentional pregnancy termination (11, 12, 13, 14).

1.3.3 Structural Aberrations

Alongside more frequent numeric aberrations (aneuploids) we ought to consider also structural aberrations, i.e. situations where the number of chromosomes is normal, but there are changes in the structural arrangement, most frequently due to breaks or, alternatively, their incorrect pairing. Consequences of such anomalies for organism are given by the size and

place the flaw occurs as well as the number and character of deformation of the cells or tissue. Unlike numeric aberrations, structural aberrations may occur at any time throughout the cells lifespan. Moreover, there are known spontaneous repair mechanisms. Structural aberrations in embryonal karyotype are more or less accidental findings, not fitting the population screening criteria due to their low incidence. More importantly, no efficient screening method has been discovered. Structural aberrations may often be accompanied by morphological deficiencies, detectable during prenatal ultrasound examination.

1.3.4 Screening

In general, screening is a statistic method used in medicine to distinguish between population with low and high risk of a particular disease. Individuals with high risk of occurrence of the particular disease based on performed screening test are offered additional, usually invasive, diagnostic examination to confirm or deny the presence of the particular disease. The basic requirements for screening are to include the entire population if possible, non-invasive character, feasibility and economical tolerability. The difference between a screening test and a diagnostic test is in lower sensitivity and specificity. As the resulting diagnostic tests are usually invasive and costly, one of the basic requirements of screening methods is the lowest possible false positivity. The goal is to detect the maximum of the screened pathological incidents with a minimum exposure of population to diagnostic testing.

1.3.5 Prenatal Aneuploidy Screening

Due to frequency and seriousness of DS, from the moment the connection of DS incidence with the mother's age was identified, conscious effort has been made to locate the population at risk. From the moment routine invasive prenatal diagnostic methods were introduced, effort has been made to screen the target group in the earliest possible stages of pregnancy.

1.3.6 Screening Methods

1.3.6.1 History

Mother's age used to be the first and only screening criterion. The risk of DS occurrence doubles at the age of 35 when compared with the average population risk, while the risks, connected with the invasive diagnostic method are almost on par with the gains of detecting

the disease. The marginal risk level for recommended embryonal karyotypisation is reached at the age of 35 at delivery. The age criterion alone can detect about 30% of the absolute number of DS affected fetuses. False positivity (FPR) is greatly influenced by the age structure of the particular pregnant population. In this country, 17% of pregnant women is older than 35, whereas 20 years ago, there were only 5% of pregnant women older than 35.

Majority of DS infants, from the absolute figure, were born to younger mothers, i.e. those from the largest group of pregnant women. Therefore, in the 1970s, a so called triple test was formed and later implemented (15, 16, 17), based on the findings that the levels of certain analytes were changed in mothers with aneuploidy fetuses. The biochemical test examines the mothers' serum, checking the levels of alpha-fetoprotein, human chorio gonadotropin and estriol. The risk of DS occurrence of 1/250 - 300 is usually considered the positive/negative margin in this test. Detection rate (DR) of this test was between 60 - 70% upon FPR between 5 - 15%. (15, 17, 18, 19) The problem of this test was its strictly biochemical nature and frequent incorrectly stated age of gravidity the results were based on. Additional problem of the test was that results were known quite late in pregnancy, in about its half. Mental strain of pregnant women connected with this type of screening was another negative aspect of this test. Therefore, a way was looked for to ensure higher sensitivity and specificity, preferably as early as towards the end of the first trimester.

1.3.6.2 Present Days

The test which met the requirements for simplicity, high sensitivity and specificity was the so called first trimester combined test. In 1990s, Nicolaides noticed that the value of temporarily increased nuchal translucency in fetuses between 11 and 14 week of pregnancy correlates with the DS risk. He complemented the morphological exam of the fetus with the biochemical exam of the mother's serum, examining the levels of pregnancy-associated plasma protein A (PAPP-A) and free beta subunit of human chorionic gonadotropin (free βhCG). With time, the test was further developed and additional ultrasound parameters, which could be either measured or visualized on the fetus, added. Today, the so called contingency test contains alongside measuring the nuchal translucency parameter (NT), PAPP-A and free βhCG also portrayal of nasal bone (NB), a tricuspid aortic valve blood flow (TCR) and ductus venosus Arantii blood flow (DV). Fetal heart rate (FHR) is also factored into the DS risk calculating

algorithm together with anamnestic data (ovulation induction, etc.). The individual listed markers are statistically independent of one another.

Methodically correct test achieves over 95% DR upon 5% FPR (20, 21, 22, 23), while assessing the risks of T18 and T13 alongside DS. High level of NT (over 95 centile) further signals other, nonspecific, potential gravidity risk factors and is often associated with many severe fetus conditions (24, 25, 26, 27, 31, 32). As will be discussed below, one of the undisputed positives of this test is its implementation into the basic algorithm of the prenatal care process. First trimester biochemical and ultrasound (visualization) screening has become the basic diagnostic method during pregnancy worldwide.

1.3.7 Screening Markers of Aneuploidy

Every used characteristics, marker has its own set likelihood ration - LR. NT, FHR, DV and biochemical markers work as quantitative parameters, continuous variables. By changing the marginal values of the individual parameters we can change both DR and FPR. Other markers, NB and TCR, are evaluated qualitatively - i.e. whether or not the marker is present or absent. DR and FPR of these markers are given by their prevalence in healthy and affected population.

Pregnancy dating, i.e. confirmation of the correct pregnancy age by ultrasound examination, influences the validity of the individual measurements or marker presence in a vital way. The basic screening parameter is thus the pregnancy age as stipulated by CRL - the crown-rump length of the fetus at rest. As a healthy fetus growth proportionally and the measured markers are bigger in bigger (older) fetuses, CRL parameter underestimation, i.e. measuring smaller sizes than they in reality are, increases FPR, just as overestimating the markers will potentially result in a false negative result. This conclusion may also be applied to early growth retardation of aneuploidy fetuses (28).

1.3.8 Screening Markers Pathophysiology

1.3.8.1 Nuchal Translucency - NT

This is the basic and probably most prominent known ultrasound screening marker of the greatest relevance (23, 24, 25, 26). If we performed the aneuploidy (T21, T18, T13) occurrence in fetus risk calculation based on NT measurements only, in relation to CRL, we

would achieve 75% DR upon FPR at 5% level of relevance. The same results would be achieved for monosomy X (60% DR) and triploidia (29, 30).

Anatomically, the measured marker is a layer of subcutaneous extracellular fluid in the fetal nuchal region, extended caudally at various distances. It has been proved that the front-back dimension of this layer directly correlates with the risk of occurrence of a number of pathological states (24, 25, 26, 27, 31, 32). The greater the value of nuchal translucency, the greater the risk of DS, fetal heart disease and premature death of the fetus in utero.

The reason for the fluid layer enlargement is not known. Several rather heterogeneous states are being considered:

1. Structural cardiac or cardiovascular changes
2. Lymphatic system development deficiencies
3. Increased intrathoracic pressure
4. Decreased fetus mobility
5. Hypoproteinaemia
6. Infection
7. Deviation in the extracellular matrix composition, predominantly in the form of flaw in the connective tissue formation, i.e. pathological collagen formation

Resulting from the heterogenous nature of the listed causes, we can speculated that their common denominator could be a collagen formation deficiency.

Distribution of the NT values in population during the gestation age between 11+3 and 13+6 has two different forms. In one group of fetuses, the NT values proportionally collide with the pregnancy age, while in the second group the average value is independent of the pregnancy age. The proportional representation of incidents in the two groups varies based on the chromosomal situation of the fetuses (33).

1.3.8.2 Nasal Bone

Differences in development and formation of nasal bone are judged based on studies of adults with DS. Radiologic and anthropometric studies confirm occurrence of NB hypoplasia

significantly more frequently than in the euploid population (34, 35). This phenomenon was proven even pre-natal both in the first and the second trimesters of pregnancy (36, 37).

Different connective tissue formation, proven in individuals with DS (38, 39, 40, 41), is considered as the cause of this developmental divergence. Inclusion of NB into the aneuploidy screening algorithm significantly increased DR (92% for DS) upon concurrent decrease of FPR to 3%.

1.3.8.3 Tricuspid Valve Blood Flow Measuring - TCR

TCR measuring is performed using Doppler ultrasound modalities (pulsed Doppler) and is viewed as borderline between the anatomic and functional examination of the fetus. As the examination is performed during the 1st trimester, it is important to note here the safety aspect of ultrasound examination in early fetal developmental stages. Fetuses exposure to acoustic energy upon use of Doppler modalities is significantly higher than upon use of the conventional B-mode. As recommended by respected authorities (International Society for Ultrasound in Obstetrics and Gynecology, European Federation of Societies for Ultrasound in Medicine and Biology), it is possible to examine fetus at the end of the 1st trimester using pulsed Doppler upon adhering to the general principles for exposure of living organisms to energies as defined for other biomedical specializations and generally referred to as ALARA (as low as reasonably achievable), i.e. only for the necessary period of time and with the lowest possible radiation energy.

Several specifics of the fetal circulatory system during prenatal period must be taken into consideration upon tricuspid aortic valve blood flow examination in the 1st trimester. The main difference is a surprisingly low ratio of contractile proteins in fetal myocardium, resulting in very low contractility and elasticity. Furthermore, unlike during the postnatal period, prenatally, cardiac output is combined, the pressure gradient is right-left and thus the right-sided circulation works with greater resistance than the left-sided circulation (42). Tricuspid valve is thus subject to greater pressures, both opening and closing, and the right myocardium is subjected to greater stress. This corresponds with the values and developmental trends of metric contraction and relaxation times when compared with the ejection times (43, 44).

Another specific factor of the first trimester is a relatively high placental resistance, resulting in the fetal hearth, in the first trimester especially, working in the top part of the Frank-Starling curve (45).

Thus, otherwise practically unmeasurable functional aberrations of the fetal myocardium and fetal central circulation per say, are detectable on the tricuspid valve, namely towards the end of the first trimester.

Statistically significantly higher incidence of tricuspid valve regurgitation has been proven in aneuploid fetuses than aneuploid fetuses (measurable tricuspid valve regurgitation at the ventricular ejection times) (22,46). Etiology of this phenomenon has not been fully clarified, but, again, a conclusion could be drawn that a flaw in the connective tissue formation and the extracellular matrix may be at its origin, most probably causing a slight myocardium dilatation and malfunctioning both of the valve and the papillary muscle. Abnormal peripheral resistance as well as abnormal elastic arteries capacity of the central circulatory system. TCR thus reflects a relatively complex set of changes to fetal bloodstream both in relation to afterload and preload.

It is apparent from the given expected mechanisms and causes that TCR is present not only in DS, T13 and T18, but also in monosomy X. It can further be expected that TCR will be detected in connection with a number of aberrations in connective tissue formation (hypothetically e.g. Maran syndrome).

By including TCR into the aneuploidy risk calculating algorithm, the DR for DS has been increased to 96% upon 3% FPR.

1.3.8.4 Ductus Venosus Arantii Blood Flow Measuring - DV

DV connects the umbilical vein to the inferior vena cava, right before it reaches the right atrium, draining about 50% of blood from the umbilical vein. The flow is primarily directed by the Eustachian cilia in the right atrium to foramen ovale and further to the left atrium, i.e. to coronary, cranial and system bloodstream. Due to significant pressure gradient between the umbilical vein and the inferior vena cava, DV is the most restrictive part of the central fetal venous system, resulting under normal circumstances in forward flow in DV for the entire heart revolution. Regurgitation in DV during atrial contraction (A-wave) is significantly more

often reported in aneuploid fetuses than euploid fetuses (20, 47). Reasons causing this anomaly have not been clearly explained, but most probably they are the same as the causes of the tricuspid regurgitation. All aspects considered, the causes could be identical to those responsible for increased NT values and those causing NB hypoplasia: flaws in connective tissue formation and extracellular matrix, in general.

1.3.8.5 Fetal Heart Rate - FHR

Aneuploid fetuses have different heart rate than euploid fetuses, in particular during the first trimester. Despite the ambivalent character of the connection, FHR can be used as auxiliary, additional marker (48). Pathophysiological grounds for different heart rate should be again looked for in the different connective tissue formation and thus in different characteristics of myocardium (41, 49).

1.3.8.6 Additional Fetal Aneuploidy Markers

Almost every finding more frequently recorded in fetuses with abnormal set of chromosomes may be considered as a fetal aneuploidy marker, including some congenital developmental defects, such as:

- Holoprosencephaly (T13 incidence risk 1:2)
- Diaphragm hernia (T18 incidence risk 1:4)
- Omphalocoele (T18 incidence risk 1:4, T13 incidence risk 1:10)
- Chamber septum defect (T21 incidence risk 1:2) (50, 51, 21).

All listed defects can be diagnosed with high sensitivity and specificity already during the first trimester of pregnancy (53, 54, 55, 56, 57).

1.3.9 Treatment of Pregnancy with Elevated Risk of Chromosomal Aberrations

Pregnant women with suspected fetal chromosomal aberrations are usually offered the option of invasive diagnostics. Towards the end of the first trimester, they are offered the option of undertaking chorionic villus biopsy, later in pregnancy, they can opt for amniocentesis. These procedures are connected with 0.5 to 1% pregnancy loss risk. There are two ways of minimizing the risk of unintentional loss of pregnancy:

1. Finding a way of making the screening methods more accurate, thus lowering the false positive ratio to minimum;
2. Improving the technique of the invasive procedures and achieving the lowest possible rate of complications.

1.4 Aneuploidy Screening Implementation into Basic Prenatal Care Algorithm

Congenital defects are still a major cause of perinatal, neonatal and infant mortality. Parents are keen to have a healthy child and usually require that their doctor answers the question whether or not the fetus' development in utero is normal. Information regarding fetal intrauterine development often helps medical personnel to plan in advance and without any delay, adequate post-delivery newborn care management. In these incidents, it is also beneficial for the family having enough time to prepare for the situation. There is a tendency to diagnose any abnormalities as early in pregnancy as possible, corresponding with the still evolving yet limited fetal surgery methods. If severe fetal defect is confirmed by tests, Czech Republic's legislation allows, if desired by the pregnant woman, premature termination of the pregnancy. Upon pregnancy termination, earlier stages represent lesser health risk - primarily to the mother's reproduction health, but also psychical and psychological strain of the pregnant women and her closest ones.

Trisomy of the 21st chromosome is especially population significant due to its impact on life of the affected individuals and their families. Aneuploidy, respectively all chromosomal defects are incurable. Alongside chromosomal defects, there are also cardiac diseases, neural tube clefts (myelomeningocele), limb development anomalies and more. Prenatal diagnostics shall thus not be limited to aneuploidy screening.

On the other hand, it is highly positive and clearly beneficial that the individual steps of the screening are interconnected, i.e. that the aneuploidy screening is an integral part of a broader detection examination, focused in general on the fetus morphology, pregnancy age determination, evaluation of a number of pregnancy-connected risks and impacts on fetus and the pregnancy course as such.

