First trimester assessment of ductus venosus in screening for fetal chromosomal and cardiac defects

Valoración del ductus venoso en el primer trimestre en el cribado de anomalías cromosómicas fetales y defectos cardiacos

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Para aspirar al grado de Doctora en Medicina y Cirugía
con Mención de Doctor Europeo

Enero 2010

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FIRST TRIMESTER ASSESSMENT OF DUCTUS VENOSUS IN SCREENING FOR FETAL CHROMOSOMAL AND CARDIAC DEFECTS

Submitted by

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For the degree of European Doctor in Medicine

January 2010

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I confirm that Dr Nerea Maiz Elizaran

has carried out under my supervision the studies presented in the Thesis: First trimester assessment of ductus venosus in screening for fetal chromosomal and cardiac defects

I have read the Thesis and I am happy for this to be presented to the Tribunal for The Degree of European Doctor in Medicine

Professor Kypros Herodotou Nicolaides
London October 2009

Al aita y a la ama

ACKNOWLEDGEMENTS

English

Thanks to Professor Nicolaides, it is difficult to explain in a few words how grateful I am. He taught me patiently, guided me during three years and was the best example of hard work, generosity and enthusiasm. Thanks for giving me a chance. Thanks for sharing the passion for Fetal Medicine.

To Professor Eduard Gratacós, tutor of this thesis, for his advice and availability.

Thanks to all the Research Fellows of the Harris Birthright Research Centre, for all the work and for being "my family" during three fantastic years. Without you this would have been impossible. To Katy, Catalina, Francisca, Walter, Themis, Nicola, Ebru, Ranjit, Marisa, Ismini,..... we shared so many things!! thanks for all the support and being my best friends. To the midwifes, to July, for making our lives easier. To the consultants, for being so patients.

Thanks to my parents and brothers for all the love and support. I owe them what I have and what I am. Thanks for being so close despite the distance.

To my friends and colleagues at Institut Dexeus and Sant Joan de Déu hospital in Barcelona, where I learned so many things about Obstetrics, about Fetal Medicine, ...but also about life! To Inés, Maite, Pilar, Yeli, Juanjo, for their friendship and great years in Barcelona. To Luis, I wish you were here to share this moment with you.

Thanks to my colleagues at Clínica del Pilar in San Sebastian for trusting me.

To Juana, Sofia, Cristina, Leyre, Ana, Esther, Maria,...for their unconditional friendship.

Spanish

Gracias al Profesor Nicolaides, es difícil expresar en pocas palabras todo mi agradecimiento. Él me enseñó y guió durante tres años, y fue el mejor ejemplo de trabajador incansable, generosidad y entusiasmo. Gracias por darme una oportunidad. Gracias por compartir la pasión por la Medicina Fetal.

Al Profesor Eduard Gratacós, tutor de esta tesis, por sus consejos y disponibilidad.

A los research fellows del Harris Birthright Research Centre, por todo su trabajo y por ser mi "familia" durante 3 años fantásticos. Sin ellos esta tesis hubiera sido imposible. A Katy, Catalina, Francisca, Walter, Themis, Nicola, Ebru, Ranjit, Marisa, Ismini,… hemos compartido tantas cosas!!! Gracias por todo el apoyo y ser mis mejores amigos. A las "midwifes", a July, por hacer nuestra vida más fácil. A los "consultants" por su paciencia.

A mis padres y mis hermanos, gracias por todo el amor y el apoyo constante. A ellos les debo lo que tengo y lo que soy. Gracias por estar tan cerca a pesar de la distancia.

A mis amigos y compañeros del Institut Dexeus y el Hospital Sant Joan de Déu donde aprendí Obstetricia, Medicina Fetal,…y aprendí de la vida!. A mis amigos Inés, Maite, Pilar, Yeli, Juanjo por su amistad y los fantásticos años en Barcelona. A Luis, ojalá estuvieras aquí para compartir este momento contigo.

Mi agradecimiento a mis compañeros de la Clínica del Pilar de San Sebastián por confiar en mí.

A Juana, Sofía, Cristina, Leyre, Ana, Esther, María,.. por su amistad incondicional.

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ABBREVIATIONS

ALARA: as low as reasonably achievable
BMI: body mass index
CHD: congenital heart defect
CI: confidence interval
CMV: cytomegalovirus
CVS: chorion villus sampling
CRL: crown-rump length
DR: detection rate
EDF: end diastolic flow
FGR: fetal growth restriction
FHR: fetal heart rate
FPR: false positive rate
hCG: human chorionic gonadotropin
LMP: last menstrual period
MoM: multiples of the median
NT: nuchal translucency
OR: odds ratio
PAPP-A: pregnancy associated plasma protein-A
PI: pulsatility index
PIV: pulsatility index for veins
PVIV: Peak velocity index for veins
SGA: small for gestational age
Tamx: time-averaged maximum velocity
TTTS: twin-to-twin transfusion syndrome
UA: umbilical artery
V_m: intensity-weighted time-average mean velocity

CHAPTER I

INTRODUCTION

1.1 DUCTUS VENOSUS

1.1.1 Anatomy

The ductus venosus is located in the fetal abdomen, connecting the intra-abdominal portion of the umbilical vein to the left side of the inferior vena cava (Figure 1.1) and following a posterior and cephalic direction (Kiserud *et al.,* 1991). It has a trumpet shape (Kiserud *et al.,* 1991), where the narrowest portion, the isthmus measurement, closer to the umbilical vein, increases from 0.5 mm at mid-gestation to no more than 2 mm during the rest of the gestation, the outlet width increases from 1.25 to 3 mm and the length of the ductus venosus from 5 to 17 mm (Kiserud *et al.*, 1991, 1994a, 1998, 2000a).

1.1.2 Functional anatomy

The reduction of the diameter from the umbilical vein to the ductus venosus produces a pressure gradient between the umbilical vein and the atrium, resulting in an acceleration of the blood flow in the ductus (Kiserud *et al.*, 1991,1994a). This blood flow across the ductus venosus reaches a high velocity that increases with gestational age, from 29 cm/s in the first trimester (Huisman *et al.*, 1993, van Splunder *et al.*, 1996) to 65 cm/s at 18 weeks and up to 75 cm/s at term (Kiserud *et al.*, 1991, 1992a; Huisman *et al.*, 1992; Bahlmann *et al.*, 2000).

The fetal inferior venous return is arranged in a Y-shaped inferior vena cava –foramen ovale unit, with an inferior vena cava-right atrium pathway and another ductus venosus-foramen ovale pathway:

(a) The ventral and rightward stream, together with the blood flow from the superior vena cava is directed towards the right atrium and right ventricle across the tricuspid valve. This way the deoxygenated blood from the abdominal inferior cava enters the right atrium.
(b) The dorsal and left side stream receives the oxygenated blood from the ductus venosus and left hepatic veins. This can be explained by a double mechanism:

1- Anatomically the position of the fetal atrial septum is further to the right than in the postnatal life, so that the inferior vena cava entrance is overriding the fetal atrial septum (Patten *et al.*, 1929). Also the orifice of the foramen ovale is tilted over the inferior vena cava entrance, receiving the blood flow for the left atrium.

2- The high volume and high velocity blood flow from the ductus venosus distend the foramen ovale-flap like a spinnaker. However, during atrial contraction the foramen ovale-flap moves towards the septum causing a substantial reduction in the foramen ovale diameter.

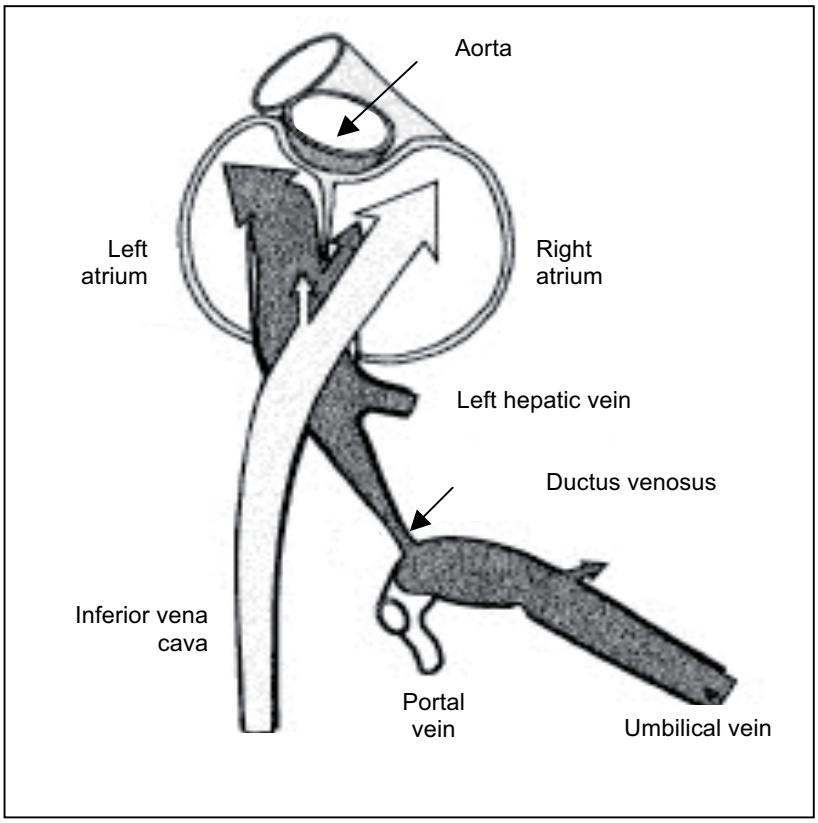

Figure 1.1 Oxygenated umbilical blood (dark gray) from the ductus venosus (DV) and left hepatic veins preferentially enters the left atrium, while deoxygenated blood (light gray) from the abdominal inferior vena cava enters the right atrium (Kiserud *et al.*, 2001).

The direction of these vessels, the high volume of oxygenated blood and the high velocity blood flow would lead this blood flow across the foramen ovale towards the left atrium (Lind *et al.*, 1949; Kiserud *et al.*, 1992b).

1.1.3 Ductus venosus shunting

Studies in animals have shown that about 50% of the blood flow from the umbilical vein is shunted to the ductus venosus (Edelstone *et al.*, 1978; Behrman *et al.*, 1970). In human fetuses, studies using ultrasound techniques have shown that about 30% of the well-oxygenated blood flow from the umbilical vein is shunted to the ductus venosus at mid-gestation, and only 20% at 30 weeks and onwards (Kiserud *et al.*, 2000a; Bellotti *et al.*, 2000).

There has been controversy in the literature whether there is a sphincter at the inlet regulating the blood flow through the ductus venosus. Some studies have shown the presence of smooth muscle fibers at the inlet of the ductus venosus (Barron, 1942; Barcroft, 1946; Barclay *et al.*, 1944; Chacko *et al.*, 1953; Ehinger *et al.*, 1968) and this has been interpreted as a sphincter. But these results have not been reproduced by other authors (Lind, 1977; Meyer *et al.*, 1966) who have interpreted these smooth muscle fibers as part of the muscular arrangement of the umbilical and portal veins.

A more recent study with immunohistochemical methods (Mavrides *et al.*, 2002) has shown a shelf rich in elastin at the level of the inlet and a narrow inlet orifice that may act to accelerate the flow from the portal sinus to a high velocity system in the ductus venosus. They also demonstrate the lack of an anatomical smooth muscle sphincter at the inlet. In this study they also found the presence of a single cell-layer of smooth muscle cells orientated longitudinally along the entire ductus venosus, and several nerve bundles and fibers along the entire ductus venosus. This would support the hypothesis that the ductus venosus is an actively regulated vessel and the changes of the diameter include the entire length of the vessel, and not only the inlet portion (Kiserud *et al.*, 2000b).

Ductus venosus shunting depends on:

1. <u>The diameter of the vessel</u>
   This is under tonic adrenergic control. Coceani *et al.* reported an α-adrenergic contraction and a β-adrenergic distension of the ductus venosus inlet (Coceani *et al.*, 1984), but posterior studies have shown that the ductus venosus is tonically

constricted, as the nitric oxide can dilate the ductus venosus, but an α_1-adrenergic agonist like phenylephrine does not produce a constriction (Kiserud *et al.*, 2000b). The ductus venosus also relaxes under the influence of prostaglandins (Adeagbo *et al.*, 1982), but the most important response is under hypoxemia, with a 60% increase of the inlet diameter, and a distension of the entire vessel (Kiserud *et al.*, 2000b).

2. Fluid dynamics: viscosity and pressure

When the blood has a high velocity, like at the ductus venosus, it has Newtonian properties, with a low viscosity, similar to water. On the other hand, at low velocities, like in the liver, the blood is non-Newtonian, with high viscosity and a low closing pressure. The pressure gradient between the abdominal portion of the umbilical vein and the inferior vena cava is responsible for the perfusion of the liver tissue and the ductus venosus.

The two pathways, the liver and the ductus venosus, will be differently affected by variations in pressure gradient and viscosity. There is a linear relationship between the pressure at the umbilical vein and the flow through the liver, but at low pressures (1 to 4 mm Hg), there is a proportionally greater fraction of umbilical vein flow directed through the ductus venosus. Similarly, with higher viscosity (high hematocrit), the reduction of blood flow to the liver is more pronounced than the one at the ductus venosus, and therefore the fraction directed to the ductus venosus is higher (Kiserud *et al.*, 1997, 2004).

1.1.4 Doppler assessment of the ductus venosus

The ductus venosus can easily be identified using color Doppler mapping as the portion with higher velocity following the umbilical vein (Figure 1.2). The ductus venosus waveform has a typical waveform where three phases can be recognized (Figure 1.2).

The highest velocity peak corresponds to the ventricular systole (S), during this phase the pressure gradient between the venous vessels and the atrium will be the highest.

The next peak of forward flow reflects the early diastole (D), with the opening of the atrioventricular valves and the early passive filling of the ventricles. The third phase, the lowest velocity forward flow corresponds to the atrial contraction (a) during the late diastole (Kiserud *et al.*, 1991).

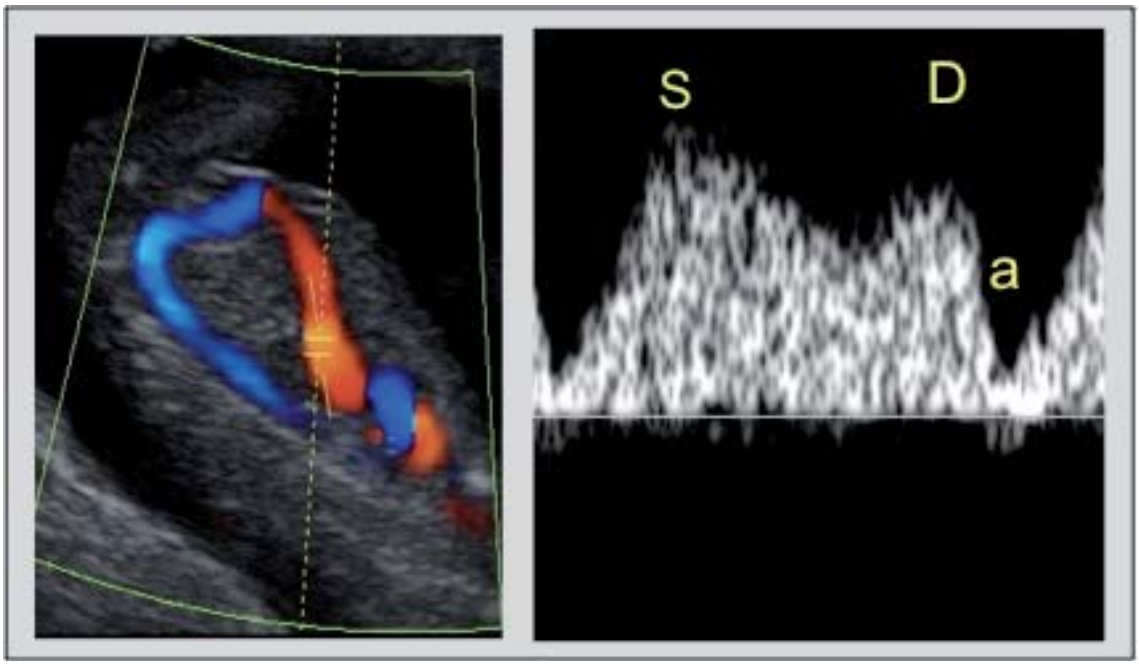

Figure 1.2 Ductus venosus color Doppler (left) and ductus venosus waveform (right) showing the three fases: (S) systole, (D) diastole, and (a) atrial contraction.

Quantitative assessment of the ductus venosus waveform: velocities and indexes.

Since 1991, when Kiserud *et al.* described the blood flow velocity pattern of the ductus venosus, many other authors have studied different parameters of the ductus venous waveform. On the second and third trimester the peak velocity during the systole (S), early diastole (D), and the atrial contraction (a) in late diastole has been measured and these variables increase with the gestational age (Huisman *et al.*, 1992; Hecher *et al.*, 1994; van Splunder *et al.*, 1996; Axt-Fliedner *et al.*, 2004; Kessler *et al.*, 2006). The intensity-weighted time-average mean velocity (V_m) (Hecher *et al.*, 1994; Axt-Fliedner *et al.*, 2004) and the time-averaged maximum velocity (Tamx) also increase with the gestational age (Kiserud *et al.* 1991; Huisman *et al.*, 1992; Hecher *et al.*, 1994; van Splunder *et al.*, 1996; Kessler *et al.*, 2006). In the first trimester the results are similar,

with a higher velocity during the systole, diastole, atrial contraction, the Vm and the Tamx (Huisman *et al.*, 1993; van Splunder *et al.*, 1996; Prefumo *et al.*, 2002; Teixeira *et al.*, 2008) as the crown-rump length (CRL) increases.

Some authors have suggested different indexes for the ductus venosus waveform assessment. Hecher *et al.* defined the pulsatility index for veins (PIV) as (S-a)/Tamx and the peak velocity index for veins (PVIV) as (S-a)/D and described the normal ranges between 20-40 weeks (Hecher *et al.*, 1994) (Figure 1.3). The PIV is one of the most used and robust parameter for clinical work (Hecher *et al.*, 1995a; Kiserud, 2003). Other indexes studied by other authors are: S/D index (Huisman *et al.*, 1992, 1993; Rizzo *et al.*, 1994a; Axt-Fliedner *et al.*, 2004), the ductus venosus index (DVI) defined as (S-a)/S (DeVore *et al.*, 1993), (S-a)/D (Hecher *et al.*, 1994) and the perfusion index (PFI) defined as Tamx/S (Kiserud *et al.*, 1992a; Kessler *et al.*, 2006).

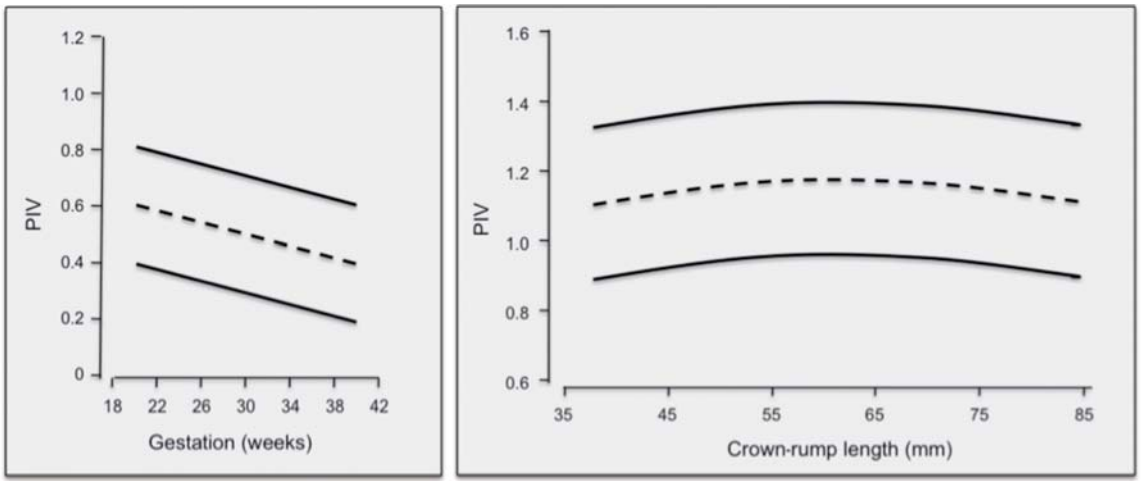

Figure 1.3 PIV between 20 and 40 weeks (Hecher *et al.*, 1994) (left) and in the first trimester (Teixeira *et al.*, 2008) (right).

In the first trimester, although initial studies showed a reduction of the PIV with gestational age (Van Splunder *et al.*, 1996), Prefumo *et al.* showed a non significant decrease of PIV with CRL (Prefumo *et al.*, 2002), and Teixeira *et al.* found a non significant increase in PIV with CRL up to a value of 63 mm, and a significant decrease thereafter (Teixeira *et al.*, 2008) (Figure 1.3).

Qualitative assessment of the ductus venosus waveform

Unlike the second and third trimester, where in normal pregnancies the flow during the atrial contraction is always forward, in the first trimester the a-wave can be zero or reversed, and this finding is more common in younger fetuses (Kiserud, 1999, 2003).

A qualitative assessment of the a-wave as positive, absent or reversed has also been described (Matias *et al.*, 1998) and used in clinical practice.

Reproducibility

Although Huisman *et al.* reported a good reproducibility of ductus venosus velocities between 12 and 15 weeks (Huisman *et al.*,1993), subsequent studies have reported contradictory results. Mavrides *et al.* found a good intraobserver repeatability of PIV, but not so good for velocity measurements, and unsatisfactory results for the interobserver reproducibility (Mavrides *et al.*, 2001a). Prefumo *et al.* found acceptable degree of intra and interobserver repeatability (Prefumo *et al.*, 2001) (Table 1.1).

Table 1.1 Intra and interobserver repeatability of Doppler measurements from the studies of Mavrides *et al.* (2001a) (M) and Prefumo *et al.* (2001) (P).

	Intraobserver						**Interobserver**					
	CV		**ICC**		**RC**		**CV**		**ICC**		**RC**	
	M	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**	**M**	**P**
PIV	8.9	10.0	0.62	0.85	1.27	0.29	11.5	8.8	0.19	0.86	1.36	0.27
S-vel	19.2	13.0	0.70	0.94	1.64	11.0	23.3	14.0	0.44	0.84	1.81	12.0
a-vel	28.5	22.0	0.69	0.94	2.03	4.40	47.2	27.0	0.20	0.87	2.99	4.40

CV= coefficient of variation, ICC= intraclass correlation coefficient, RC= repeatability coefficient, PIV= pulsatility index for veins, S-vel= systolic velocity, a-vel= atrial contraction velocity.

Both studies showed a good agreement for the classification of normal/abnormal flow based on the classification of forward/reversed flow during the atrial contraction with an intraobserver κ-coefficient 0.82 and 1.0 and interobserver κ-coefficient of 0.51 and 1.0 respectively for the studies of Mavrides *et al.* and Prefumo *et al.*. However, some authors (Hecher, 2001a) have criticised these studies as they were performed in low-risk populations and therefore, the number of abnormal waveforms was small.

More recently Borrell *et al*. studied the repeatability in a high-risk population and found a good intra and interobserver reliability for PIV and good intraobserver but moderate interobserver reliability for the a-wave (Borrell *et al.*, 2007).

1.2 DUCTUS VENOSUS AND CHROMOSOMAL ABNORMALITIES

1.2.1 Overview

Chromosomal abnormalities are major causes of perinatal death and childhood handicap. Consequently, the diagnosis of chromosomal disorders constitutes the most frequent indication for invasive prenatal diagnosis. However, invasive testing by amniocentesis or chorionic villous sampling is associated with a risk of miscarriage of about 1% and therefore these tests are carried out only in pregnancies considered to be at high-risk for chromosomal defects (Tabor *et al.*, 1986; Alfirevic *et al.*, 2003).

1.2.2 Assessment of risk for chromosomal abnormalities

Background or *a priori* risk

While every woman is at risk of having a baby with a chromosome defect, a woman's individual chance will depend on her background or *a priori* risk (which depends on maternal age and gestational age) and the results of any ultrasound or biochemical screening tests carried out during the course of the pregnancy. Every time a test is carried out the *a priori* risk is multiplied by the likelihood ratio of the test to calculate a new risk, which then becomes the *a priori* risk for the next test.

Maternal age and gestation

The most common chromosomal defects are trisomies 21, 18 and 13. The risk for all three chromosomal defects increases with maternal age (Tables 1.2, 1.3 and 1.4) (Snijders *et al.*, 1995, 1999). Additionally, because fetuses with these trisomies are more likely to die in utero than normal fetuses, the risk decreases with gestation. The rates of fetal death between 12 weeks and term are about 30% for trisomy 21 and about 80% for trisomies 18 and 13.

Turner syndrome, unlike trisomies, is unrelated to maternal age. The prevalence is about 1 per 1500 at 12 weeks, 1 per 3000 at 20 weeks and 1 per 4000 at 40 weeks. For the other sex chromosome abnormalities (47,XXX, 47,XXY and 47,XYY), there is no significant change with maternal age and since the rate of intrauterine lethality is not higher than in chromosomally normal fetuses the overall prevalence (about 1 per 500) does not decrease with gestation.

Triploidy is highly lethal and thus very rarely observed in live births; the prevalence at 12 and 20 weeks is about 1 per 2000 and 1 per 250, 000, respectively.

In the early 1970's about 5% of pregnant women were 35 years or older and this group contained about 30% of the total number of fetuses with trisomy 21. Therefore, screening on the basis of maternal age was associated with a 5% screen positive rate and a 30% detection rate.

In recent years the trend to delayed childbearing has resulted in a significant increase in the number of pregnant women ≥ 35 years (20%). If all these women were to undergo invasive testing, approximately 50% of the total number of fetuses with trisomy 21 would be detected but at a greatly increased false positive rate.

Table 1.2 Estimated risk for trisomy 21 in relation to maternal age and gestation

Maternal age (ys)	*Gestational age*					
	10 weeks	**12 weeks**	***14 weeks***	**16 weeks**	***20 weeks***	**40 weeks**
20	1/983	1/1068	1/1140	1/1200	1/1295	1/1527
25	1/870	1/946	1/1009	1/1062	1/1147	1/1352
30	1/576	1/626	1/668	1/703	1/759	1/895
31	1/500	1/543	1/580	1/610	1/658	1/776
32	1/424	1/461	1/492	1/518	1/559	1/659
33	1/352	1/383	1/409	1/430	1/464	1/547
34	1/287	1/312	1/333	1/350	1/378	1/446
35	1/229	1/249	1/266	1/280	1/302	1/356
36	1/180	1/196	1/209	1/220	1/238	1/280
37	1/140	1/152	1/163	1/171	1/185	1/218
38	1/108	1/117	1/125	1/131	1/142	1/167
39	1/82	1/89	1/95	1/100	1/108	1/128
40	1/62	1/68	1/72	1/76	1/82	1/97
41	1/47	1/51	1/54	1/57	1/62	1/73
42	1/35	1/38	1/41	1/43	1/46	1/55
43	1/26	1/29	1/30	1/32	1/35	1/41

Table 1.3 Estimated risk for trisomy 18 in relation to maternal age and gestation

Maternal age (yrs)	*Gestational age*					
	10 weeks	12 weeks	14 weeks	16 weeks	20 weeks	40 weeks
20	1/1993	1/2484	1/3015	1/3590	1/4897	1/18013
25	1/1765	1/2200	1/2670	1/3179	1/4336	1/15951
30	1/1168	1/1456	1/1766	1/2103	1/2869	1/10554
31	1/1014	1/1263	1/1533	1/1825	1/2490	1/9160
32	1/860	1/1072	1/1301	1/1549	1/2490	1/7775
33	1/715	1/891	1/1081	1/1287	1/1755	1/6458
34	1/582	1/725	1/880	1/1047	1/1429	1/5256
35	1/465	1/580	1/703	1/837	1/1142	1/4202
36	1/366	1/456	1/553	1/659	1/899	1/3307
37	1/284	1/354	1/430	1/512	1/698	1/2569
38	1/218	1/272	1/330	1/393	1/537	1/1974
39	1/167	1/208	1/252	1/300	1/409	1/1505
40	1/126	1/157	1/191	1/227	1/310	1/1139
41	1/95	1/118	1/144	1/171	1/233	1/858
42	1/71	1/89	1/108	1/128	1/175	1/644
43	1/53	1/66	1/81	1/96	1/131	1/481

Table 1.4 Estimated risk for trisomy 13 in relation to maternal age and gestation

Maternal age (ys)	*Gestational age*					
	10 weeks	12 weeks	14 weeks	16 weeks	20 weeks	40 weeks
20	1/6347	1/7826	1/9389	1/11042	1/14656	1/42423
25	1/5621	1/6930	1/8314	1/9778	1/12978	1/37567
30	1/3719	1/4585	1/5501	1/6470	1/8587	1/24856
31	1/3228	1/3980	1/4774	1/5615	1/7453	1/21573
32	1/2740	1/3378	1/4052	1/4766	1/6326	1/18311
33	1/2275	1/2806	1/3366	1/3959	1/5254	1/15209
34	1/1852	1/2284	1/2740	1/3222	1/4277	1/12380
35	1/1481	1/1826	1/2190	1/2576	1/3419	1/9876
36	1/1165	1/1437	1/1724	1/2027	1/2691	1/7788
37	1/905	1/1116	1/1339	1/1575	1/2090	1/6050
38	1/696	1/858	1/1029	1/1210	1/1606	1/4650
39	1/530	1/654	1/784	1/922	1/1224	1/3544
40	1/401	1/495	1/594	1/698	1/927	1/2683
41	1/302	1/373	1/447	1/526	1/698	1/2020
42	1/227	1/280	1/335	1/395	1/524	1/1516
43	1/170	1/209	1/251	1/295	1/392	1/1134

Fetal nuchal translucency

Nicolaides *et al.* noted that in fetuses with chromosomal defects there is increased subcutaneous accumulation of fluid behind the fetal neck in the first trimester of pregnancy and introduced the term nuchal translucency (NT) (Nicolaides *et al.*, 1992) (Figure 1.4).