Ultrasound fetus examination during the first trimester has, in general, become the basic prenatal screening during pregnancy. Detecting the risk population, in regards to potential aneuploidy, is only its part.

Characteristics, use to set the aneuploidy risk factor are at the same time general markers, pointing to a fetus handicap or abnormal pregnancy course. For example, NT increased above 99 percentile (>3.5 mm) significantly increases the risk of congenital defect occurrence

(Tab 2).

Interconnection of the aneuploidy screening with morphological or functional fetal congenital defects screening reflects the philosophy behind prenatal screening.

Alongside the above describe screening, ultrasound also works as a mean of diagnostics of morphological (anatomical) congenital defects. In regards to aneuploidy, ultrasound is only a part of the screening algorithm - detecting and determining the high risk and low risk population. In this regard, it is the goal of diagnostics to determine the individual's karyotype, i.e. providing clear evidence of different number of *chromosomes* in the nuclei of the fetus' cells.

NT values (mm)	**Serious Heart Disease Occurrence (%)**
>3.5	1-2
3.5 - 4.5	3
4.5 - 5.4	7
5.4 - 6.4	20
>6.5	30

Tab. 2: Congenital heart disease occurrence in relation to NT value (58, 59).

1.5 Aneuploidy Diagnostics

1.5.1 Methods of Obtaining Samples for Laboratory Analysis

Today, we are only able to obtain samples from the fetus using invasive methods: by CVS - Chorion Villi Sampling, AMC - amniocentesis and PUBS - Percutaneous Umbilical Blood Sampling (cordocentesis). Additional invasive prenatal exams are indicated very sporadically - e.g. fetal skin or fetal liver sampling. Fetal endoscopy is currently also rarely performed, mostly indicated to correct fetal circulation upon twin transfusion syndrome (with monochorial, biamnial pregnancies). The indicated method is usually based on the pregnancy age and the best sample for diagnosing the expected pathology.

Fetus' karyotype from chorion villus sample or amniocytes is usually know within 10 - 21 days from sampling.

1.5.2 Amniocentesis (Amniotic Cavity Puncture)

Since the 19th century, amniotic cavity puncture has been a method used for possible polyhydramnios treatment. Later, the method was also used for amniography. Application of pharmaceutics or simple drainage of the amniotic fluid were methods used for premature pregnancy termination. In the middle of the 20th century, amniocentesis started being used to determine the level of bilirubinoids, as part of fetus alloimmunization treatment (60, 61). It is important to note here that until the end of the 1970s, genetics was subject to severe ideological scrutiny in the former Soviet Bloc of countries and in fact stopped developing. For example, in 1951, Hašek states that: "The number of chromosomes in human individuals oscillates between 30 and 70." (Mendel - Morganism in relation to socialistic science. Socialist Academy Library, Osvěta publishing house, 1951, p. 18). In 1956, Fuch and Riis (62, 63) first determined the fetus' sex using an amniotic fluid sample. C complete fetus' karyotype was first assembled in the 1960s, while prenatal diagnostics of DS, using this method, was first successfully executed in 1968. First amniocentesis in our country was performed in 1971 (64). Effort was made to perform the amniotic fluid sampling as early as possible, preferably towards the end of the first trimester. This method was called an 'early amniocentesis' during the 1980s. Randomized studies proved that early amniocentesis (performed before completion of the week 15 of pregnancy) represents higher risk of

pregnancy loss, makroglosia and pes equinovarus. Intraamnial fluid sampling in early first trimester may cause limb reduction defects, most probably as a result of early trauma caused to the amnion (65, 66, 67). Today, amniocentesis is performed post completion of week 15 of pregnancy.

The used instruments also developed in time to today-used needles of 0.9 mm in diameter and 120 mm in length. Thinner needles could be used, but it has not yet been proven that such alternative decreases the exam-connected risk (68). Whereas, it is a proven fact that the thickness of the sampling needle corresponds with the level of trauma caused to the amnion (69).

1.5.3 Chorion Villus Sampling

Chorion Villi Sampling, as an earlier-performed alternative to amniotic fluid sampling has been know since the 1980s. Chorion biopsy was developed as an alternative to amniocentesis as it proved problematic to cultivate amniocytes from samples taken towards the end of the first trimester and in early second trimester and it was difficult to puncture the amniotic cavity as ultrasound machines with high resolutions were available. Later, it was determined that chorion villi sampling prior completion of week 10 of pregnancy is technically possible, but connected with the risk of limb reduction deformities of the fetus (70). Therefore, the chorion biopsy has become the commonly accepted first choice upon assembling the fetus' karyotype post 10 week of pregnancy till the moment a risk-free amniocentesis can be performed (i.e. till completion of week 15).

Originally, several millimeters in diameter thick needles were used to collect the sample. The exam was painful and the greater needle thickness was connected with higher risk of amnion traumatization.

In time, the technique was improved and still thinner, and thus safer, sample-harvesting, punction needles used. In our country, the pre-1898 situation was also influenced by unavailability of the needles. Development of laboratory examination methods also played its part, allowing for decreasing the amount of the tested material upon upkeep of the diagnostic quality.

1.5.4 PUBS - Percutaneous Umbilical Blood Sampling (Cordocentesis)

Fetal umbilical blood sampling, using transabdominal punction, is usually performed post completion of the week 18 to 20 of pregnancy, with the main limitation being the umbilical vein visibility and lumen. The most common indication for the exam is that of an Rh incompatible fetus. The method is also used for harvesting samples of the fetal blood for karyotyping, in particular during later stages of pregnancy or in cases where it is required to confirm or deny an atypical finding of the fetus' karyotype. The exam connected risk is the same as with amniocentesis if performed by an experienced professional.

1.5.5 Invasive Diagnostic Methods Safety

The exam (sample harvesting) connected risk is compatible with all three described sample harvesting methods, quoted at the rate of 0.5 - 1% of lost pregnancies, referring to the time, rather than cause, interconnection. Today, it is not possible for ethical reasons to execute a randomized prospective study to collect data regarding the safety of the individual methods. Therefore, we still quote the studies performed in the past, which do not necessarily fully reflect today's situation (11, 12, 13, 14).

The invasive exam risks are tightly connected with the reasons for the exam indication, erudition of the professional performing the exam and the used instruments. The indication factor works quite paradoxically - the more accurate the reasons for the exam, the higher the risk of the examined population. As a result, higher rate of complications can be expected. For example, a positive first trimestral contingency test (high NT value, low PAPP-A value, alternatively also high free βhCG value) may, alongside other signs, be potential miscarriage symptoms. Erudition of the exam-performing specialist is also a very important and undisputable factor. The trauma level of the fetal membrane may be directly connected with the incidence of complications after performed-amniocentesis.

The vacuum tube sampling concept, developed as part of this work, allows to further decrease the diameter of the punction needles and also increase the comfort of the exam-performing specialist. The concept thus definitely promotes greater safety of the invasive prenatal examination.

The diagnostics is performed in vitro, by laboratory examination of the harvested samples: DNA analysis and cultivation (cytogenetic) examination.

Despite the tendencies to limit the amniotic fluid examination to mere DNA analysis, cytogenetic examinations remains the golden industry standard.

2. Specialized Part

2.1 Hypothesis and Goals

The work focuses on two areas:

A: Determining the relations between fraction shortening of the left and right ventricles and fetal chromosomal complement.

B: Determining the reliability of a new method of harvesting amniotic fluid samples and performing chorion biopsy using vacuum tubes.

A: The goal of the first and general part of the work was to determine the relations between fraction shortening of the left and right ventricles and fetal chromosomal complement observed during an ultrasound examination of fetuses at the end of the first trimester. The following questions were asked:

1. Is it possible not only to measure, but also to evaluate the usual hemodynamic parameters, i.e. fraction shortening of the left and right ventricles, already at the end of the first trimester?

2. Are there statistically significant differences in these parameters between the euploid and aneuploid population?

3. What is the relation between fraction shortening of the left and right ventricles and the tricuspid valve regurgitation phenomenon?

B: The subsequent part of the work focuses on questions related to improvements and simplification of invasive prenatal diagnostic methods - amniotic fluid harvesting and chorion

biopsy - the two basic methods used to determine the karyotype of the fetus. The goal of this part of the work was to determine the reliability of a new method of harvesting amniotic fluid samples and performing chorion biopsy employing a modification designed by us, using vacuum tubes.

3. Measuring Fraction Shortening of the Left and Right Ventricles in Fetuses with Unknown Karyotype

3.1 Objective

This work attempted to evaluate the relation between fraction shortening of the left and right ventricles and a fetal chromosomal complement, using ultrasound examination of fetus at the end of first trimester of pregnancy.

3.2 Materials and Used Methodology

The values of LV and RV fraction shortening in fetuses with unknown karyotype, without a visible heart defect, were measured during the period of CRL between 45 - 84 mm. This study was designed to compare first trimester SFRV and SFLV values between euploid fetuses and fetuses with trisomy 21. SFRV in fetuses with normal contingency test results, without elevated aneuploidy risk, without heart defect, with and without detected tricuspid regurgitation, were further compared and contrasted.

The ultrasound examination was performed either at the time of the first trimester combined screening or before chorionic villus sampling (CVS). Each fetus had a CRL measurement obtained in a standard manner.

The technique used to measure the SFRV is essentially identical to the one used to measure the SFLV. The heart was imaged in one of two ways: the two ventricles were either viewed in the long axis with the face of the transducer being approximately parallel to the ventricular septum (Figure 1), or in the short axis view (Figure 2).

The image of the fetal chest was significantly magnified so that the fetal heart filled approximately 75% of the image. An M-mode cursor was then placed through the two ventricles at a right angle to the ventricular septum beneath the level of the A-V valves

(Figures 3 and 4). The appropriate M-mode images were obtained using a 7 MHz abdominal probe [M7C, Vivid7 Dimension, Logic 9(GE)] by a single experienced operator (M.B.) and stored.

From the saved M-mode image, the maximum diastolic size values of the left (LVDD) and right (RVDD) ventricle and the minimum systolic size values of the left (LVSD) and right (RVSD) ventricle in the same heart cycle were measured. The SF value was calculated as a relative shortening using the following formula: [(VDD-VSD)/VDD]*100.

Evaluation of the fetal heart anatomy and function has become an integral part of obstetrical ultrasound examination.

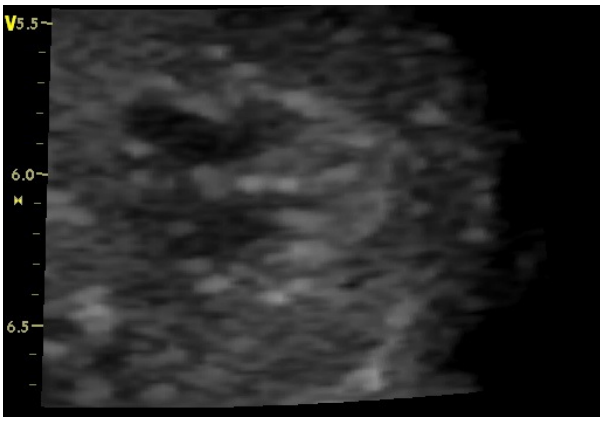

Figure 1: Four chamber view, 90 degree angle insonance in relation to the chamber septum

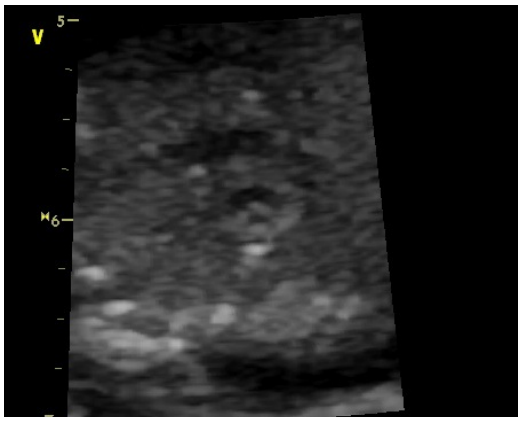

Figure 2: Short axis view

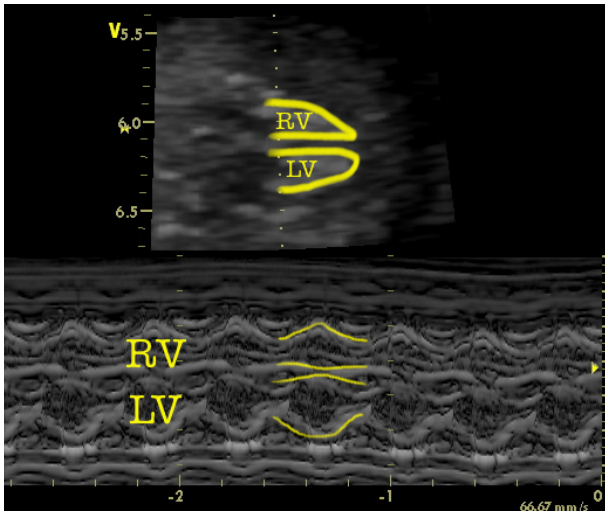

Figure 3: SF measuring scheme in 4-chambre view

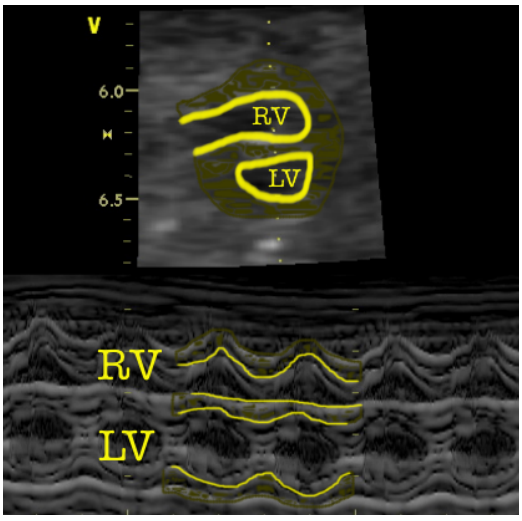

Figure 4: SF measuring scheme in SAX

3.3 Results

We examined 58 fetuses that fit the study criteria. In two of the cases, appropriate images could not be obtained. Out of the 56 fetuses that were examined successfully, 49 were chromosomally normal and 7 had trisomy 21. The SFLV values in the euploid fetuses were statistically smaller than in fetuses with trisomy 21: 38.00 (95% CI: 33.72-42.27) versus 52.07 (43.72-56.13) ($p < 0.05$). There was also a significant difference in the nuchal translucency (NT) measurements between the two groups: 1.78 (95% CI: 1.08-2.48) in the euploid population versus 5.06 (95% CI: 3.61-6.71) in the fetuses with trisomy 21 ($p < 0.05$). The two groups did not differ in CRL measurements [euploid: 66.81 mm (95% CI: 58.28-75.35 mm) versus trisomy 21: 74.68 mm [95% CI: 65.23-79.59 mm ($p = 0.05$)], LVDD measurements [euploid: 3.35 mm (95% CI: 2.67-4.03 mm) versus trisomy 21: 3.66 mm (95% CI: 2.69-4.06 mm) ($p = 0.19$)], and LVSD measurements [euploid: 2.09 mm (95% CI: 1.58-2.60 mm) versus trisomy 21: 1.78 mm (95% CI: 1.17-2.20 mm) ($p =0.28$)]. Out of the 28 cases where the time to obtain the SFLV was measured, two cases (7.14%) required less than 60 s, 22 cases (78.57%) between 60 and 120 s, and in two cases (7.14%) 240 s were needed. Examination in the remaining two cases (7.14%) did not yield an acceptable M-mode image even after a prolonged examination.

A total of 62 fetuses examined between September 2008 and February 2010 were included in the study. Of those, four either had suboptimal images for the SFRV measurement or the images could not be obtained at all. Of the remaining 58 fetuses that were included in the study, 49 had a normal chromosomal complement and 9 had trisomy 21. The comparison between the two populations revealed a significantly larger SFRV in the fetuses with trisomy 21 (mean: 48.6 mm; range: 36-56.25 mm) as compared to the euploid fetuses (mean: 34.11 mm; range: 22.73-43.48 mm) ($p < 0.0001$). The medians were similar: 50.0 and 34.6 for trisomy 21 and euploid fetuses, respectively. A significant difference was also noted between the two groups in the nuchal translucency (NT) measurements. The trisomy 21 fetuses had larger NT measurements (mean: 5.36 mm; range: 3.2-8.9 mm) as compared to the euploid fetuses (mean: 1.78 mm; range: 1.2-6.1 mm) ($p < 0.0001$). Overall, the CRL measurements were slightly larger in the trisomy 21 group (mean: 72.9 mm; range: 61-80 mm) than in the

euploid group (mean: 66.8 mm; range: 45.2-83.1 mm) (p = 0.041). There was no statistical difference in the RVSD measurements between the two groups (mean: 1.56 mm; range: 1.2-2.3 mm in fetuses with trisomy 21 and mean: 1.67 mm; range: 1.3-2.4 mm in the euploid group) (p = 0.17). However, there was a significant difference in the RVDD measurements with the trisomy 21 group being larger (mean: 3.08 mm; range: 2.2-4.7 mm) than the euploid group (mean: 2.54; range: 1.9-3.6 mm) (p = 0.03).

A total of 69 women were enrolled in the study. Their fetuses were divided into two groups: one where tricuspid regurgitation was absent (TR [-]) (n=44) and one where tricuspid regurgitation was present (TR [+]) (n=25).The two groups were similar with respect to maternal age (TR [-]: mean 31.39 years (range: 21-39); TR [+]: mean 31.96 years (range: 25-43) (p=0.84)), CRL measurement (TR [-]:mean 71.13 mm (range: 58.0-84.1); TR [+]: mean 61.97 mm (range: 49.2-82.3) (p=0.56)).

The RVDD measurements in the TR [-] group had a mean of 2.73 mm and a range of 1.7-3.7 mm. The RVDD measurements in the TR [+] group had a mean of 2.95 mm and a range of 2.2-4.4 mm. The RVSD measurements in the TR [-] group had a mean of 1.75 mm and a range of 1.0-2.4 mm. The RVSD measurements in the TR [+] group had a mean of 1.88 mm and a range of 1.2-2.9 mm. The RVDD and RVSD increased linearly with gestational age in both groups: RVDD (r=0.37) RVSD (r=0.36) in TR [-] fetuses; RVDD (r=0.21) RVSD (r=0.20) in TR [+] fetuses. The regression line, which best described the mean RVDD according to gestational age in the TR [-] group was y = x (0.047)-0.59. The regression line for the mean RVSD according to age in the TR [-] group was y = x (0.03)-0.41. The calculated mean delta RVDD was 0.013 mm (range: -0.98-0.8) in the TR [-] group and 0.29 mm (range: -0.61-1.94) in the TR [+] group which is significantly larger (<0.05). The mean calculated delta RSVD was 0.035 mm (range: -0.67-0.67) in the TR [-] group and 0.17 mm (range: -0.35-0.88) in the TR [+] group. There appears to be a trend for the RVSD to be greater in the TR [+] group but it did not achieve statistical significance (p=0.13).

The mean SFRV in the TR [-] group was 36.07 (range: 30.00-41.94) and was 36.35 (range: 20.00-52.00) in the TR [+] group. The SFRV values were found to be independent of gestational age (TR [-]: r=0.001; TR [+]: r=0.01). The SFRV values were similar in both groups (p=0.84).

The mean NT measurements were 1.98 mm (range: 1.4-3.2) in the TR [-] group and 2.02 mm (range: 12-2.9) in the TR [+] group. The NT measurements increased with gestational age in both groups (TR [-]: r=0.28; TR [+]: r=0.30). The regression line that best described this association in the TR [-] group was y = x (0.023) + 0.32. The delta NT in the TR [-] group was 0.04 mm (range: -0.58-0.48) and 0.11 mm (range: -0.25-0.96) in the TR [+] group. There was no statistical difference between the two groups (p=0.41).

3.4 Discussion

We know and have supported by a series of measuring a number of differences in fetal blood circulation between euploid and aneuploidy fetuses. Among these differences are, for example, tricuspid regurgitation or pathological flows in ductus venosus Arantii in aneuploidy fetuses, even upon absence of anatomical heart defect.

The reason for these differences in not clear. Some theories expect that the different composition of the extracellular matrix, primarily collagen, results in different characteristics of the bloodstream. A theoretical proof should also be the different contractility and elasticity of myocardium. Another theory talks about pathological development of tricuspid valve, resulting in its either temporary or permanent (in particular towards the end of the first trimester) insufficiency (22, 38, 39, 40, 41).

Both these theoretical suppositions - composition and developmental defect of the extracellular matrix and developmental defect of the tricuspid valve - could result in dilatation of the right ventricle, alternatively of both atriums. During the prenatal developmental stages, fetal heart works with combined output.

It can thus be expected that, similarly as the most frequent sign of heart failure - the SFLV change - appears long before emergence of mitral insufficiency and thus before the LV dilatation manifestation, SFRV, eventually also SFLV, could be a more frequent and more sensitive marker of potential changes to myocardial development, than till now applied TCR measuring.

The determinants of the systolic function are well known and include preload (initial sarcomere length), afterload (downstream resistance), efficiency of the contractility of the myofibrils, heart rate and the availability of calcium for binding to contractile proteins.

Measurements of EDVI, ESVI, EF and SFLV in adults with Down syndrome appear to be different from chromosomally normal individuals, suggesting a difference in systolic function (Hamada et al., 1993; Russo et al., 1998). It is reasonable to investigate whether these differences exist during the fetal period as well. Accurate volumetric measurements of the cardiac ventricles would be difficult if not impossible to obtain in the first trimester; therefore, SFLV only was used in our study.

Our findings regarding pathophysiology of fetal central circulation system do not have to be connected with diagnostics of chromosomal defects only. It is a question for additional studies to determine whether the changes, leading in some unaffected fetuses to TCR manifestation, are not connected with placental defects and thus significant in etiology of preeclampsia or premature delivery.

Limitations of the presented results of functional measuring of fetal myocardium was the size of the examined group and the possibility of an intraindividual error (as all measurements were supplied by one individual).

3.5 Conclusion

3.5.1 SF Measuring During First Trimester

We have confirmed that it is possible to routinely measure SF during the first trimester of pregnancy. Since 2008 we have been routinely measuring SFLV and SFRV in first trimester fetuses. We have publish a number of these measuring as part of studies, evaluating the SFLV and SFRV relation to aneuploidy fetuses and possible etiology of TCR development (71, 72, 73). We have found that measurement of SFLV in the first trimester is feasible and, after allowing time to acquire experience with the procedure, adds very little time to the ultrasound examination.

3.5.2 Statistically Significant Differences in SFLV, resp. SFRV, in Euploid vs Aneuploid Fetuses

We also found a significant difference in the SFLV values between euploid fetuses and the fetuses with trisomy 21 at 11 weeks to 13 weeks 6 days of gestation, suggesting a difference in the left ventricular performance between the two groups. SFLV is increased in trisomy 21 fetuses, which suggests an improved left ventricular performance in that group. These

findings are in line with those of Huggon *et al*. who studied 159 normal fetuses and 142 fetuses with Down syndrome at the same gestational age as in our study (Huggon *et al*., 2004). In their study, the myocardial performance index (MPI) was found to be significantly decreased in trisomy 21 fetuses, also suggesting better ventricular function (MPI is inversely proportional to SFLV). Similar findings are seen in individuals with Down syndrome postnatally (Hamada *et al*., 1993; Russo *et al*., 1998). There appears to be a statistically significant difference in the SFRV values between fetuses with trisomy 21 and euploid fetuses. We have previously demonstrated a similar difference in the measurements of the left ventricular systolic function using the same approach. M-mode evaluation of the ventricular function has the potential to be an additional screening test for trisomy 21.

3.5.3 Interconnection betweem Right Ventricle Dilatation and Tricuspid Regurgitation

Irrespective of the exact etiology for TR, our data suggests that an association between the presence of TR and relative right ventricular enlargement exists. Whereas a causative link between the two cannot be drawn, this study does provide support for the idea that ventricular size plays a role in the genesis of tricuspid regurgitation in otherwise normal fetuses. In this study, we limited the presence of confounding variables by including only low risk patients that were chromosomally and structurally normal with normal nuchal translucency measurements.

4. Verification of Amniotic Fluid Sample Harvesting and Chorion Biopsy Reliability upon Use of Vacuum Tubes

4.1 Objective

The second goal of this work was to determine the reliability of a new method of harvesting amniotic fluid samples and performing chorion biopsy using vacuum tubes.

4.2 Methodology

Amniotic fluid has traditionally been obtained with a syringe. There are two major disadvantages of the classic amniotic fluid aspiration with the syringe. The operator is holding the ultrasound probe in his left hand and the needle in his right hand, while the assistant

aspirates the amniotic fluid with a syringe. During aspiration, the assistant easily interferes with the operator and can cause unwanted displacement of the needle. Aspirated amniotic fluid is usually redistributed into two containers and shipped to the laboratory. The manipulation of amniotic fluid under non-laboratory conditions is potentially risky.

Transabdominal CVS is a technique very similar to amniocentesis, as a needle is inserted into the uterus through the abdominal wall under aseptic conditions, but instead of directing the needle to a free pocket of amniotic fluid, it is passed through the long axis of the chorionic tissues (Smidt-Jensen *et al*., 1986). Identically to amniotic fluid, chorionic tissue is also traditionally obtained with a syringe using hand aspiration (Alfirevic and von Dadelszen, 2003). The classic syringe aspiration technique thus presents the same risk of two potential problems as amniotic fluid sampling: unwanted displacement of the needle and the manipulation of chorionic tissue sample under somewhat non-sterile conditions as the aspirated villi are usually transferred from the syringe to a container and transported to the laboratory. The manipulation of chorionic tissue under non-laboratory conditions carries the potential risk of contamination and spillage.

The vacuum tube technique addresses both of these disadvantages. Aspiration is 'automatic' due to negative pressure in the tube. There is no further manipulation of the specimen in the tube before it arrives at the laboratory.

The system for amnio-vacucentesis consists of a needle for amniocentesis, vacutainer, one-use holder and adapter (Becton Dickinson No 364815 and No 36730) and a 10 ml vacuum tube without additives, with silicone-coated interior (Becton Dickinson No 368430). After inserting the amniocentesis needle under ultrasound guidance to the amniotic cavity the holder is attached to the needle and the 10 ml vacutainer is inserted in the holder. The vacutainer produces negative pressure, which allows automatic aspiration of amniotic fluid. If necessary, a second vacutainer is used to obtain desired amount of amniotic fluid.

For the chorionic villus vacu-sampling, we puncture through the skin with the CVS needle. We traverse the uterine wall and continue further tangentially to the uterine cavity through the long axis of the thickest portion of the chorionic tissue. Upon reaching the most distal end of the chorion, the stylet is removed and an assistant attaches the vacuum holder with adaptor and vacuum tube to the needle. The attachment generates negative pressure; the operator

slowly withdraws the needle, passing all the way back through the chorionic tissue and finally removing the needle from the uterus and the puncture site. Negative pressure of the vacuum continues as the needle is withdrawn through the maternal tissues. Once the needle is removed from the patient, we add 2 mL culture medium into the vacuum tube by puncturing the rubber stopper using the same needle.

4.3 Discussion

Despite the decreasing tendency of the invasive exams and their gradually changing spectrum, invasive diagnostics still remains one of the most reliable methods of prenatal aneuploidy diagnostics. Complications, recorded in connection with invasive prenatal diagnostics, are connected with the erudition of the exam-performing specialist (operator) and the number of performed exams. There are two ways to minimizing the risk of pregnancy losses: increased precision of the screening methods, resulting in decreased number of invasive exams, and improvement of the invasive exam technique, resulting in lesser interindividual error and shorter teaching interval. Increased comfort of the exam-performing specialist in general leads to better operation results and often is connected with significant improvement of the patient's comfort.

It is expected that decreased false positive rate, in theory at limits under 0.1, and consecutive increase of the detection potential above 0.99 could put the screening potential on par with the potential of diagnostics. In this regard, one of the priorities is the decreased number of invasive prenatal exams to a minimum. As discussed above, these exams represent a potential risk of pregnancy loss or pregnancy complication (around 0.5%) (11, 12, 13, 14).

To what extent will the screening and later probably even the diagnostics of aneuploidy move to the level of non-invasive fetal DNA testing from the mother's peripheral blood, is difficult to estimate at this point. Currently, these methods cannot be considered as diagnostics due to their sensitivity, specificity, and most importantly, the considered option of false negativity (74, 75, 76).

4.4 Conclusion

We have designed and published a more comfortable way of performing transabdominal punction of chorion and amniotic fluid. We have published 377 evaluated samplings of the

chorionic tissue and 1219 samplings of the amniotic fluid. The number of routinely performed exams in praxis is a lot higher as in the clinical praxis only vacuum tube sampling is now performed. The presented data is being further reconfirmed by reports from the clinical praxis. Significantly shorter 'teaching intervals' can be listed as one of the undisputable advantages of the system. The advantages of implementing the vacuum sampling system for prenatal invasive diagnostics were tested in praxis and published as a "by product" of this work, concluding that alongside the increased comfort of the operator, and thus also the patient, one of the most significant and undisputable advantages of the system is its enclosed nature, preventing any potential contamination of the samples during harvesting and manipulation outside the laboratory environment. A very small amount of complications both in regards to pregnancy and the laboratory was recorded.