Subsequent studies involving more than 200,000 pregnancies and including about 900 fetuses with trisomy 21 have demonstrated that screening at 11-13 weeks by a combination of maternal age and fetal NT can detect more than 75% of fetuses with trisomy 21 at an FPR of 5% (Nicolaides, 2004).

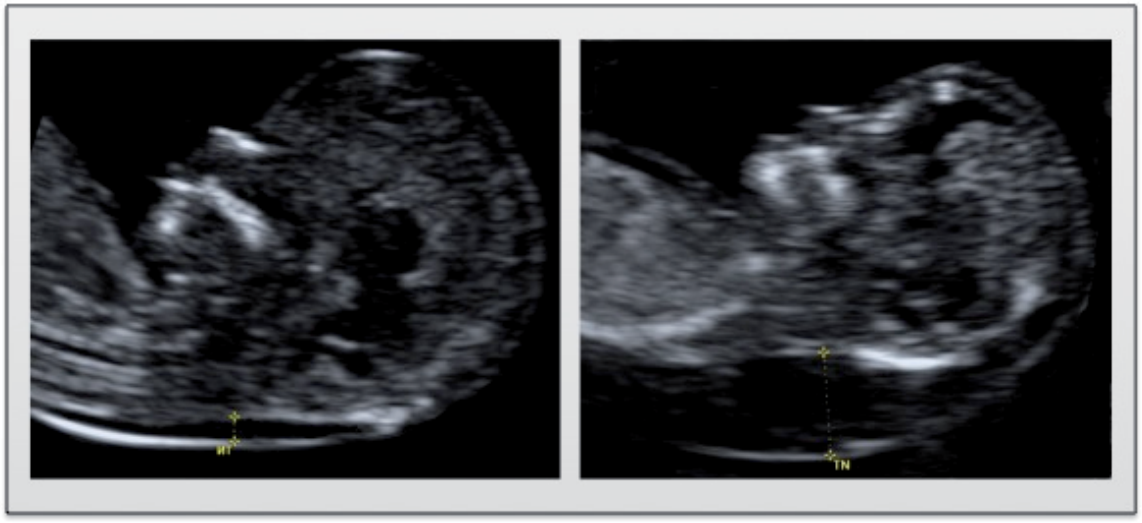

Figure 1.4 Ultrasound picture of 12-weeks euploid fetus with normal NT thickness (left) and trisomy 21 fetus (right) demonstrating increased subcutaneous accumulation of fluid behind the neck.

Maternal serum biochemistry

In trisomy 21 pregnancies at 11-13 weeks, the maternal serum concentration of free ß-hCG is higher than in chromosomally normal fetuses, whereas PAPP-A is lower (about 2 multiples of the median [MoM] and about 0.5 MoM, respectively) (Brizot *et al.*, 1994, 1995; Spencer *et al.*, 1999, 2003a; Bindra *et al.*, 2002). However, in trisomy 21, the deviation from normal in PAPP-A is lower and in free ß-hCG is higher with advancing gestation, and these temporal changes with gestation should be taken into account in the calculation of risk (Spencer *et al.*, 2002). There is no significant association between fetal NT and maternal serum free ß-hCG or PAPP-A in either trisomy 21 or chromosomally normal pregnancies, and therefore the ultrasononographic and

biochemical markers can be combined to provide more effective screening than either method individually. Several prospective studies on more than 100,000 pregnancies, including more than 500 fetuses with trisomy 21, have demonstrated that for a 5% false-positive rate the detection rate of trisomy 21 by the first-trimester combined test is 90% (Nicolaides, 2004; Nicolaides *et al.*, 2005).

1.2.3 Abnormal flow in the ductus venosus

A high proportion of fetuses with trisomy 21 and other chromosomal abnormalities have increased impedance to flow in the Ductus venosus at 11-13 weeks of gestation. In the combined data from seven studies, abnormal ductal blood flow was observed in 5.2% of euploid fetuses and 70.8%, 89.3%, 81.8% and 76.9% of fetuses with trisomies 21, 18 and 13 and Turner syndrome, respectively (Table 1.5) (Matias *et al.*, 1998; Antolín *et al.*, 2001; Murta *et al.*, 2002; Zoppi *et al.*, 2002; Borrell *et al.*, 2003; Toyama *et al.*, 2004; Prefumo *et al.*, 2005).

Table 1.5 Studies reporting on the incidence of abnormal flow in the ductus venosus in the first-trimester in euploid fetuses and in those with trisomy 21, 18, 13, Turner syndrome and other chromosomal abnormalities. Abnormal flow was defined as absent or reverse α-wave or pulsatility index for veins above the 95th centile.

Author	N	Normal	Trisomy 21	Trisomy 18	Trisomy 13	Turner syndrome
Matias *et al.* 1998	486	13/423 (3.1%)	35/38 (92.1%)	12/12 (100%)	5/7 (71.4%)	2/3 (66.7%)
Antolin *et al*. 2001	924	39/911 (4.3%)	5/7 (71.4%)	3/3 (100%)	-	1/1 (100%)
Murta *et al.* 2002	372	7/343 (2.0%)	18/18 (100%)	1/1 (100%)	2/2 (100%)	2/2 (100%)
Zoppi *et al.* 2002	325	38/292 (13.0%)	14/20 (70.0%)	6/7 (85.7%)	1/1 (100%)	1/3 (33.3%)
Borrell *et al.* 2003	3,382	162/3,249 (5.0%)	36/48 (75.0%)	-	-	-
Toyama *et al*. 2004	1,097	69/1,075 (6.4%)	5/7 (71.4%)	3/5 (60.0%)	1/1 (100%)	4/4 (100%)
Prefumo *et al.* 2005	572	26/497 (5.2%)	18/47 (38.3%)	-	-	-
Total	7,158	354/ 6,790 (5.2%)	131/185 (70.8%)	25/28 (89.3%)	9/11 (81.8%)	10/13 (76.9%)

There is uncertainty as to whether the incidence of abnormal ductal blood flow is associated with the other first-trimester sonographic and biochemical markers of chromosomal abnormalities and the extent to which assessment of the ductus venosus would improve the performance of combined first-trimester screening.

1.3 DUCTUS VENOSUS AND CARDIAC DEFECTS

1.1.1 Overview

Abnormalities of the heart and great arteries are the commonest congenital defects and are associated with a high proportion of stillbirths and over half the deaths from congenital malformations in childhood.

Fetal echocardiography during the second trimester of pregnancy is an accurate method of assessing cardiac anatomy and diagnosing fetal cardiac defects. However, echocardiography is time consuming, requires sophisticated ultrasound equipment and expertise, and it is consequently limited to specialist centres. The majority of patients examined in these centres are referred on the basis of being at 'high-risk'. Selection of a 'high-risk' group has traditionally been based on maternal medical and obstetric history and includes such factors as maternal diabetes mellitus, ingestion of teratogens or a family history of congenital heart disease. Although in these patients the risk of cardiac defects is increased, the majority of congenital cardiac defects arise from the low-risk group.

In the late 1980's it was suggested that during the routine second trimester scan an attempt is made to obtain the four-chamber view of the heart and those with suspicious findings should be referred for fetal echocardiography. The results of screening studies in unselected populations have demonstrated the feasibility of incorporating the four-chamber view in the routine scan but the sensitivity of this scan in identifying major cardiac defects is only about 25% (Tegnander *et al.*, 1995). The inclusion of views of the great vessels may improve the sensitivity to about 50% but the expertise necessary for this examination is not widely available.

An overall detection rate of major CHD of 25% has been reported in the United Kingdom (UK) and in other European countries (Bull, 1999; Garne *et al.*, 2001). Successful identification of CHD in the routine anomaly scan has a large variation in different centres, which might be firstly, due to the different policies for screening of fetal abnormalities in different centres or regions, and secondly due to the different

skills of the sonographer involved. Experience of the sonographers and specific training programmes in fetal echocardiography for obstetric sonographers have proved to increase the detection rate for CHD (Hunter *et al.*, 2000; Carvalho *et al.*, 2002a; Tegnander *et al.*, 2006).

Prenatal diagnosis of congenital heart defects allows planning the delivery in a specialized unit, in the best conditions, especially for those fetuses with abnormalities that require a medical or surgical treatment immediately after birth, like the ductal-dependent defects. Prenatal diagnosis of these defects improves preoperative and postoperative mortality in these infants (Bonnet *et al.*, 1999; Khoshnood *et al.*, 2005). Another potential benefit might be reduction of neurological damage secondary to hemodynamic compromise in the infant, although no study has assessed this long-term effect (Allan, 2007).

1.3.2 Prevalence of congenital cardiac defects

Congenital malformations account for a 15% and 23% of the stillbirths and neonatal deaths respectively (CEMACH report, 2008). Congenital heart disease is the commonest form of severe congenital abnormality resulting in stillbirth, neonatal and childhood death and is a major cause of childhood morbidity (Table 1.6) (OPCS, 1997).

Table 1.6 Congenital abnormalities listed as the main cause of stillbirth (SIB), neonatal (NND) or childhood (CHD) death in 1996 (OPCS, 1997).

Congenital abnormality	SB n(%)	NND n(%)	CHD n(%)	Total n(%)
Heart and circulation	51 (15.6%)	228 (39.2%)	238 (53.1%)	517 (38.1%)
Central nervous system	93 (28.4%)	66 (11.3%)	55 (12.3%)	214 (15.8%)
Respiratory system	6 (1.8%)	105 (18.0%)	12 (2.7%)	123 (9.1%)
Musculoskeletal abnormalities	19 (5.8%)	55 (9.5%)	19 (4.2%)	93 (6.9%)
Genitourinary abnormalities	11 (3.4%)	25 (7.6%)	4 (0.9%)	40 (2.9%)
Facial clefts / upper GI abnormalities	10 (3.1%)	14 (2.4%)	27 (6.0%)	51 (3.8%)
Chromosomal abnormalities	62 (19.0%)	41 (7.0%)	45 (10.0%)	148 (10.9%)
Others / unspecified	75 (22.9%)	48 (8.2%)	48 (10.7%)	171 (12.6%)
Total	327	582	448	1357

The prevalence of congenital heart disease is about 5-10 per 1,000 live births and the commonest are ventricular septal defects (Mitchell *et al.*, 1971; EURO-PERISTAT project, 2008).

1.3.3 Screening for fetal cardiac defects

Despite the high mortality of the CHD, the overall detection rate is about 25% (Bull, 1999; Garne *et al.*, 2001).

In the early 80's specialist in fetal echocardiography focused on fetuses with an increased risk of having a congenital heart disease. These high-risk groups included family history of congenital heart disease, maternal diabetes, fetal arrhythmia, fetal hydrops, extracardiac fetal abnormalities and teratogenic drugs.

Family history of congenital cardiac defects

Studies of families with a high prevalence of congenital heart defects support a multifactorial inheritance model participating in the etiology of congenital heart diseases. The theoretical risk of recurrence in sibs calculated for this type of inheritance was 1 to 5%, which was similar to the one observed in this study (Nora, 1968). Furthermore, the risks for some specific defects have been reported to be higher, in particular the abnormalities of the left side of the heart (Allan *et al.*, 1986). The risk of recurrence also increases with the number of siblings affected (Nora, 1968; Allan *et al.*, 1986).

Maternal diabetes mellitus

The introduction of insulin together with improved medical, obstetric and neonatal care has lead to a dramatic improvement in both maternal and perinatal mortality in women with diabetes mellitus. The incidence of congenital malformations in this group has, however, remained similar and consequently congenital abnormalities have become a major cause of perinatal morbidity and mortality in babies born to diabetic mothers (Yang *et al.*, 2006).

Congenital heart disease is one of the commonest types of malformation affecting diabetic pregnancies, with an incidence around 5% (Ramos-Arroyo *et al.*, 1992; Yang *et al.*, 2006; Macintosh *et al.*, 2006). The incidence of congenital abnormalities, including congenital heart defects, in offspring of women with type 2 diabetes and gestational diabetes is also increased (Schaefer-Graf *et al.*, 2000). Increasing hyperglycemia at diagnosis or presentation for care is associated with a higher risk of abnormalities (Miller *et al.*, 1981; Schaefer-Graf *et al.*, 2000).

Despite the high incidence of cardiac defects in diabetic women, the management of pregnant women with diabetes in the UK does not routinely include screening for cardiac defects. A recent study in England, Wales and Northern Ireland has shown that only about 55% of these abnormalities are detected antenatally (Macintosh *et al.*, 2006).

Maternal ingestion of known teratogens

A number of drugs have been implicated as the primary cause of various congenital malformations including congenital heart disease. Providing exact risks for individual compounds is complicated by selective reporting, incomplete data regarding the true number of infants affected and the number of infants exposed, and a possible teratogenic effect of the maternal condition that is being treated.

Some strongly teratogenic compounds, such as thalidomide, where the incidence of congenital heart disease is 10% if ingested between days 20 and 36 after conception, have been withdrawn from the market (Nora *et al.*, 1978). Others, such as alcohol, where 25% of infants affected by fetal alcohol syndrome have congenital heart disease, don't lend themselves to prenatal screening (Jones *et al.*, 1973; Nora *et al.*, 1978; Zierler, 1985). A study with 90 cono-truncal heart abnormalities showed a higher prevalence of maternal alcohol consumption during the first trimester in infants with this heart defect (Tikkanen *et al.*, 1992), but in other studies this association failed to show significance (Adams *et al.*, 1989; Shaw *et al.*, 1992; Carmichael *et al.*, 2003).

Epileptic patients lend themselves to a higher level of antenatal surveillance. This medical disorder is encountered relatively frequently by the obstetrician (0.5 % of women

of child bearing age), and patients are readily identified. The inherent maternal risk of stopping medication has placed a greater emphasis on prenatal diagnosis of any teratogenic effect, although the exact risk is poorly defined. A higher prevalence of physical abnormalities in babies of mothers with epilepsy is associated with the use of anticonvulsivant drugs during pregnancy, rather than with epilepsy itself (Holmes *et al.*, 2001).

A prospective study in the UK with more than 3600 women on antiepileptic drugs during the pregnancy showed a prevalence of 4.2% (95% CI, 3.6-5.0%) of major congenital malformation, with a higher rate for those women in polytherapy than monotherapy, 6% vs. 3.7%. In those on monotherapy, the risk of major congenital malformation was higher for valproate, and in women with polytherapy, also in those combinations containing valproate (Morrow *et al.*, 2006). The specific risk for cardiac defects is unclear. In the study by Morrow, the prevalence of cardiac defects was 0.7%, 0.7%, 0.6% and 1.2% for valproate, carbamazepine, lamotrigine and phenytoin respectively (Morrow *et al.*, 2006). On the other hand, Arpino *et al.*, in a case-control study, showed an increased risk for cardiac defects with phenobarbital (OR 2.20, 95%CI 1.2-4.0, p=0.009) and valproate (OR 1.63, 95%CI 1.0-2.7, p=0.049) (Arpino *et al.*, 2000), and Canger *et al.*, in a prospective observational study found an increased risk with barbiturics (p<0.0009) (Canger *et al.*, 1999).

In the 1970s a strong association was suggested between maternal lithium treatment and congenital heart defects, most commonly Ebstein's anomaly (Nora *et al.*, 1974). More recent studies have shown lower risks (Cohen *et al.*, 1994). Four case-control studies of Ebstein's anomaly involving 207 affected children, found that none of the mothers had taken lithium during the pregnancy (Källén, 1988; Sípek, 1989; Zalstein *et al.*, 1990; Edmonds *et al.*, 1990). In two cohort studies, one of them showed no significant association between lithium intake and risk of cardiac malformations, although the only cardiac abnormality in the 138 exposed women was an Ebstein's anomaly (Jacobson *et al.*, 1992), and the other one found a RR of 7.7 (95%CI, 1.2 - 59.9) and none of the four fetuses with a heart defect had an Ebstein's anomaly (Källén *et al.*, 1983).

Screening through risk factors in the maternal history

Sharland *et al*. reported a series of 222 cases of CHD diagnosed prenatally after referral for fetal echocardiography. High risk pregnancies were referred either for maternal reasons such as a family history of CHD, maternal diabetes or exposure to cardiac teratogens, or for fetal indications such as the detection of hydrops, extracardiac abnormalities, a fetal arrhythmia or an abnormality of the four chamber view of the heart on a routine scan. Although the majority of referrals were for historic indications (67.49%) the majority of CHD was found in fetuses with an abnormal four chamber view (59.46%), and only 10.8% in the high risk group (Sharland *et al.*, 1991). This clearly demonstrated that the historic indications for referral for fetal echocardiography were of less importance in the prenatal diagnosis of congenital heart disease than the sonographic detection of fetal abnormalities. The four chamber view was soon recognised as an important method of screening for CHD and became an integral part of the obstetric anomaly scan.

Screening by nuchal translucency

In 1996 Hyett *et al.* (1996) found an association between NT thickness and CHD in postmortem examination of 21 chromosomally normal fetuses with increased NT after termination of the pregnancy. Subsequently, Hyett *et al.*, (1997a) reported a higher prevalence of CHDs in 1427 chromosomally normal fetuses with increased NT and suggested measurement of NT thickness as a screening method for CHD. A retrospective study of 29,154 screened patients with 50 major cardiac defects, found a detection rate of 56.0% (95% CI 42.0-70.0%) for a false positive rate of 5% (Hyett *et al.* 1999). Subsequent studies have confirmed the utility of this measurement as a marker for CHDs (Makrydimas *et al.*, 2003) with a wide range of detection rates (17% to 56%) (Hyett *et al.*, 1999; Mavrides *et al.*, 2001b).

A meta analysis on a larger number of CHD (n=830) showed a detection rate 35.5% for an NT≥2.5 mm (Makrydimas *et al.*, 2005). The prevalence of CHD is doubled when the NT measurement is between the 95th and the 99th centile, and increases exponentially with the NT thickness for NT measurement above 3.5 mm (Atzei *et al.*, 2005) (Figure 1.5).

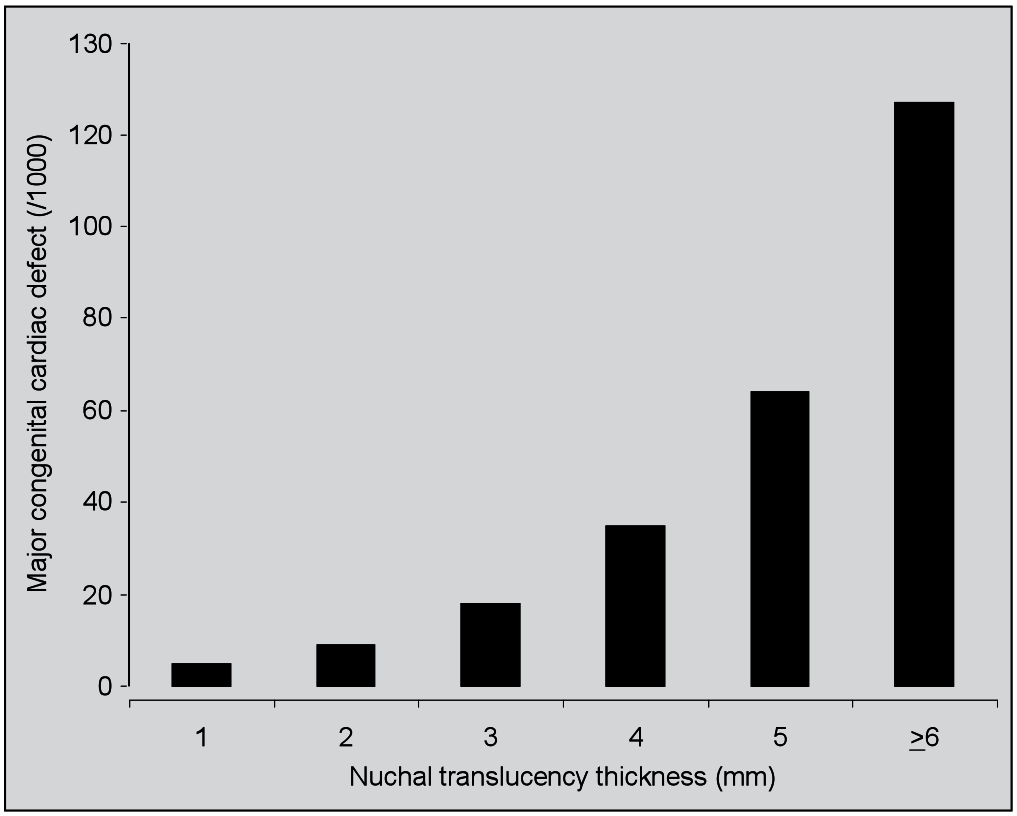

Figure 1.5 Prevalence of CHD increases exponentially with nuchal translucency thickness (Atzei *et al.* 2005).

1.3.4 Ductus venosus and cardiac defects

Abnormal flow in the ductus venosus at 11-13 weeks of gestation is associated with increased risk for chromosomal abnormalities and cardiac defects (Matias *et al.*, 1998, 1999; Borrell *et al.*, 1998; Bilardo *et al.*, 2001; Murta *et al.*, 2002; Zoppi *et al.*, 2002; Haak *et al.*, 2003; Favre *et al.*, 2003; Toyama *et al.*, 2004). In the combined data from seven studies that examined ductus venosus waveforms in 600 chromosomally normal fetuses with increased NT thickness (above the 95th centile) a major cardiac defect was observed in 29 (4.8%) fetuses and 28 (96.6%) of these had abnormal Doppler waveforms in the ductus venosus (Table 1.7). However, there were wide variations between the individual studies in the number of cases examined (16-142), the prevalence of cardiac defects (1.7-9.5%), and the incidence of abnormal ductus venosus waveforms in those without cardiac defects (0-40%).

Table 1.7 Studies reporting on the relationship between ductus venosus waveforms and major cardiac defects in euploid fetuses with nuchal translucency thickness above the 95th centile.

Author	Total	Cardiac defects	Abnormal ductus venosus flow	
			No cardiac defects	Cardiac defects
Matias *et al*. 1999	142	7 (4.9%)	4/135 (3.0%)	7/7 (100%)
Bilardo *et al.* 2001	69	4 (6.8%)	19/65 (29.2%)	4/4 (100%
Murta *et al.* 2002	16	1 (6.3%)	0/15 (0.0%)	1/1 (100%)
Zoppi *et al.* 2002	115	2 (1.7%)	30/113 (26.5%)	2/2 (100%)
Haak *et al.* 2003	22	2 (9.1%)	8/20 (40.0%)	2/2 (100%
Favre *et al.* 2003	95	9 (9.5%)	20/86 (23.3%)	9/9 (100%)
Toyama *et al.* 2004	141	4 (2.8%)	23/137 (16.8%)	3/4 (75%)
Total	600	29 (4.8%)	104/571 (18.2%)	28/29 (96.6%)

1.4 DUCTUS VENOSUS AND FETAL GROWTH RESTRICTION (FGR)

1.4.1 Overview

Fetal growth and weight at birth depends on the genetically predetermined growth potential, and is modulated by four variables: maternal health, fetal health, placental health and external factors (Gardosi *et al.*, 1992; Baschat, 2004a).

Rather than a disease on its own, fetal growth restriction is a sign or a manifestation of an underlying disease. It can be the only sign or it might be associated with other abnormalities or signs. There is a wide range of conditions that can lead to a fetal growth restriction:

1- Maternal diseases: hypertensive disease, renal disease, thrombophilia, antiphospholipid syndrome, vascular disease
2- Fetal abnormalities such as chromosomal abnormalities or genetic syndromes, congenital structural abnormalities.
3- Placental disease: placental insufficiency
4- External factors: infections (toxoplasmosis, cytomegalovirus (CMV) and other viral infections), smoking, drugs

1.4.2 Diagnostic approach to FGR

Fetal growth restriction is defined as an estimated weight (calculated using an algorithm that combines head circumference, abdominal circumference and femur length) below the 10th centile or an abdominal circumference below the 5th, 3rd or 2nd centile. Abdominal circumference is the measurement with a highest detection rate (Tamura *et al.*, 1980), which might be improved by serial measurements at least 14 days apart (Divon *et al.*, 1986).

The differential diagnosis of small for gestational age fetuses includes (Figure 1.6):

- **Wrong dates.** The fetus is anatomically normal, the measurements are symmetrically small, amniotic fluid, fetal activity and placental Doppler are normal. A repeat scan in two weeks shows normal growth velocity.

- **Normal small**, which constitute about 80% of the SGA. The fetus is anatomically normal, the measurements symmetrically small, the amniotic fluid, fetal activity and placental Doppler are normal. A repeat scan two weeks apart shows a decrease in growth velocity. These fetuses are not at increased risk of perinatal death.

- **Starving small**. Accounting for about 15% of the total. These fetuses are anatomically normal and the measurements are asymmetrically small, with the abdomen and femur being more affected than the head. The amniotic fluid and fetal activity are reduced. The Doppler studies show an increased pulsatility index (PI) in the uterine arteries and/or the umbilical arteries, reduced PI in the middle cerebral artery, and the ductus venosus flow may be abnormal. The fetal heart rate pattern may also be abnormal. A repeat scan in two weeks shows a marked decreased growth velocity.

- **Abnormal small**, accounting for about 5% of the total. The fetus may have abnormalities suggestive of aneuploidies, genetic syndromes or viral infections. The measurements may be symmetrically small or asymmetrical decreased in

the head and limbs. The amniotic fluid volume and fetal activity may be decreased, increased or normal. The fetal and placental Doppler indices, and the fetal heart rate pattern may be normal or abnormal. A repeat scan in two weeks may show a decrease in growth velocity.

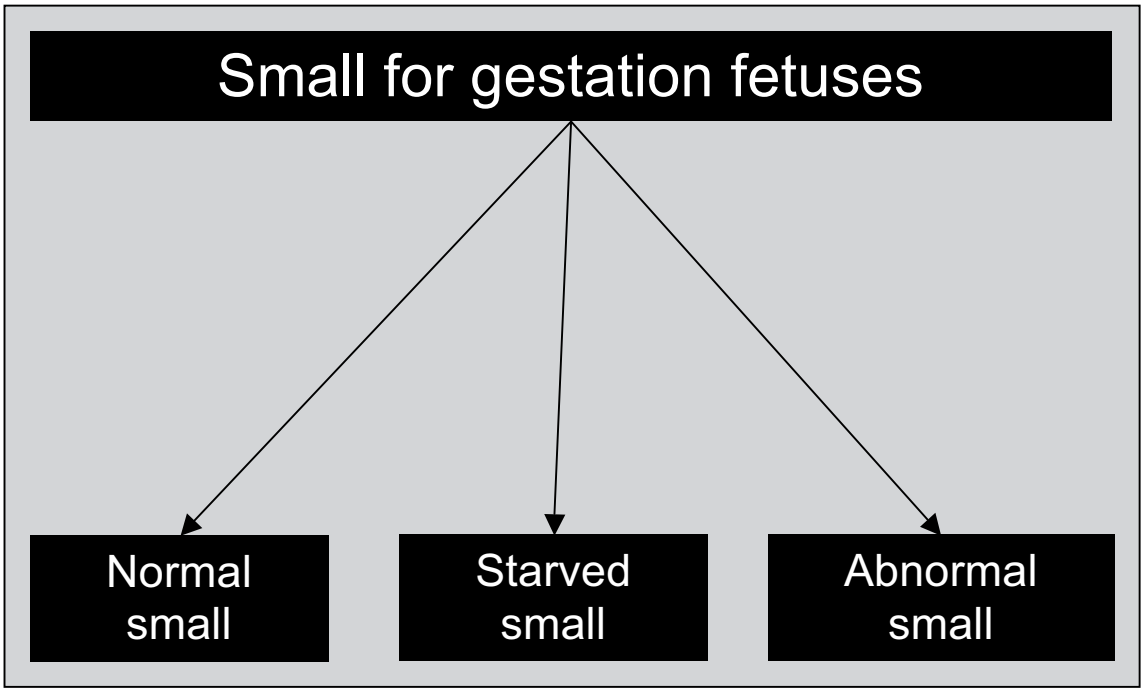

Figure 1.6 Differential diagnosis of small for gestational age fetuses.

It is necessary to identify those small fetuses that are normally grown from the growth restricted fetuses with an underlying condition. A complete evaluation of the maternal history, fetal anatomy, amniotic fluid volume, placental appearance and Doppler examination of the blood flows is necessary for the differential diagnosis. Placental disease and fetal abnormalities are responsible for the vast majority of the fetal growth restrictions (Khoury *et al.*, 1988; Snijders *et al.*, 1993; Sickler *et al.*, 1997; Odegård *et al.*, 2000). A detailed study of the fetal anatomy may identify other structural abnormalities, skeletal dysplasias, aneuploidy markers or signs of viral infections. Increased amniotic volume will point towards a chromosomal abnormality or viral infection, whereas normal or reduced amniotic fluid is compatible with placental insufficiency (Sickler *et al.*, 1997). Further test such as fetal karyotyping, maternal

serology (TORCH), or maternal thrombophilia studies may confirm some of the underlying causes of FGR if these are suspected.

An accurate dating of the pregnancy is basic, in order to use appropriate reference ranges. The last menstrual period (LMP) is used to date the pregnancy, unless the mother has irregular periods, is unsure about the LMP, or was on contraceptive drugs or breastfeeding less than 3 cycles before getting pregnant. In these cases the pregnancy dating is based on ultrasound biometries, preferably on the fist trimester scan as estimation of the gestational age based on ultrasound has a larger predictive error as the pregnancy advances (7 days in the first, 14 days in the second and 21 days in the third trimester).