5. Conclusions

A: The first and general part of the work focuses on the relations between fraction shortening of the left and right ventricles and fetal chromosomal complement observed during an ultrasound examination of fetuses at the end of the first trimester. We have confirmed that:

1. It is possible to measure and evaluate hemodynamic parameters of fetal hearts - i.e. fraction shortening of the left and right ventricles as early as at the end of the first trimester.
2. Differences in the values of the fraction shortening of the left and right ventricles measured in euploid and aneuploid fetuses are statistically significant. It would thus be possible to modify the risk of incidence of certain aneuploidy types, primarily trisomy 21, measuring these parameters.
3. Tricuspid valve regurgitation at the end of the first trimester is connected with dilatation of the right ventricle.

B: In the second part of the work, we have proved that the method of amniotic fluid harvesting and performing chorion biopsy using vacuum tubes does not differ in reliability and safety from the currently used method. We have proved that the new method designed by us significantly lowers the teaching interval and has the potential to reduce the number of complications connected with these invasive examinations.

Abbreviations

AMC	amniocenthesis
CRL	crown-rump lenght
CVS	chorionic villi sampling
DNA	deoxy ribo nucleotic acid
DR	detection rate
DR	detection rate
DS	Down syndrom
DV	ductus venosus
FHR	fetal heart rate
FPR	false positiv rate
LR	likelihood ratio
LV	left ventricle
LVDD	left ventricle diastolic diameter
LVSD	left ventricle systolic diamter
NB	nasal bone
NT	nuchal translucency
PAPP-A	pregnancy associated protein A
PUBS	percutaneous umbilical blood sampling
RV	right ventricle
RVDD	right ventricle diastolic diameter
RVSD	right ventricle systolic diameter
SF	shortening fraction
SFLV	left ventricle shortening fraction
SFRV	right ventricle shortening fraction
T13	trisomy 13
T18	trisomy 18
T21	trisomy 21
TCR	tricuspid regurgitation
βhCG	free beta subunit of human chorionic gonadotropin

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b) without impact factor

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2. Secondary Sources

a) with impact factor

CALDA, P., NOVOTNÁ, M., ČUTKA, D., **BŘEŠŤÁK, M**., HAŠLÍK, L., GOLDOVÁ, B., VÍTKOVÁ, I., VANĚČKOVÁ, M., SEIDL, Z. A case of an epignathus with intracranial extension appearing as a persistently open mouth at 16 weeks and subsequently diagnosed at 20 weeks of gestation. *Journal of Clinical Ultrasound*. 2011, vol. 39, issue 3, s. 164-168. DOI: 10.1002/jcu.20762. **(IF - 0,808)**

b) without impact factor, reviewed

CALDA, P., FISCHEROVA, D., **BŘEŠŤÁK, M**. Doporučení k posouzení komplikací vývoje plodu v I. trimestru (zamlklý potrat, ektopická gravidita, těhotenství neznámé lokalizace). *Actual Gyn.* 2013, č. 5, s. 78-80.

CALDA, P., **BŘEŠŤÁK, M**. Doporučení k provádění rutinního ultrazvukového vyšetření v těhotenství. *Actual Gyn.* 2012, č. 4, s. 22-30.

CALDA, P., **BŘEŠŤÁK, M**., DOSEDLA, E., VALTROVA, H. Nové možnosti včasné UZ diagnostiky a prevence rozštěpových vad u plodu. *Česká gynekologie.* 2010, 75(4), s. 379.

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Addenda

OBSTETRICS

Amniovacucentesis vs standard syringe technique for amniocentesis: experience with 1219 cases

Pavel Calda, MD, PhD; Miroslav Brestak, MD

OBJECTIVE: The aim of the study was to compare amniovacucentesis to the usual syringe use for amniotic fluid aspiration.

STUDY DESIGN: We compared 2 groups of procedures: 1117 amniocenteses performed with the usual syringe technique and 1219 amniovacucenteses.

RESULTS: The numbers of needle insertions, unsuccessful amniocyte cultures, and miscarriage up to 21 days after the procedure were statistically not significant ($P > .01$) comparing the 2 techniques.

CONCLUSION: The vacuum tube serves as an automated aspiration tool alternative. The major subjective differences between the 2 methods are the operator's comfort and dexterity during sampling and the absence of an extra manipulation of the amniotic fluid after aspiration.

Key words: amniocentesis, automatic aspiration, instrumentation, methods, prenatal diagnosis, vacuum tube

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Amniotic fluid has traditionally been obtained with a syringe aspiration technique, generally requiring 2 hands to obtain the fluid and a skilled sonographer to concurrently visualize the needle passage.[1] We developed and tested a new method using vacuum tubes, eliminating some manipulation with the needle and the sample and leaving the operator's other hand available for the concurrent proprioreceptive needle guidance with the transducer.

In the majority of amniocenteses, the visualization of needle passage is successful. However, nearly all physicians have had the experience of losing visualization of the needle tip and, despite directives to the assistant to reposition, have resorted to guide the assistant's hand on the transducer to revisualize the needle. This breaks sterile technique and can result in a failed aspiration. We have named this alternative procedure amniovacucentesis, wherein the physician uses 1 hand for the needle passage and has the other hand providing guidance with the transducer.

The aim of the study was to determine whether amniovacucentesis was as successful and efficient as the usual syringe amniotic fluid aspiration method.

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Materials and Methods

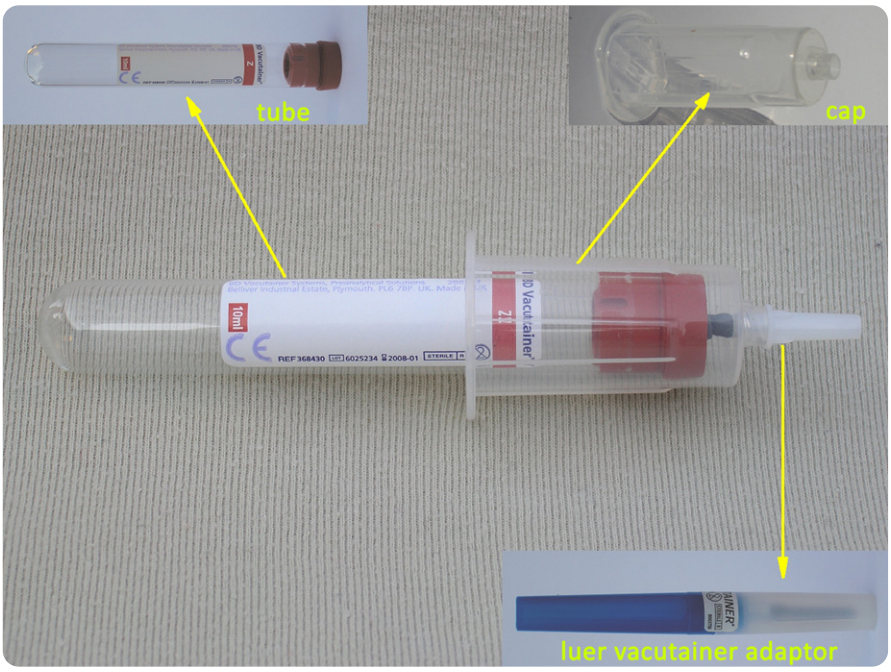
The system for amniovacucentesis consists of a needle/stylet for amniocentesis, vacuum tubes, a 1-use holder and adapter (Becton Dickinson cat. nos. 364815 and 367300; Becton Dickinson, Franklin Lakes, NJ), and a 10-mL silicone-coated interior tube without additives (Vacutainer; Becton Dickinson cat. no. 368430) (Figure 1). The amniocentesis needle/stylet is inserted under ultrasound guidance into the amniotic cavity; the stylet is removed; the holder is attached to the needle; and the 10-mL vacuum tube is inserted into the holder. The vacuum tube produces negative pressure, which allows automated aspiration of amniotic fluid (Figure 2). If necessary several vacuum tubes may be used to obtain the desired amount of amniotic fluid. We used 0.9 × 120-mm needles for both techniques.

The indications for amniocentesis in both groups were advanced maternal age (35.1% vs 34.2%), a positive second-trimester screening (39.1% vs 41.2%), abnormal ultrasound findings (15.9% vs 17.3%, respectively) genetic risk (3% vs 2.3%), or anxiety or other (6.9% vs 5%).

We compared 2 groups of amniocentesis procedures performed in our unit: 1117 amniocenteses in singletons performed with the usual syringe technique during the period between January 2004-December 2005 (2 skilled operators, Z.Z., P.C.) and 1219 amniovacucenteses in singletons performed by 3 skilled operators (P.C., M.B., Z.Z.) in the period between January 2006-February 2008 in our unit.

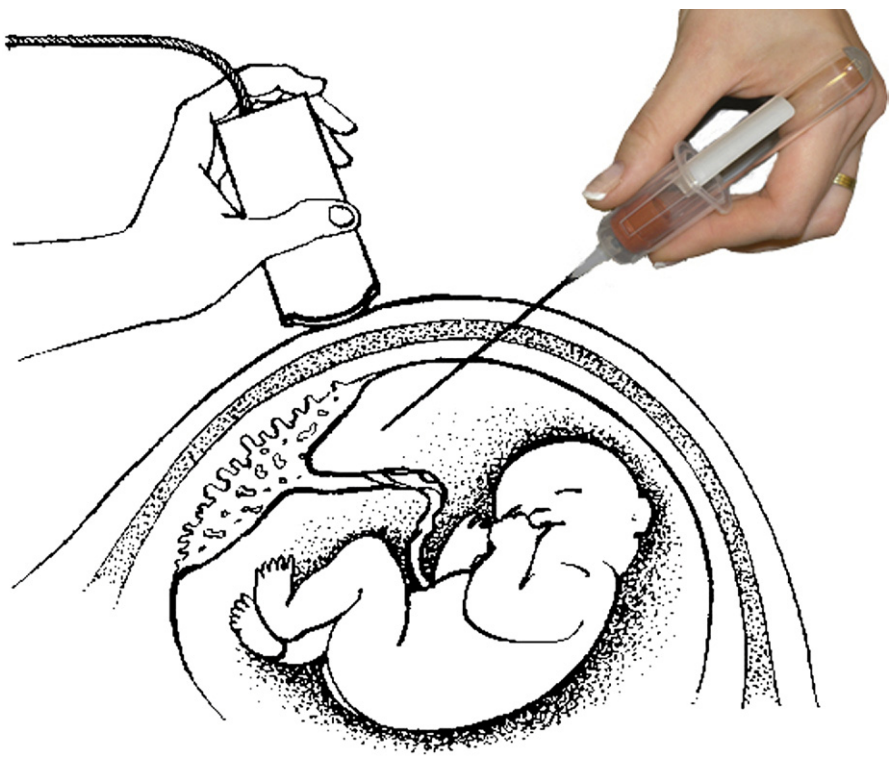
In both groups, we recorded the number of needle insertions, the amount of amniotic fluid obtained, the percentage of unsuccessful amniocyte cultures, and repeated usage of vacuum tubes in the latter group. We excluded twin pregnancies from the evaluation, because the number of needle insertions can vary according to the anatomic situation and does not reflect the method used. We subjected the data from the 2 methods to statistical analysis with Student *t* test. The study was exempt from institutional review board approval, because the research was conducted in a commonly accepted setting.

FIGURE 1
The unit for vacucentesis



Calda. Amniovacucentesis vs standard syringe technique for amniocentesis. Am J Obstet Gynecol 2009.

FIGURE 2
Vacuum tube use in practice



Calda. Amniovacucentesis vs standard syringe technique for amniocentesis. Am J Obstet Gynecol 2009.

RESULTS

In the first period, 2004-2005, there were 7 cases (0.57%) in the syringe-technique group in which a second insertion of the needle was necessary. Unsuccessful amniocyte cultures occurred in 6 cases (0.54%). In the second period, 2006-2008, using the new vacuum tube technique, a second insertion of the needle was necessary in 5 cases (0.43%). In 28 cases (2.43%), it was necessary to reinsert the vacuum tube because of obstruction of the needle. In all cases the required amount of 16-20 mL of amniotic fluid was obtained. In 2 cases, amniocyte culture was unsuccessful. The numbers of needle insertions, unsuccessful amniocyte cultures, and miscarriage up to 21 days after the procedure were statistically not significant ($P > .01$) comparing the 2 techniques.

COMMENT

There are other descriptions of using vacuum or negative pressure for amniotic fluid aspiration. Dolinger and Donnenfeld[2] used a vacuum bottle aspiration technique for removal of large volumes of amniotic fluid. Robinson et al[3] reported use of an adapter, which converted a standard syringe to 1 permitting aspiration. Brestak et al[4] reported using a vacuum tube during genetic amniocentesis.[5]

Both the syringe and vacuum tube methods were similar in the percentages of necessary needle reinsertions and unsuccessful amniocyte cultures. Our culture failure rates and number of needle reinsertions are comparable with those reported in the literature.[6] The percentage of miscarriages caused by premature rupture of membranes, infection, or membrane disruption was similar in both groups (Table).

Sixteen milliliters of amniotic fluid is usually requested by our laboratory for standard genetic amniocentesis. For fetal lung maturity assays or other needs, smaller vacuum tube sizes can be substituted to obtain lesser amounts of amniotic fluid.

Data were collected over sequential time frames, and an added operator (M.B.) performed only amniovacucen-

TABLE
Comparison of syringe and vacuum tube techniques

Variable	Period 2004-2005 Syringe technique	Period 2006-2008 Vacuum tube technique	Statistical significance
Maternal age (mean)	31.6	32.1	> .01 (NS)
Gestational age (mean)	17	16.8	> .01 (NS)
Amniocenteses in singletons, n	1117	1219	—
Needle reinsertions	7 (0.57%)	5 (0.43%)	> .01 (NS)
Unsuccessful amniocyte culture	6 (0.54%)	2 (0.16%)	> .01 (NS)
Vacuum tube reinsertion	[a]	28 (2.43 %)	—
Miscarriage up to 21 d after procedure	4 (0.36%)	5 (0.41%)	> .01 (NS)

NS, not significant.

[a] Not relevant.

Calda. Amniovacucentesis vs standard syringe technique for amniocentesis. Am J Obstet Gynecol 2009.

tesis. One might argue that the operators would become more experienced over time, if the second method had been statistically superior. In reality, all 3 operators have performed more than 2000 amniocenteses each, antecedent to the study, and all 3 should be considered very experienced.

Amniovacucentesis was also efficient with oligohydramnios. In our experience, manual aspiration would not provide a better means for vacuum control, albeit we did not collect data to support this statement.

For initially bloody taps, all that was required was insertion of a fresh vacuum tube when the fluid cleared.

The cost of the 2 vacuum tubes, the needle adapter, and holder is approximately 31 cents (US) and equal to the cost of one 20-mL syringe and 2 transport bottles at 36 cents. Recently, Borrell et al[7] published a research letter using our method of amniovacucentesis in 202 cases, validating advantages of this method. Their results are in accordance with our findings.