When FGR is suspected and other causes have been excluded, Doppler ultrasound provides useful information about the feto-placental vascular function (Baschat *et al.*, 2000). Combination of Doppler with fetal biometries identifies those fetuses at increased risk for adverse outcome from placental insufficiency (Hecher *et al.*, 1992; Ott, 2000). In growth restricted fetuses presenting before 34 weeks of gestation, umbilical artery Doppler is frequently abnormal (Hecher *et al.*, 1992; Baschat *et al.*, 2000). After 34 weeks umbilical artery Doppler could be normal but redistribution may still occur (Hershkovitz *et al.*, 2000). Therefore, beyond 34 weeks attention should focus on the middle cerebral artery PI. Randomised trials and meta-analyses have shown that the use of the umbilical artery Doppler to differentiate the constitutionally small fetuses from the FGR, in need for surveillance and possible intervention, results in a significant reduction in perinatal mortality and unnecessary obstetric interventions (Neilson *et al.*, 2000; Westergaard *et al.*, 2001).

1.4.3 FGR due to placental insufficiency

Fetal growth restriction due to placental insufficiency is associated with a bad short-term and long-term prognosis. These fetuses are at substantially increased risk of perinatal mortality and long-term morbidity, including neurodevelopmental delay (Taylor *et al.*, 1989; Hecher *et al.*, 1992; McIntire *et al.*, 1999; Bernstein *et al.*, 2000; Bukowski, 2004). In addition, FGR is associated with increased susceptibility to type 2 diabetes mellitus, hypertension, dyslipidemia, obesity and cardiovascular disease in adulthood. This group

of conditions is also known as metabolic syndrome. These diseases may be a consequence of 'programming', where a stimulus or an insult at a critical period of the fetal life alters the structure, physiology and metabolism of the developing organism (Barker, 1992; Eleftheriades *et al.*, 2006).

Fetal cardiovascular changes

In FGR due to placental insufficiency there is an increased impedance to flow at the uterine arteries (high PI), which reflects an increased spiral artery blood flow resistance in the maternal compartment of the placenta (Papageorghiou *et al.*, 2001). In the fetal compartment, there is an increased PI at the umbilical artery, secondary to the decreased umbilical artery end diastolic velocity (UA EDV) (Rigano *et al.*, 2001). When 30% of fetal villous vessels are abnormal, umbilical artery end-diastolic velocity decreases and the PI increases (Giles *et al.*, 1985), and when 60 to 70% is damaged, absent or reversed end-diastolic flow may be seen (Morrow *et al.*, 1989).

In response to the associated hypoxemia there is redistribution of the fetal circulation, where the fetus increases the blood supply to the brain, myocardium and adrenal glands, and reduces the perfusion to kidneys, gastrointestinal tract and extremities.

Fetal circulatory response can be divided in early and late response (Hecher *et al.*, 2001b; Ferrazzi *et al.*, 2002). Increased placental flow resistance increases right ventricle afterload, resulting in a shift of the blood flow towards the left heart. The increase in the left heart output increases the blood flow to the upper part of the body. These findings can be documented with Doppler indices by a decrease in the ratio of cerebral and umbilical arteries (cerebroplacental Doppler ratio) (Gramellini *et al.*, 1992). Cerebral blood flow also increases by an active autoregulation response reducing the blood flow resistance. This results in a reduction of the middle cerebral artery PI (brain sparing) (Wladimiroff *et al.*, 1986). During this period, there is a fetal hypoxemia, but the pH is usually maintained within the normal range (Akalin-Sel *et al.*, 1994; Baschat, 2003).

Late circulatory changes are a consequence of a further metabolic and circulatory deterioration. When there is a failure of the physiologic adaptation to hypoxemia, cardiac compromise is manifested as an increased impedance to the ductus venosus flow, with

absent or reversed flow during atrial contraction, as a result of the high pressure at the right atrium (Hecher *et al.*, 1996). Further deterioration leads to holosystolic tricuspid insufficiency, leading, finally, to fetal demise (Rizzo *et al.*, 1994b). Elevation of venous Doppler indices shows an increased risk of fetal academia (Baschat *et al.*, 2004b).

Fetal biophysical changes

Early and late changes can also be observed in fetal behavioural response to placental insufficiency. In fetal growth restriction there is a delayed maturation of the fetal heart rate (FHR) control: delayed decline of the average baseline heart rate with advancing gestational age, decreased short-term and long-term variability and delayed reactivity maturation (Visser *et al.*, 1990). Once fetal hypoxemia is established, there is a decline in amniotic fluid volume and a reduction of the global fetal activity. With increasing hypoxemia there is a reduction of fetal breathing and gross body movements (Ribbert *et* al., 1993a,b). With the development of acidemia there is a loss of fetal movements and tone, and fetal heart rate decelerations and loss of short-term variability can be documented (Ribbert *et al.*, 1990; Vintzileos *et al.*, 1991; Manning *et al.*, 1993; Baschat *et al.*, 2001).

Antenatal surveillance of FGR due to placental insufficiency

FGR fetuses are at increased risk of adverse outcome and complications associated to prematurity, especially for those delivered before 32 weeks (Bernstein *et al.*, 2000; Baschat *et al.*, 2007).

Determination of the monitoring intervals and timing for intervention are key elements to reduce perinatal mortality and morbidity (Divon *et al.*,1989). Antenatal surveillance should integrate Doppler parameters with the biophysical profile, as they provide information about the fetal deterioration rate and the acid-base fetal status respectively (Baschat *et al.*, 2001).

The monitoring interval should be inversely related to the severity of the fetal compromise and delivery should be considered when intrauterine risks exceed neonatal risk for adverse outcome.

It is important to know the changes preceding stillbirth:

- Stillbirth before 34 weeks: there is a major increase in the umbilical artery and ductus venosus PI and decrease in the amniotic fluid volume, fetal tone and movements. Weekly umbilical artery Doppler is suggested, and the monitoring interval should be shortened if there is an increase in umbilical artery or ductus venosus PI, or a decrease in amniotic fluid volume with daily scans if necessary.

- Stillbirth after 34 weeks: there is a decrease in the middle cerebral artery PI, fetal heart rate variability and amniotic fluid volume.

Delivery should be considered as the prematurity-related risks decrease or if there is a fetal deterioration, increasing substantially the risk of brain damage or intrauterine demise. Between 24 and 28 weeks the chance of intact survival increases 2% per day in utero, and about 1% until 32 weeks.

Therefore, the threshold for delivery will change with gestational age (Baschat *et al.*, 2007):

- Before 28 weeks: reversed a-wave in the ductus venosus and reversed end-diastolic flow in the umbilical artery and <2 cm deepest pool of amniotic fluid and no fetal movements.

- 28-30 weeks: reversed a-wave in the ductus venosus or reversed end diastolic flow in the umbilical artery and <2 cm deepest pool of amniotic fluid and no fetal movements.

- 31-33 weeks: absent end-diastolic flow in the umbilical artery or absent a-wave in the ductus venosus or <2 cm deepest pool of amniotic fluid and no fetal movements.

- After 34 weeks: high PI in the umbilical arteries or high PI in the ductus venosus or low PI in the middle cerebral artery or amniotic fluid index below the 5th centile.

1.4.4 Role of the ductus venosus in FGR

In 1994 two studies (Kiserud *et al.*, 1994b; Rizzo *et al.*, 1994a) showed a lower velocity during the atrial contraction in fetuses with growth restriction and this was more pronounced in fetuses with higher pulsatility index at the umbilical artery or if there was an absent or reversed end diastolic flow at the umbilical artery. In 1995 Hecher *et al.* found a higher PIV in compromised growth restricted fetuses before 32 weeks, but there was no difference between the compromised and no compromised fetuses after 32 weeks (Hecher *et al.*, 1995a). A higher pulsatility index of the ductus venosus is a marker for fetal acidosis in growth restricted fetuses (Hecher *et al.*, 1995b; Baschat *et al.*, 2004b).

In growth restricted fetuses there is an increased cardiac afterload due to high placental resistance and a peripheral fetal arterial vasoconstriction (Hecher *et al.*, 1995 b). In the presence of fetal hypoxemia and acidemia the myocardium has a reduced compliance (Rizzo *et al.*, 1991). The combination of an increased cardiac afterload and a myocardial dysfunction leads to an increased end-diastolic ventricular pressure resulting in an abnormal blood flow at the ductus venosus, with higher pulsatility, mainly due to a reduction of the a-wave velocity and in the most severe cases an absent or reversed a-wave (Hecher *et al.*, 1995a; Rizzo *et al.*, 1996).

Ductus venosus Doppler is a useful parameter to determine the timing of delivery. In severe growth restricted fetuses before 32 weeks, there is an increased umbilical artery pulsatility index, a middle cerebral artery pulsatility index, and reduced amniotic fluid index for four or five weeks before delivery (Figure 1.7). These parameters show changes about two weeks before delivery, but the ductus venosus Doppler and short term variation of the heart rate only become abnormal a few days before delivery (Hecher *et al.*, 2001b; Baschat *et al.*, 2001). A more recent study has also shown that the ductus venosus is a good predictor of neonatal outcome in growth restricted fetuses before 33 weeks (Baschat *et al.*, 2007).

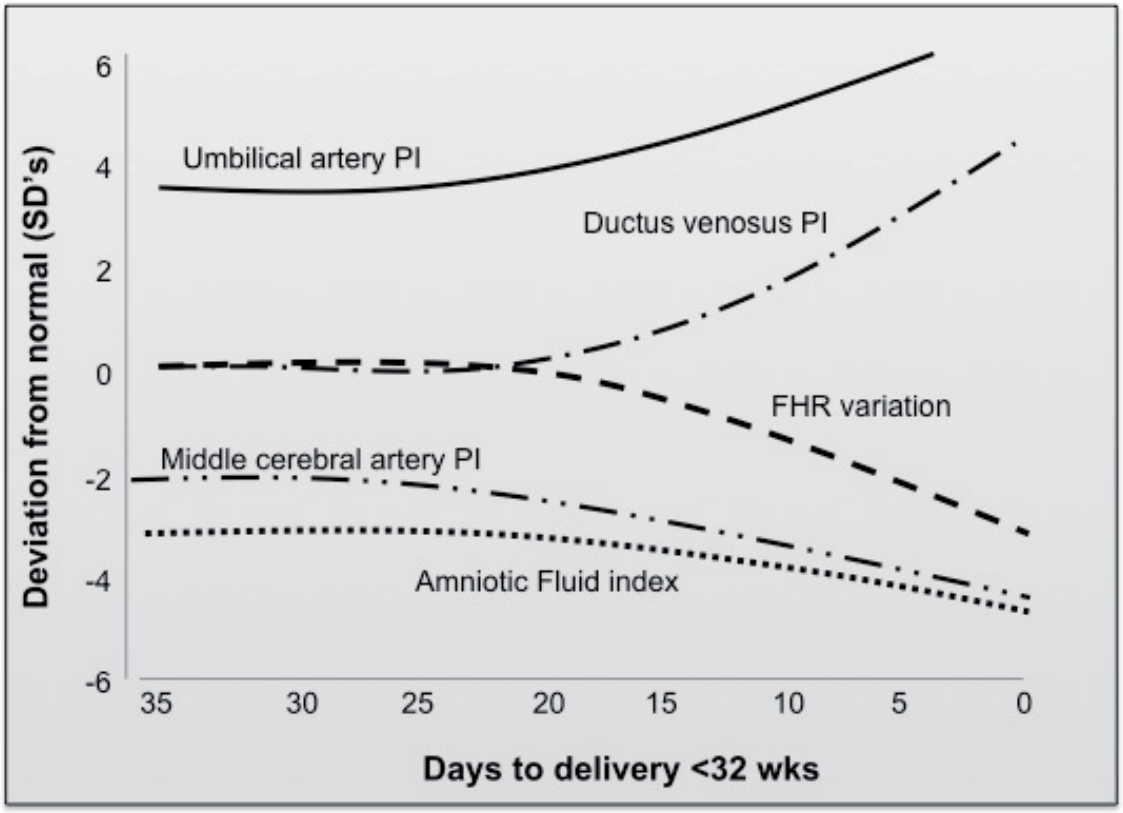

Figure 1.7 Sequence of changes of the umbilical artery, middle cerebral artery and ductus venosus Doppler, amniotic fluid index and fetal heart rate in growth restricted fetuses delivered before 32 weeks. Hecher *et al.*, 2001b.

1.5 DUCTUS VENOSUS AND ADVERSE PREGNANCY OUTCOME

1.5.1 Pregnancy loss

Other adverse pregnancy outcomes include pregnancy loss, either due to miscarriage (fetal death before 24 weeks) or fetal loss (death at or after 24 weeks) prior to labor. The risk of fetal loss increases with advanced gestational and maternal age, obesity, parity, smoking and poor obstetric history (Cnattingius *et al.*, 2002; Fretts, 2005).

First trimester screening for pregnancy loss

First trimester markers for chromosomal abnormalities are also associated to an increased risk for fetal death (Wald *et al.*,2003a; Goetzl *et al.*,2004). Several studies

have demonstrated an association between low PAPP-A and pregnancy loss before and after 24 weeks (Westergaard *et al.*, 1983; Ong *et al.*, 2000; Yaron *et al.*, 2002a; Wald *et al.*, 2003a; Goetzl *et al.*, 2004; Smith *et al.*, 2004; Dugoff *et al.*, 2004; Spencer *et al.*, 2006) (Figure 1.8).

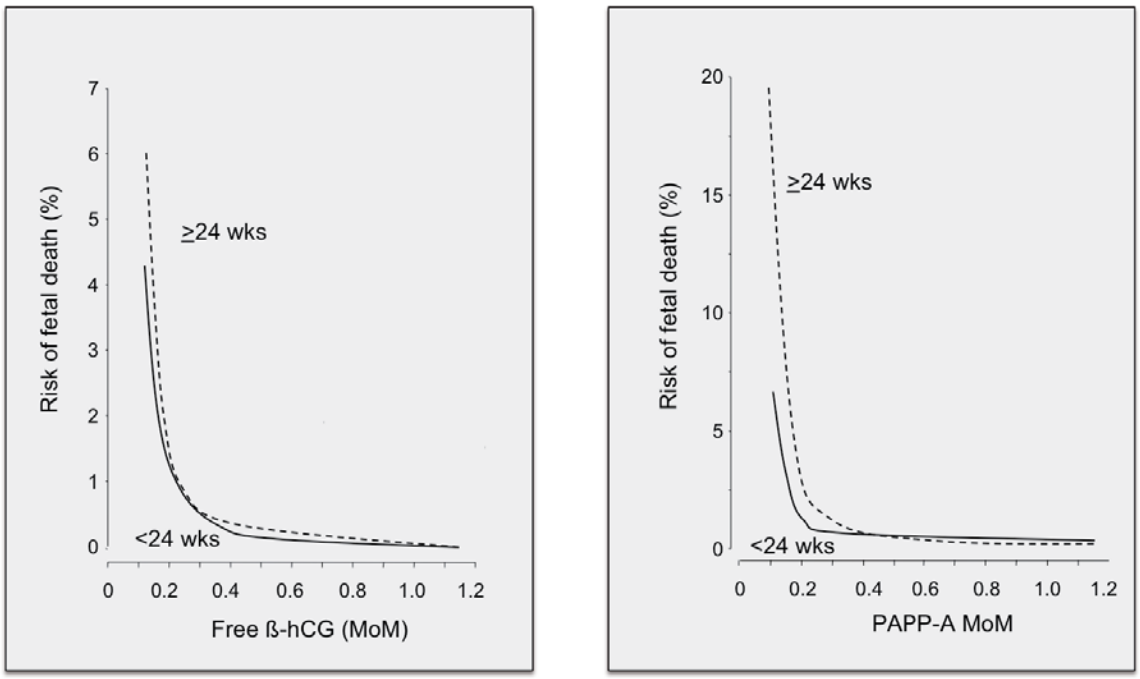

Figure 1.8 Relationship between maternal serum free β-hCG (left) and PAPP-A (right) with the risk of pregnancy loss before 24 weeks (solid line), and at or after 24 weeks (interrupted line) (Spencer *et al.*, 2006).

Low levels of free β-hCG have also been associated with an increased risk of miscarriage (Yaron *et al.*, 2002b; Wald *et al.*, 2003a; Goetzl *et al.*, 2004; Dugoff *et al.*, 2004; Spencer *et al.*, 2006) (Figure 1.8) and intrauterine death at or after 24 weeks although some studies have failed to find this association (Ong *et al.*, 2000; Smith *et al.*, 2004). These low levels of PAPP-A and free β-hCG are presumably indicating an impaired placentation that will lead to an early or late fetal death (Spencer *et al.*, 2006).

Increased NT is also associated with fetal death, and the risk increases exponentially with the NT thickness. The majority of fetuses die before 24 weeks (Souka *et al.*, 2001; Michailidis *et al.*, 2001; Wald *et al.*, 2003a; Goetzl *et al.*, 2004; Dugoff *et al.*, 2004). The results are contradictory for fetal death at or after 24 weeks, as some studies found a

significant association (Souka *et al.*, 2001; Michailidis *et al.*, 2001; Wald *et al.*, 2003a) but not others (Goetzl *et al.*, 2004; Dugoff *et al.*, 2004) (Figure 1.9).

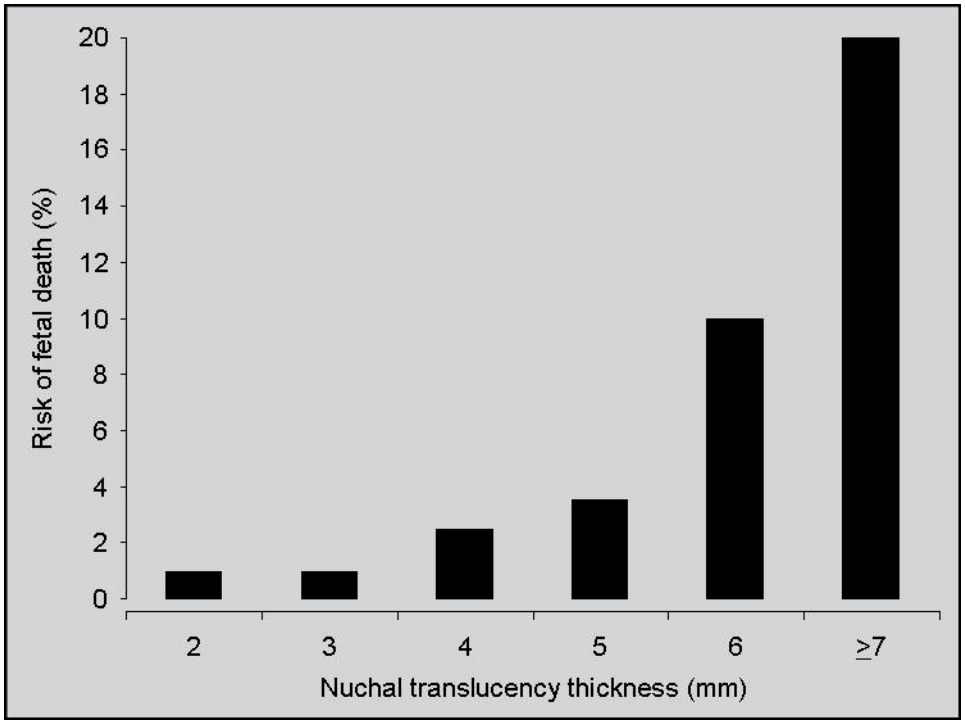

Figure 1.9 Relationship between nuchal translucency thickness and the risk of fetal death (Souka *et al.*, 2005)

Ductus venosus in the first trimester and pregnancy loss

There is also some evidence that abnormal flow in the ductus venosus is associated with an increased risk for fetal death. A first-trimester screening study reported that the prevalence of abnormal flow in the ductus venosus in the cases resulting in fetal death was 22.2% (4 of 18) compared to 5.9% (61 of 1,041) in the normal outcome group (Toyama *et al.*, 2004). However, abnormal flow was observed in 4 of the 8 fetal deaths presenting with NT above the 95th centile and in none of the 10 with normal NT. A case-control study of fetuses with NT below the 95th centile reported that in 10 of 42 (23.8%) cases with abnormal flow in the ductus venosus there was an adverse outcome, including perinatal death and chromosomal, cardiac or non-cardiac abnormalities (Oh *et*

al., 2007). In contrast, an adverse outcome was observed in only 1 of 83 (1.2%) controls with normal flow in the ductus venosus.

1.5.2 Major abnormalities

Major fetal abnormalities are defined as those requiring medical and/or surgical treatment or conditions associated with mental handicap. Despite most of ultrasonographic features have been described more than 20 years ago, there is a continuing problem to detect a large percentage of major defects (Garne *et al.*, 2005) (Figure 1.10).

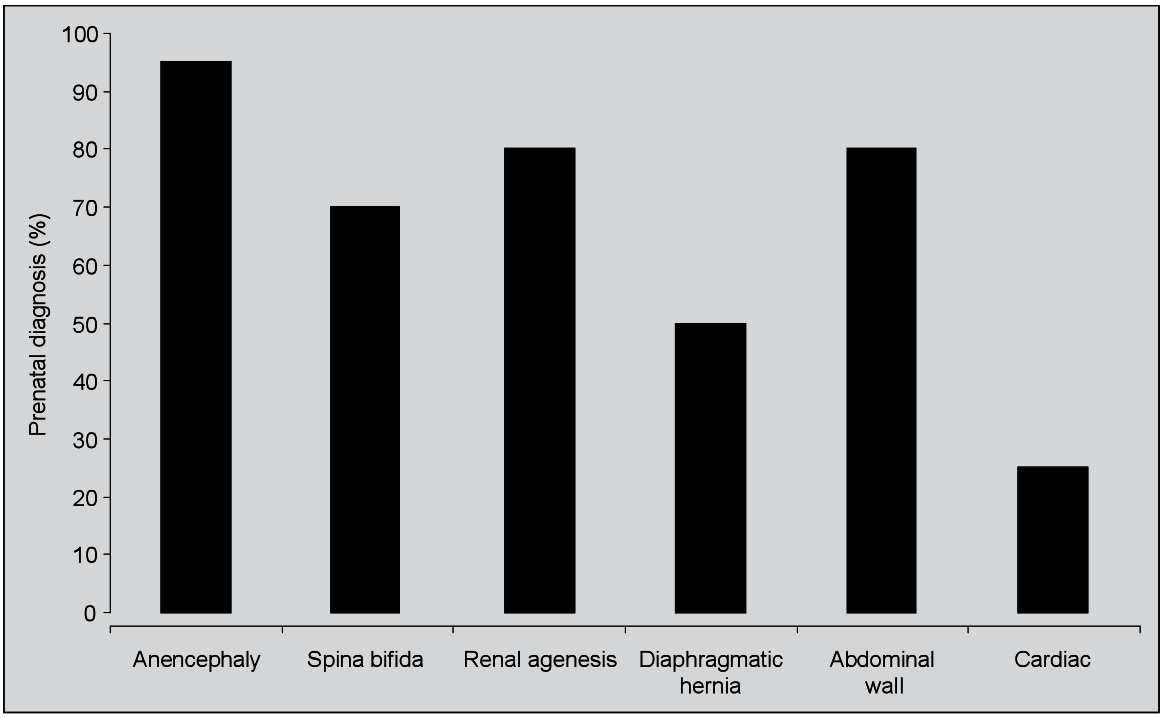

Figure 1.10 Detection rates of major congenital abnormalities in a multicentric European study (Garne *et al.*, 2005)

First trimester screening for major abnormalities

At 11-13 weeks a detailed anatomy exam of the fetus is feasible. Successful visualization of the fetal anatomy depends on the fetal CRL, maternal body mass index (BMI), and availability of transvaginal probe (Souka *et al.*, 2004). Combined data from six studies has shown that more than half of the major structural abnormalities can be diagnosed in the first trimester (Table 1.8; Hernádi *et al.*, 1997; Economides *et al.*, 1998; Carvalho *et*

al., 2002b; Chen *et al.*, 2004; Souka *et al.*, 2006; Becker *et al.*, 2006). There are wide variations between the individual studies in the detection rates (from 38% to 84%) and adequate examination time and a proper training of the sonographers appear to be factors improving the sensitivity of the scan (Taipale *et al.*, 2003, 2004; Souka *et al.*, 2006).

Table 1.8 Studies reporting on the detection rate for major structural abnormalities prenatally and in the first trimester of pregnancy

			Ultrasound detection rate	
Author	**n**	**Major defects (%)**	**TOTAL**	**First trimester**
Hernádi *et al*. 1997	3,991	49 (1.2%)	39 (79.6%)	20 (40.8%)
Economides *et al.* 1998	1,632	13 (0.8%)	10 (76.9%)	7 (53.9%)
Carvalho *et al.* 2002b	2,853	66 (2.3%)	52 (78.8%)	25 (37.9%)
Chen *et al.* 2004	1,609	26 (1.6%)	20 (76.9%)	14 (53.8%)
Souka *et al.* 2006	1,148	14 (1.2%)	13 (92.9%)	7 (50%)
Becker *et al.* 2006	3,094	86 (2.8%)	81 (94.2%)	72 (83.7%)
TOTAL	**14,327**	**254 (1.8%)**	**215 (84.7%)**	**145 (57.1%)**

Screening for major abnormalities by nuchal translucency

In chromosomally normal fetuses the risk of major structural defects is increased in fetuses with increased NT (Souka *et al.*, 2001; Michailidis *et al.*, 2001). The prevalence of major abnormalities increases with increasing NT, from 1.6% in when the NT is below the 95th centile, to 2.5% for NT between the 95th and the 99th centile, and exponentially thereafter to about 45% in those with NT of 6.5 mm or more (Souka *et al.*, 2005).

A wide range of conditions have been reported in fetuses with increased NT. Different mechanism have been suggested for this association (Souka *et al.*, 2001):

1- Cardiac failure, in association with cardiac defects
2- Venous congestion of the head and neck, in association with the constriction of superior mediastinal compression, in diaphragmatic hernia, narrow chest in skeletal dysplasia, or constriction of the upper part of the body in amnion rupture sequence.

3- Failure of the lymphatic drainage, due to an abnormal anatomy of the lymphatic system or to reduced fetal movements in neuromuscular fetal disorders.
4- Altered composition of the fetal dermis,
5- Fetal anemia and hypoproteinemia,
6- Congenital infection,

In the combined data from four studies, the detection rate of NT thickness for structural abnormalities is 35% (Table 1.9). The wide range of detection rates (from 12.8% to 68.6%) might be due to the different definitions of major structural abnormalities. Becker *et al.* excluded abnormalities that would be compatible with a normal life such as unilateral renal agenesis, cleft lip or polydactyly, which are unlikely to have an increased NT thickness, and they did not exclude chromosomal abnormalities (Becker *et al.* 2006).

Table 1.9 Studies reporting on the detection rate for major structural abnormalities prenatally by first trimester nuchal translucency thickness

Author	n	Major abnormalities (%)	NT Cut-off	NT Detection rate
Michailidis *et al.* 2001	6,606	125 (1.9%)	95th centile	16 (12.8%)
Chen *et al.* 2004	1,503	26 (1.7%)	95th centile	7 (26.9%)
Souka *et al.* 2006	1,148	14 (1.2%)	95th centile	5 (35.7%)
Becker *et al.* 2006	3,094	86 (2.8%)	2.5 mm	59 (68.6%)
TOTAL	**12,351**	**251 (2.0%)**		**87 (34.7%)**

In the same study, Becker *et al.* found that the detection rate of major abnormalities was markedly higher (98.3%, 58/59) in the group of fetuses with NT ≥ 2.5 mm than in the group with NT < 2.5 mm (51.9%, 14/27) (Becker *et al.* 2006).

Screening for major abnormalities by ductus venosus flow

Some of the studies assessing the association of abnormal ductus venosus flow and chromosomal abnormalities or cardiac defects have reported non-cardiac structural abnormalities in fetuses with abnormal ductus venosus flow (Table 1.10).

Table 1.10 Different non-cardiac structural abnormalities reported in fetuses with abnormal ductus venosus flow

Author	Abnormality
Matias *et al.* 1999	Chrondrodysplasia punctata
Bilardo *et al.* 2001	Multiple congenital abnormalities (Fryns syndrome)
	Adult polycystic kidney disease
Oh *et al.* 2007	Omphalocele, dolicocephaly, bilateral club feet
	Omphalocele
	Multicystic renal dysplasia
	Unilateral renal agenesis

1.6 DUCTUS VENOSUS AND TWIN PREGNANCIES

1.6.1 Overview

About 1% of the pregnancies are twin pregnancies, with two-thirds being dizygotic and one-third monozygotic. All dizygotic pregnancies are dichorionic. In monozygotic twins, two thirds are monochorionic and one-third dichorionic, depending if the splitting of the single embryonic mass into two happens within three days post conception (dichorionic) or after (monochorionic). Therefore, all monochorionic twins are monozygotic, and six out of seven dichorionic are dizygotic.

The prevalence of twin pregnancies has increased in the last twenty years, especially for dizygotic twins, due to the increase in use of assisted reproduction techniques, and to increasing maternal age. The prevalence of dizygotic twins is higher in Black than White women, it increases with maternal age, and with the use of ovulation drugs. The prevalence of monozygotic twins is not influenced by the ethnic group or maternal age, but it is about 2-3 times higher in women having *in vitro* fertilization.

Chorionicity can be determined reliably by ultrasound examination at 11 to 14 weeks of pregnancy; in dichorionic twins there is an extension of placental tissue into the base of the inter-twin membrane, referred to as the lambda sign (Sepulveda *et al.*, 1996) (Figure 1.11).

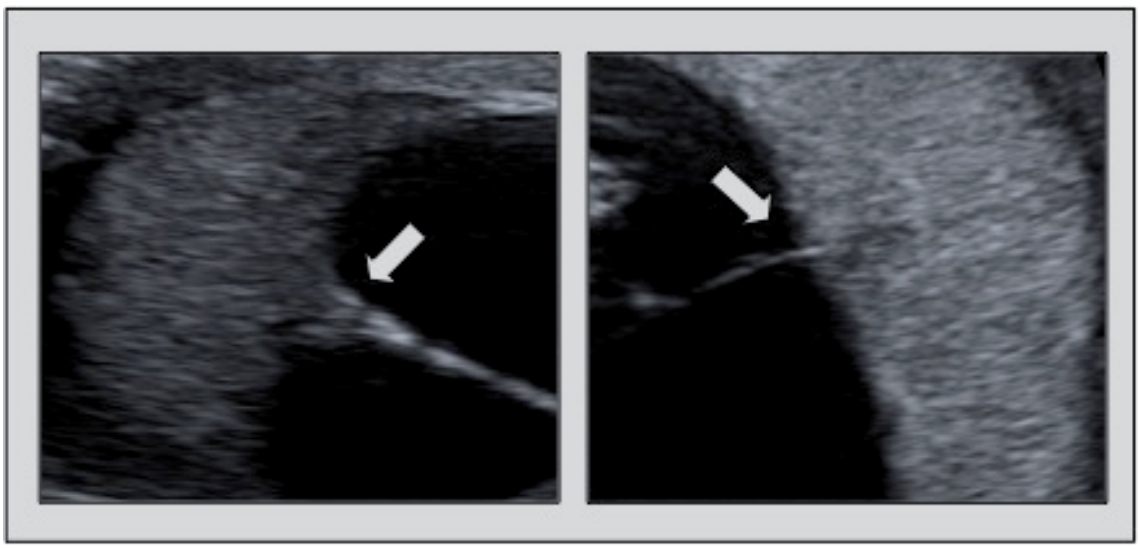

Figure 1.11 Dichorionic pregnancy with extension of placental tissue into the base of the inter-twin membrane, lambda sign (left), and monochorionic pregnancy with absence of placental tissue in the base of the inter-twin membrane (right).

1.6.2 Pregnancy complications in twins

Chorionicity, rather than zygosity will determine the pregnancy outcome (Sebire *et al.*, 1997a) (Table 1.11).

Table 1.11 Prevalence of pregnancy complications in singleton and twin pregnancy according to chorionicity (Sebire *et al.*, 1997a).

	Singletons	Dichorionic	Monochorionic
Miscarriage (11-23 weeks)	1%	2%	10%
Perinatal death (> 23 weeks)	0.5%	2%	4%
Fetal growth restriction	5%	20%	30%
Preterm delivery	1%	5%	10%
Major defects	1%	1%	4%

In monochorionic twins the rate of pregnancy loss between 10 and 23 weeks is about 5 times higher than in dichorionic twins. This is likely to be due to severe early onset of twin-to-twin transfusion syndrome. The increased perinatal mortality in twins is mainly

due to prematurity, which is about 5 times higher in dichorionic twins than in singletons, and ten times higher in monochorionics. One of the causes of the higher perinatal mortality, and also increased prematurity rate in monochorionic twins is twin-to-twin transfusion syndrome.

In twin pregnancies, the risk of fetal growth restriction in at least one of the fetuses is about four times higher than in singleton pregnancies. The chance of a growth restriction of both twins is about four times higher in monochorionic (7.5%) compared to dichorionic twins (1.7%) (Sebire *et al.*, 1997a). Fetal growth restriction in dichorionic twins might be due to a discordance in the effectiveness of trophoblastic invasion of the maternal spiral arteries and therefore a placental impairment. In monochorionic twins three principal factors influence the fetal growth: the degree of placental sharing, the quality of implantation of each placental portion and the angioarchitecture (Lewi *et al.*, 2003).

Selective FGR in monochorionic twins

Severe selective FGR in monochorionic twins has a higher morbidity in monochorionics when compared to dichorionic twin pregnancies (Victoria *et al.*, 2001). Selective FGR in monochorionic twins has been classified in three groups according to the umbilical artery Doppler in the smaller twin: type I, when there is a positive flow; type II, if there is a persistent absent or reversed end diastolic flow; and type III when there is an intermittent absent or reversed end diastolic flow (Gratacós *et al.*, 2007). The three groups have a different clinical behaviour, and patterns of placental anastomoses (Table 1.12).

Table 1.12 Different clinical behaviour, prognosis and pattern of placental anastomoses according to the classification of the selective FGR in monochorionic twins (Gratacós *et al.*, 2007).

Parameter	Type I	Type II	Type III
***In-utero* deterioration of FGR fetus**	0%	90%	10.8%
Unexpected intrauterine fetal death of small twin	2.6%	0%	15.4%
Parenchimal brain damage of large twin	0%	3.3%	19.7%
Arterio-arterial anastomoses > 2 cm	70%	18%	98%

FGR= fetal growth restriction. Umbilical artery Doppler in the smaller twin: Type I: positive end-diastolic flow; Type II= persistent absent or reversed end-diastolic flow; Type III= intermittent absent or reversed end-diastolic flow.

In the type III group, there is a higher risk of neurological damage in the large twin, irrespective of whether there is an intrauterine death of the co-twin or not (Gratacós *et al.*, 2004).

1.6.3 Twin to twin transfusion syndrome (TTTS)

The higher morbidity and mortality in monochorionic twins is a consequence of the presence of vascular anastomoses in 96% of the cases. There are three types of anastomoses: arterio-arterial, arterio-venous and venous-venous. In about 15% of monochorionic twins there is a chronic imbalance in the net flow across the anastomoses, usually due to the presence of arterio-venous anastamoses, with blood flow in only one direction (Denbow *et al.*, 2000).

In TTTS there is hypovolemia, oliguria and oligohydramnios in the 'donor' twin, resulting in a 'stuck twin' and the other twin, the 'recipient', will have hypervolemia, polyuria and polyhydramnios, leading many times to a circulatory volume overload and hydrops.
The natural history of TTTS is variable, and it is difficult to predict which cases will remain stable and which will progress to a severe TTTS. Quintero *et al*. (Quintero *et al.,* 1999) suggested a classification based on ultrasound findings and with prognostic significance (Table 1.13).

Table 1.13 Staging of TTTS based on sonographic and Doppler findings (Quintero *et al.*, 1999).

Stage	Poly / Oligo hydramnios	Absent bladder in donor	Abnormal Doppler	Hydrops	Fetal death
I	+	-	-	-	-
II	+	+	-	-	-
III	+	+	+	-	-
IV	+	+	+	+	-
V	+	+	+	+	+

Polyhydramnios= deepest pool > 8 cm; oligohydramnios= deepest pool < 2cm; Abnormal Doppler: absent or reversed end-diastolic flow at the umbilical artery, or reversed a-wave at the ductus venosus or pulsatile umbilical venous flow

Untreated TTTS has a poor outcome, with a high mortality of about 70-80%. Although different treatment options have been used, amnioreduction, septostomy and fetoscopic laser coagulation of the vascular anastomoses, the latter has shown to be the best treatment, with survival rate of 76% compared to 56% with amnioreduction (p=0.009), and brain damage of 6% compared to 14% with amnioreduction (p=0.02) (Senat *et al.*, 2004).

Role of the ductus venosus in TTTS

The presence of an abnormal flow at the ductus venosus in monochorionic twin pregnancies affected with a TTTS was firstly described by Hecher *et al.*, who found an absence or reversal blood flow during the atrial contraction in 13.2% (5 of 38) recipients and 10.5% (4 of 38) donors, and also a higher PIV in recipients (Hecher *et al.*, 1995c).

The recipient fetus has a congestive heart failure due to a hypervolemia and increased preload. The ventricular pump function can be affected as a consequence of the cardiac hypertrophy secondary to the hypervolemia.

In the donor there is a double mechanism to explain the abnormal ductus venosus waveforms: the hypovolemia and the high placental resistance. On the one hand there is a hypovolemia with a decrease in venous return. As a consequence of the low umbilical venous pressure the pressure wave during atrial contraction is propagated to the ductus venosus and the umbilical vein. On the other hand, in these fetuses there is an increased cardiac afterload as a consequence of the increased placental vascular resistance (Giles *et al.*, 1990). This is the same mechanism that has been found in fetuses with a selective FGR (Hecher *et al.*, 1995a; Rizzo *et al.*, 1996).

Ductus venosus flow Doppler has been included as one of the criteria at the staging of TTTS (Quintero *et al.*, 1999), as previously described.

The ductus venosus a-wave is also a prognostic factor in those cases of TTTS treated with fetoscopic laser. Absent or reversed a-wave before the procedure, either in the donor or in the recipient, is associated with a higher mortality in the recipient fetus

(Zikulnig *et al.*, 1999). Reversed a-wave in the donor after the procedure shows a trend towards predicting fetal demise in the donor (Martínez *et al.*, 2003).

Prediction of TTTS

An additional complication of TTTS, especially in the most advanced stages, when there is severe polyhydramnios, is preterm labour. Identifying the pregnancies at a higher risk of developing a TTTS would be helpful in order to monitor these pregnancies closer and diagnose and treat the TTTS at early stages. Different parameters have been used trying to predict the monochorionic twins that will develop a TTTS.

Increased NT (>95th centile) in at least one of the fetuses at 11-14 weeks has been associated with an increased risk of TTTS (likelihood ratio 3.5), although the detection rate is only about 30% with a false positive rate of 10% (Sebire *et al.*, 2000). The increased NT might be secondary to an early cardiac dysfunction due to the hypervolemic congestion in the recipient twin. Similarly, inter-twin discordance in NT thickness may help to identify pregnancies at a higher risk. If the discordance is 20% or more 30% of pregnancies will be complicated with an early fetal death or a TTTS, while if the discordance is less than 20% the chances are 10% (Kagan *et al.*, 2007).

Inter-twin membrane folding at 15-17 weeks is seen in about one third of monochorionic twins, and from this 43-53% progress to severe TTTS requiring fetoscopic laser (Sebire *et al.*, 1998, 2000). The membrane folding would be reflecting a disparity in amniotic fluid due to a TTTS. The likelihood ratio of the membrane folding for the subsequent development of severe TTTS is 4.2 (95%CI 3.0-6.0) (Sebire *et al.*, 2000).

Pathologic evaluation of the placenta in monochorionic diamniotic pregnancies showed that velamentous cord insertion is more common in those pregnancies that developed TTTS than in those that didn't (63.6% vs. 18.5%, $p<0.01$) (Fries *et al.*, 1993). Furthermore, TTTS cases are more likely to have discordant cord insertion, central/peripheral (Fick *et al.*, 2006). Velamentous cord insertion can be reliably diagnosed by ultrasound in the first and second trimester (Sepulveda *et al.*, 2003, 2006), and further studies are needed to determine its value in the prediction of TTTS.

A small study suggested ductus venosus flow in the first trimester as a predictor of TTTS. In a series of 11 monochorionic twin pregnancies severe TTTS developed in the two cases with abnormal ductus venosus waveforms in the recipient fetus at 11-13 weeks of gestation and in none of the cases with normal ductus venosus flow patterns (Matias *et al.*, 2000).

1.6.4 Screening for chromosomal abnormalities in twin pregnancies

Screening by maternal age

In twin pregnancies zygosity, rather than chorionicity, determines the risk for chromosomal abnormalities and whether the two fetuses may be or not concordant for the aneuploidy. In monozygotic twins the risk that at least one of the fetuses is affected by a chromosomal abnormality is the same as in singleton pregnancies and in the vast majority of these cases both fetuses are affected.

In dizygotic twins the maternal age-related risk for each fetus is the same as in singletons, and therefore the risk that at least one of the fetuses has a chromosomal abnormality is the twice as high as in singleton pregnancies.

Screening by nuchal translucency

In dichorionic twin pregnancies the detection rate for trisomy 21 by measurement of the NT is similar to those in singleton pregnancies, 75-80%, with a false positive rate of 5% per fetus or 10% per pregnancy (Sebire *et al.*, 1996a; Chasen *et al.*, 2007). An additional advantage is that NT measurement gives individual risks for each of the fetuses, and therefore in cases with a discordancy for chromosomal abnormalities it may identify the fetus with a higher risk. In monochorionic twins the false positive rate is higher than in dichorionic twins, 8% per fetus or 14% per pregnancy. One possible mechanism is that increased NT may be an early sign of TTTS (Sebire *et al.*, 1997b). The risk for trisomy 21 for each fetus based on fetal NT is estimated and the average of the two risks is used for the whole pregnancy (Sebire *et al.*, 1996a).

Screening by maternal serum biochemistry

In twin pregnancies addition of the first trimester biochemistry markers to the NT measurement improves the detection rate for Down syndrome (Spencer, 2000; Spencer *et al.*, 2003b; Wald *et al.*, 2003b; Chasen *et al.*, 2007) and reduces the false positive rate (Gonce *et al.*, 2005). Although initial studies suggested that chorionicity does not influence the biochemical markers levels, a more recent and larger study has shown that there is significant difference in the distribution of PAPP-A between monochorionic and dichorionic, but not for the free β-hCG (Spencer *et al.*, 2008).

Amniocentesis and chorion villus sampling (CVS) in twin pregnancies

In twin pregnancies, invasive testing has some specific considerations: a) the fetal loss rate may be higher than in singleton, b) there is a risk of sampling twice the same twin, and c) the fetuses might be discordant for a chromosomal abnormality, in which case the best moment for a selective fetocide should be considered. The procedure-related fetal loss for amniocentesis in twin pregnancies is about 2% (Weisz *et al.*, 2005). Reliable karyotype can be obtained with different techniques: two uterine entries using two different needles or a single uterine entry using a single needle (Sebire *et al.*, 1996b). Fetal loss following CVS in twin pregnancies is similar to second trimester amniocentesis (Weisz *et al.*, 2005). In about 1% of cases there may be a diagnostic error, either due to sampling the same placenta twice or due to cross-contamination.

In fetuses discordant for chromosomal abnormalities selective fetocide of the affected twin carries a risk of miscarriage and preterm delivery for the other twin. The risk for these complications is related to the gestational age at fetocide, there is a three-fold increased risk of both miscarriage before 24 weeks and preterm delivery before 33 weeks (Evans *et al.*, 1994).

In twin pregnancies screening in the first trimester by combination of NT, PAPP-A and free β-hCG identifies accurately the fetuses at high risk for chromosomal abnormalities. In these cases a CVS can be performed to diagnose the aneuploidy, allowing a safer selective fetocide before 16 weeks.

1.7 HYPOTHESIS OF THE THESIS

The ductus venosus has an important role in directing oxygenated blood from the placenta towards the fetal heart and brain. Its haemodynamics can be influenced by many conditions or situations that affect the cardiac afterload or preload, like fetal structural abnormalities, placental insufficiency or twin-to-twin transfusion syndrome.

The hypothesis of the studies in this thesis is that flow through the ductus venosus can be assessed routinely at 11-13 weeks of gestation and that abnormal flow at this scan can help identify fetal chromosomal and structural defects as well as adverse pregnancy outcome.

1.8 AIMS OF THE THESIS

The aim of this thesis is to examine if the routine assessment of the ductus venosus flow at the 11-13 weeks scan improves the prediction for chromosomal abnormalities, cardiac defects and adverse pregnancy outcomes.

The specific aims of each study were:

Aims of the first study

- To determine the number of scans necessary for training of sonographers to examine accurately the ductus venosus at 11-13 weeks.

Aims of the second study

- To assess the independent contribution of the ductus venosus flow in the screening for major cardiac defects in chomosomally normal fetuses with increased NT thickness at 11-13 weeks.

Aims of the third study

- To assess the performance of a specific algorithm which combines assessment of ductus venosus flow with maternal age, fetal NT thickness, FHR and maternal

serum free ß-hCG and PAPP-A in screening for trisomies 21, 18 and 13 and Turner syndrome.

- To examine the performance of two different screening strategies, one assessing the ductus venosus flow in all the patients, and two in a two-stage strategy, only in those with an intermediate risk of one in 51 to 1 in 1000 after the first stage.

Aims of the fourth study

- To estimate the independent contribution of abnormal flow in the ductus venosus at 11-13 weeks of gestation in the prediction of major fetal abnormalities and fetal death.

Aims of the fifth study

- To examine the prevalence of abnormal ductus venosus flow in monochorionic and dichorionic twin pregnancies.

- To examine the association between abnormal flow in the ductus venosus with adverse pregnancy outcome in twin pregnancies.

CHAPTER II

PUBLISHED STUDIES

STUDY 1 Maiz N, Kagan KO, Milovanovic Z, Celik E, Nicolaides KH.
Learning curve for Doppler assessment of ductus venosus flow at 11-13 weeks.
Ultrasound Obstet Gynecol 2008; 31:503-6

STUDY 2 Maiz N, Plasencia W, Dagklis T, Faros E, Nicolaides KH.
Ductus venosus Doppler in fetuses with cardiac defects and increased nuchal translucency thickness.
Ultrasound Obstet Gynecol 2008;31:256-60.

STUDY 3 Maiz N, Valencia C, Kagan KO, Wright D, Nicolaides KH.
Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation.
Ultrasound Obstet Gynecol 2009;33:512-7

STUDY 4 Maiz N, Valencia C, Emmanuel EE, Staboulidou I, Nicolaides KH.
Screening for adverse pregnancy outcome by ductus venosus Doppler at 11-13 weeks of gestation.
Obstet Gynecol 2008;112:598-605.

STUDY 5 Maiz N, Staboulidou I, Leal AM, Minekawa R, Nicolaides KH.
Ductus venosus Doppler at 11-13 weeks in the prediction of outcome in twin pregnancies
Obstet Gynecol 2009; 113:860-5.

STUDY 1

Learning curve for Doppler assessment of ductus venosus flow at 11-13 weeks.

Maiz N, Kagan KO, Milovanovic Z, Celik E, Nicolaides KH.
Ultrasound Obstet Gynecol 2008; 31:503-6.

Ultrasound Obstet Gynecol 2008; **31**: 503–506
Published online 10 March 2008 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.5282

Learning curve for Doppler assessment of ductus venosus flow at 11 + 0 to 13 + 6 weeks' gestation

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KEYWORDS: Doppler ultrasound; ductus venosus; first-trimester screening; trisomy 21

ABSTRACT

Objective *To determine the number of scans necessary for training sonographers to examine accurately the ductus venosus at 11 + 0 to 13 + 6 weeks' gestation.*

Methods *Ten sonographers with prior extensive experience in the measurement of nuchal translucency thickness were given practical training in the accurate assessment of the ductus venosus. They were then asked to examine the ductus venosus during the routine 11 + 0 to 13 + 6 weeks' scan. Each scan was assessed by an experienced sonographer and classified as being successful or unsuccessful (failure to obtain a waveform, poor quality image with contamination or wrong classification of the A-wave). Each sonographer performed a total of 300 examinations, the data were analyzed in 15 groups of 20 examinations and in each group the percentage of unsuccessful examinations was calculated.*

Results *In the total 3000 cases examined by the 10 sonographers there were 2849 (95.0%) successful examinations and 151 unsuccessful, including 104 failures to obtain a waveform, 30 cases where the quality of the image was considered to be inadequate and 17 cases in which the classification of the A-wave was wrong. The overall frequency of unsuccessful examinations decreased significantly with the number of scans carried out ($r = 0.982$, $P < 0.0001$). The sonographers required an average of 80 examinations before they could successfully examine the ductus in at least 19 of a group of 20 scans. Although one of the 10 trainees achieved this standard within the first block of 20 scans some of the sonographers required training in 100 cases.*

Conclusion *Competence in Doppler assessment of the ductus venosus is achieved only after extensive supervised training. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

The ductus venosus is a unique shunt directing well-oxygenated blood from the umbilical vein to the coronary and cerebral circulation by preferential streaming through the foramen ovale into the left atrium. Blood flow in the ductus has a characteristic waveform, with high velocity during ventricular systole (S-wave) and diastole (D-wave), and forward flow during atrial contraction (A-wave). A high proportion of fetuses with trisomy 21 have increased impedance to flow in the ductus venosus at 11 + 0 to 13 + 6 weeks of gestation, manifested as absent or reversed A-wave (Table 1)[1–8].

However, assessment of the A-wave can be technically difficult because of the small size of the ductus venosus and contamination of the flow pattern from this vessel by signals from the adjacent umbilical vein, hepatic vein or inferior vena cava.

The aim of this study was to determine the number of scans necessary for training sonographers to examine accurately the ductus venosus at 11 + 0 to 13 + 6 weeks' gestation.

METHODS

This was a prospective study involving 10 sonographers who had received The Fetal Medicine Foundation Certificates of Competence in the 11 + 0 to 13 + 6 weeks' scan and in uterine and umbilical artery Doppler. The sonographers were given practical training in the accurate assessment of the ductus venosus by fulfilling the following criteria (Figures 1 and 2):

(a) the examination should be undertaken during fetal quiescence;
(b) the magnification of the image should be such that the fetal thorax and abdomen occupy the whole screen;

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Accepted: 1 January 2008

Table 1 Studies reporting on the incidence of abnormal flow in the ductus venosus (DV) in first-trimester trisomy 21 and non-trisomy 21 fetuses

Reference	GA (weeks)	No. of fetuses (% with abnormal DV)	
		Trisomy 21	Non-trisomy 21
Matias *et al.*[1] (1998)	10 to 13 + 6	38 (92.1)	448 (3.1)
Antolin *et al.*[2] (2001)	10 to 13 + 6	7 (71.4)	917 (4.3)
Murta *et al.*[3] (2002)	10 to 13 + 6	18 (100)	354 (1.7)
Zoppi *et al.*[4] (2002)	10 to 13 + 6	20 (70.0)	305 (13.0)
Borrell *et al.*[5] (2003)	10 to 13 + 6	48 (75.0)	3334 (5.0)
Toyama *et al.*[6] (2004)	11 to 13 + 6	7 (71.4)	1090 (3.1)
Prefumo *et al.*[7] (2005)	11 to 14 + 1	47 (38.3)	581 (5.2)
Borrell *et al.*[8] (2005)	10 to 13 + 6	25 (76.0)	3706 (5.0)
Total		210 (71.4)	10 735 (5.0)

Abnormal flow was defined as absent or reversed A-wave or pulsatility index for veins above the 95th centile. GA, gestational age.

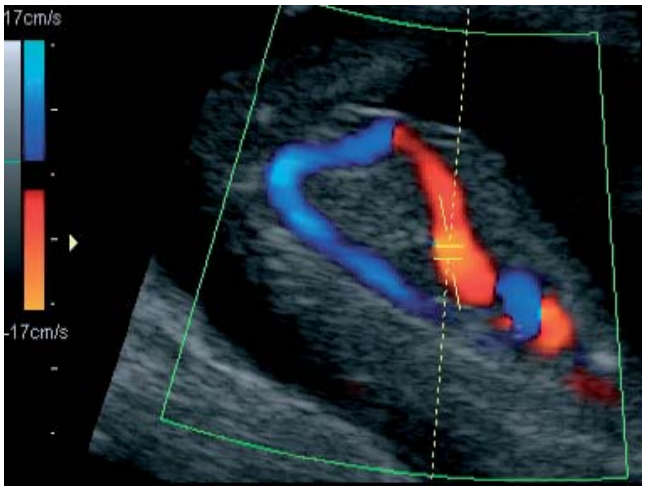

Figure 1 Right ventral mid-sagittal ultrasound image of the fetal trunk demonstrating the position of the pulsed Doppler gate for the study of ductus venosus blood flow.

(c) a right ventral mid-sagittal view of the fetal trunk should be obtained and color flow mapping should be undertaken to demonstrate the umbilical vein, ductus venosus and fetal heart;
(d) the pulsed Doppler sample should be small (0.5–1 mm) to avoid contamination from the adjacent veins, and it should be placed in the yellowish aliasing area, which is the portion immediately above the umbilical sinus;
(e) the insonation angle should be less than 30°;
(f) the filter should be set at a low frequency (50–70 Hz) so that the A-wave is not obscured; and
(g) the sweep speed should be high (2–3 cm/s) so that the waveforms are spread allowing better assessment of the A-wave.

When these criteria are satisfied, it is possible to assess the A-wave and determine qualitatively whether the flow is positive, absent or reversed.

The sonographers were asked to examine the ductus venosus during the routine 11 + 0 to 13 + 6-week scan, which is carried out transabdominally, and either to record that they were unable to do so or, if they were successful, to obtain a hard record of the waveform and to classify this according to the A-wave into normal (positive) or abnormal (absent or reversed). On a daily basis one of two highly trained sonographers examined all the images and determined whether the quality was adequate and whether the original diagnosis of normal or abnormal A-wave was correct. Consequently, each scan was assessed by the experienced sonographers as being successful or unsuccessful (failure to obtain a waveform, poor quality image with contamination or wrong classification of the A-wave).

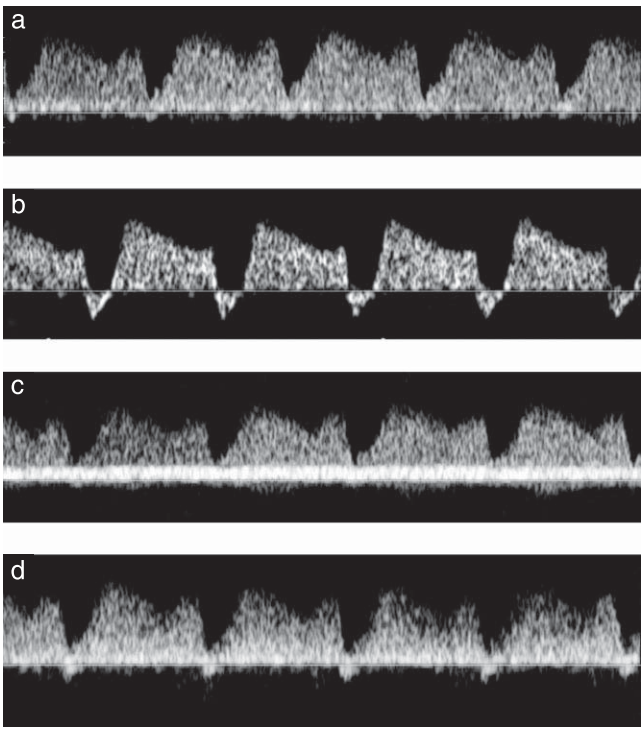

Figure 2 Flow velocity waveforms from the ductus venosus in 12-week fetuses: (a) normal waveform with positive A-wave; (b) reversed A-wave; (c) contamination from the umbilical vein, which could lead to the erroneous diagnosis of positive A-wave; and (d) contamination from the hepatic vein, which could lead to the erroneous diagnosis of reversed A-wave.

Each sonographer performed a total of 300 examinations, the data were analyzed in 15 groups of 20 examinations and in each group the percentage of unsuccessful examinations was calculated. Regression analysis was used to examine whether successful examination of the ductus was significantly related to fetal crown–rump length (CRL) in mm, maternal ethnic origin (Caucasian, Afro-Caribbean, Asian, Oriental or mixed), body mass index (BMI) in kg/m^2 and number of scans (in chronological order from 1 to 300 for each sonographer).

RESULTS

The 10 sonographers carried out a combined total of 3000 scans at a mean gestational age of 12 (range, 11 + 0 to 13 + 6) weeks; the median fetal CRL was 63.4 (range,

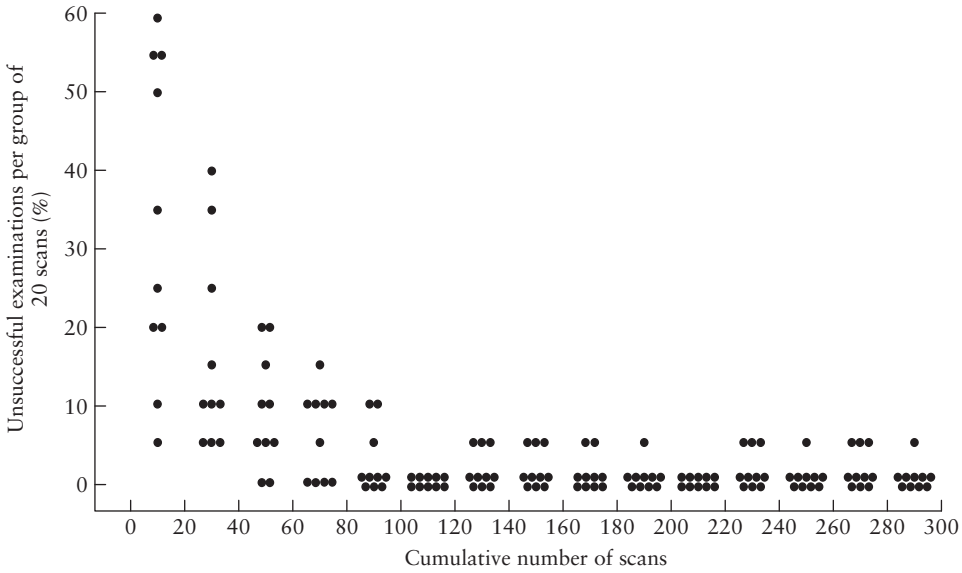

Figure 3 Frequency of unsuccessful examinations in the Doppler assessment of the ductus venosus by 10 sonographers in consecutive groups of 20 scans.

Table 2 Regression analysis in the prediction of adequate assessment of the ductus venosus

	Univariate analysis		*Multivariate analysis*	
Prediction	*Odds ratio (95% CI)*	P	*Odds ratio (95% CI)*	P
Crown–rump length (mm)	1.03 (1.006–1.052)	0.014	1.02 (0.996–1.045)	0.106
Body mass index (kg/cm^2)	0.98 (0.950–1.016)	0.302	—	—
Cumulative number of scans	1.02 (1.019–1.027)	< 0.0001	1.02 (1.019–1.027)	< 0.0001
Ethnic origin				
Caucasian	1			
Afro-Caribbean	1.09 (0.714–1.656)	0.697	—	—
Asian	1.43 (0.577–3.547)	0.440	—	—
Oriental	2.52 (0.345–18.367)	0.363	—	—
Mixed	0.66 (0.302–1.458)	0.307	—	—

45–84) mm and the median nuchal translucency (NT) thickness was 1.8 (range, 0.9–9.3) mm. The ethnic origin of the women was Caucasian in 2124 (70.8%) cases, Afro-Caribbean in 594 (19.8%), Asian in 138 (4.6%), Oriental in 48 (1.6%) and mixed in 96 (3.2%). The median maternal BMI was 24.5 (range, 15.4–53.5) kg/m^2.

In the total 3000 cases examined by the 10 trainee sonographers there were 2849 (95.0%) successful examinations (A-wave positive, $n = 2681$, absent, $n = 57$, reversed, n = 111) and 151 unsuccessful, including 104 failures to obtain a waveform, 30 cases where the quality of the image was considered to be inadequate and 17 cases in which the classification of the A-wave was wrong.