There are 2 occasional problems with the classic amniotic fluid aspiration syringe technique: unwanted displacement of the needle and the manipulation of amniotic fluid under somewhat nonsterile conditions. There are 2 common setups when performing amniocentesis: first, the operator is holding the ultrasound probe in 1 hand and the needle in the other hand while the assistant aspirates the amniotic fluid with a syringe. During aspiration, the assistant can easily interfere with the operator, causing unwanted displacement of the needle. The other option is for the physician to insert the needle and aspirate the amniotic fluid using 2 hands, while being shown the needle placement by the sonographer.

In this method, the manipulation of the syringe can cause unwanted displacement of the needle. The amniotic fluid is thereafter injected from the syringe to other containers and transported to the laboratory. Although it rarely happens, this transfer of amniotic fluid under nonsterile conditions carries the potential risks of contamination and spillage, whereas direct aspiration into the vacuum tube obviates the possibility. The aspiration is automated because of negative pressure in the tube, and there is no transfer of the fluid prior to arrival at the laboratory.

The differences of studied parameters of both methods, the usual syringe and new vacuum tube aspiration, were not significant. But we encourage others to try this alternative automated vacuum tube aspiration technique. Like us, they might find this method preferable and convenient, with fewer instances of lost visualization and frustration with less skilled, steady, and reliable assistants. ■

ACKNOWLEDGMENTS

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RESEARCH LETTER

Chorionic villus vacu-sampling in 377 consecutive cases

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KEY WORDS: chorion villus sampling; invasive procedure; vacuum tube; fetal ultrasound; fetal imaging; general cytogenetics; prenatal cytogenetics; maternal serum screening

Chorionic villus sampling (CVS) is the method of choice for obtaining fetal tissue for prenatal diagnosis before 15 weeks of pregnancy. CVS can be performed using either a transabdominal or a transcervical approach. Transabdominal CVS is a technique very similar to amniocentesis, as a needle is inserted into the uterus through the abdominal wall under aseptic conditions, but instead of directing the needle to a free pocket of amniotic fluid, it is passed through the long axis of the chorionic tissues (Smidt-Jensen *et al*., 1986). Both amniotic fluid and chorionic tissue are traditionally obtained with a syringe using hand aspiration (Alfirevic and von Dadelszen, 2003). We first reported on the use of a vacuum tube with negative pressure for amniotic fluid aspiration (Brestak and Calda, 2006) and our successful experience has been replicated by groups (Borrell *et al*., 2008).

We (Calda *et al*., 2007) as well as others (Battagliarin *et al*., 2009) have also successfully applied the same negative pressure vacuum tube system to CVS, a technique that we have named 'chorionic villus vacu-sampling'. We now report our experience with this technique at our center.

From January 2006 to November 2008, 376 chorionic villus vacu-sampling procedures were performed by two skilled operators (PC and MB) in singleton gestations and one in a monochorionic twin gestation. Included were pregnancies at <15.9 weeks with an indication for karyotyping. There were no exclusion criteria.

For our chorionic villus vacu-sampling, we employed a 120 mm 18G needle (Somatex®) with Luer lock, a single use vacuum tube holder, an adaptor and a 10 mL green-top sterile vacuum glass tube with sodium heparin (17 IU/mL) (BD Vacutainer Plasma Tube® Catalog # 368 480). The vacuum tube, holder and adaptor have long been utilized for blood sampling from peripheral veins (Figure 1). The vacuum tube serves as an automated aspiration tool and if needed, it allows for up to five tube reinsertions without loss of negative pressure (Calda *et al*., 2007). At our institution, the cost of one tube, adaptor and holder (0.27 USD) are similar to the cost of one 20 mL syringe and one transport bottle ($0.125 + 0.125 = 0.25$ USD).

For the procedure, we first inject local anesthesia (5 mL 1% mesocain) to the designated puncture site. Then, we puncture through the skin with the CVS needle. We traverse the uterine wall and continue further tangentially to the uterine cavity through the long axis of the thickest portion of the chorionic tissue. Upon reaching the most distal end of the chorion, the stylet is removed and an assistant attaches the vacuum holder with adaptor and vacuum tube. The attachment generates negative pressure; the operator slowly withdraws the needle, passing all the way back through the chorionic tissue and finally removing the needle from the uterus and the puncture site (Figure 2). Negative pressure of the vacuum continues as the needle is withdrawn through the maternal tissues. Once the needle is removed from the patient, we add 2 mL culture medium into the vacuum tube by puncturing the rubber stopper using the same needle. The amount of aspirated villi in the vacuum tube is easily visible in the vacuum tube and can be qualitatively estimated. The minimum amount of villi required for analysis was 3 mg. The vacuum tube with the villi was sent to the laboratory. Quantitative fluorescent polymerase chain reaction (QF-PCR) for trisomy 21, 18 and 13 and full karyotype cultures were performed. All patients signed an informed consent for the CVS procedure and laboratory examination of the villi. No specific consent was deemed necessary for the use of vacuum tube during the procedure. We recorded the number of needle insertions, the percentage of unsuccessful chorionic villi QF-PCR examinations and cultures. All patients with normal fetal morphology in the first trimester and normal karyotype were appointed for ultrasound scan in the 22nd week. The outcomes of pregnancies after the procedure were available until the 22nd week.

The indications for CVS were positive first trimester combined test (56%), advanced maternal age (24%), a combination of maternal age and genetic risk (8%), abnormal ultrasound findings (8.5%) and genetic risk (3.5%). The median gestational age at the time of CVS was 13.0 weeks (range 10.0–15.9 weeks). In all cases, we obtained a sufficient amount of villi for analysis during one pass of the needle and we did not need to reinsert the vacuum tube into the holder

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Figure 1—The system for chorion villi vacu-sampling. The adaptor (blue) is attached to the holder (transparent cap). After removal of the stylet, the adaptor with holder is attached to the needle. The needle with adaptor and cap is inserted to the furthest extent of the chorionic plate (without the vacuum tube)

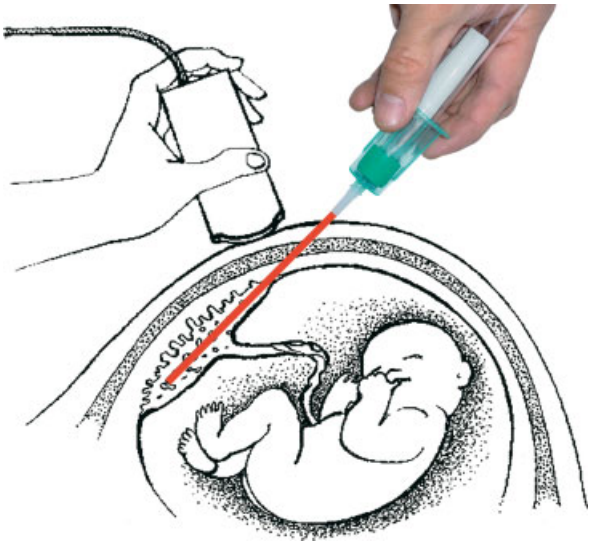

Figure 2—Chorion villi vacu-sampling. The green vacuum tube is attached to the holder after the needle reaches the furthest extent of the chorionic plate. Vacuum tube produces negative pressure during the slow removal of the needle through the chorionic plate

(Table 1). Fast QF-PCR analysis was successful in all cases. The median culture time was 11 days (range 10–29 days). A normal karyotype was obtained in 298 (79%) cases, an abnormal karyotype in 65 (17.24%) and other chromosomal rearrangements in 12 (3.18%) cases. Confined placental mosaicism occurred in 3 of 377 cases (0.79%). There were two (0.53%) unsuccessful chorionic villi cultures, albeit the QF-PCR results revealed a normal karyotype. The amount of villi in both cases was sufficient (i.e. more than 10 mg).

Table 1—The weight of villi aspirated during single pass of the needle through the chorion (qualitative assessment) (Newport *et al*., 1986)

Amount of villi mg	N	%
≤5	20	5.3
6–10	126	33.4
11–15	112	29.7
16–20	61	16.2
>20	58	15.4

A total of 103 patients requested termination of pregnancy: 77 with fetal chromosomal abnormalities, 26 with a normal karyotype, but with structural fetal malformations detected at ultrasound (anencephalus, renal agenesis, non-immune fetal hydrops, cystic hygroma, omphalocele, cleft lip and palate). Follow-up to 22 weeks was available in all the remaining 274 continuing gestations. There were two fetal losses before 22 weeks (0.73%). The first one was diagnosed following vaginal bleeding on the third day after sampling at 12+ weeks, having had a positive combined test (1 : 50 risk for trisomy 21) as indication for CVS. The second loss was diagnosed at 20 weeks 3 days. The indication for CVS was advanced maternal age of 39 years. As the pregnancies were not followed after the 22nd week, no information was available on rate of obstetric complications after 22 weeks.

Our results demonstrate the technical feasibility of chorionic villus vacu-sampling, with 100% adequate yield with a single pass, a 5.3% retrieval of <5 mg and a fetal loss rate before 22 weeks of 0.73%. Such results compare favorably with those of the only other published series using a similar technique (Battagliarin *et al*., 2009). That randomized study found no significant differences in the size of chorionic samples between use of CVS vacu-sampling compared with the classic syringe and hand-grip device to create vacuum. However, 16% of vacuum-obtained samples yielded 5 mg or less. Moreover, a second needle insertion was required more frequently with the use of vacuum (9% vs 1%); both techniques required a median number of four passes of the needle within the chorion. All three fetal losses within 4 weeks of the procedure occurred in the group undergoing traditional technique.

Our technique is slightly different from Battagliarin *et al*.: we used a 10 mL vacuum tube, an 18 gauge needle, and the needle was placed fully forward through the chorion frondosum and removed once only, slowly, to obtain the sample. Battagliarin *et al*. used a 4 mL vacuum tube, a 20 gauge needle and several passes through the chorion. Differences in success rates of the two techniques may be related to the needle gauge. An 18 gauge needle seems necessary to get our results. The quantity of chorionic villi aspirated is related to the

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needle size: Cochrane *et al*. (2003) in a randomized trial, obtained an average of 1.5 mg more sample using an 18 gauge needle compared with a 20 gauge needle (95% CI: 0.8–2.3 mg). We tested several other needles and gauges from different manufacturers in cases of planned pregnancy termination. Our needle choices [Somatex® 18G/120 mm and Cook® 18G/160 mm (JS-171 660)] allowed aspiration of enough material (more than 3 mg chorionic villi) with a single pass through the chorion frondosum. We decided to use the Somatex® needle because it is less expensive.

The classic syringe aspiration technique presents the risk of two potential problems: unwanted displacement of the needle and the manipulation of chorionic tissue sample under somewhat non-sterile conditions. First, the operator is holding the ultrasound probe in one hand and the needle in the other hand, whereas the assistant aspirates the chorionic tissue with a syringe. During aspiration, the assistant may interfere with the operator's steady hold, causing unwanted displacement of the needle. The second disadvantage is that the aspirated villi are usually transferred from the syringe to a container and transported to the laboratory. The manipulation of chorionic tissue under non-laboratory conditions carries the potential risk of contamination and spillage. The vacuum tube technique addresses both of these disadvantages. Aspiration is 'automatic' due to negative pressure in the tube. There is no further manipulation of the specimen in the tube before it arrives at the laboratory.

Ultrasound guided aspiration can be performed using either percutaneous/transabdominal or the transvaginal/transcervical approach. The choice of the approach and the choice of instruments tend to be based upon the operator's personal preference and experience (Alfirevic and von Dadelszen 2003). We have not used the transcervical approach, but would surmise that use of the vacuum tube instead of a syringe could be used successfully also for a transcervical CVS.

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Left ventricle shortening fraction: a comparison between euploid and trisomy 21 fetuses in the first trimester

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Objectives Measurement of the shortening fraction of the left ventricle (SFLV) is an objective way to assess systolic performance. The aim of the study was to compare first trimester SFLV values in euploid fetuses to those in fetuses with trisomy 21.

Methods We measured SFLV in 56 fetuses from 11 weeks to 13 weeks 6 days. The left ventricular diastolic diameter (LVDD) and left ventricular systolic diameter (LVSD) were measured offline, and SFLV was calculated. The data were analyzed using Mann–Whitney *U* test.

Results We found a significant difference in the SFLV measurements between the group of 49 euploid fetuses and the 7 fetuses with trisomy 21 [38.00 (95% CI: 33.72–42.27) vs 49.93 (95% CI: 43.72–56.13)] ($p < 0.05$). There was also a significant difference in the nuchal translucency measurements between the two groups: 1.78 mm (95% CI: 1.08–2.48 mm) in the euploid population versus 5.06 mm (95% CI: 3.61–6.71 mm) in the fetuses with trisomy 21 ($p < 0.05$). There were no significant differences between the group of euploid fetuses and the group of trisomy 21 fetuses in the following parameters: CRL (chorionic villus sampling), LVDD and LVSD.

Conclusions SFLV is a well-defined, simple measurement of systolic function of the fetal myocardium. SFLV values in fetuses with trisomy 21 appear to be significantly higher than in euploid fetuses. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: trisomy 21; first trimester; marker; shortening fraction of the left ventricle

INTRODUCTION

Shortening fraction of the left ventricle (SFLV) is felt to be an indicator of cardiac contractility. It may be measured in the fetus using M-mode ultrasound and has been shown to be fairly constant in the second and third trimesters (DeVore *et al*., 1984; Agata *et al*., 1991; Hsieh *et al*., 2000; DeVore, 2005).

Studies comparing myocardial function in adults with Down syndrome to chromosomally normal individuals have shown major differences in myocardial function (Hamada *et al*., 1993; Russo *et al*., 1998). Indices of left heart function point to a better performance in individuals with Down syndrome: the end diastolic volume index (EDVI) and end systolic volume index (ESVI) tend to be decreased, and the ejection fraction (EF) and mean velocity of circumferential fiber shortening (mean Vcf) tend to be increased. Measurement of the SFLV is an objective way to assess systolic function (DeVore *et al*., 1984; Agata *et al*., 1991; Hamada *et al*., 1993; Russo *et al*., 1998; Hsieh *et al*., 2000; DeVore, 2005). The aim of the study was to assess whether SFLV values in euploid fetuses are different from those with trisomy 21 in the first trimester.