The overall frequency of unsuccessful examinations decreased significantly with the number of scans carried out ($r = 0.982$, $P < 0.0001$; Figure 3). Regression analysis demonstrated that successful examination of the ductus depended on the experience of the sonographer (defined by the cumulative number of scans) but not fetal CRL, maternal ethnic origin or BMI (Table 2).

Successful examination of at least 19 of the 20 cases was first achieved (and remained so in all subsequent groups) within the first group of 20 scans by one sonographer, after the first 40 scans by a further two, after 60 scans by a total of five and after 100 scans by all.

DISCUSSION

The findings of this study demonstrate that competence in Doppler assessment of the ductus venosus is achieved only after extensive supervised training. Sonographers with prior extensive experience in the 11 + 0 to 13 + 6 weeks' scan required an average of 80 examinations before they could successfully examine the ductus in at least 19 of a group of 20 scans. Although one of the 10 trainees achieved this standard within the first block of 20 scans, some of the sonographers required training in 100 cases. This finding is compatible with the results of two previous studies reporting that competence in the measurement of NT thickness and assessment of the nasal bone in the first-trimester scan is also achieved after a minimum of 80 scans[9,10].

The study has also shown that the impact of factors which could pose technical difficulties in achieving a

successful examination of the ductus venosus, such as high maternal BMI or low fetal CRL is insignificant compared to the level of training of the sonographers.

The high association between abnormal flow waveforms in the ductus venosus with chromosomal abnormalities makes it likely that this sonographic marker will be incorporated in first-trimester screening for trisomy 21[1–8]. Abnormal flow waveforms are found in about 70% of trisomy 21 fetuses and in 5% of chromosomally normal fetuses (Table 1). Consequently, in screening for trisomy 21 the patient-specific risk is increased by a factor of about 14-fold (70% ÷ 5%) if the A-wave is abnormal and it is reduced by a factor of 3-fold (95% ÷ 30%) if it is normal. Such a major effect on the estimated risk for trisomy 21, and inevitably on the decision for or against invasive testing, necessitates accurate assessment of the A-wave and avoidance of contamination from adjacent vessels. An absent or reversed A-wave can be wrongly classified as positive if there is contamination from the umbilical vein, and a positive A-wave can be erroneously considered to be reversed if there is contamination from the hepatic vein or inferior vena cava.

Sonographers undertaking risk assessment by Doppler examination of the ductus venosus should receive appropriate training and certification of their competence in performing such a scan. As shown in this study a good sonographer experienced in NT scanning needs to perform on average 80 scans in order to achieve this level of competence.

ACKNOWLEDGMENT

This study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116)

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STUDY 2

Ductus venosus Doppler in fetuses with cardiac defects and increased nuchal translucency thickness.

Maiz N, Plasencia W, Dagklis T, Faros E, Nicolaides KH.
Ultrasound Obstet Gynecol 2008;31:256-60.

Ultrasound Obstet Gynecol 2008; **31**: 256–260
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.5262

Ductus venosus Doppler in fetuses with cardiac defects and increased nuchal translucency thickness

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KEYWORDS: cardiac defects; Doppler ultrasound; ductus venosus; first-trimester screening; nuchal translucency thickness

ABSTRACT

Objective *To examine the possible role of Doppler ultrasound assessment of ductus venosus blood flow in screening for major cardiac defects in chromosomally normal fetuses with increased nuchal translucency (NT) thickness at 11 + 0 to 13 + 6 weeks' gestation.*

Methods *Ductus venosus blood flow velocity waveforms were obtained immediately before chorionic villus sampling for fetal karyotyping in fetuses with NT thickness of 3.5 mm or more at 11 + 0 to 13 + 6 weeks of gestation. In the chromosomally normal group fetal echocardiography was performed by a specialist pediatric cardiologist at 11 + 0 to 13 + 6 weeks and/or 18–22 weeks' gestation.*

Results *Major cardiac defects were diagnosed in 16 (8.4%) of the 191 chromosomally normal fetuses. Reversed or absent flow in the ductus venosus during atrial contraction was observed in 11 of the 16 (68.8%) fetuses with cardiac defects and in 40 of the 175 (22.9%) with no cardiac defects. Multivariate analysis demonstrated that the prevalence of an abnormal A-wave in the ductus venosus in fetuses without major cardiac defects increased with fetal NT thickness (odds ratio (OR), 1.463; 95% CI, 1.183–1.809;* $P < 0.0001$*) but in those with cardiac defects it did not change significantly with NT thickness (OR, 2.054; 95% CI, 0.573–7.360;* $P = 0.269$*). The likelihood ratio for a major cardiac defect when the ductus venosus flow was abnormal decreased with fetal NT thickness from 4.58 at NT 3.5 mm to 2.47 for NT 5.5 mm, and the likelihood ratio when the ductus venosus flow was normal increased from 0.37 at NT 3.5 mm to 0.43 for NT 5.5 mm.*

Conclusion *In chromosomally normal fetuses with increased NT the finding of an absent or reversed A-wave in the ductus venosus is associated with a three-fold increase in the likelihood of a major cardiac defect, whereas the finding of normal ductal flow is associated with a halving in risk for such defects. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Abnormal flow in the ductus venosus at 11 + 0 to 13 + 6 weeks of gestation is associated with an increased risk for chromosomal abnormalities and cardiac defects[1–9]. In the combined data from seven studies that examined ductus venosus waveforms in 600 chromosomally normal fetuses with increased nuchal translucency (NT) thickness (above the 95th centile) a major cardiac defect was observed in 29 (4.8%) fetuses, and 28 (96.6%) of these had abnormal Doppler waveforms in the ductus venosus (Table 1)[3–9]. However, there were wide variations between the individual studies in the number of cases examined (16–142), the prevalence of cardiac defects (1.7–9.5%), and the incidence of abnormal ductus venosus waveforms in those without cardiac defects (0–40%).

The aim of this study was to examine further the possible role of Doppler ultrasound assessment of ductus venosus blood flow in screening for major cardiac defects in chromosomally normal fetuses with increased NT thickness at 11 + 0 to 13 + 6 weeks' gestation.

METHODS

In this prospective study ductus venosus blood flow velocity waveforms were obtained immediately before chorionic villus sampling for fetal karyotyping in fetuses with NT thickness of 3.5 mm or more at 11 + 0 to 13 + 6 weeks of gestation, between November 2005 and August 2007. The Doppler studies were undertaken by

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Accepted: 31 December 2007

Table 1 Previous studies that examined the relationship between ductus venosus waveforms and major cardiac defects in chromosomally normal fetuses with nuchal translucency thickness above the 95th centile

			Abnormal ductus venosus flow	
Reference	*Total* (n)	*Cardiac defects* (n (%))	*No cardiac defects* (n (%))	*Cardiac defects* (n (%))
Matias *et al.* 1999[3]	142	7 (4.9)	4/135 (3.0)	7/7 (100)
Bilardo *et al.* 2001[4]	69	4 (5.8)	19/65 (29.2)	4/4 (100
Murta *et al.* 2002[5]	16	1 (6.3)	0/15 (0.0)	1/1 (100)
Zoppi *et al.* 2002[6]	115	2 (1.7)	30/113 (26.5)	2/2 (100)
Haak *et al.* 2003[7]	22	2 (9.1)	8/20 (40.0)	2/2 (100)
Favre *et al.* 2003[8]	95	9 (9.5)	20/86 (23.3)	9/9 (100)
Toyama *et al.* 2004[9]	141	4 (2.8)	23/137 (16.8)	3/4 (75)
Total	600	29 (4.8)	104/571 (18.2)	28/29 (96.6)

sonographers with experience in this technique and the following criteria were fulfilled: (a) the examinations were undertaken during fetal quiescence; (b) the magnification of the image was such that the fetal thorax and abdomen occupied the whole screen; (c) a right ventral mid-sagittal view of the fetal trunk was obtained and color flow mapping was used to demonstrate the umbilical vein, ductus venosus and fetal heart; (d) the pulsed Doppler sample was small (0.5–1.0 mm) to avoid contamination from the adjacent veins and it was placed in the yellowish aliasing area, which is the portion immediately above the umbilical sinus; (e) the insonation angle was less than 30°; (f) the filter was set at a low frequency (50–70 Hz) to allow visualization of the whole waveform; and (g) the sweep speed was high (2–3 cm/s) so that the waveforms were widely spread, allowing better assessment of the A-wave[10]. Waveforms were assessed qualitatively and considered to be abnormal if the A-wave was absent or reversed (Figure 1).

In this study we report the findings in fetuses with a normal karyotype. In these cases fetal echocardiography was performed transabdominally by a specialist pediatric cardiologist at 11 + 0 to 13 + 6 weeks and/or 18–22 weeks. Fetal echocardiography included assessment of the position of the heart, the four-chamber view, the outflow tracts, the duct and the arch.

Statistical analysis

Logistic regression analysis was performed to determine the significance of the contribution of fetal NT thickness and crown–rump length (CRL) in the prediction of the prevalence of an abnormal A-wave in the ductus venosus in fetuses with and without major cardiac defects.

RESULTS

Successful visualization of the ductus venosus and assessment of the A-wave was achieved in 191 (98.5%) of the 194 chromosomally normal fetuses examined. The median maternal age was 32 (range, 17–44) years, the median CRL was 65.3 (range, 45–84) mm and the median NT thickness was 4.0 (range 3.5–13.0) mm. An absent or reversed A-wave was observed in 51 (26.7%) of the fetuses, and the prevalence increased with fetal NT ($P < 0.0001$) and decreased with CRL ($P = 0.004$). The median pulsatility index for veins in those with an abnormal A-wave was 1.97 (range, 1.45–2.86) and

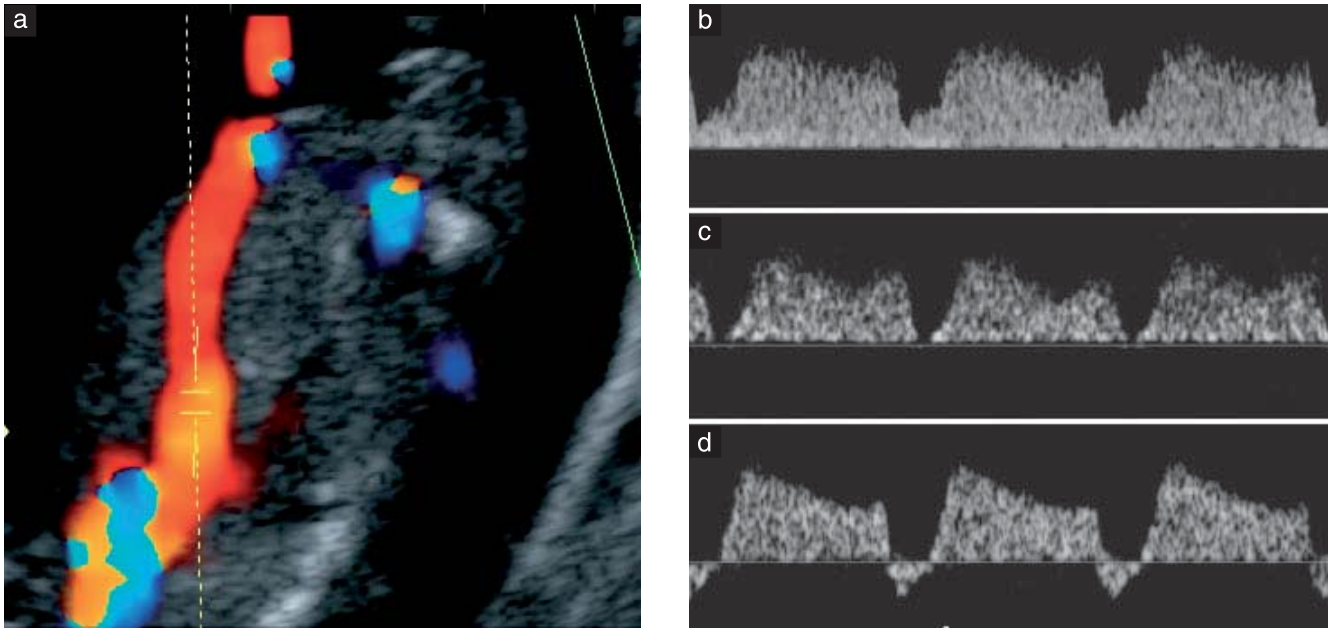

Figure 1 Mid-sagittal view of the fetal trunk demonstrating, with color flow mapping, the umbilical vein, ductus venosus and fetal heart (a) and ductus venosus flow velocity waveforms showing a normal waveform (b), absent A-wave (c) and reversed A-wave (d).

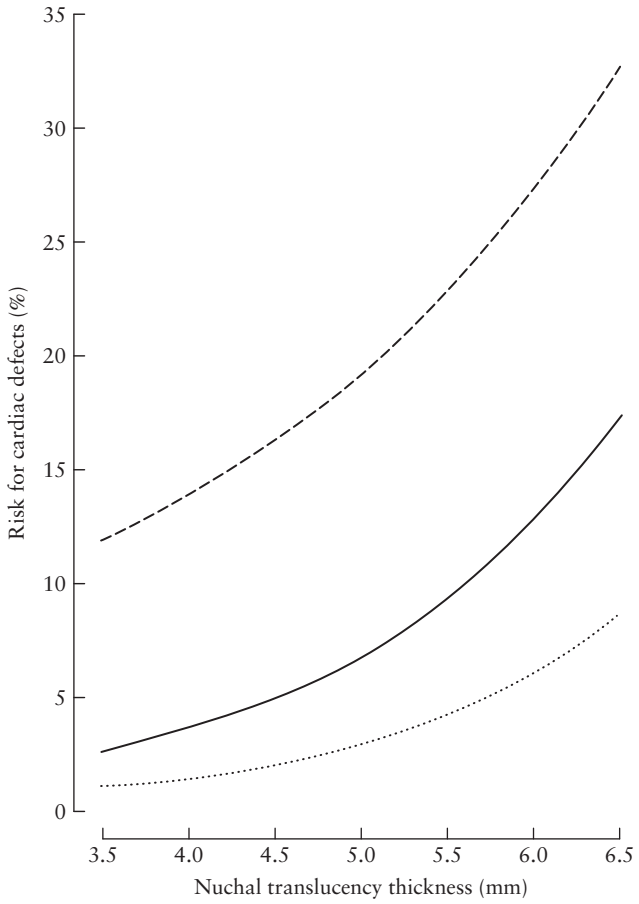

Figure 2 Relationship between nuchal translucency (NT) thickness in chromosomally normal fetuses and risk of major cardiac defects. The *a priori* NT-related risk[11] (———) is multiplied by the positive and negative likelihood ratios for abnormal (– – – – –) and normal (·····) A-waves in the ductus venosus, respectively, to derive the adjusted risk.

Table 2 Ductus venosus Doppler findings in fetuses with cardiac defects

Cardiac defect	*Ductus venosus A-wave*	*CRL (mm)*	*NT (mm)*
Tricuspid atresia	Reversed	54.5	9.1
Tricuspid atresia	Reversed	69.0	13.0
Tricuspid dysplasia	Reversed	53.2	4.3
Hypoplastic left heart syndrome	Reversed	45.3	3.5
Double outlet right ventricle	Reversed	81.0	5.5
Double outlet right ventricle	Reversed	55.4	3.8
Double outlet right ventricle	Reversed	70.2	5.8
Pulmonary atresia	Reversed	66.9	4.6
Pulmonary atresia, tricuspid dysplasia	Positive	64.2	5.5
Pulmonary stenosis	Positive	74.4	4.7
Tetralogy of Fallot, pulmonary atresia	Positive	77.0	3.8
Coarctation of the aorta	Positive	51.1	3.5
Coarctation of the aorta	Positive	69.8	4.0
Left atrial isomerism (heart block, VSD)	Reversed	60.7	4.0
Atrioventricular septal defect	Reversed	49.0	6.1
Transposition of the great arteries	Reversed	47.1	5.1

CRL, crown–rump length; NT, nuchal translucency thickness; VSD, ventricular septal defect.

in those with a normal A-wave it was 1.03 (range, 0.54–1.49).

Fetal echocardiography was performed both at 11 + 0 to 13 + 6 weeks and at 18–22 weeks in 127 cases. There was agreement between the two scans for absence of cardiac defects in 121 cases and presence of cardiac defects in five cases (coarctation of the aorta in two, double outlet right ventricle in one, pulmonary stenosis in one and tricuspid atresia in one). There was an additional case where the heart was thought to be normal at 13 weeks, coarctation of the aorta was suspected at 20 weeks but the heart was found to be normal at birth. There were 14 cases in which fetal echocardiography was carried out only in the first trimester because the pregnancies resulted in fetal death or termination at the request of the parents. In this group, major cardiac defects were suspected in six cases (hypoplastic left heart in one, pulmonary atresia in two, tricuspid atresia in one, tricuspid dysplasia in one, ventricular septal defect with left isomerism and heart block in one). There were 50 cases in which fetal echocardiography was carried out only in the second trimester, and in this group a major cardiac defect was diagnosed in five cases (double outlet right ventricle in two, transposition of the great arteries in one, atrioventricular septal defect in one and tetralogy of Fallot in one).

In total, major cardiac defects were diagnosed in 16 (8.4%) of the 191 fetuses (Table 2). An abnormal A-wave in the ductus venosus was identified in 11 of the 16 (68.8%) fetuses with cardiac defects and in 40 of the 175 (22.9%) with no cardiac defects.

Multivariate analysis demonstrated that in fetuses with major cardiac defects the prevalence of an abnormal A-wave in the ductus venosus was not significantly associated with fetal NT thickness (odds ratio (OR), 2.054; 95% CI, 0.573–7.360; $P = 0.269$) or CRL (OR, 0.930; 95% CI, 0.834–1.038; $P = 0.196$). In fetuses without major cardiac defects the prevalence of an abnormal A-wave in the ductus venosus was significantly associated with fetal NT thickness (OR, 1.463; 95% CI, 1.183–1.809; $P < 0.0001$) but not CRL (OR, 0.962, 95% CI, 0.925–1.0; $P = 0.053$).

The likelihood ratio for cardiac defects with an abnormal A-wave in the ductus venosus was derived by dividing the prevalence of abnormal Doppler findings in fetuses with cardiac defects by that in fetuses without cardiac defects. In fetuses with cardiac defects

Table 3 Relationship between fetal nuchal translucency (NT) thickness and likelihood ratios (LR) for major cardiac defects depending on the findings of ductus venosus Doppler ultrasonography

	Prevalence (%) of abnormal A-wave when cardiac defect is:		*Major cardiac defect*		
NT (mm)	*Present*	*Absent*	*LR positive*	*LR negative*	*a priori risk (%)*
3.5	68.8	15.0	4.58	0.37	2.6
4.0	68.8	17.7	3.89	0.38	3.5
4.5	68.8	20.7	3.32	0.39	4.9
5.0	68.8	24.1	2.85	0.41	6.7
5.5	68.8	27.9	2.47	0.43	9.2

To derive the patient-specific risk for cardiac defects the appropriate LR was multiplied by the *a priori* risk reported in a previous study on the relationship between fetal NT and major cardiac defects[11].

the prevalence of an abnormal A-wave was 68.8%. In fetuses without cardiac defects the prevalence (%) of an abnormal A-wave was: $(\text{odds}/1 + \text{odds}) \times 100$, where $\text{odds} = \exp Y$ or $Y = \text{Log}_e(\text{odds})$, $= -3.104 + (0.391 \times \text{NT in mm})$. In chromosomally normal fetuses with increased NT when the A-wave in the ductus venosus is abnormal the risk that the fetus has a major cardiac defect is derived by multiplying the *a priori* prevalence of cardiac defects, which increases with fetal NT[11], by the positive likelihood ratio (Figure 2, Table 3). When the A-wave in the ductus venosus is normal the risk that the fetus has a major cardiac defect is derived by multiplying the *a priori* prevalence of cardiac defects by the negative likelihood ratio (Figure 2, Table 3)[10].

DISCUSSION

The findings of this study confirm the high association between abnormal ductus venosus flow at 11 + 0 to 13 + 6 weeks' gestation and major cardiac defects. In chromosomally normal fetuses with increased NT the finding of an absent or reversed A-wave in the ductus venosus is associated with a three-fold increase in the likelihood of a major cardiac defect, whereas the finding of normal ductal flow is associated with a halving in risk for such defects.

The prevalence of major cardiac defects in our chromosomally normal fetuses with NT of 3.5 mm or more (8.4%) was 17 times higher than the estimated 0.5% in chromosomally normal fetuses at this gestation[12], confirming that high NT is associated with increased risk for cardiac defects[13–17]. A fetal echocardiographic study of 6921 chromosomally normal fetuses reported that the prevalence of major cardiac defects increases with fetal NT thickness from about 0.5% in those with NT below the median, to 1% for NT between the median and less than the 95th centile, 2% for NT between the 95th and 99th centiles, and exponentially thereafter to 3.5, 6.5 and 12.5 for NT of 3.5–4.4, 4.5–5.4 and ≥ 5.5 mm, respectively (Figure 2)[11].

In the assessment of individual risk for major cardiac defects the *a priori* NT-dependent risk can be multiplied by the appropriate likelihood ratio depending on whether the A-wave in the ductus venosus is normal or abnormal. For example, in a fetus with NT thickness of 3.5 mm the risk for major cardiac defects is 2.6%, and this is increased to 11.9% if the a-wave is abnormal and reduced to 1.0% if the flow is normal, and in a fetus with NT thickness of 5.5 mm the *a priori* risk is 9.2%, and this is increased to 22.7% if the a-wave is abnormal and reduced to 4.0% if the flow is normal.

A meta-analysis of studies examining the screening performance of NT thickness for the detection of cardiac defects in fetuses with normal karyotype reported that the detection rates were about 37% and 31% for the respective NT cut-offs of the 95th and 99th centiles[17]. This compares favorably with the less than 10% detection associated with a policy of screening by maternal factors, such as diabetes mellitus, exposure to teratogens or family history of cardiac defects[18]. The findings of our study and those of previous reports[3–9] that 68.8 and 96.6%, respectively, of fetuses with major cardiac defects have abnormal flow in the ductus venosus suggest that Doppler assessment of this vessel may provide an effective method for early screening for such defects. However, the studies examining the association between ductus venosus flow and cardiac defects are essentially confined to fetuses with increased NT, and it is therefore uncertain whether in fetuses with cardiac defects and normal NT the A-wave in the ductus venosus is also abnormal. Consequently, population-based studies in which there is no preselection by measurement of the NT thickness are needed to assess the true effectiveness of first-trimester screening for major cardiac defects by Doppler assessment of the ductus venosus.

ACKNOWLEDGMENT

This study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116). We are grateful to Drs Lindsey Allan and Ian Huggon for carrying out the fetal cardiac scans.

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STUDY 3

Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation.

Maiz N, Valencia C, Kagan KO, Wright D, Nicolaides KH.
Ultrasound Obstet Gynecol 2009;33:512-7.

Ultrasound Obstet Gynecol 2009; **33**: 512–517
Published online 31 March 2009 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.6330

Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation

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KEYWORDS: chromosomal abnormality; ductus venosus; first trimester; screening; trisomy 21

ABSTRACT

Objectives *To investigate the performance of first-trimester screening for aneuploidies by including assessment of ductus venosus flow in the combined test of maternal age, fetal nuchal translucency thickness, fetal heart rate, and serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A.*

Methods *Screening by the combined test was performed in singleton pregnancies, including 19 614 with euploid fetuses, 122 with trisomy 21, 36 with trisomy 18, 20 with trisomy 13 and eight with Turner syndrome. In all cases the a-wave in the fetal ductus venosus flow was assessed. We examined the performance of two screening strategies: first, assessment of the a-wave in all patients and, second, first-stage screening using the combined test in all patients followed by second-stage assessment of the a-wave only in those with an intermediate risk of one in 51 to one in 1000 after the first stage*

Results *Reversed a-wave was observed in 3.2% of the euploid fetuses, and in 66.4%, 58.3%, 55.0% and 75.0% of fetuses with trisomies 21, 18 and 13 and Turner syndrome, respectively. Inclusion of ductus venosus flow in all pregnancies would detect 96%, 92%, 100% and 100% of trisomies 21, 18 and 13 and Turner syndrome, respectively, at a false-positive rate of 3%. The same detection rates were achieved with the two-stage strategy at a false-positive rate of 2.6%, in which it was necessary to assess the ductus venosus in only 15% of the total population.*

Conclusions *Assessment of ductus venosus flow improves the performance of first-trimester screening for aneuploidies.*

INTRODUCTION

A high proportion of fetuses with trisomy 21 and other chromosomal abnormalities have increased impedance to flow in the ductus venosus at 11–13 weeks of gestation. In the combined data from seven studies, abnormal ductal blood flow was observed in 5.2% of euploid fetuses, and 70.8%, 89.3%, 81.8% and 76.9% of fetuses with trisomies 21, 18 and 13 and Turner syndrome, respectively (Table 1)[1–7]. There is uncertainty about whether the incidence of abnormal ductal blood flow is associated with the other first-trimester sonographic and biochemical markers of chromosomal abnormalities, and the extent to which assessment of the ductus venosus would improve the performance of combined first-trimester screening.

Screening for trisomy 21 by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11–13 weeks is associated with a detection rate of about 90% for a false-positive rate of 5%[8,9]. A beneficial consequence of screening for trisomy 21 is the early diagnosis of other major chromosomal abnormalities, including trisomies 18 and 13 and Turner syndrome. Although all four chromosomal abnormalities are associated with increased fetal NT there are some differences in their distribution of maternal age, serum PAPP-A and serum free β-hCG. We have recently reported the development of specific algorithms for trisomy 21, trisomy 18 and trisomy 13, which, in addition to maternal age, fetal NT and serum free β-hCG and PAPP-A, also use fetal heart rate (FHR)[10]. When all three algorithms are used in combination the detection rates of trisomies 21, 18 and 13 and Turner syndrome are 91%, 97%, 94%

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Accepted: 7 January 2009

Table 1 Studies reporting on the incidence of abnormal flow in the ductus venosus in the first trimester in euploid fetuses, and in those with trisomies 21, 18 and 13, Turner syndrome and other chromosomal abnormalities

Reference	n	*Normal*	*Trisomy 21*	*Trisomy 18*	*Trisomy 13*	*Turner syndrome*	*Other*
Matias *et al.* 1998[1]	486	13/423 (3.1)	35/38 (92.1)	12/12 (100)	5/7 (71.4)	2/3 (66.7)	3/3 (100)
Antolin *et al.* 2001[2]	924	39/911 (4.3)	5/7 (71.4)	3/3 (100)	—	1/1 (100)	1/2 (50.0)
Murta *et al.* 2002[3]	372	7/343 (2.0)	18/18 (100)	1/1 (100)	2/2 (100)	2/2 (100)	3/6 (50.0)
Zoppi *et al.* 2002[4]	325	38/292 (13.0)	14/20 (70.0)	6/7 (85.7)	1/1 (100)	1/3 (33.3)	1/2 (50.0)
Borrell *et al.* 2003[5]*	3382	162/3249 (5.0)	36/48 (75.0)	—	—	—	—
Toyama *et al.* 2004[6]	1097	69/1075 (6.4)	5/7 (71.4)	3/5 (60.0)	1/1 (100)	4/4 (100)	2/5 (40.0)
Prefumo *et al.* 2005[7]*	572	26/497 (5.2)	18/47 (38.3)	—	—	—	—
Total	7158	354/6790 (5.2)	131/185 (70.8)	25/28 (89.3)	9/11 (81.8)	10/13 (76.9)	10/18 (55.6)

Values are *n* (%). *These papers do not provide specific data for other chromosomal abnormalities.

and 100%, respectively, for an overall false-positive rate of 3.1%[10].

The aims of this study were, first, to derive a specific algorithm that combines assessment of ductus venosus flow with maternal age, fetal NT, FHR and maternal serum free β-hCG and PAPP-A and, second, to examine the performance of such an algorithm in screening for trisomies 21, 18 and 13 and Turner syndrome. We examined the performance of two screening strategies: first, integrated first-trimester screening including assessment of the ductus venosus in all patients and, second, first-stage screening of all patients using maternal age, fetal NT, FHR and maternal serum free β-hCG and PAPP-A followed by second-stage assessment of ductus venosus flow only in those with an intermediate risk of one in 51 to one in 1000 after the first stage.

METHODS

This was a prospective screening study for trisomy 21 in singleton pregnancies by a combination of maternal age, fetal NT thickness and maternal serum free β-hCG and PAPP-A in a one-stop-clinic for first-trimester assessment of risk (OSCAR) at 11 + 0 to 13 + 6 weeks of gestation[8,9]. Transabdominal ultrasound examination was performed to diagnose any major fetal defects and for measurement of fetal crown–rump length (CRL), NT and FHR[10]. Ductus venosus blood flow velocity waveforms were also routinely obtained by sonographers who had received the appropriate Fetal Medicine Foundation Certificate of Competence in this assessment[11]. Automated machines that provide reproducible results within 30 min were used to measure PAPP-A and free β-hCG (Delfia Xpress System, Perkin Elmer, Waltham, MA, USA).

Maternal demographic characteristics, ultrasonographic measurements and biochemical results were recorded in a computer database. Karyotype results and details on pregnancy outcomes were added to the database as soon as they became available. A search of the database was done to identify all singleton pregnancies in which first-trimester combined screening was carried out between January 2006 and May 2007.

In the ductus venosus studies the following criteria were fulfilled[11]: (1) the examinations were undertaken during fetal quiescence; (2) the magnification of the image was such that the fetal thorax and abdomen occupied the whole screen; (3) a right ventral mid-sagittal view of the fetal trunk was obtained and color flow mapping was used to demonstrate the umbilical vein, ductus venosus and fetal heart; (4) the pulsed Doppler sample was small (0.5–1.0 mm) to avoid contamination from the adjacent veins and it was placed in the aliasing area, which is the portion immediately above the umbilical sinus; (5) the insonation angle was less than 30°; (6) the filter was set at a low frequency (50–70 Hz) to allow visualization of the whole waveform; and (7) the sweep speed was high (2–3 cm/s) so that the waveforms were widely spread, allowing better assessment of the a-wave. Waveforms were assessed qualitatively, and considered to be abnormal if the a-wave was reversed and normal if it was present or absent (Figure 1).