METHODS

The study population included consecutive first trimester fetuses between 11 weeks and 13 weeks 6 days of gestation in which the chromosomal status was determined by chorionic villus sampling (CVS). The ultrasound examination was performed either at the time of the first trimester combined screening test or prior to CVS. In each case, the decision to preform the CVS was based on an increased risk on the first trimester combined screen. The heart was imaged in one of two ways: the two ventricles were either viewed in the long axis with the face of the transducer being approximately parallel to the ventricular septum (Figure 1), or in the short axis view (Figure 2). The image of the heart was magnified, so it filled at least 75% of the image. An M-mode cursor was then placed through the two ventricles at a right angle to the ventricular septum beneath the level of the A–V valves (Figures 1 and 2). The appropriate M-mode images were obtained using a 7 MHz abdominal probe [M7C, Logic 9(GE)] by a single experienced operator (M.B.) and stored. We determined the time required to

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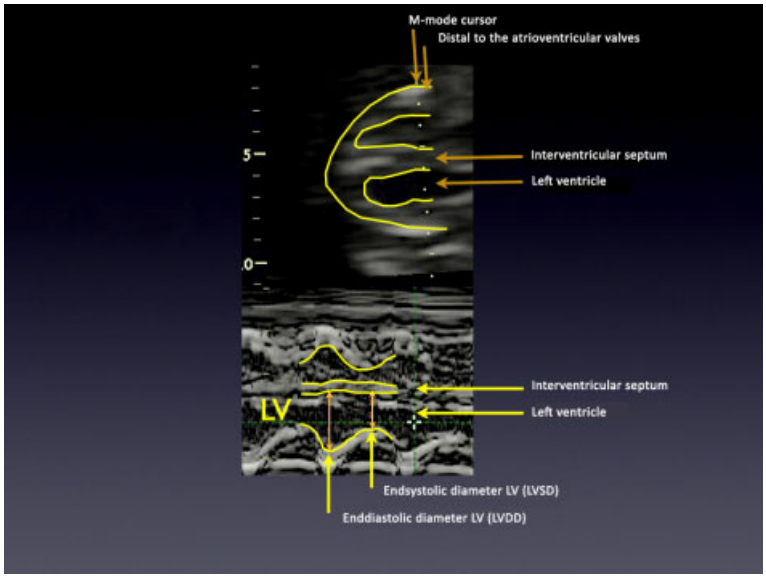

Figure 1—Fetal heart and the orientation of the M-mode cursor, placed perpendicular to the interventricular septum, just below the tips of the atrioventricular valves

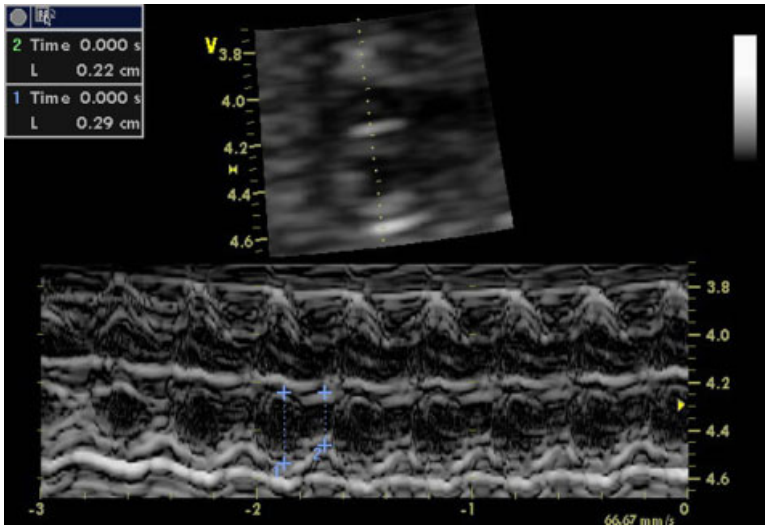

Figure 2—Fetal heart and the orientation of the M-mode cursor in the short axis view

obtain an appropriate image in the last 28 cases. The reason for using the most recent cases for this purpose was to allow a reasonable period to attain proficiency in this technique. The left ventricular diastolic diameter (LVDD) and left ventricular systolic diameter (LVSD) were measured offline. The SFLV was calculated using the following formula: [(LVDD − LVSD)/LVDD] × 100. The fetal chromosomal status was not known at the time of the measurements. Multiple gestations and fetuses with an apparent heart defect were excluded from the study. Each patient signed a consent for the ultrasound examination.

RESULTS

Between September 2008 and February 2009, we examined 58 fetuses that fit the study criteria. In two of the cases, appropriate images could not be obtained. Out of the 56 fetuses that were examined successfully, 49 were chromosomally normal and 7 had trisomy 21. The SFLV values in the euploid fetuses were statistically smaller than in fetuses with trisomy 21: 38.00 (95% CI: 33.72–42.27) versus 52.07 (43.72–56.13) ($p < 0.05$). There was also a significant difference in the nuchal translucency (NT) measurements between the two groups: 1.78 (95% CI: 1.08–2.48) in the euploid population versus 5.06 (95% CI: 3.61–6.71) in the fetuses with trisomy 21 ($p < 0.05$). The two groups did not differ in CRL measurements [euploid: 66.81 mm (95% CI: 58.28–75.35 mm) versus trisomy 21: 74.68 mm [95% CI: 65.23–79.59 mm ($p = 0.05$)], LVDD measurements [euploid: 3.35 mm (95% CI: 2.67–4.03 mm) versus trisomy 21: 3.66 mm (95% CI: 2.69–4.06 mm) ($p = 0.19$)], and LVSD measurements [euploid: 2.09 mm (95% CI: 1.58–2.60 mm) versus

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trisomy 21: 1.78 mm (95% CI: 1.17–2.20 mm) ($p = 0.28$)].

Out of the 28 cases where the time to obtain the SFLV was measured, two cases (7.14%) required less than 60 s, 22 cases (78.57%) between 60 and 120 s, and in two cases (7.14%) 240 s were needed. Examination in the remaining two cases (7.14%) did not yield an acceptable M-mode image even after a prolonged examination.

STATISTICS

The statistical comparison was performed using Mann–Whitney U test. The null hypothesis was rejected for $p < 0.05$.

DISCUSSION

Background

The determinants of the systolic function are well known and include preload (initial sarcomere length), afterload (downstream resistance), efficiency of the contractility of the myofibrils, heart rate and the availability of calcium for binding to contractile proteins. Measurements of EDVI, ESVI, EF and SFLV in adults with Down syndrome appear to be different from chromosomally normal individuals, suggesting a difference in systolic function (Hamada *et al*., 1993; Russo *et al*., 1998). It is reasonable to investigate whether these differences exist during the fetal period as well. Accurate volumetric measurements of the cardiac ventricles would be difficult if not impossible to obtain in the first trimester; therefore, SFLV only was used in our study. The feasibility of performing of SFLV measurements has been demonstrated in the second and third trimesters (DeVore *et al*., 1984; Agata *et al*., 1991; Hsieh *et al*., 2000; DeVore, 2005). The measurement is relatively simple as it involves measuring just the left ventricular diameter in diastole and in systole. The use of a ratio, rather than absolute distances, compensates for some of the variability of measurements that may result from slight differences in the angle of insonation.

In this study, we found that measurement of SFLV in the first trimester is feasible and, after allowing time to acquire experience with the procedure, adds very little time to the ultrasound examination. We also found a significant difference in the SFLV values between euploid fetuses and the fetuses with trisomy 21 at 11 weeks to 13 weeks 6 days of gestation, suggesting a difference in the left ventricular performance between the two groups. SFLV is increased in trisomy 21 fetuses, which suggests an improved left ventricular performance in that group. These findings are in line with those of Huggon *et al*. who studied 159 normal fetuses and 142 fetuses with Down syndrome at the same gestational age as in our study (Huggon *et al*., 2004). In their study, the myocardial performance index (MPI) was found to be significantly decreased in trisomy 21 fetuses, also suggesting better ventricular function (MPI is inversely proportional to SFLV). Similar findings are seen in individuals with Down syndrome postnatally (Hamada *et al*., 1993; Russo *et al*., 1998).

Theoretically, most of the known qualities of the myocardium in fetuses with trisomy 21 would be expected to lead to an impaired left ventricular performance. The myocardium contains both structural and ultrastructural abnormalities. There is an increase in cell size and a reduced cell number per unit area (Recalde *et al*., 1986). The composition of the connective tissue in individuals with Down syndrome also differs from that in euploid individuals. Genes for several matrix-related proteins, particularly collagen VI and XVIII are located on chromosome 21 (Vis *et al*., 2009). Gittenberger-de Groot *et al*. (2003) found higher concentrations of collagen VI in hearts of fetuses with trisomy 21 as compared to euploid fetuses. Carvalhaes *et al*. (2006) found that collagen XVIII is localized not only in various basement membranes but is also highly expressed throughout the connective tissue core of the endocardial cushions and forms A–V valve leaflets. It seems feasible that collagen XVIII, or one or more of its proteolytic fragments may play a role in the migration, proliferation and differentiation of connective tissue cells. It would be reasonable to expect that the changes in the composition of the extracellular matrix not only results in the congenital structural heart defects frequently seen in trisomy 21 (Freeman *et al*., 2008) but may also adversely affect the contractile properties of the fetal myocardium.

It has been shown that adult individuals with Down syndrome have a consistently lower blood pressure, both systolic and diastolic (Richards and Enver, 1979; Russo *et al*., 1998). It is hypothesized that the apparently better performance of the left ventricle found in adults with Down syndrome is due to this reduction in afterload. Whether the same mechanism is responsible for the findings in fetuses with trisomy 21 in our study is difficult to prove. However, this is a plausible theory that would reconcile the apparently contradictory findings of improved left ventricular performance in the setting of an abnormal myocardium. A physiologic finding that may support this assertion indirectly is the finding of slightly increased heart rate in first trimester fetuses with trisomy 21 (Liao *et al*., 2000); decreased peripheral resistance may lead to tachycardia.

Limitations

This pilot study is designed to answer two basic questions: whether measuring SFLV in the first trimester is feasible, and whether there is a difference in SFLV measurements between euploid fetuses and fetuses with trisomy 21 based on evaluations of a single experienced operator. As such, it has a number of limitations. The study does not address the inter- and intra-observer variabilities of this measurement. It also does not determine whether the SFLV values change with gestational age, nuchal translucency measurement and fetal heart rate. Further studies are needed to address the outstanding

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issues as well as the potential value of this measurement in screening for trisomy 21.

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Shortening fraction of the right ventricle: a comparison between euploid and trisomy 21 fetuses at week 11 to week 13 and 6 days of gestation

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Objectives This study was designed to compare the first trimester shortening fraction of the right ventricle (SFRV) values between euploid fetuses and fetuses with trisomy 21.

Methods SFRV was measured in 58 first trimester fetuses between September 2008 and February 2010. The stored M-mode images were used to obtain the right ventricular diastolic diameter (RVDD) and right ventricular systolic diameter (RVSD) measurements offline.

Results The SFRV values were found to be significantly greater in the 9 fetuses with trisomy 21 as compared to the group of 49 euploid fetuses (mean: 48.6 mm; range: 36–56.25 mm vs mean: 34.11 mm; range: 22.73–43.48 mm) ($p < 0.0001$). The RVDD measurements were also found to be significantly greater in the fetuses with trisomy 21 than in the euploid fetuses (mean: 3.08 mm; range: 2.2–4.7 mm vs mean: 2.54 mm; range: 1.9–3.6 mm) ($p = 0.03$).There was no difference in the RVSD measurements between the two groups [mean: 1.56 mm; range: 1.2–2.3 mm (trisomy 21 fetuses) vs mean: 1.67 mm; range: 1.3–2.4 mm (euploid fetuses)] ($p = 0.17$).

Conclusions The SFRV values in fetuses with trisomy 21 appear to be significantly greater than in the euploid fetuses. The RVDD also appears to be greater in fetuses with trisomy 21 than in the euploid fetuses.

KEY WORDS: trisomy 21; first trimester; marker; shortening fraction of the right ventricle

INTRODUCTION

There is evidence that the cardiac performance in individuals with trisomy 21 is different from euploid individuals. This appears to be the case in both postnatal life (Hamada *et al*., 1993; Russo *et al*., 1998) and in fetuses (Huggon *et al*., 2004; Calda *et al*., 2010).

Measurement of both the shortening fraction of the left ventricle (SFLV) and the shortening fraction of the right ventricle (SFRV) has been used to objectively evaluate cardiac contractility during the ventricular systole (DeVore *et al*., 1984; Hsieh *et al*., 2000; DeVore, 2005). The feasibility of performing these measurements in the fetus has been demonstrated in all three trimesters (DeVore *et al*., 1984; Agata *et al*., 1991; Hsieh *et al*., 2000; DeVore, 2005; Calda *et al*., 2010). Using M-mode ultrasound, these measurements appear to be fairly constant and reproducible.

The difference between the myocardial performance in Down syndrome and euploid individuals was initially demonstrated in adult subjects. Indices of ventricular function such as end diastolic volume index (EDVI), end systolic volume index (ESVI), ejection fraction (EF), and mean velocity of circumferential fiber shortening (mean VcF) all suggest better cardiac performance of the left ventricle in individuals with Down syndrome (Hamada *et al*., 1993; Russo *et al*., 1998).

We have demonstrated a difference in the SFLV between euploid fetuses and fetuses with trisomy 21 in the first trimester. In this study, we investigated whether such a difference also exists in SFRV measurements between the two groups.

METHODS

The study consisted of unselected first trimester fetuses at week 11 to week 13 and 6 days of gestation [crown-rump length (CRL): 45–84 mm]. The ultrasound examination was performed either at the time of the first trimester combined screening or before chorionic villus sampling (CVS). Each fetus had a CRL measurement obtained in a standard manner. In each fetus, the chromosomal status was determined by the CVS. The technique used to measure the SFRV is essentially identical to the one used to measure the SFLV (Calda *et al*., 2010). The image of the fetal chest was significantly magnified so that the fetal heart filled approximately 75% of the image. The heart was insonated in one of two ways. One

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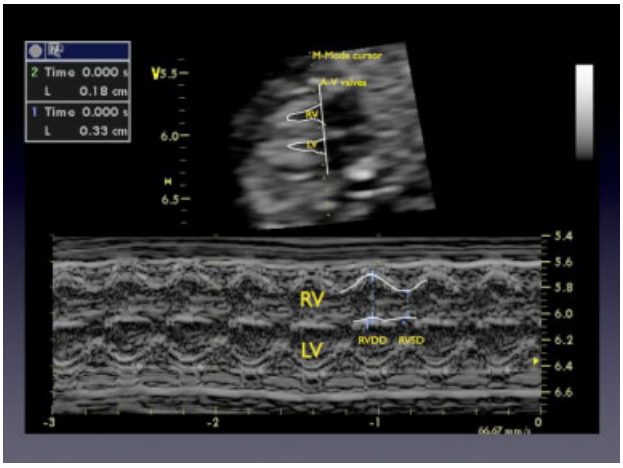

Figure 1—A four chamber image with an accompanying M-mode image (RV, right ventricle; LV, left ventricle; RVDD, right ventricular diastolic diameter; RVSD, right ventricular systolic diameter)

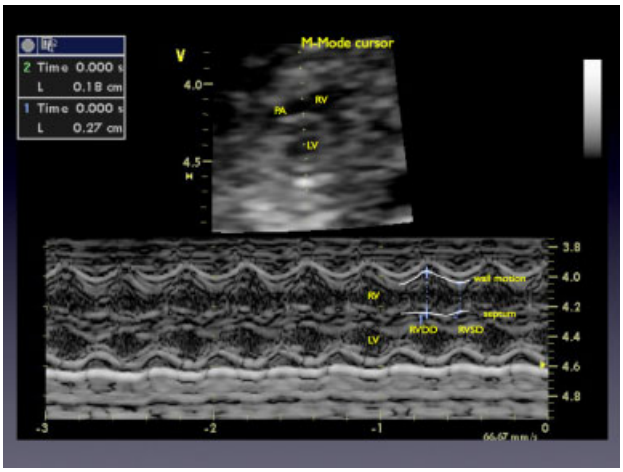

Figure 2—A short axis view of the heart below the level of the atrioventricular valves with an accompanying M-mode image (RV, right ventricle; LV, left ventricle; RVDD, right ventricular diastolic diameter; RVSD, right ventricular systolic diameter; PA, pulmonary artery)

consisted of a four chamber heart view. In this view, the face of the transducer needs to be as parallel to the long axis of the ventricular septum as possible (Figure 1). The other one is a transverse (short axis) view transecting the two ventricles immediately below the level of the atrioventricular valves (Figure 2). In this view, the angle of insonation should be as close to 90° to the long axis of the ventricular septum as possible. In both cases, an M-mode cursor is placed through the cardiac ventricles immediately beneath the atrioventricular valves at a right angle to the ventricular septum.