Statistical analysis

The performance of contingent screening was assessed by applying the findings of ductus venosus Doppler imaging to the group with a total risk for trisomies 21, 18 and 13 and Turner syndrome of between one in 51 and one in 1000 based on the combined test including maternal age, fetal NT, FHR, free β-hCG and PAPP-A[10]. Screen positivity was defined as either a risk of one in 50 or higher on the basis of the combined test, or a risk of one in 100 or higher after inclusion of ductus venosus flow in the intermediate group with risks between one in 51 and one in 1000.

In order to modify the risk from the combined test on the basis of the findings of ductus venosus Doppler imaging we used multiple logistic regression to model the

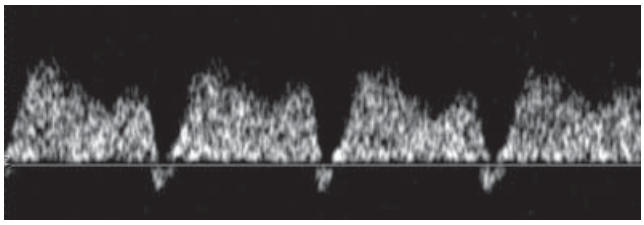

Figure 1 Reversed a-wave in the ductus venosus in a fetus with trisomy 21 at 12 weeks of gestation.

conditional probability of reversed a-wave in the ductus venosus given fetal karotype, fetal NT, free β-hCG and PAPP-A, and covariates representing ethnicity and maternal smoking status. Bayes' theorem was applied to produce risks of trisomy 21, trisomy 18, trisomy 13 and Turner syndrome. This enabled us to examine the performance of a screening policy where combined test and ductus venosus Doppler imaging is used in all pregnancies.

Screening performance was assessed by calculating the proportions with risks above a given threshold after adjustment for maternal age according to the distribution of pregnancies in England and Wales in 2000–2002 (Office for National Statistics, 2000–2002)[12].

RESULTS

Study population

The search of the database identified 21 141 singleton pregnancies. In 1110 (5.3%) cases the outcome was not available, in 188 (0.9%) cases one of the covariates was missing, and in 43 (0.2%) cases there was a chromosomal abnormality other than trisomies 21, 18 or 13, or Turner syndrome. Thus, our study population consisted of 19 614 pregnancies with a normal karyotype or delivery of a phenotypically normal baby (euploid group), 122 cases of trisomy 21, 36 cases of trisomy 18, 20 cases of trisomy 13 and eight cases of Turner syndrome. The characteristics of the study population are summarized in Table 2.

Fetal nuchal translucency, heart rate and maternal serum biochemistry

The distributions of fetal NT, FHR and maternal serum free β-hCG and PAPP-A in fetuses with trisomies 21, 18 and 13 and Turner syndrome are shown in Table 3.

Table 2 Characteristics of 19 800 patients

Variable	*Median (range) or* n *(%)*
Maternal characteristics	
Age (years)	34.5 (14.1–50.1)
Weight (kg)	64.0 (34.0–165.0)
Spontaneous conception	19 038 (96.2)
Smoker	1145 (5.8)
Ethnicity	
Caucasian	15 850 (80.1)
Afro-Caribbean	2148 (10.8)
East Asian	271 (1.4)
South Asian	1031 (5.2)
Mixed	500 (2.5)
Gestational age	
11 + 0 to 11 + 6 weeks	1477 (7.5)
12 + 0 to 12 + 6 weeks	11 495 (58.1)
13 + 0 to 13 + 6 weeks	6828 (34.5)
Crown–rump length (mm)	63 (45.0–84.0)
Karyotype	
Normal	19 614 (99.1)
Trisomy 21	122 (0.6)
Trisomy 18	36 (0.2)
Trisomy 13	20 (0.1)
Turner syndrome	8 (0.04)

Table 3 Crown–rump length (CRL), fetal nuchal translucency thickness (NT), fetal heart rate (FHR), serum pregnancy-associated plasma protein-A (PAPP-A) and serum free β-human chorionic gonadotropin (β-hCG) in chromosomally normal and abnormal fetuses

Variable	*Median (range)*
CRL (mm)	
Normal karyotype	63.2 (45.0–84.0)
Trisomy 21	63.1 (47.4–84.0)
Trisomy 18	55.1 (45.0–70.4)
Trisomy 13	57.0 (45.5–82.9)
Turner syndrome	62.0 (45.0–69.7)
Deviation from expected fetal NT (mm)	
Normal karyotype	0.1 (−1.0 to 8.5)
Trisomy 21	1.4 (−0.4 to 11.2)
Trisomy 18	2.6 (−0.4 to 9.3)
Trisomy 13	3.1 (0.0 to 6.3)
Turner syndrome	8.5 (1.5 to 10.4)
PAPP-A (MoM)	
Normal karyotype	1.0 (0.2–3.3)
Trisomy 21	0.5 (0.06–2.2)
Trisomy 18	0.2 (0.03–3.9)
Trisomy 13	0.3 (0.1–0.6)
Turner syndrome	0.5 (0.3–0.8)
Free β-hCG (MoM)	
Normal karyotype	1.0 (0.1–29.4)
Trisomy 21	2.0 (0.1–7.0)
Trisomy 18	0.2 (0.02–4.8)
Trisomy 13	0.4 (0.2–1.1)
Turner syndrome	1.2 (0.3–2.0)
Deviation from expected FHR (bpm)	
Normal karyotype	−0.1 (−32.4 to 45.4)
Trisomy 21	0.6 (−21.6 to 17.8)
Trisomy 18	−3.4 (−17.4 to 10.5)
Trisomy 13	18.4 (10.5 to 32.0)
Turner syndrome	2.1 (−3.9 to 9.5)

MoM, multiples of the median.

Ductus venosus flow

Reversed a-wave in the ductus venosus flow was observed in 3.2% (622/19 614) of the euploid fetuses, in 66.4% (81/122), 58.3% (21/36) and 55.0% (11/20) of fetuses with trisomies 21, 18 and 13, respectively, and in 75.0% (6/8) of fetuses with Turner syndrome.

Logistic regression analysis demonstrated highly significant ($P < 0.0001$) effects on the prevalence of reversed a-wave from maternal Afro-Caribbean ethnicity, fetal NT, fetal CRL, serum PAPP-A and fetal karyotype (Table 4). The effects of maternal age, weight, smoking status and serum free β-hCG were not significant ($P > 0.05$). FHR had a small but significant effect ($P = 0.002$) and was negatively associated with reversed DV flow.

Risk distribution and test performance

The total risk for trisomies 21, 18, 13 and Turner syndrome, according to maternal age, fetal NT, FHR, serum PAPP-A and serum free β-hCG, after standardization for the maternal age distribution of pregnancies in England and Wales in 2000–2002, was one in 50 or higher in 1.5% of the euploid pregnancies, and in

Table 4 Fitted logistic regression model for presence of reversed ductus venosus flow

Parameter	*Coefficient*	*Standard error*	Z	P	*OR (95% CI)*
Constant	−1.73803	0.33612	−5.17	< 0.0001	
Nuchal translucency (mm)	0.53657	0.05760	9.31	< 0.0001	1.71 (1.53–1.91)
Crown–rump length (mm)	−0.04447	0.00543	−8.19	< 0.0001	0.96 (0.95–0.97)
Log PAPP-A MoM	−0.87863	0.16062	−5.47	< 0.0001	0.42 (0.30–0.57)
Trisomy 21/euploid	3.11399	0.22851	13.63	< 0.0001	22.51 (14.38–35.23)
Trisomy 13/euploid	1.66997	0.54401	3.07	0.0021	5.31 (1.83–15.43)
Trisomy 18/euploid	1.77208	0.43844	4.04	< 0.0001	5.88 (2.49–13.89)
Turner/euploid	0.78711	1.11791	0.70	0.4814	2.20 (0.25–19.65)
Black/White ethnicity	1.07125	0.09719	11.02	< 0.0002	2.92 (2.41–3.53)

MoM, multiples of the median; OR, odds ratio; PAPP-A, pregnancy-associated plasma protein-A.

Table 5 Detection rates for given false-positive rates (FPRs) in screening by maternal age, fetal nuchal translucency thickness (NT), fetal heart rate, maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), with and without ductus venosus (DV) Doppler, standardized to the maternal age distribution of pregnancies in England and Wales in 2000–2002[12]

	Detection rate (%)							
	Trisomy 21		*Trisomy 18*		*Trisomy 13*		*Turner syndrome*	
FPR (%)	*Without DV*	*With DV*	*Without DV*	*With DV*	*Without DV*	*With DV*	*Without DV*	*With DV*
1.0	80	88	84	84	100	100	100	100
2.0	86	94	88	88	100	100	100	100
3.0	91	96	100	92	100	100	100	100
4.0	93	96	100	92	100	100	100	100
5.0	94	97	100	92	100	100	100	100
2.6	96		92		100		100	

The last row gives the results of contingent screening in which DV Doppler is carried out only in those with risk estimates of one in 51 to one in 1000 after first-line screening by maternal age, fetal NT, fetal heart rate and maternal serum free β-hCG and PAPP-A.

Table 6 Distribution of risk and effectiveness of contingent screening

	First stage			*Second stage*	
Fetal karyotype	*≥ 1 in 50 (%)*	*1 in 51 to 1 in 1000 (%)*	*< 1 in 1000 (%)*	*≥ 1 in 100 (%)*	*Total (%)*
Euploid	1.5	14.7	83.8	1.1	2.6
Trisomy 21	85.3	13.4	1.2	10.5	95.9
Trisomy 18	88.5	11.5	0.0	3.2	91.7
Trisomy 13	100	0.0	0.0	0.0	100.0
Turner syndrome	100	0.0	0.0	0.0	100.0

In the first stage the patients are divided into three risk categories after screening by maternal age, fetal nuchal translucency thickness, fetal heart rate, maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A. The patients with a risk of one in 50 or more are considered to be screen positive and those with a risk of less than one in 1000 are screen negative. The patients with an intermediate risk of one in 51 to one in 1000 have second-stage screening with fetal ductus venosus Doppler which modifies their risk. If the adjusted risk is one in 100 or more the patients are considered to be screen positive and those with a risk of less than one in 100 are screen negative. The last column lists the overall detection rates at a false-positive rate of 2.6%. All percentages are adjusted according to the maternal age distribution of pregnancies in England and Wales in 2000–2002[12].

85.3%, 88.5%, 100% and 100% of those with trisomies 21, 18 and 13 and Turner syndrome, respectively. Total risks of one in 51 to one in 1000 were found in 14.7% of the euploid pregnancies, and 13.4%, 11.5%, 0% and 0% of those with trisomies 21, 18 and 13 and Turner syndrome, respectively. Total risks of less than one in 1000 were found in 83.8% of the euploid pregnancies, and 1.2%, 0%, 0% and 0% of those with trisomies 21, 18 and 13 and Turner syndrome, respectively.

The performance of screening is shown in Tables 5 and 6. For a fixed false-positive rate of 3% the standardized detection rate was 91% for trisomy 21, whereas for trisomy 18, trisomy 13 and Turner syndrome it was 100%. Assessment of the ductus venosus flow in all pregnancies would increase the detection rate of trisomy

21 to 96%, and those for trisomy 18, trisomy 13 and Turner syndrome would be 92%, 100% and 100%.

A contingent policy (where screen positivity is defined as either a first-stage total risk of one in 50 or higher based on maternal age, fetal NT, FHR, serum PAPP-A and serum free β-hCG, or a risk of one in 100 or higher after assessment of the a-wave in the ductus venosus flow in those cases where the first-line total risk is between one in 51 and one in 1000) would detect 96% of all cases with trisomy 21 for a false-positive rate of 2.6%, and the respective detection rates for trisomy 18, trisomy 13 and Turner syndrome would be 92%, 100% and 100%, respectively (Table 6).

DISCUSSION

The findings of this prospective screening study demonstrate that reversed a-wave in the ductus venosus at 11–13 weeks is found in about 3% of euploid fetuses, in 65% of fetuses with trisomy 21, in about 55% of those with trisomies 13 and 18, and in 75% of those with Turner syndrome. Inclusion of ductus venosus flow in first-trimester screening by maternal age, fetal NT, FHR and maternal serum free β-hCG and PAPP-A would detect about 96% of trisomy 21 fetuses at a false-positive rate of about 2.5%.

There is a theoretical risk of thermal damage to the developing fetus from the use of color and pulsed Doppler examination. However, such theoretical risk applies only to transvaginal sonography before 10 weeks and in any case there is no epidemiological or other evidence to support such an assertion[13]. In our study the ultrasound examinations were performed transabdominally after 11 weeks and we used the as low as reasonably achievable (ALARA) principle with output settings of the machines resulting in thermal index and mechanical index values below 0.6.

The prevalence of reversed flow in the ductus venosus during atrial contraction (a-wave) is affected not only by the fetal karyotype but also by maternal ethnicity (being higher in black than in white women); it is also inversely related to fetal CRL and serum PAPP-A and increases with fetal NT. We used logistic regression analysis to take into account these factors in the development of an algorithm for the calculation of risks for chromosomal abnormalities. Reversed flow in the venous system is observed when atrial contraction occurs against a ventricle of high end-diastolic pressure. There are three factors that may explain the CRL-related decrease in the prevalence of reversed a-wave: first, improved ventricular filling and decreased myocardial stiffness with advancing gestation, second, decrease in placental resistance and therefore cardiac afterload and, third, improvement in renal function to counteract any tendency to fluid retention[14–17]. Similarly, the association between the prevalence of reversed a-wave with low PAPP-A and black ethnicity may be explained by increased cardiac afterload due to impaired placentation and therefore increased placental resistance. We have reported previously that low PAPP-A and black ethnicity are associated with increased risk of fetal growth restriction, development of pre-eclampsia and fetal death[18–20]. The association between reversed a-wave and increased NT may be explained by the coincidence of cardiac defects or transient cardiac dysfunction.

Effective first-trimester screening for chromosomal abnormalities is provided by a combination of maternal age, fetal NT and maternal serum free β-hCG and PAPP-A[10]. The estimated detection rate of trisomy 21 was 91% for a false-positive rate of 3.1%[10]. As demonstrated in this study, assessment of ductus venosus flow improves the performance of first-trimester combined screening by increasing the estimated detection rate to about 96% and reducing the false-positive rate to about 2.5%. We investigated two strategies for assessment of ductus venosus flow. In the first approach the ductus venosus can be examined in all cases with the advantage of not only improving the performance of screening for chromosomal abnormalities, but also identifying pregnancies at increased risk of fetal cardiac defects and fetal death[18]. Because assessment of ductus venosus flow is time consuming and requires appropriately trained sonographers, the alternative strategy is to reserve this examination for the subgroup of pregnancies with an intermediate risk (between one in 51 and one in 1000) after combined fetal NT, FHR, free β-hCG and PAPP-A screening, which constitutes only one sixth of the total population. Similar two-stage strategies have been advocated for assessment of flow across the tricuspid valve and in examination for the presence or absence of the nasal bone[9,21,22].

Sonographers undertaking risk assessment by Doppler examination of the ductus venosus should receive appropriate training and certification of their competence in performing such a scan, and should adhere to a series of strict criteria for obtaining the appropriate waveform. We have previously shown that sonographers with extensive experience in the 11–13-week scan require an average of 80 examinations to achieve this level of competence[11].

ACKNOWLEDGMENT

This study was supported by a grant from The Fetal Medicine Foundation (UK charity number 1037116).

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STUDY 4

Screening for adverse pregnancy outcome by ductus venosus Doppler at 11-13 weeks of gestation.

Maiz N, Valencia C, Emmanuel EE, Staboulidou I, Nicolaides KH
Obstet Gynecol 2008;112:598-605.

Screening for Adverse Pregnancy Outcome by Ductus Venosus Doppler at 11–13^{+6} Weeks of Gestation

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OBJECTIVE: To estimate the independent contribution of abnormal flow in the ductus venosus at 11 to 13^{+6} weeks of gestation in the prediction of major fetal abnormalities and fetal death.

METHODS: This was a prospective assessment of singleton pregnancies by maternal history, serum free β-hCG, pregnancy-associated plasma protein A (PAPP-A), fetal nuchal translucency thickness, and ductus venosus Doppler. The patients were subdivided into five groups: normal outcome (n=10,120), miscarriage or fetal death (n=185), abnormal karyotype (n=95), and major cardiac (n=20) or noncardiac defect (n=70). Regression analysis was performed to determine the significance of the contribution to adverse outcome of reversed a-wave in the ductus venosus, maternal characteristics, fetal delta nuchal translucency, maternal serum log PAPP-A multiples of the median, and log free β-hCG multiples of the median.

RESULTS: The prevalence of reversed a-wave was significantly higher in the groups with miscarriage or fetal death (10.8%), abnormal karyotype (62.1%), and fetal cardiac defect (25.0%) than in the normal outcome group (3.7%), but not noncardiac defect (4.3%). An adverse outcome was observed in 2.7% of the fetuses with nuchal translucency at or below the 95th centile (in 2.6% of those with normal a-wave and in 7.0% of those with reversed a-wave) and in 19.3% of the fetuses with nuchal translucency above the 95th centile (in 8.9% of those with normal a-wave and in 70.9% of those with reversed a-wave).

CONCLUSION: Reversed a-wave is associated with increased risk for chromosomal abnormalities, cardiac defects, and fetal death. However, in about 80% of cases with reversed a-wave, the pregnancy outcome is normal. *(Obstet Gynecol 2008;112:598–605)*

LEVEL OF EVIDENCE II

Abnormal flow in the ductus venosus at 11–13^{+6} weeks of gestation is associated with increased risk for trisomy 21 and other chromosomal abnormalities.[1–8] In euploid fetuses, abnormal flow in the ductus venosus is associated with an increased risk for cardiac defects.[3,4,6,9–13] However, most studies examining such an associated are confined to fetuses with increased nuchal translucency thickness. There is also some evidence that abnormal flow in the ductus venosus is associated with an increased risk for fetal death. A first-trimester screening study reported that the prevalence of abnormal flow in the ductus venosus in the cases resulting in fetal death was 22.2% (four of 18) compared with 5.9% (61 of 1,041) in the normal outcome group.[6] However, abnormal flow was observed in four of the eight fetal deaths presenting with nuchal translucency above the 95th centile and in none of the 10 with normal nuchal translucency.

A case–control study of fetuses with nuchal translucency below the 95th centile reported that in 10 of 42 (23.8%) cases with abnormal flow in the ductus venosus there was an adverse outcome, including perinatal death and chromosomal, cardiac or noncardiac abnormalities.[14] In contrast, an adverse outcome was observed in only one of 83 (1.2%) controls with normal flow in the ductus venosus.

The aim of this screening study involving more than 10,000 pregnancies, in which maternal charac-

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Supported by a grant from the Fetal Medicine Foundation (Charity No: 1037116).

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Financial Disclosure
The authors have no potential conflicts of interest to disclose.

ISSN: 0029-7844/08

teristics, serum free β-hCG and pregnancy-associated plasma protein A (PAPP-A), and fetal nuchal translucency thickness as well as the flow in the fetal ductus venosus were assessed at 11 to 13^{+6} weeks of gestation, was to estimate the independent contribution of abnormal flow in the ductus venosus in the prediction of major fetal abnormalities and fetal death.

MATERIALS AND METHODS

This was a prospective screening study for trisomy 21 in singleton pregnancies by a combination of maternal age, fetal nuchal translucency thickness, and maternal serum free β-hCG and PAPP-A in a one-stop-clinic for first trimester assessment of risk at 11 to 13^{+6} weeks of gestation.[15] Transabdominal ultrasound examination was performed to diagnose any major fetal defects and for measurement of fetal crown-rump length (CRL) and nuchal translucency thickness.[16] Ductus venosus blood flow velocity waveforms were also routinely obtained by ultrasonographers who had received the appropriate Fetal Medicine Foundation Certificate of Competence in this assessment. Automated machines that provide reproducible results within 30 minutes were used to measure PAPP-A and free β-hCG (Delfia Express System, Perkin Elmer, Waltham, MA). Patients were asked to complete a questionnaire on maternal age, ethnic origin (White, Black, Indian or Pakistani, Chinese, or Japanese, and Mixed), cigarette smoking during pregnancy (yes or no), parity (parous or nulliparous if no delivery beyond 23 weeks), and mode of conception (spontaneous, including those receiving ovulation induction drugs, or assisted reproduction by in vitro fertilization [IVF]). The maternal weight and height were measured and the body mass index (BMI) was calculated in kilograms per square meter. Written informed consent was obtained from each patient participating in the study, which was approved by King's College Hospital Research Ethics Committee.

Maternal demographic characteristics, ultrasonographic measurements, and biochemical results were recorded in a computer database. Karyotype results, details on fetal abnormalities diagnosed during subsequent ultrasound examinations or postnatally, and pregnancy outcomes were obtained from cytogenetic laboratories, hospital maternity records, the patients themselves, or their general medical practitioners, and these data were added into our database as soon as they became available.

In the ductus venosus studies the following criteria were fulfilled[17]: 1) the examinations were undertaken during fetal quiescence; 2) the magnification of the image was such that the fetal thorax and abdomen occupied the whole screen; 3) a right ventral mid sagittal view of the fetal trunk was obtained and color flow mapping was used to demonstrate the umbilical vein, ductus venosus, and fetal heart; 4) the pulsed Doppler sample was small (0.5–1.0 mm) to avoid contamination from the adjacent veins, and it was placed in the yellowish aliasing area, which is the portion immediately above the umbilical sinus; 5) the insonation angle was less than 30 degrees; 6) the filter was set at a low frequency (50–70 Hz) to allow visualization of the whole waveform; and 7) the sweep speed was high (2–3 cm/s) so that the waveforms were widely spread, allowing better assessment of the a-wave. Waveforms were assessed qualitatively and considered to be abnormal if the a-wave was reversed.

A search of the database was done to identify all singleton pregnancies in which first trimester combined screening was carried out from March 2006 to May 2007. The patients were subdivided into 1) a normal outcome group of live births with no obvious chromosomal abnormalities or fetal defects; 2) miscarriage before 24 weeks of gestation or fetal death at or after 24 weeks; 3) abnormal karyotype; and 4) fetal defect. In the last group we included only cases in which the defect would result in severe handicap or the need for surgical or medical treatment. We did not include conditions like polydactyly or mild ventriculomegaly or pyelectasia.

Analysis of variance (with the Bonferroni post-hoc test) and χ^2 were used to compare the demographic characteristics of the normal outcome group with each of the adverse outcome groups. The measured free β-hCG and PAPP-A were converted into a multiples of the median (MoM) for gestational age adjusted for maternal weight, ethnicity, smoking status, method of conception, parity, and machine for the assays.[18] The distributions of PAPP-A MoM and free β-hCG MoM were made Gaussian after logarithmic transformation. The measured nuchal translucency was expressed as a difference from the normal mean for gestation (delta value).[19]

Comparisons between each of the pregnancy outcomes and the normal outcome group was by analysis of variance (with Bonferroni post-hoc test) for log PAPP-A MoM and log free β-hCG MoM, Kruskal-Wallis test (with Dunn's post-hoc test) for the fetal delta nuchal translucency and the χ^2 and Fisher exact test for the prevalence of reversed a-wave. Multiple logistic regression analysis in the whole population was used to determine the significant contributors to reversed a-wave in the fetal ductus venosus from maternal characteristics, different outcome groups, fetal CRL, delta nuchal translucency,

Table 1. Demographic Characteristics in the Different Outcome Groups

	Normal Outcome (n=10,120)	Abnormal Karyotype (n=95)	Fetal Death (n=185)	Fetal Defect (n=90)
Maternal age (y)	32.2 (16–49)	37.2 (19.0–46.5)*	32.7 (16.0–44.0)	31.7 (18.2–43.2)
Body mass index	24.6 (14.4–59.2)	24.9 (22.2–28.6)	26.8 (17.4–48.9)*	24.8 (18.1–47.2)
Crown–rump (mm)	63.9 (45.0–84.0)	61.7 (47.4–79.7)*	63.8 (48.0–84.0)	63.1 (46.5–84)
Ethnicity				
White	7,117 (70.3)	75 (78.9)	88 (47.6)*	68 (75.6)
Black	1,972 (19.5)	12 (12.6)	85 (45.9)*	13 (14.4)
Indian or Pakistani	518 (5.1)	4 (4.2)	3 (1.6)†	5 (5.6)
Chinese or Japanese	150 (1.5)	1 (1.1)	1 (0.5)	1 (1.1)
Mixed	363 (3.6)	3 (3.2)	8 (4.3)	3 (3.3)
Cigarette smoker	855 (8.4)	7 (7.4)	16 (8.6)	11 (12.2)
Conception by in vitro fertilization	150 (1.5)	1 (1.1)	5 (2.7)	4 (4.4)

Data are median (range) or n (%).
* $P<.001$ compared with the normal outcome group.
† $P<.05$ compared with the normal outcome group.

maternal serum log MoM PAPP-A, and log MoM free β-hCG. Similarly, multiple regression analysis was performed to determine the significance of the contribution to fetal death, fetal chromosomal abnormality or defect of reversed a-wave, maternal characteristics, fetal CRL, delta nuchal translucency, maternal serum log MoM PAPP-A, and log MoM free β-hCG.

RESULTS

During the study period, first-trimester combined screening was carried out in 11,093 cases, and in 11,005 (99.2%) of these there was successful visualization of the ductus venosus and assessment of the a-wave. In 493 cases there was loss to follow-up, and 22 women chose to have pregnancy termination for psychosocial indications. In the remaining 10,490 pregnancies, the median maternal age was 32 (range 16–49) years, the median CRL was 63.8 (range 45–84) mm and the median delta nuchal translucency thickness was 0.07 (range −0.9 to 11.2) mm.

There were 10,120 pregnancies resulting in live births with no obvious chromosomal abnormalities or fetal defects (normal outcome group). In 185 pregnancies there was miscarriage before 24 weeks or fetal death at or after 24 weeks (fetal death group). In 95 cases the fetal karyotype, determined by chorionic villous sampling, amniocentesis, or postnatally, was abnormal including trisomy 21 in 45, trisomy 18 or 13 in 28, and other defects (triploidy, sex chromosome aneuploidies, deletions, translocations, mosaicism) in 22. Fetal defects were detected in 90 cases, including 20 with cardiac defects (coarctation of the aorta in six, Ebstein anomaly in three, atrioventricular or ventricular septal defect in three, double-outlet right ventricle in two, transposition of the great arteries in two, and one case each of tetralogy of Fallot, pulmonary atresia, pulmonary stenosis, and Scimitar syndrome) and 70 with noncardiac defects (ventriculomegaly of more than 12 mm in five, agenesis of the vermis in one, neural tube defects in three, facial cleft in nine, diaphragmatic hernia or cystic adenomatoid malformation of the lungs in eight, esophageal or duodenal atresia in two, abdominal wall defect in six, obstructive uropathy multicystic kidneys, polycystic kidneys or renal agenesis in 20, talipes in 13, skeletal dysplasia in 3). The demographic characteristics of the outcome groups are summarized in Table 1.

Reversed a-wave was observed in 458 (4.4%) of the fetuses, and the prevalence was significantly higher in the groups with miscarriage or fetal death (10.8%), abnormal karyotype (62.1%), and fetal cardiac defect (25.0%), but not noncardiac defect (4.3%) than in the normal outcome group (3.7%) (Table 2). There were no significant differences between miscarriages and late fetal deaths in prevalence of reversed a-wave ($P=.907$), log MoM PAPP-A ($P=.713$), or log MoM free β-hCG ($P=.539$), and therefore the data of the two subgroups were combined.

Logistic regression analysis demonstrated that significant contribution to reversed a-wave was provided by the different outcome groups (fetal death, odds ratio [OR] 2.222; 95% confidence interval [CI] 1.359–3.635; $P<.001$; abnormal karyotype OR 17.514; 95% CI 9.752–31.455; $P<.001$; fetal cardiac defect OR 6.793; 95% CI 2.296–20.104; $P<.001$), but also by fetal CRL (OR 0.967; 95% CI 0.954–0.980; $P<.001$), and delta nuchal translucency (OR 1.387; 95% CI 1.183–1.627; $P<.001$), Black ethnicity (OR 2.425; 95% CI 1.952–3.014; $P<.001$) and serum log MoM PAPP-A (OR 0.434; 95% CI 0.295–0.640; $P<.001$) but not maternal age ($P=.118$), BMI

Table 2. Prevalence of Reversed a-Wave in the Ductus Venosus, Median (Range) Delta Nuchal Translucency, Pregnancy-Associated Plasma Protein A (Multiples of the Median) and Free ß-hCG (Multiples of the Median) in Each Outcome Group

Outcome	n	Reversed a-Wave	Delta NT (mm)	PAPP-A (MoM)	ß-hCG (MoM)
Normal outcome	10,120	371 (3.7)	0.07 (–0.9 to 5.0)	1.01 (0.06–7.87)	0.97 (0.13–11.9)
Fetal death	185	20 (10.8)*	0.08 (–0.7 to 3.9)	0.87 (0.07–4.03)*	0.87 (0.18–7.95)
Miscarriage	127	13 (10.2)*	0.06 (–0.7 to 3.9)	0.88 (0.07–4.03)*	0.86 (0.20–7.95)
Death at more than 24 wk	58	7 (12.1)†	0.08 (–0.6 to 1.2)	0.85 (0.15–3.71)	0.90 (0.18–4.19)
Abnormal karyotype	95	59 (62.1)*	2.13 (–0.5 to 11.2)†	0.45 (0.02–3.93)*	1.37 (0.02–6.77)
Trisomy 21	45	29 (64.4)*	1.83 (–0.1 to 11.2)†	0.55 (0.14–2.17)*	2.03 (0.75–5.41)*
Trisomy 18 or 13	28	19 (67.9)*	3.68 (–0.3 to 9.4)†	0.24 (0.03–3.93)*	0.20 (0.02–2.38)*
Other	22	11 (50.0)*	2.11 (–0.5 to 10.1)†	0.67 (0.02–1.57)*	1.26 (0.10–6.77)
Fetal defect	90	8 (8.9)†	0.19 (–0.4 to 6.2)†	0.87 (0.11–2.7)	0.86 (0.16–5.69)
Cardiac defect	20	5 (25.0)*	0.57 (–0.3 to 3.8)†	0.79 (0.28–1.97)	0.81 (0.47–3.17)
Noncardiac defect	70	3 (4.3)	0.15 (–0.4 to 6.2)	0.89 (0.11–2.7)	0.89 (0.16–5.69)

NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein A; MoM, multiples of the median.
Data are n (%) or median (range).
* $P<.001$ compared with the normal outcome group.
† $P<.05$ compared with the normal outcome group.

($P=.748$), smoking ($P=.708$), serum log MoM free β-hCG ($P=.414$) or fetal noncardiac defects ($P=.997$).

An adverse outcome (fetal death, abnormal karyotype, or fetal defect) occurred in 370 (3.5%) of the 10,490 cases. Logistic regression analysis demonstrated that significant contribution to adverse outcome was provided by reversed a-wave (OR 3.648; 95% CI 2.639–5.042; $P<.001$), fetal delta nuchal translucency (OR 2.929; 95% CI 2.473–3.468; $P<.001$), maternal serum log MoM PAPP-A (OR 0.180; 95% CI 0.118–0.275; $P<.001$), Black ethnicity (OR 2.027; 95% CI 1.560–2.634; $P<.001$) and maternal BMI (OR 1.024; 95% CI 1.003–1.045; $P<.025$), but not maternal age ($P=.051$), smoking ($P=.616$), serum log MoM free β-hCG ($P=.529$), or fetal CRL ($P=.885$).

Reversed a-wave was observed in 3.7% (372 of 9,976) of the fetuses with nuchal translucency at or below the 95th centile and in 16.7% (86 of 514) with nuchal translucency above the 95th centile (Table 3). An adverse outcome was observed in 2.7% of the fetuses with nuchal translucency at or below the 95th centile (in 2.6% of those with normal a-wave and in 7.0% of those with reversed a-wave) and in 19.3% of

Table 3. Prevalence of Different Outcome Groups in Relation to Ductus Venosus a-Wave and Fetal Nuchal Translucency Thickness

	Total			Nuchal Translucency Thickness 95th Centile or Less			Nuchal Translucency Thickness More Than 95th Centile		
		Ductus Venosus a-Wave			Ductus Venosus a-Wave			Ductus Venosus a-Wave	
Outcome	N	Normal	Reversed	n	Normal	Reversed	n	Normal	Reversed
Total	10,490	10,032	458	9,976	9,604	372	514	428	86
Normal outcome	10,120 (96.5)	9,749 (97.2)	371 (81.0)*	9,705 (97.3)	9,359 (97.4)	346 (93.0)	415 (80.7)	390 (91.1)	25 (29.1)
Fetal death	185 (1.8)	165 (1.6)	20 (4.4)*	175 (1.8)	158 (1.6)	17 (4.6)*	10 (1.9)	7 (1.6)	3 (3.5)†
Abnormal karyotype	95 (0.9)	36 (0.4)	59 (12.9)*	22 (0.2)	16 (0.2)	6 (1.6)*	73 (14.2)	20 (4.7)	53 (61.6)*
Trisomy 21	45 (0.4)	16 (0.2)	29 (7.3)*	7 (0.1)	4 (0.0)	3 (0.8)*	38 (7.4)	12 (2.8)	26 (30.2)*
Trisomy 18 or 13	28 (0.3)	9 (0.1)	19 (4.9)*	8 (0.1)	6 (0.1)	2 (0.5)†	20 (3.9)	3 (0.7)	17 (19.8)*
Other	22 (0.2)	11 (0.1)	11 (2.9)*	7 (0.1)	6 (0.1)	1 (0.3)	15 (2.9)	5 (1.2)	10 (11.6)*
Fetal defect	90 (0.9)	82 (0.8)	8 (1.7)†	74 (0.7)	71 (0.7)	3 (0.8)	16 (3.1)	11 (2.6)	5 (5.8)*
Cardiac defect	20 (0.2)	15 (0.2)	5 (1.3)*	12 (0.1)	12 (0.1)	0 (0)	8 (1.6)	3 (0.7)	5 (5.8)*
Noncardiac defect	70 (0.7)	67 (0.7)	3 (0.8)	62 (0.6)	59 (0.6)	3 (0.8)	8 (1.6)	8 (1.9)	0 (0)

Data are n or n (%).
* $P<.001$ compared with those with normal and those with reversed a-wave.
† $P<.05$ compared with those with normal and those with reversed a-wave.

the fetuses with nuchal translucency above the 95th centile (in 8.9% of those with normal a-wave and in 70.9% of those with reversed a-wave).

Reversed a-wave in the fetal ductus venosus was observed in 64.4% of fetuses with trisomy 21, 67.9% of fetuses with trisomies 18 or 13, and 50% of fetuses with other chromosomal abnormalities. All three groups were associated with increased nuchal translucency and decreased PAPP-A. In trisomy 21, free β-hCG was increased; in trisomies 18 and 13 it was decreased, and in the other chromosomal abnormalities it was not significantly different from normal (Table 2).

Logistic regression analysis demonstrated that significant contribution to fetal chromosomal abnormality was provided by reversed a-wave, as well as maternal age, fetal delta nuchal translucency and maternal serum log MoM PAPP-A, but not maternal serum log MoM free β-hCG, ethnicity, BMI, smoking, or fetal CRL (Table 4). The apparent lack of significant contribution from log MoM free β-hCG is because in this analysis we considered all chromosomal abnormalities together; in trisomy 21 the levels were significantly increased, and in trisomies 18 and 13 they were decreased.

Reversed a-wave was observed in 25.0% of fetuses with cardiac defects. Logistic regression analysis demonstrated that significant contribution to fetal cardiac defect was provided by reversed a-wave in the fetal ductus venosus and fetal delta nuchal translucency, but not maternal age, ethnicity, BMI, smoking, log MoM PAPP-A, log MoM free β-hCG, or fetal CRL (Table 4).

The prevalence of reversed a-wave in fetuses with noncardiac defects was not significantly higher than in the normal outcome group (4.3% compared with 3.7%, $P=.744$). Logistic regression analysis demonstrated that significant contribution to fetal noncardiac defects was provided by fetal delta nuchal translucency, but not reversed a-wave, maternal age, ethnicity, BMI, smoking, log MoM PAPP-A, log MoM free β-hCG, or fetal CRL (Table 4).

Logistic regression analysis demonstrated that significant contribution to fetal death was provided by reversed a-wave in the fetal ductus venosus, as well as Black ethnicity, BMI, and maternal serum log MoM PAPP-A, but not maternal age, smoking, serum log MoM free β-hCG, fetal delta nuchal translucency, or CRL (Table 4).

The patient-specific risk for fetal death (%) was calculated from the formula: odds/(1+odds), where odds=e^Y. Y was derived from the multiple regression analysis above: Y=–5.324+(0.807 if reversed a-wave, 0 if normal a-wave)+0.033×BMI in Kg/m^2+(1.144 if Black, 0 if other ethnic origin)–1.238×log MoM PAPP-A; $R^2=0.062$, $P<.001$. Figure 1 illustrates how the PAPP-A–related risk of fetal death in women of Black and White ethnicity is modified by the findings of the fetal ductus venosus Doppler.

DISCUSSION

At 11–13^{+6} weeks of gestation, approximately 4% of fetuses demonstrate reversed a-wave in the flow velocity waveform from the ductus venosus. This abnormal flow pattern is associated with increased risk for chromosomal abnormalities, cardiac defects, and fetal death.

The finding that the risk for chromosomal abnormalities is increased with maternal age, fetal nuchal

Table 4. Multiple Logistic Regression Analysis to Predict Each of the Adverse Outcome Groups

	Abnormal Karyotype		Fetal Cardiac Defect		Fetal Noncardiac Defect		Fetal Death	
Contributor	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Ductus venosus a-wave	19.018 (9.624–37.582)	<.001	7.008 (2.363–20.780)	<.001		.784	2.242 (1.373–3.659)	.001
Delta nuchal translucency (mm)	7.910 (5.586–11.201)	<.001	3.777 (2.591–5.506)	<.001	2.285 (1.685–3.098)	<.001	–	.548
Crown-rump length		.519	–	.228	–	.644	–	.771
Maternal age (y)	1.201 (1.122–1.285)	<.001	–	.472	–	.861	–	.781
Body mass index (kg/m^2)	–	.760	–	.433	–	.090	1.034 (1.007–1.060)	.012
Ethnicity					–			
White	1	–	1	–	1	–	1	–
Black	–	.078	–	.252	–	.452	3.139 (2.286–4.310)	<.001
Indian or Pakistani	–	.546	–	.993	–	.527	–	.228
Chinese or Japanese	–	.650	–	.996	–	.943	–	.586
Mixed	–	.681	–	.890	–	.716	–	.176
Cigarette smoker		.706	–	.803	–	.191	–	.923
Log MoM PAPP-A	0.004 (0.001–0.011)	<.001	–	.480	–	.089	0.290 (0.164–0.511)	<.001
Log MoM free ß-hCG	–	.175	–	.858	–	.064	–	.127

OR, odds ratio; CI, confidence interval; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein A.

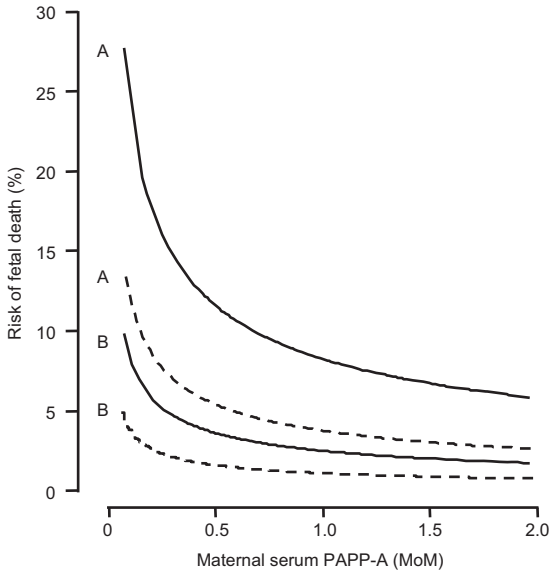

Fig 1. Relationship between maternal serum pregnancy-associated plasma protein A multiples of the median and risk for subsequent fetal death. The *solid lines* are for reversed a-wave in the ductus venosus flow pattern, and the *interrupted lines* are for normal a-wave. *A,* Black women. *B,* White women. PAPP-A, pregnancy-associated plasma protein A; MoM, multiples of the median.

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translucency, altered concentrations of maternal serum PAPP-A, and free β-hCG is compatible with the results of extensive previous studies.[15,16,20] In addition, we observed reversed a-wave in the ductus venosus in about 65% of fetuses with trisomies 21, 18, and 13 and in 50% of those with other abnormalities. In eight previous studies on a combined total of 10,945 pregnancies, abnormal flow in the fetal ductus venosus was observed in 5% (range 1.7–13.0%) of euploid fetuses and in 72.1% (range 38.3–100%) of the 210 with trisomy 21.[1–8] The incorporation of flow in the ductus venosus in the risk algorithms for first-trimester screening of chromosomal abnormalities is the subject of a separate study.

The findings that the prevalence of major cardiac defects increased with fetal nuchal translucency and was higher in those with reversed a-wave than in those with normal ductus venosus flow are compatible with previous reports.[3,4,6,9–13,21,22] A major limitation of our study was the method of diagnosing or excluding cardiac defects, which was based on antenatal ultrasonographic assessment or neonatal clinical examination. Ideally the antenatal findings of either absence or presence of a cardiac defect should have been validated by specialist examination of all infants that were liveborn and by postmortem examination in all cases of pregnancy termination or perinatal death. This methodologic problem is the most likely explanation for the overall prevalence of major cardiac defects (0.2%) being lower than the estimated 0.5% in chromosomally normal fetuses at this gestation.[23] Despite this limitation, the proportion of our fetuses with cardiac defects presenting with nuchal translucency above the 95th centile (eight of 20) was similar to the results of a meta-analysis, which reported that in 37% of euploid fetuses with cardiac defects the fetal nuchal translucency were above the 95th centile.[22]

In fetuses with increased nuchal translucency, the prevalence of abnormal flow in the ductus venosus is higher in those with cardiac defects than in those with no cardiac defects. In our euploid fetuses with nuchal translucency above the 95th centile, reversed a-wave was observed in 62.5% (five of eight) fetuses with cardiac defects, compared with 6.0% (25 of 415) in the normal outcome group. In seven previous studies on a combined total of 600 euploid fetuses with nuchal translucency above the 95th centile, there were 29 cases of cardiac defects, and abnormal flow in the ductus venosus was observed in 96.6% (range 75–100%) of fetuses with cardiac defects, compared with 18.2% (range 0–40%) in those with no cardiac defects.[3,4,6,9–12] In another study of 191 euploid fetuses with nuchal translucency above the 99th centile, including 16 with cardiac defects, abnormal flow in the ductus venosus was observed in 68.8% of fetuses with cardiac defects, compared with 22.9% in those with no cardiac defects.[13] In the majority of fetuses with cardiac defects and normal nuchal translucency, in contrast to those with increased nuchal translucency, the flow pattern in the ductus venosus is normal. Abnormal flow in the ductus was not observed in any of the 12 such cases in our study or the four cases in the combined data from two previous studies.[6,12] The consequence of this finding is that despite previous expectations,[13] first-trimester screening by Doppler of the ductus venosus is unlikely to improve the overall detection rate of cardiac defects achieved by screening with nuchal translucency alone.

The finding that the prevalence of noncardiac defects increased with fetal nuchal translucency thickness is compatible with the results of previous studies showing that with increasing fetal nuchal translucency thickness there is an increase in the prevalence of a wide variety of fetal malformations, dysplasias, deformations, disruptions, and genetic syndromes.[24] Although the prevalence of reversed a-wave increased with increasing fetal nuchal translucency thickness,

the prevalence of reversed a-wave in fetuses with noncardiac defects was not significantly higher than in the normal outcome group. Similarly, in one previous screening study by Toyama et al,[6] abnormal flow in the ductus venosus was observed in 11.1% (one of nine) of fetuses with noncardiac defects, which was not significantly higher than the prevalence of 5.9% (61 of 1,041) in the normal outcome group.

The prevalence of reversed a-wave in fetal deaths was three times higher than in the normal outcome group, irrespective of fetal nuchal translucency thickness. In a previous smaller screening study, the prevalence of abnormal flow in the ductus venosus was similarly higher in the cases resulting in fetal death than in the normal outcome group (22.2% compared with 5.9%), but in all their fetal deaths with abnormal flow there was increased nuchal translucency thickness.[6] We found that additional independent risk factors for fetal death included low maternal serum PAPP-A and Black ethnicity. The association between low PAPP-A, which presumably implies impaired placentation, and both early and late fetal death is compatible with the results of previous studies.[25–30] As demonstrated in our study, this PAPP-A–related patient-specific risk for fetal death can be modified by the results of the assessment of the fetal ductus venosus. In the second and third trimesters of pregnancy, abnormal flow in the ductus venosus is associated with fetal compromise,[31] and this seems to be true also for the first trimester.

There is a theoretical risk of thermal damage to the developing fetus from the use of color and pulsed Doppler examination. However, such theoretical risk applies only to transvaginal ultrasonography before 10 weeks, and in any case there is no epidemiologic or other evidence to support such assertion.[32] In our study, the ultrasound examinations were performed transabdominally after 11 weeks, and we used the "as low as reasonably achievable" principle, with output settings of the machines resulting in thermal index and mechanical index values below 0.6.

Reversed a-wave in the ductus venosus is associated with increased risk for chromosomal abnormalities, cardiac defects, and fetal death. However, in approximately 80% of cases with reversed a-wave, the pregnancy outcome is normal. The rate of adverse outcome is not only associated with reversed a-wave but also with certain maternal characteristics, altered levels of serum metabolites, and fetal nuchal translucency. The rate of adverse outcome in fetuses with reversed a-wave increases from less than 10% in those with normal nuchal translucency to more than 70% in those with high nuchal translucency.

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STUDY 5

Ductus venosus Doppler at 11-13 weeks in the prediction of outcome in twin pregnancies

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Obstet Gynecol 2009;113:860-5.

Ductus Venosus Doppler at 11 to 13 Weeks of Gestation in the Prediction of Outcome in Twin Pregnancies

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OBJECTIVE: To examine the independent contribution of abnormal flow in the ductus venosus at 11 to 13 weeks of gestation in the prediction of adverse pregnancy outcome in relation to chorionicity.

METHODS: This was a prospective study in 516 dichorionic and 179 monochorionic twin pregnancies in which the fetal ductus venosus flow was assessed at 11 0/7 to 13 6/7 weeks of gestation. The prevalence of reversed a-wave in the fetal ductus venosus was compared between monochorionic and dichorionic pregnancies and between those with and without pregnancy complications. Comparisons between each of the pregnancy outcomes and the normal outcome group and between monochorionic and dichorionic pregnancies were made using the Mann-Whitney U-test for continuous variables and the χ^2 test and Fisher exact test for categorical variables.

RESULTS: The prevalence of reversed a-wave in at least one of the fetuses was significantly higher in monochorionic than in dichorionic pregnancies (18.4% compared with 8.3%, $P<.001$) and in pregnancies complicated by miscarriage (28.6%, $P=.005$), fetal aneuploidy (70.0%, $P<.001$), and twin–twin transfusion syndrome (38.5%, $P<.001$) compared with the pregnancies with two healthy live births (7.7%). Pregnancy outcome was normal in 33 of the 43 (76.7%) dichorionic and in 14 of the 33 (42.4%) monochorionic twins with reversed a-wave in at least one of the fetuses.

CONCLUSION: In twins, reversed a-wave in the ductus venosus at 11 to 13 weeks of gestation is associated with increased risk for aneuploidies, miscarriage, and development of severe twin–twin transfusion syndrome. However, in about 75% of dichorionic twins and 40% of monochorionic twins with reversed a-wave, the pregnancy outcome is normal.

(Obstet Gynecol 2009;113:860–5)

LEVEL OF EVIDENCE: II

In singleton pregnancies at 11–13 weeks of gestation, about 4% of fetuses demonstrate reversed a-wave in the flow velocity waveform from the ductus venosus. This abnormal flow pattern is associated with increased risk for chromosomal abnormalities, cardiac defects, and fetal death.[1] In a prospective study in singleton pregnancies, reversed a-wave was observed in 3.7% of 10,120 with normal outcome, 10.8% of the 185 resulting in miscarriage or fetal death, 62.1% of the 95 with abnormal karyotype, 25% of the 20 with major cardiac defect, and 4.3% of the 70 with noncardiac defect.[1]

Studies of ductus venosus flow in twin pregnancies have focused on the association of abnormal flow as an early ultrasound marker of severe twin–twin transfusion syndrome. In a series of 11 monochorionic twin pregnancies, severe twin–twin transfusion syndrome developed in the two cases with abnormal ductus venosus waveforms in the recipient fetus at 11–13 weeks of gestation and in none of the cases with normal ductus venosus flow patterns.[2]

The aim of this observational study of 179 monochorionic and 516 dichorionic twin pregnancies undergoing ultrasound examination at 11–13 weeks of gestation was to examine whether the value of ductus

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Supported by a grant from the Fetal Medicine Foundation (Charity No: 1037116).

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Financial Disclosure
The authors did not report any potential conflicts of interest.

ISSN: 0029-7844/09

venosus flow in the prediction of adverse pregnancy in twins is similar to that in singleton pregnancies and the interaction between flow and chorionicity in the prediction of outcome.

MATERIALS AND METHODS

This was a prospective study in diamniotic twin pregnancies examined at 11 0/7 to 13 6/7 weeks of gestation as part of our policy of screening for chromosomal abnormalities by a combination of maternal age and fetal nuchal translucency thickness.[3] The inclusion criteria were diamniotic twin pregnancies with two live fetuses at 11–13 weeks. Transabdominal ultrasound examination was performed to diagnose any major fetal defects and for measurement of the crown-rump length (CRL) and nuchal translucency thickness of each twin. Ductus venosus blood flow velocity waveforms also were obtained routinely by sonographers who had received the appropriate Fetal Medicine Foundation Certificate of Competence in this assessment, but the results of ductus flow were not used in the clinical management of the patients. The pregnancies were diagnosed as being dichorionic or monochorionic by the presence or absence, respectively, of placental tissue in the base of the inter-twin membrane (lambda sign).[4] Gestational age was calculated from the last menstrual cycle unless there was a discordance of more than 6 days from the gestation estimated from the CRL of the fetus with the longest measurement. In each fetus, ductus venosus flow also was assessed.

The Doppler studies were undertaken during fetal quiescence. The magnification of the image was such that the fetal thorax and abdomen occupied the whole screen. A right ventral midsagittal view of the fetal trunk was obtained, and color flow mapping was used to demonstrate the umbilical vein, ductus venosus, and fetal heart. The pulsed Doppler sample was small (0.5–1.0 mm) to avoid contamination from the adjacent veins, and it was placed in the portion immediately above the umbilical sinus. The insonation angle was less than 30°, the filter was set at a low frequency (50–70 Hz) to allow visualization of the whole waveform, and the sweep speed was high (2–3 cm/s) so that the waveforms were spread widely, allowing better assessment of the a-wave. The waveforms were considered to be abnormal if the a-wave was reversed.[5]

Our policy for the follow-up of monochorionic twins includes ultrasound examinations at 16–18 weeks of gestation and monthly thereafter, unless there is evidence of twin–twin transfusion syndrome, in which case the frequency of examinations is increased as necessary. In cases of severe twin–twin transfusion syndrome, endoscopic laser coagulation of the communicating placental vessels is performed.[6] The indications for such treatment are 1) ultrasound diagnosis of hydramnios in one twin and anhydramnios in the other and 2) absent or reversed end diastolic flow in either the umbilical artery or ductus venosus in one or both fetuses. In dichorionic twins, ultrasound examinations are performed routinely at 16–18 weeks and monthly thereafter.

Patients were asked to complete a questionnaire on maternal age, ethnic origin (white, African, Indian or Pakistani, Chinese or Japanese, and mixed), method of conception (natural or by in vitro fertilization [IVF]), and cigarette smoking during pregnancy (yes or no). Maternal weight and height were measured, and body mass index (BMI) was calculated as kg/m^2. Written informed consent was obtained from each patient participating in the study, which was approved by the King's College Hospital Research Ethics Committee.

Demographic details, ultrasound findings, fetal interventions, and pregnancy outcome were entered into a computer database. Pregnancy outcome was obtained from the maternity units or from the patients themselves.

In each pregnancy, the inter-twin discordance in nuchal translucency and CRL was calculated as the difference in each measurement between the two fetuses (largest-smallest) expressed as a percentage of the bigger measurement.

Comparisons between each of the pregnancy outcomes and the normal outcome group and between monochorionic and dichorionic pregnancies was by the Mann-Whitney U-test for continuous variables and the χ^2 test and Fisher exact test for categorical variables. Kolmogorov-Smirnov test was used to test for normality of the distributions of maternal age and BMI. We used post hoc Bonferroni correction for the multiple comparisons, dividing the critical value for significance (.05) by the number of tests we had conducted, which was four for dichorionic and six for monochorionic twins.

Multiple logistic regression analysis in the whole population was used to determine the significant contributors to reversed a-wave in the fetal ductus venosus from maternal characteristics, chorionicity, different outcome groups, and inter-twin discordance in CRL and nuchal translucency. Similarly, multiple logistic regression analysis was performed to determine the significance of the contribution to each outcome group of reversed a-wave and inter-twin

discordance in CRL and nuchal translucency and maternal characteristics.

The statistical software package SPSS 12.0 (SPSS Inc., Chicago, IL) was used for all data analyses.

RESULTS

During the study period (January 2006 to January 2008), we prospectively examined 733 diamniotic twin pregnancies with two live fetuses at 11 0/7 to 13 6/7 weeks of gestation and successfully obtained ductus flow waveforms from both fetuses in 728 (99.3%) cases. We excluded 33 (4.5%) cases for which there was a missing outcome. In the remaining 695 pregnancies, 516 were dichorionic and 179 were monochorionic. Measurement of fetal nuchal translucency was carried out in all fetuses. The median maternal age was 33.3 (interquartile range 29–36) years, the median gestational age was 89 (interquartile range 86–92) days, and the median BMI was 25.0 (interquartile range 22.5–28.3). In 555 (79.9%) cases, the mother was white, in 92 (13.2%) she was African, in 30 (4.3%) Indian or Pakistani, in 7 (1.0%) Chinese or Japanese, and in 11 (1.6%) of mixed ethnic origin. The maternal demographic characteristics in monochorionic and dichorionic twin pregnancies are shown in Table 1.

The prevalence of reversed a-wave in at least one of the fetuses was significantly higher in pregnancies complicated by miscarriage (28.6%, 95% confidence interval [CI] 13.6–50.2%, $P=.005$), fetal aneuploidy (70.0%, 95% CI 39.2–89.7%, $P<.001$), and twin–twin transfusion syndrome (38.5%, 95% CI 22.4–57.5%, $P<.001$) compared with the pregnancies with two healthy live births (7.7%, 95% CI 5.8–10.1%).

Table 1. Demographic Characteristics in the Dichorionic and Monochorionic Twin Pregnancies

	Dichorionic (n=516)	Monochorionic (n=179)
Maternal age (y)	33.5 (29.7–36.7)	31.9 (27.7–36.5)*
Body mass index (kg/m^2)	25.0 (22.5–28.3)	25.2 (22.4–28.4)
Ethnic origin		
White	412 (79.8)	143 (79.9)
African	71 (13.8)	20 (11.2)
Indian or Pakistani	22 (4.3)	9 (5.0)
Chinese or Japanese	3 (0.6)	4 (2.2)
Mixed	8 (1.6)	3 (1.7)
Conception by in vitro fertilization	168 (32.6)	11 (6.1)†

Data are median (interquartile range) or n (%).
Comparisons by χ^2 test for categorical variables and, for continuous variables, *t*-test for independent samples for maternal age and Mann-Whitney U-test for maternal BMI.
* $P<.05$.
† $P<.01$.

Pregnancy outcome was normal in 565 of the 619 (91.3%, 95% CI 88.8–93.3%) pregnancies with normal a-wave in both twins and in 47 of the 76 (61.8%, 95% CI 50.6–72.0%) of the pregnancies with reversed a-wave in at least one of the fetuses. Pregnancy outcome was normal in 33 of the 43 (76.7%, 95% CI 62.1–87.0%) dichorionic and in 14 of the 33 (42.4%, 95% CI 27.2–59.2%) monochorionic twins with reversed a-wave in at least one of the fetuses.

Of the 516 dichorionic twin pregnancies, there were 484 (93.8%, 95% CI 91.4–95.7%) that resulted in the live birth of two healthy babies, 10 (1.9%, 95% CI 0.9–3.5%) that resulted in miscarriage, eight (1.6%, 95% CI 0.7–3.0%) in which there was death of one fetus and live birth of the other, nine (1.7%, 95% CI 0.8–3.3%) in which one of the fetuses had a chromosomal abnormality (five with trisomy 21 and one each of trisomy 18, trisomy 13, triploidy, and partial deletion of chromosome 18) and the other twin was euploid, and five (1.0%, 95% CI 0.4–2.3%) in which one of the fetuses was healthy and the other had a major defect (one case each of Dandy Walker malformation with major ventricular septal defect, spina bifida, severe ventriculomegaly, transposition of the great arteries, and atrioventricular septal defect).

Reversed a-wave in the ductus venosus was observed in 47 (4.6%, 95% CI 3.4–6.0%) of the 1,032 fetuses and in at least one of the twins in 43 (8.3%, 95% CI 6.1–11.1%) of the pregnancies (Table 2). The prevalence of reversed a-wave was significantly higher in the pregnancies with chromosomal abnormalities than in those with normal outcome (66.7% compared with 6.8%).

Of the 179 monochorionic twin pregnancies, there were 128 (71.5%, 95% CI 64.3–78.0%) that resulted in the live birth of two healthy babies, 11 (6.2%, 95% CI 3.1–10.7%) that resulted in miscarriage, six (3.4%, 95% CI 1.2–7.2%) in which there was death of one fetus and either live birth of the other (n=5) or termination of pregnancy because the cotwin developed intraventricular and periventricular hemorrhage, one (0.6%, 95% CI 0.01–3.1%) in which the fetuses had trisomy 21, three (1.7%, 95% CI 0.4–4.8%) in which one of the fetuses was healthy and the other had a major defect (one case each of transposition of the great arteries, tetralogy of Fallot, and pulmonary atresia), 26 (14.5%, 95% CI 9.7–20.6%) that developed severe twin–twin transfusion syndrome (laser endoscopic surgery was carried out in 24 cases, and there was fetal death before surgery

Table 2. Prevalence of Reversed a-Wave in the Different Outcome Groups

		Reversed a-Wave in the Ductus Venosus		
	n	Pregnancies	One Fetus	Both Fetuses
Dichorionic				
Total	516	43 (8.3, 6.2–11.1)	39 (7.6, 5.6–10.2)	4 (0.8, 0.2–2.1)
Live birth/live birth	484	33 (6.8, 4.9–9.5)	30 (6.2, 4.4–8.7)	3 (0.6, 0.1–1.9)
Death of one fetus	8	1 (12.5, 0.1–49.2)	1 (12.5, 0.1–49.2)	–
Aneuploidy one fetus	9	6 (66.7, 35.1–88.3)*	6 (66.7, 35.1–88.3)*	–
Major defect one fetus	5	1 (20.0, 2.0–64.0)	1 (20.0, 2.0–64.0)	–
Miscarriage	10	2 (20.0, 4.6–52.1)	1 (10, 0.0–42.6)	1 (10, 0.0–42.6)
Monochorionic				
Total	179	33 (18.4, 13.4–24.8)	21 (11.7, 7.7–17.3)	12 (6.7, 3.8–11.5)
Live birth/live birth	128	14 (10.9, 6.5–17.6)	9 (7.0, 3.6–13.0)	5 (3.9, 1.4–9.1)
Death of one fetus	6	1 (16.7, 1.1–58.2)	1 (16.7, 1.1–58.2)	–
Aneuploidy both fetuses	1	1 (100, 16.8–100)	–	1 (100, 16.8–100)
Major defect one fetus	3	2 (66.7, 20.2–94.4)	2 (66.7, 20.2–94.4)	–
Miscarriage	11	4 (36.4, 15.0–64.8)	2 (18.2, 4.0–48.9)	2 (18.2, 4.0–48.9)
Twin–twin transfusion	26	10 (38.5, 22.4–57.5)†	6 (23.1, 10.7–42.4)	4 (15.4, 5.5–34.2)
Selective growth restriction	4	1 (25.0, 3.4–71.1)	1 (25.0, 3.4–71.1)	–

Data are n (%, 95% confidence interval) unless otherwise specified.
χ^2 test with Bonferroni correction for comparison of each group with the normal-outcome group.
* $P<.013$.
† $P<.008$.

could be performed in two cases), and four (2.2% 95% CI 0.6–5.6%) in which there was a selective severe intrauterine growth restriction for which an endoscopic laser surgery was performed.