All examinations and measurements were performed by a single experienced operator (M. B.) with an M7C abdominal probe using Logiq 9 [GE]. The M-mode images were stored. The right ventricular diastolic diameter (RVDD) and right ventricular systolic diameter (RVSD) were measured offline. The operator was blinded to the chromosomal status of the fetus at the time of the measurements. The SFRV was calculated using the following formula: $[(\text{RVDD}-\text{RVSD})/\text{RVDD}] \times 100$. Exclusion criteria were multiple gestations and fetuses with an evident cardiac defect. An informed consent for the ultrasound examination, which had been approved by an institutional review board, was signed by each patient.

Statistical analysis

The data was analyzed using Mann–Whitney U test. The null hypothesis was rejected for $p < 0.05$.

RESULTS

A total of 62 fetuses examined between September 2008 and February 2010 were included in the study. Some of these fetuses are the same as used in our previous publications dealing with SFLV (Calda *et al*., 2010). Of those, four either had suboptimal images for the SFRV measurement or the images could not be obtained at all. Of the remaining 58 fetuses that were included in the study, 49 had a normal chromosomal complement and 9 had trisomy 21. The comparison between the two populations revealed a significantly larger SFRV in the fetuses with trisomy 21 (mean: 48.6 mm; range: 36–56.25 mm) as compared to the euploid fetuses (mean: 34.11 mm; range: 22.73–43.48 mm) ($p < 0.0001$) (Figure 3). The medians were similar: 50.0 and 34.6 for trisomy 21 and euploid fetuses, respectively. A significant difference was also noted between the two groups in the nuchal translucency (NT) measurements. The trisomy 21 fetuses had larger NT measurements (mean: 5.36 mm; range: 3.2–8.9 mm) as compared to the euploid fetuses (mean: 1.78 mm; range: 1.2–6.1 mm) ($p < 0.0001$). Overall, the CRL measurements were slightly larger in the trisomy 21 group (mean: 72.9 mm; range: 61–80 mm) than in the euploid group (mean: 66.8 mm; range: 45.2–83.1 mm) ($p = 0.041$). There was no statistical difference in the RVSD measurements between the two groups (mean: 1.56 mm; range: 1.2–2.3 mm in

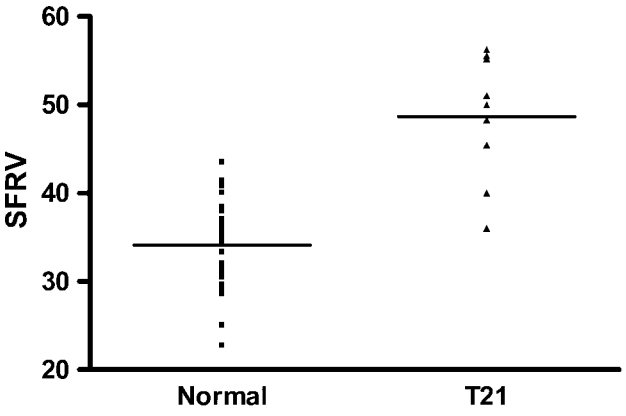

Figure 3—Graph of the shortening fraction of the right ventricle (SFRV) values (y-axis) for euploid and trisomy 21 fetuses. Means are represented by the horizontal lines

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fetuses with trisomy 21 and mean: 1.67 mm; range: 1.3–2.4 mm in the euploid group) ($p = 0.17$). However, there was a significant difference in the RVDD measurements with the trisomy 21 group being larger (mean: 3.08 mm; range: 2.2–4.7 mm) than the euploid group (mean: 2.54; range: 1.9–3.6 mm) ($p = 0.03$).

DISCUSSION

Background

There are a number of determinants of ventricular systolic function. These include preload (initial sarcomeres length), afterload (downstream resistance), efficiency of contractility of the myofibrils, availability of calcium to bind to contractile proteins, and heart rate. Ventricular performance may be evaluated noninvasively using ultrasound.

It has been shown that EDVI, ESVI, EF, and SFLV values in adults with Down syndrome are significantly different in comparison to euploid individuals (Hamada *et al.*, 1993; Russo *et al.*, 1998). Counterintuitively, these studies suggest that the myocardial performance is better in individuals with Down syndrome. Huggon *et al.* (2004) evaluated the left ventricular performance between week 11 and week 13 and 6 days of gestation by measuring the myocardial performance index (MPI). Their results were also suggestive of better cardiac performance in fetuses with trisomy 21. Using SFLV measurements, we have shown a similar difference between trisomy 21 fetuses and euploid fetuses at week 11 to week 13 and 6 days of gestation (Calda *et al.*, 2010).

The right ventricular performance in adolescent and adult individuals with Down syndrome has not been studied as extensively as has been in the left ventricular performance. Furthermore, postnatally the pulmonary circulation in individuals with Down syndrome is often significantly affected by changes in the pulmonary vasculature. Increased pulmonary resistance is present in a large percentage of these individuals even at a very young age (Yamaki *et al.*, 1983; Wilson *et al.*, 1993; Kawai *et al.*, 1995; Suzuki *et al.*, 2000; Shah *et al.*, 2004; Cua *et al.*, 2007). This appears to be due to both primary changes in these vessels (persistence of fetal double capillary network, reduction of the cross-sectional area of the vascular bed, thin tunica media in the pulmonary arteries, and impaired endothelial function) (Chi, 1975; Hals *et al.*, 1993; Yamaki *et al.*, 1993; Cappelli-Bigazzi *et al.*, 2004) and secondary to a reduction in the number of alveoli (Cooney and Thurlbeck, 1982). This is exacerbated by the chronic upper airway obstruction due to a number of midface, oral, nasopharyngeal, laryngeal, and tracheal abnormalities that are commonly found in persons with Down syndrome (Loughlin *et al.*, 1981; Levine and Simpser, 1982; Jacobs *et al.*, 1996; Levanon *et al.*, 1999). The increase in pulmonary artery pressure leads to changes in the right ventricular function. Therefore, it is difficult to discern to what extent the right ventricular performance in individuals with trisomy 21 is affected by primary changes within the myocardium themselves as opposed to the abnormalities in the afterload at the level of the pulmonary vasculature.

In postnatal life, the right and left sides of the heart function as two pumps in a series; the right ventricle moves blood through the pulmonary circulation and the left ventricle moves blood through the systemic circulation. However, *in utero* the fetal heart functions under essentially univentricular conditions with the left and right ventricles pumping in parallel. Only a small portion (approximately 10–15%) of blood exiting the right ventricle enters the pulmonary circulation (Rudolph, 1979). The rest is channeled through the ductus arteriosus into the systemic circulation. Therefore, the status of the systemic vasculature has a similar effect on both the left and right ventricular performance.

In this study, we used shortening fraction as a measurement of choice to evaluate the right ventricular function. We have shown previously that the shortening fraction is an easily obtainable measurement. Furthermore, we did find a difference in this parameter on the left side of the heart between the euploid and Down syndrome groups previously (Calda *et al.*, 2010). The measurement is obtained using M-mode ultrasound and involves measuring the right ventricular diameter during end diastole and during end systole. The shortening fraction is the ratio of the two measurements. The fact that a ratio rather than absolute values is used compensates to some extent for slight inconsistencies in the angles of insonation that are inevitable while performing this measurement. We estimated the time required to obtain and appropriate image to measures the ventricular dimensions in our previous publication dealing with the SFLV. We found that in 87% of the cases, the image could be obtained in 2 min or less (Calda *et al.*, 2010).

We found two significant differences between the groups of fetuses with trisomy 21 and euploid fetuses: both the SFRV and the RVDD appear to be larger in the fetuses with Down syndrome. The increased SFRV suggests improved ventricular performance in trisomy 21 fetuses, which is a similar result to what we found in the left ventricle at the same gestational age. It is somewhat surprising that the ventricular myocardial performance is better in fetuses with trisomy 21 than in their euploid counterparts in light of the existence of an extensive body of evidence pointing toward the fact that the myocardial structure in trisomy 21 is abnormal. The number of cells per unit area is decreased and the cells are increased in size (Recalde *et al.*, 1986). The composition of the connective tissue is also abnormal. Several genes for the matrix-related proteins are located on chromosome 21 and the concentration of their products is increased in trisomy 21 (Gittenberger-de Groot *et al.*, 2003; Carvalhaes *et al.*, 2006; Vis *et al.*, 2009). The two that follow this pattern and have been studied extensively are collagen VI and XVIII (Vis *et al.*, 2009). Collagen XVIII is of particular interest as it is an important component of the connective tissue core and basement membranes throughout the myocardium, the endocardial cushion, and the atrioventricular leaflets. It also may be involved in cell migration (Vis *et al.*,

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2009). As such, abnormalities in this collagen may not only influence the function of the heart but may also be in part responsible for the congenital structural defects which are commonly seen in trisomy 21 (Freeman *et al*., 2008).

Literature dealing with adult cardiovascular systems may provide us with one possible explanation for the better than expected myocardial performance in individuals with Down syndrome. It appears that the peripheral vascular resistance (PVR) is decreased in these individuals (Richards and Enver, 1979; Pitetti *et al*., 1992). As PRV is a significant determinant of myocardial performance, its decrease may lead to an improvement in the ventricular performance. Since *in utero* both the left and the right ventricles are pumping against the same (systemic) vascular resistance, this may serve as an explanation for the similar findings in the left and the right ventricles. Furthermore, a difference has been found in heart rates of euploid fetuses and those that have trisomy 21 at week 11 to week 13 and 6 days of gestation (Liao *et al*., 2000). The trisomy 21 fetuses were noted to have a somewhat increased heart rate. One possible explanation for this finding is a relatively low PVR.

The finding that the RVDD in fetuses with trisomy 21 is larger than in euploid fetuses may be explainable by the above-mentioned abnormalities of the ventricular wall structure. It is conceivable that these abnormalities may lead to a relative dilatation of the right ventricular cavity. This finding is in line with those of DeVore (2001) in second trimester fetuses. He found that the right-to-left chamber disproportion of the heart (the right being larger than the left) was a significant indicator of aneuploidy (likelihood ratio of 36.9).

There was a slight difference in the CRL measurements between the two groups ($p = 0.41$). While this may have had some effect on the results, given the relatively minor degree of difference, we suspect that this effect is not great.

Limitations

This pilot study was designed to evaluate the feasibility of measuring the SFRV between week 11 and week 13 and 6 days of gestation and to see if there is a difference between the SFRV values of euploid fetuses and those with trisomy 21. The ultrasound studies and measurements were performed by a single operator with extensive experience in first trimester ultrasound in general and M-mode evaluations of cardiac dimensions specifically. The study achieved its two goals and produced the unexpected finding of increased RVDD measurements in fetuses with trisomy 21. However, it has a number of limitations. It does not evaluate inter- or intra-observer variability of the measurements. The numbers are too small to address whether SFRV values or RVDD measurements change with gestational age, NT measurement, and fetal heart rate. These outstanding issues as well as whether these parameters are useful as markers in first trimester screening for trisomy 21 need to be addressed in further studies.

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ORIGINAL ARTICLE

Comparison of right ventricular measurements and SFRV in fetuses with and without tricuspid regurgitation at 11+0 and 13+6 weeks' gestation

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Abstract

Objectives: To compare right ventricular dimensions and systolic shortening fraction of the right ventricle (SFRV) in fetuses with tricuspid regurgitation (TR [+]) to those without tricuspid regurgitation (TR [−]).

Methods: Unselected patients presenting for first trimester screening between 11 + 0 and 13 + 6 weeks' gestation were examined for the presence or absence of fetal tricuspid regurgitation using a standard approach. Only euploid fetuses without structural anomalies were included in the study. The heart was examined with the aid of M-mode using a previously described method. The right ventricular diastolic diameter (RVDD) and right ventricular systolic diameter (RVSD) were measured on stored M-mode images and the SFRV was calculated using the following formula [(RVDD−RVSD)/RVDD] × 100.

Results: A total of 69 fetuses ($n = 44$ (TR [−]); $n = 25$ (TR [+])) were examined. The two groups were similar in maternal age, gestational age and nuchal translucency (NT) measurements. The SFRV was noted not to change with gestational age and there was no statistical difference between the two groups. Both the RVDD and the RVSD increased with gestational age. The calculated delta RVDD was statistically larger in the TR [+] group (mean: 0.29, CI 95%: 0.054–0.532) than the TR [−] group (mean: 0.013, CI 95%: −0.128 to 0.154) ($p < 0.05$). This was not true for the delta RVSD: TR [+] (mean: 0.17, CI 95%: 0.015–0.325) versus TR [−] group (mean: 0.035, CI 95%: −0.061 to 0.131). However, there was a trend towards larger RVSD in the TR [+] group ($p = 0.13$).

Conclusions: The presence of TR appears to be associated with an increased RVDD in normal fetuses between 11 + 0 and 13 + 6 weeks' gestation.

Keywords

First trimester, size of the right cardiac ventricle, tricuspid regurgitation

History

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What's already known:

Right ventricular enlargement is the most common cause for tricuspid regurgitation in postnatal life.

Tricuspid regurgitation can be detected in some normal fetuses between 11 + 0 and 13 + 6 weeks' gestation.

What's new:

We have shown that right ventricular diastolic dimensions are larger in normal fetuses with tricuspid regurgitation than in those without tricuspid regurgitation between 11 + 0 and 13 + 6 weeks' gestation.

Introduction

Evaluation of the fetal heart anatomy and function has become an integral part of obstetrical ultrasound examination. This includes checking for the presence or absence of tricuspid regurgitation.

Tricuspid regurgitation can be seen in many normal fetuses. It is present in approximately 1% of euploid fetuses between 11 + 0 and 13 + 6 weeks' gestation. However, its prevalence is increased in the presence of certain fetal anomalies such as fetal aneuploidy and congenital heart disease [1].

The etiology for the association between fetal aneuploidy such as trisomy 21 and tricuspid regurgitation (TR) in the absence of a cardiac defect is unclear. One theory is that the formation of the tricuspid valve (TCV) leaflets is defective resulting in an incomplete closure. This may be a result of abnormal genetic polymorphisms such as BMPR2 mutation, which is associated with congenital heart disease and abnormalities of pulmonary circulation later on in life [2,3].

The second postulated mechanism is that the lumen of the right ventricle is enlarged in trisomy 21, which may lead to the dilatation of the tricuspid valve annulus and result in regurgitation [4].

We have shown previously that the dimensions of the ventricular lumen can be measured objectively between

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$11+0$ and $13+6$ weeks gestation using M-mode. This has been utilized to compare the shortening fractions of both the left and right ventricles (SFLV and SFRV) in euploid and trisomy 21 fetuses [5,6].

In this study, we investigated the relationship between the size of the right ventricular cavity and the presence or absence of tricuspid regurgitation. We focused on chromosomally normal fetuses with no detectable cardiac defects. In this manner, we sought to establish whether such relationship exists after the removal of confounding variables.

Methods

This study was performed at the Charles University, Czech Republic. It included unselected fetuses at $11–13+6$ weeks' gestation (CRL: 45–84 mm). The patients were women who presented for routine first trimester screening. The following fetal parameters were examined and recorded: crown-rump length (CRL) measurement, nuchal translucency (NT) measurement and Doppler evaluation of the tricuspid valve for the presence or absence of TR. The Fetal Medicine Foundation protocol was followed in each case [7].

Specifically, the examination of the tricuspid valve using pulsed Doppler was done by obtaining an apical view of the four chamber heart so that the fetal chest occupied the majority of the image. The angle formed by the long axis of the ventricular septum and the Doppler beam had to be 30 degrees or less. A relatively wide Doppler gate was placed over the tricuspid valve and several consecutive waveforms were obtained. The diagnosis of TR was made if flow across the valve exceeding 60 cm/s in velocity was noted during ventricular systole. The duration of the flow had to be in excess of 50% of the systole.

The right ventricular measurements were obtained using the technique, which we described previously [5,6]. All examinations and measurements were performed using a M7C abdominal probe (Vivid 7 Dimension, General Electric) by a single operator (M.B.), who has extensive experience in this technique. Briefly, the magnification used was such that the heart filled approximately 75% of the entire image. The heart was insonated in one of two ways. One consisted of a four chamber view where the face of the transducer was parallel to the longitudinal axis of the ventricular septum (approximately 90 degree angle of insonation). The second approach involves obtaining a short-axis view of the heart again making certain that the angle formed by the ultrasound beam and the longitudinal axis of the ventricular septum was approximately 90 degrees. In both views, the M-mode cursor was placed within the ventricles immediately below the level of the atrio-ventricular valves (Figure 1). The images were stored electronically. Right ventricular diastolic diameter (RVDD) and right ventricular systolic diameter (RVSD) were measured off-line and recorded. Additionally, SFRV was calculated using the following formula: $[(\text{RVDD} - \text{RVSD})/\text{RVDD}] \times 100$ for each fetus.

Maternal age was recorded. Normal karyotype was determined antenatally using chorionic villus sampling in seven of the 25 fetuses with TR and in one of the fetuses without TR. In the remainder, normalcy of outcome was determined based on the results of subsequent ultrasound studies and a normal neonatal examination.

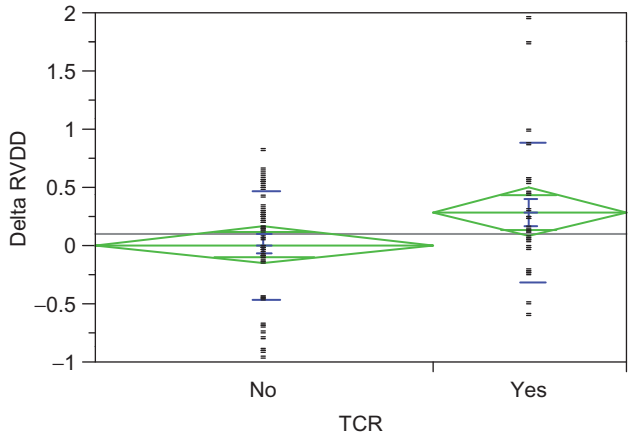

Figure 1. A graph of delta RVDD distributions in fetuses without tricuspid regurgitation (TCR ''no'', $n=44$) and with tricuspid regurgitation (TCR ''yes'', $n=25$) ($p<0.05$).

Exclusion criteria included multiple gestations, fetal anomalies, fetal aneuploidy and nuchal translucency measurement $\geq$3.5 mm. An informed consent for the ultrasound examination was signed by each patient.

Statistical analysis

The dependency of the right ventricular measurements on gestational age was documented using Pearson's *r*-test. Regression lines of the means based on the RVDD and RVSD data of the fetuses without TR were generated. Individual measurements were then subtracted from the gestational age adjusted means to calculate delta RVDD and delta RVSD in both the population of fetuses with and without TR. These were then compared using two-tailed *t*-test. The same technique was employed to compare the NT measurements in the two populations. Two tailed *t*-test was used to compare non-gestational age-dependent data (GraphPad Software, La Jolla, CA). Null hypothesis was rejected for $p<0.05$.

Results

A total of 69 women were enrolled in the study. Their fetuses were divided into two groups: one where tricuspid regurgitation was absent (TR [−]) ($n=44$) and one where tricuspid regurgitation was present (TR [+]) ($n=25$). The two groups were similar with respect to maternal age (TR [−]: mean 31.39 years (range: 21–39); TR [+]: mean 31.96 years (range: 25–43) ($p=0.84$)), CRL measurement (TR [−]: mean 71.13 mm (range: 58.0–84.1); TR [+]: mean 61.97 mm (range: 49.2–82.3) ($p=0.56$)).

The RVDD measurements in the TR [–] group had a mean of 2.73 mm and a range of 1.7–3.7 mm. The RVDD measurements in the TR [+] group had a mean of 2.95 mm and a range of 2.2–4.4 mm. The RVSD measurements in the TR [–] group had a mean of 1.75 mm and a range of 1.0–2.4 mm. The RVSD measurements in the TR [+] group had a mean of 1.88 mm and a range of 1.2–2.9 mm.

The RVDD and RVSD increased linearly with gestational age in both groups: RVDD ($r=0.37$) and RVSD ($r=0.36$) in

TR [−] fetuses; RVDD ($r = 0.21$) and RVSD ($r = 0.20$) in TR [+] fetuses. The regression line, which best described the mean RVDD according to gestational age in the TR [−] group was $y = x$ (0.047) – 0.59. The regression line for the mean RVSD according to age in the TR [−] group was $y = x$ (0.03) − 0.41. The calculated mean delta RVDD was 0.013 mm (CI 95%: −0.128 to 0.154) in the TR [−] group and 0.29 mm (CI 95%: 0.054–0.532) in the TR [+] group, which is significantly larger (<0.05) (Figure 2). The mean calculated delta RSVD was 0.035 mm (CI 95%: −0.061 to 0.131) in the TR [−] group and 0.17 mm (CI 95%: 0.015–0.325) in the TR [+] group. There appears to be a trend for the RVSD to be greater in the TR [+] group but it did not achieve statistical significance ($p = 0.13$) (Figure 3).

The mean SFRV in the TR [−] group was 36.07 (range: 30.00–41.94) and was 36.35 (range: 20.00–52.00) in the TR [+] group. The SFRV values were found to be independent of gestational age (TR [−]: $r = 0.001$; TR [+]: $r = 0.01$). The SFRV values were similar in both groups ($p = 0.84$).

The mean NT measurements were 1.98 mm (range: 1.4–3.2) in the TR [−] group and 2.02 mm (range: 12–2.9) in the TR [+] group. The NT measurements increased with gestational age in both groups (TR [−]: $r = 0.28$; TR [+]: $r = 0.30$). The regression line that best described this association in the TR [−] group was $y = x$ (0.023) + 0.32. The delta NT in the TR [−] group was 0.04 mm (CI 95%: −0.041 to 0.130) and 0.11 mm (CI 95%: −0.016 to 0.227) in the TR [+] group. There was no statistical difference between the two groups ($p = 0.41$).

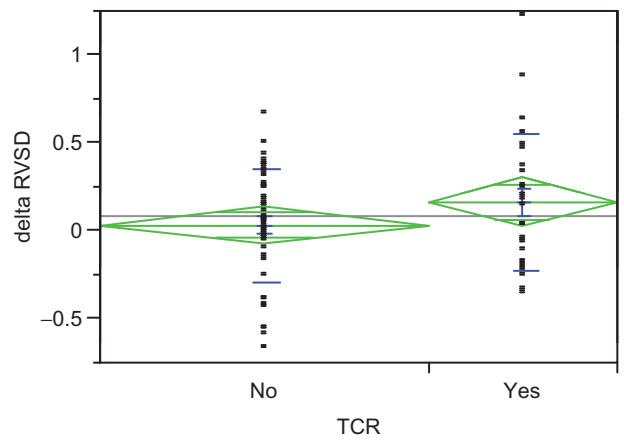

Figure 2. A graph of delta RVSD distributions in fetuses without tricuspid regurgitation (TCR ''no'') and with tricuspid regurgitation (TCR ''yes'') ($p = 0.13$).

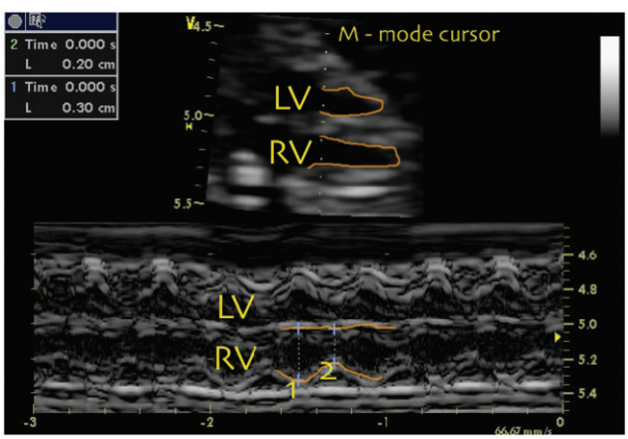

Figure 3. Four chamber heart view with M-mode (RV = right ventricle, LV = left ventricle, measurement #1 = RVDD, measurement #2 = RVSD).

Discussion

Tricuspid regurgitation is significantly more common in fetuses, which are affected by aneuploidy and/or those with cardiac defects (Table 1); therefore, Doppler evaluation of the tricuspid valve in the first trimester has become an important tool in screening for both of these conditions [1,8].

In the post-natal life, the most common etiology for TR is right ventricular enlargement and subsequent dilatation of the tricuspid valve annulus [9]. However, *in utero* the exact etiology for TR is not clear. In this study, we have shown that in otherwise normal first trimester fetuses, the dimensions of the right ventricle are greater in the presence of tricuspid regurgitation. This is especially true of the diastolic diameter, which achieved statistical significance. Even though the size difference of the right ventricle in systole did not reach statistical significance, a trend toward larger size appears to be present in those fetuses that have tricuspid regurgitation.

This study does not address the etiology for the apparent right ventricular enlargement in normal fetuses with TR. However, postulated mechanisms may be divided into two general categories: primary (intracardiac) and secondary (extracardiac). Under the primary heading, one could postulate that the fetuses with TR have delayed development of either the connective tissue of the heart or its muscle. This in turn could lead to any of the following or a combination there of: larger than usual dimensions of the ventricular lumen, more pliable than normal papillary muscles and chordae tendinae, and a delay in the formation of the tricuspid annulus and valves. Even though the human heart attains its four chamber appearance by the end of the 8th week of development (10 weeks' gestation), it is believed that ultrastructural changes continue to occur throughout pregnancy. For example, the percentage of the number of binucleated cardiomyocytes, a measure of cardiomyocyte maturation, increases during gestation in both the human and the sheep [10,11]. In the sheep model, it has also been shown that there is an increase in certain adult troponin isoform proteins such as troponin I and C and there is a change in sensitivity to

Table 1. Prevalence of TR in association with various fetal conditions.

Fetal findings	Tricuspid regurgitation (*n*)
Euploidy, no CHD	0.9% (181/19 614)*
Trisomy 21	55.7% (68/122)*
Trisomy 18	33.3% (12/36)*
Trisomy 13	30.0% (6/20)*
Monosomy X	37.5% (3/8)*
CHD, no aneuploidy	32.9% (28/85)†

CHD = multiple types of congenital heart disease.
*Kagan et al. [1].
†Pereira et al. [8].

Ca^{++} [11,12]. Changes in myocardial contractility associated with advancing gestational age have been documented directly in the sheep model and indirectly in human fetuses [12,13]. Normal biological variability in the progression of these and other ultrastructural changes may result in variability of the effectiveness of the tricuspid valve closure.

The mechanism of TR due to extracardiac (secondary) causes is related to an increase in downstream resistance. Changes in downstream (i.e. systemic) resistance affect both the left and right sides of the heart. This is due to the fact that the fetal cardiac ventricles operate as an essentially parallel system; there are both intracardiac (foramen ovale) and extracardiac (ductus arteriosus) connections between the systemic and pulmonary circulatory systems.

Variability in downstream resistance may be caused by any number of factors. However, two structures that have the potential to affect vascular resistance in a major and sustained manner are the aortic isthmus and the placenta. It has been shown that the relative size of the aortic isthmus changes throughout gestation [13,14]. Since it is such a major conduit of blood in the systemic circulation, its biological variability alone may significantly contribute to the intracardiac pressures. It should be kept in mind that change in pressure is related to the square of the change in diameter; therefore, even small changes in the isthmic diameter cause significant changes in the vessel pressure. The placenta receives approximately 40% of the cardiac output. The placental resistance progressively decreases during the course of the pregnancy. Some biological variability in this process is expected and may be accompanied by variable downstream resistance.

Conclusion

Regardless of the exact etiology for TR, our data suggest that there is an association between the presence of TR and relative right ventricular enlargement in the first trimester. Whereas a causative link between the two cannot be drawn, this study does provide support for the idea that ventricular size plays a significant role in the genesis of tricuspid regurgitation in otherwise normal fetuses. In this study, we limited the presence of confounding variables by including only low risk patients that were chromosomally and structurally normal with normal nuchal translucency measurements. The major limitation of the study is a relatively small number of subjects.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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