Reversed a-wave in the ductus venosus was observed in 45 (12.6%, 95% CI 9.3–16.5%) of the 358 fetuses and in at least one of the twins in 33 (18.4%, 95% CI 13.0–24.9%) of the pregnancies (Table 2). The prevalence of reversed a-wave was significantly higher in the pregnancies resulting in twin-to twin transfusion syndrome than in those with normal outcome (38.5% compared with 10.9%).

The prevalence of reversed a-wave in the ductus venosus was significantly higher in all monochorionic twins than in all dichorionic twins (18.4% compared with 8.3%, $P<.001$) but not in the pregnancies with normal outcome (10.9% compared with 6.8%, $P=.171$). Logistic regression analysis in the total population of 695 twin pregnancies demonstrated that, in addition to chorionicity, significant contributors to reversed a-wave in the ductus venosus were provided by inter-twin discordance in CRL and nuchal translucency, fetal aneuploidy, and development of severe twin–twin transfusion syndrome (Table 3).

Multiple logistic regression analysis to predict pregnancies with at least one fetal aneuploidy demonstrated significant contribution by reversed a-wave in at least one of the fetuses (odds ratio [OR] 11.27, 95% CI 2.32–54.73, $P=.003$) and inter-twin discordance in nuchal translucency (OR 1.07, 95% CI 1.03–1.11, $P<.001$) but not chorionicity ($P=.413$), maternal age ($P=.354$), ethnicity ($P=.587$), BMI ($P=.979$), conception by IVF ($P=.830$), smoking ($P=.997$), or inter-twin discordance in CRL ($P=.056$).

In the total of 695 pregnancies, there were 21 (3.0%, 95% CI 2.0–4.6%) miscarriages. Multiple lo-

Table 3. Multiple Logistic Regression Analysis for the Prediction of Reversed a-Wave in the Ductus Venosus

	OR (95% CI)	P
Monochorionicity	1.93 (1.05–3.55)	.034
Crown-rump length discordance	1.11 (1.05–1.18)	<.001
Nuchal translucency discordance	1.03 (1.01–1.05)	.001
Outcome groups		
Live birth/live birth	1	–
Fetal death	0.59 (0.10–3.52)	.563
Aneuploidy	11.72 (2.32–59.32)	.003
Major defect	4.26 (0.77–23.73)	.098
Miscarriage	2.85 (0.98–8.33)	.055
Twin–twin transfusion	3.67 (1.30–10.35)	.014
Selective growth restriction	1.56 (0.15–16.52)	.714
Ethnic origin		
White	1	–
African	1.13 (0.57–2.25)	.718
Indian or Pakistani	0.88 (0.26–3.00)	.843
Chinese or Japanese	1.38 (0.16–11.62)	.770
Mixed	0.86 (0.10–6.56)	.856
Maternal age	1.00 (0.96–1.05)	.968
Body mass index	0.96 (0.90–1.01)	.112
In vitro fertilization	0.96 (0.55–1.66)	.873

OR, odds ratio; CI, confidence interval.

gistic regression analysis demonstrated that significant contribution to miscarriage was provided by reversed a-wave in at least one of the fetuses (OR 3.38, 95% CI 1.16–9.83, P=.025), monochorionicity (OR 4.02, 95% CI 1.60–10.11, P=.003), inter-twin discordance in nuchal translucency (OR 1.04, 95% CI 1.00–1.07, P=.03), and maternal African ethnic group (OR 4.64, 95% CI 1.68–12.80, P=.003) but not inter-twin discordance in CRL (P=.074), maternal age (P=.094), BMI (P=.629), conception by IVF (P=.163), or smoking (P=.855).

In the total of 695 pregnancies, there were six (0.9%, 95% CI 0.4–1.9%) with major cardiac defects in one of the fetuses. The prevalence of reversed a-wave in the pregnancies with cardiac defects (50%, three of six) was not significantly higher than in the pregnancies with normal outcome (7.7%, 47 of 612) (P<.008). For this difference to have been significantly different with a power of 80% we would have needed to examine seven pregnancies with fetal cardiac defects and 748 with normal outcome.

In the total of 179 monochorionic pregnancies, there were 26 (14.5%, 95% CI 10.1–20.5%) that developed severe twin–twin transfusion syndrome. In the group with reversed a-wave in at least one of the fetuses, the prevalence of severe twin–twin transfusion syndrome was 30.3% (95% CI 17.3–47.5%, 10 of 33) compared with 11.0% (95% CI 6.8–17.2%, 16 of 146) in those with normal a-wave in both fetuses (P=.01). The mean inter-twin discordance in nuchal translucency was 19.6% (95% CI 10.9–28.3%) in the twin–twin transfusion syndrome group and 16.7% (95% CI 14.4–19.0%) in the non–twin–twin transfusion syndrome group (P=.780). Multiple logistic regression analysis demonstrated that significant contribution to severe twin–twin transfusion syndrome was provided by reversed a-wave in at least one of the fetuses (OR 5.09, 95% CI 1.94–13.37, P=.001) but not inter-twin discordance in nuchal translucency (P=.161) or CRL (P=.175), maternal age (P=.108), ethnicity (P=.999), BMI (P=.490), conception by IVF (P=.347), or smoking (P=.327).

DISCUSSION

The findings of this study in twin pregnancies that the prevalence of reversed a-wave in the ductus venosus at 11–13 weeks of gestation is more common in fetuses with aneuploidies and miscarriage are compatible with the data in singleton pregnancies.[1] An additional factor affecting the prevalence of reversed flow in twins is chorionicity, being more common in monochorionic pregnancies and in particular those that subsequently develop severe twin–twin transfusion syndrome.

Studies in singleton pregnancies have shown that abnormal flow in the ductus venosus is a good marker for aneuploidies and improves the performance of first-trimester screening for trisomy 21 provided by a combination of maternal age, fetal nuchal translucency thickness, and maternal serum free ß–hCG and pregnancy-associated plasma protein A.[1,7–9] In our study of twins, there was a similarly high prevalence of reversed a-wave in aneuploid compared with euploid fetuses as in singleton pregnancies,[1] and assessment of the ductus venosus flow improved the prediction of aneuploidies provided by screening with fetal nuchal translucency alone. Nevertheless, this study of twins is too small, in comparison with that of singletons, to provide accurate prediction of the estimated improvement in the performance of screening with the inclusion of ductus venosus flow.

In our study of twins, as in our previous study in singletons,[1] the rate of miscarriage in those with reversed a-wave and in those of African ethnicity was increased. The miscarriage rate was also higher in monochorionic than in dichorionic twins, which may be due to early twin–twin transfusion syndrome before 16 weeks, when endoscopic laser surgery can be performed. Another possible explanation for the association of early fetal death and miscarriage with reversed a-wave is that this abnormal flow pattern could be the consequence of impaired placentation leading to increased cardiac afterload.

The prevalence of reversed a-wave in fetuses from singleton pregnancies and dichorionic twins is similar, but in monochorionic twins it is substantially higher. In one third of monochorionic twins with reversed a-wave, there was subsequent development of severe twin–twin transfusion syndrome, and, therefore, the prevalence of abnormal flow was increased compared with dichorionic twins, even in those monochorionic twins that did not develop severe twin–twin transfusion syndrome. Thus, in monochorionic twins examined at 11–13 weeks, the rate of subsequent development of severe twin–twin transfusion syndrome is about 15%; this is increased to 30% if there is reversed a-wave in at least one of the fetuses and reduced to about 10% in those with normal a-wave in both fetuses. Consequently, ductus venosus flow at 11–13 weeks helps to modify the risk for subsequent development of severe twin–twin transfusion syndrome and the intensity of monitoring of such pregnancies. It is possible that, in the pregnancies we studied with abnormal Doppler and normal outcome, there was spontaneous resolution of twin–twin trans-

fusion syndrome between the first and second trimesters, and, in contrast, in those with normal Doppler and subsequent twin–twin transfusion syndrome, the hemodynamic abnormality developed during the second trimester.

In twins, reversed a-wave in the ductus venosus at 11–13 weeks of gestation is associated with increased risk for aneuploidies, miscarriage, and development of severe twin–twin transfusion syndrome. However, in about 75% of dichorionic twins and 40% of monochorionic twins with reversed a-wave, the pregnancy outcome is normal.

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CHAPTER III

RESULTS SUMMARY

3.1 Learning curve for Doppler assessment of ductus venosus flow at 11-13 weeks

Ten sonographers with prior extensive experience in the measurement of nuchal translucency thickness were given practical training in the accurate assessment of the ductus venosus. They were then asked to examine the ductus venosus during the routine 11-13 weeks scan. Each scan was assessed by an experienced sonographer and classified as being successful or unsuccessful (failure to obtain a waveform, poor quality image with contamination or wrong classification of the a-wave). Each sonographer performed a total of 300 examinations, the data were analyzed in 15 groups of 20 examinations and in each group the percentage of unsuccessful examinations was calculated. In the total 3000 cases examined by the 10 sonographers there were 2849 (95.0%) successful examinations and 151 unsuccessful, including 104 failures to obtain a waveform, 32 cases where the quality of the image was considered to be inadequate and 15 cases in which the classification of the a-wave was wrong. The overall frequency of unsuccessful examinations decreased significantly with the number of scans carried out ($r=0.982$, $p<0.0001$). Regression analysis demonstrated that successful examination of the ductus venosus depended on the experience of the sonographer (defined by the cumulative number of scans) but not fetal crown-rump length, maternal ethnic origin or body mass index. The sonographers required an average of 80 examinations before they could successfully examine the ductus in least 19 of a group of 20 scans. Although one of the 10 trainees achieved this standard within the first block of 20 scans some of the sonographers required training in 100 cases.

3.2 Ductus venosus Doppler in fetuses with cardiac defects

Ductus venosus blood flow velocity waveforms were obtained immediately before chorion villous sampling for fetal karyotyping in fetuses with NT thickness of 3.5 mm or more at 11-13 weeks of gestation. In the chromosomally normal group fetal echocardiography was also performed by a specialist pediatric cardiologist at 11-13 weeks and/or 18-22 weeks. Major cardiac defects were diagnosed in 16 (8.4%) of the

191 fetuses. Reverse or absent flow during atrial contraction was observed in 11 of the 16 (68.8%) fetuses with cardiac defects and in 40 of the 175 (22.9%) with no cardiac defects. Multivariate analysis demonstrated that the prevalence of abnormal a-wave in the ductus venosus in fetuses without major cardiac defects increased with fetal NT thickness (Odds ratio [OR], 1.463; 95% CI, 1.183-1.809; p<0.0001) but not CRL (OR, 0.962, 95%CI, 0.925-1.0; p=0.053). In those with cardiac defects the prevalence of abnormal a-wave did not change significantly with NT thickness (OR, 2.054; 95% CI, 0.573-7.360; p=0.269) or CRL (OR, 0.930, 95%CI, 0.834-1.038; p=0.196). The likelihood ratio for a major cardiac defect when the ductus venosus flow was abnormal decreased with fetal NT thickness from 4.58 at NT of 3.5 mm to 2.47 for NT of 5.5 mm and the likelihood ratio when the ductus venosus flow was normal increased from 0.37 at NT of 3.5 mm to 0.43 for NT of 5.5 mm.

3.3 Ductus venosus Doppler in screening for chromosomal abnormalities

Screening by the combined test was performed in singleton pregnancies, including 19,614 with euploid fetuses, 122 with trisomy 21, 36 with trisomy 18, 20 with trisomy 13 and 8 with Turner syndrome. In all cases the a-wave in the fetal ductus venosus flow was assessed. We examined the performance of two screening strategies: firstly, assessment of the a-wave in all patients and secondly, first-stage screening using the combined test in all patients followed by second-stage assessment of the a-wave only in those with an intermediate risk of 1 in 51 to 1 in 1,000 after the first-stage. Logistic regression analysis demonstrated that the prevalence of reversed a-wave significantly increased with fetal NT (p<0.0001), decreased with fetal CRL and maternal serum PAPP-A, and was more common in Black ethnic group. Reversed a-wave was observed in 3.2% of the euploid fetuses and in 66.4%, 58.3%, 55.0% and 75.0% of fetuses with trisomies 21, 18 and 13 and Turner syndrome, respectively. Inclusion of ductus venosus flow in all pregnancies would detect 96%, 92%, 100% and 100% of trisomies 21, 18 and 13 and Turner syndrome, respectively, at a false positive rate of 3%. The same detection rates were achieved with the two-stage strategy at a false

positive rate of 2.6% in which it was necessary to assess the ductus venosus in only 15% of the total population.

3.4 Ductus venosus in screening for adverse pregnancy outcome

Prospective assessment of singleton pregnancies by maternal history, serum free ß-hCG and pregnancy-associated plasma protein A (PAPP-A) and fetal nuchal translucency (NT) thickness and ductus venosus Doppler. The patients were subdivided into five groups: normal outcome (n=10,120), miscarriage or fetal death (n=185), abnormal karyotype (n=95), major cardiac (n=20) or non-cardiac defect (n=70). Regression analysis was performed to determine the significance of the contribution to adverse outcome of reversed a-wave in the ductus venosus, maternal characteristics, fetal delta NT, and maternal serum log PAPP-A MoM and log free ß-hCG. The prevalence of reversed a-wave was significantly higher in the groups with miscarriage or fetal death (10.8%), abnormal karyotype (62.1%) and fetal cardiac defect (25.0%) but not non-cardiac defect (4.3%) than in the normal outcome group (3.7%). Logistic regression analysis demonstrated that significant contribution to fetal chromosomal abnormality was provided by reversed a-wave, as well as maternal age fetal delta NT and maternal serum log MoM PAPP-A. Significant contribution to fetal cardiac defect was provided by reversed a-wave in the fetal ductus venosus and fetal delta NT. Significant contribution to fetal death was provided by reversed a-wave in the fetal ductus venosus, as well as Black ethnicity, BMI and maternal serum log MoM PAPP-A, An adverse outcome was observed in 2.7% of the fetuses with NT at or below the 95th centile (in 2.6% of those with normal a-wave and in 7.0% of those with reversed a-wave) and in 19.3% of the fetuses with NT above the 95th centile (in 8.9% of those with normal a-wave and in 70.9% of those with reversed a-wave).

3.5 Ductus venosus Doppler at 11-13 weeks in the prediction of outcome in twin pregnancies

This was a prospective study in 516 dichorionic and 179 monochorionic twin pregnancies in which the fetal ductus venosus flow was assessed at 11-13 weeks of

gestation. The prevalence of reversed a-wave in the fetal ductus venosus was compared between monochorionic and dichorionic pregnancies and between those with and without pregnancy complications. The prevalence of reversed a-wave in at least one of the fetuses was significantly higher in monochorionic than in dichorionic pregnancies (18.4% vs. 8.3%, $p<0.001$) and in pregnancies complicated by miscarriage (28.6%, $p=0.005$), fetal aneuploidy (70.0%, $p<0.001$) and twin-to-twin-transfusion syndrome (38.5%, $p<0.001$) compared to the pregnancies with two healthy live births (7.7%). In dichorionic twins the prevalence of reversed a-wave was significantly higher in the pregnancies with chromosomal abnormalities than in those with normal outcome (66.7% vs 6.8%). In monochorionic twins the prevalence of reversed a-wave was significantly higher in the pregnancies resulting in twin-to twin transfusion syndrome than in those with normal outcome (38.5% vs 10.9%). Logistic regression analysis in the total population of 695 twin pregnancies demonstrated that in addition to chorionicity significant contributors to reversed a-wave in the ductus venosus were provided by inter-twin discordance in CRL and nuchal translucency, fetal aneuploidy and development of severe twin-to-twin transfusion syndrome. Pregnancy outcome was normal in 33 of the 43 (76.7%) dichorionic and in 14 of the 33 (42.4%) monochorionic twins with reversed a-wave in at least one of the fetuses.

CHAPTER IV

DISCUSSION

The studies in this thesis have to a large extent fulfilled the aims as outlined in chapter 1.8.

The first study demonstrated that competence in Doppler assessment of the ductus venosus is achieved only after extensive supervised training. Sonographers with prior extensive experience in the 11-13 weeks scan require an average of 80 examinations before they could successfully examine the ductus in least 19 of a group of 20 scans. Although one of the 10 trainees achieved this standard within the first block of 20 scans some of the sonographers required training in 100 cases.

The findings of the second study demonstrated that there is a high association between abnormal ductus venosus flow at 11-13 weeks of gestation and major cardiac defects. In chromosomally normal fetuses with increased fetal NT the finding of absent or reversed a-wave in the ductus venosus is associated with a three fold increase in the likelihood of a major cardiac defect, whereas the finding of normal ductal flow is associated with a halving in risk for such defects. In the assessment of individual risk for major cardiac defects the *a priori* NT-dependent risk can be multiplied by the appropriate likelihood ratio depending on whether the a-wave in the ductus venosus is normal or abnormal. However, the studies examining the association between ductus venosus flow and cardiac defects are essentially confined to fetuses with increased NT and it is therefore uncertain whether in fetuses with cardiac defects and normal NT the a-wave in the ductus venosus is also abnormal. Consequently, population-based studies in which there is no pre selection by measurement of the NT thickness are needed to assess the true effectiveness of first trimester screening for major cardiac defects by Doppler assessment of the ductus venosus.

The findings of the third study demonstrated that reversed a-wave in the ductus venosus at 11-13 weeks is found in about 3% of euploid fetuses, in 65% of fetuses with trisomy 21, in about 55% of those with trisomies 13 and 18 and in 75% of Turner syndrome. As demonstrated in this study assessment of ductus venosus flow improves the performance of first-trimester combined screening by increasing the estimated detection rate to about 96% and reducing the false positive rate to about 2.5%. We demonstrated two strategies for assessment of ductus venosus flow. In the first approach the ductus venosus can be examined in all cases with the advantage of not

only improving the performance of screening for chromosomal abnormalities but also identifying pregnancies at increased risk of fetal cardiac defects and fetal death (Maiz *et al.*, 2008a). The alternative strategy, since assessment of ductus venosus flow is time-consuming and requires appropriately trained sonographers, is to reserve this examination to the subgroup of pregnancies with an intermediate-risk (between 1 in 51 and 1 in 1000) after combined fetal NT, FHR, free ß-hCG and PAPP-A screening, which constitutes only one sixth of the total population. Similar two stage strategies have also been advocated for assessment of flow across the tricuspid valve and in the examination for the presence or absence of the nasal bone (Nicolaides *et al.*, 2005; Falcon *et al.*, 2006; Cicero *et al.*, 2006).

The objective of the fourth study was to examine the independent contribution of abnormal flow in the ductus venosus at 11-13 weeks of gestation in the prediction of major fetal abnormalities and fetal death. The study showed that reversed a-wave in the flow velocity waveform from the ductus venosus is associated with increased risk for chromosomal abnormalities, cardiac defects and fetal death. However, in about 80% of cases with reversed a-wave the pregnancy outcome is normal. The rate of adverse outcome is not only associated with reversed a-wave but also with certain maternal characteristics, altered levels of serum metabolites and fetal NT. The rate of adverse outcome in fetuses with reversed a-wave increases from less than 10% in those with normal NT to more than 70% in those with high NT.

The findings of the fifth study demonstrated that in twin pregnancies as in singleton pregnancies the prevalence of reversed a-wave in the ductus venosus at 11-13 weeks increases with fetal nuchal translucency thickness, decreases with CRL and is more common in fetuses with aneuploidies, major cardiac defects and miscarriage. An additional factor affecting the prevalence of reversed flow in twins is chorionicity, being more common in monochorionic pregnancies and in particular those that subsequently develop severe twin-to-twin transfusion syndrome. In twins reversed a-wave in the ductus venosus at 11-13 weeks is associated with increased risk for aneuploidies, cardiac defects, miscarriage and development of severe twin-to-twin transfusion syndrome. However, in about 75% of dichorionic twins and 40% of monochorionic twins with reversed a-wave the pregnancy outcome is normal.

CHAPTER V

CONCLUSIONS

7.1 Conclusions of the first study

Competence in ductus venosus assessment can be achieved after extensive supervised training.

7.2 Conclusions of the second study

In chromosomally normal fetuses with increased NT, reversed or absent a-wave in the ductus venosus is associated with a three-fold increase in the risk of cardiac defects, whereas if it is positive the risk is halved.

7.3 Conclusions of the third study

Assessment of the ductus venosus flow improves the performance of the first trimester screening for chromosomal abnormalities, either when it is assessed in all the fetuses, or in a two-stage strategy, only in those with an intermediate risk of one in 51 to one in 1000 after the first stage.

7.4 Conclusions of the fourth study

Reversed a-wave in the ductus venosus is associated with adverse pregnancy outcome such as chromosomal abnormalities, cardiac defects and fetal death.

7.5 Conclusions of the fifth study

Reversed a-wave in the ductus venosus is more common in monochorionic than in dichorionic twin pregnancies.

In twins, reversed a-wave in the ductus venosus is associated with increased risk of chromosomal abnormalities and miscarriage, and also with twin-to-twin transfusion syndrome.

7.6 Final conclusions

After an extensive supervised training, ductus venosus flow assessment can be incorporated into the first trimester scan, where it improves the performance of screening for chromosomal defects and cardiac defects, and it helps to identify the fetuses with a higher risk of death. Similarly, in twin pregnancies ductus venosus assessment identifies the pregnancies with a higher risk of having a fetus with an aneuploidy, those with a higher risk of miscarriage, and those that will subsequently develop twin-to-twin transfusion syndrome.

CHAPTER VI

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CHAPTER VII

SUMMARY IN SPANISH

1. INTRODUCCIÓN

Anatomía funcional del ductus venoso

El ductus venoso comunica la porción intra-abdominal de la vena umbilical con la parte izquierda de la vena cava (Kiserud *et al.*, 1991). Hay una reducción del diámetro entre la vena umbilical y el ductus venoso, dando lugar a un gradiente de presiones entre la vena umbilical y la aurícula, que resulta en una aceleración del flujo sanguíneo en el ductus (Kiserud *et al.*, 1991, 1994a). El ductus venoso juega un papel fundamental en la oxigenación de los principales órganos fetales, dirigiendo la sangre oxigenada procedente de la placenta hacia el corazón izquierdo a través del foramen oval.

Doppler del ductus venoso

El ductus venoso se identifica fácilmente mediante el uso del Doppler color como la porción de alta velocidad que sigue a la vena umbilical. La onda de velocidad del ductus venoso tiene una morfología característica en la que se reconocen tres fases. La de más alta velocidad, que corresponde con la sístole ventricular (S), durante esta fase el gradiente de presión entre los vasos venosos y la aurícula es la más alta. El siguiente pico de flujo anterógrado refleja la diástole precoz (D), con la apertura de las válvulas auriculo-ventriculares y el llenado pasivo de los ventrículos. La tercera fase, la de flujo anterógrado con velocidad más baja, se corresponde con la contracción atrial (a), durante la diástole tardía (Kiserud *et al.*, 1991). A diferencia del segundo y tercer trimestre, donde en las gestaciones normales el flujo durante la contracción atrial es siempre anterógrado, en el primer trimestre el flujo de la onda-a puede ser ausente o reverso, y este hallazgo es más común en los fetos más jóvenes (Kiserud *et al.*, 1999, 2003).

Flujo en el ductus venoso y anomalías cromosómicas

Una alta proporción de fetos con trisomía 21 y otras cromosomopatías tienen una alta impedancia al flujo en el ductus venoso a las 11-13 semanas de gestación. En los datos combinados de siete estudios, se observó un flujo ductal anormal en 5,2% de los fetos cromosómicamente normales y en un 70,8%, 89,3%, 81,8% y 76,9% de fetos con trisomías 21, 18, 13 y síndrome de Turner respectivamente (Matias *et al.*, 1998;

Antolín *et al.,* 2001; Murta *et al.,* 2002; Zoppi *et al.,* 2002; Borrell *et al.,* 2003; Toyama *et al.,* 2004; Prefumo *et al.,* 2005). Se desconoce si la incidencia del flujo ductal anómalo se asocia con otros marcadores ecográficos y bioquímicos de cromosomopatías, y hasta qué punto la incorporación de la valoración del ductus venoso mejoraría la eficacia del screening combinado de primer trimestre.

Flujo en el ductos venoso y cardiopatías

Las anomalías cardiacas y de las grandes arterias son los defectos congénitos más comunes, y son causa de un elevado porcentaje de las muertes intrauterinas, y de más de la mitad de las muertes por malformación congénita durante la infancia. El flujo anormal en el ductos venoso a las 11-13 semanas de gestación se asocia a un elevado riesgo de anomalías cromosómicas y de defectos cardiacos (Matias *et al.,*1998; Matias *et al.,*1999; Borrell *et al.*1998; Bilardo *et al.,* 2001; Murta *et al.,* 2002; Zoppi *et al.,* 2002; Haak *et al.,* 2003; Favre *et al.,* 2003; Toyama *et al.,* 2004). En los datos combinados de siete estudios que examinaron la onda de flujo en 600 fetos cromosómicamente normales y con translucencia nucal (TN) aumentada (por encima del 95º percentil) se observó un defecto cardiaco mayor en 29 (4,8%) fetos, y de éstos 28 (96,6%) tenían una onda de flujo anormal en el ductos venoso. Algunos estudios que valoraban la asociación entre la onda de flujo anormal en el ductos venoso con cromosomopatías o defectos cardiacos han hallado también anomalías estructurales no cardiacas en fetos con onda de flujo anormal en el ductos venoso (Matias *et al.,* 1999; Bilardo *et al.,* 2001; Oh *et al.,* 2007).

Flujo en el ductos venoso y muerte fetal

En el crecimiento intrauterino restringido (CIR) por insuficiencia placentaria hay una hipoxemia y una redistribución de la circulación fetal con un aumento del aporte al cerebro, miocardio y glándulas adrenales, y reducción de la perfusión a los riñones, tracto gastrointestinal y extremidades. Cuando hay un fallo de la adaptación fisiológica a la hipoxemia, el fallo cardiaco se manifiesta como un aumenta de la impedancia al flujo sanguíneo en el ductos venoso, con flujo ausente o reverso durante la contracción atrial como resultado del aumento de presión en la aurícula derecha (Hecher *et al.*, 1996). Hay indicios de que el flujo anormal en el ductos venoso durante el primer trimestre se asocia a un mayor riesgo de muerte fetal. Un estudio de screening informó que la

prevalencia del flujo anormal en el ductos venoso en casos que resultaron en muerte fetal era de 22,2% (4 de 18), a diferencia con un 5,9% (61 de 1.041) en el grupo con un resultado perinatal normal (Toyama *et al.*, 2004). Sin embargo, el flujo anormal se observó en 4 de las 8 muertes fetales que presentaban una TN por encima del 95º percentil y en ninguna de las 10 con TN normal. Un estudio de casos y controles en fetos con la TN por debajo del 95º percentil encontró que en 10 de 42 (23,8%) casos con un flujo anormal en el ductos venoso había un resultado perinatal adverso, incluyendo muerte perinatal, anomalías cromosómicas, anomalías cardiacas y extra-cardiacas. (Oh *et al.*, 2007). Por otro lado, tan sólo se observó un resultado perinatal adverso en uno de 83 (1,2%) de los controles con flujo normal en el ductos venoso.

Flujo en el ductos venoso y gestaciones gemelares

En embarazos gemelares el riesgo de aborto, prematuridad y restricción de crecimiento es más alto que en embarazos únicos (Sebire *et al.*, 1997a). Los embarazos gemelares monocoriales presentan otras complicaciones adicionales como el síndrome de transfusión feto-fetal (STFF) y la restricción selectiva de crecimiento intrauterino (CIRs). Un pequeño estudio sugirió el flujo en el ductos venoso en el primer trimestre como predictor del STFF. En una serie de 11 embarazos gemelares monocoriales, se desarrolló un STFF en los dos únicos casos con flujo anormal en el ductus venoso en el receptor a las 11-13 semanas, y en ninguno de los casos con flujo normal en el ductos venoso (Matías *et al.*, 2000). No hay estudios que estudien la aplicación del ductos venoso en otras complicaciones de embarazos gemelares.

2. HIPÓTESIS DE LA TESIS

La hipótesis de los estudios de esta tesis es que el flujo sanguíneo en el ductus venoso puede ser valorado de forma rutinaria a las 11-13 semanas de embarazo y que el flujo anormal en esta ecografía puede ayudar a identificar aquellos fetos con cromosomopatías, anomalías estructurales y resultado adverso del embarazo tanto en embarazos.

3. OBJETIVOS DE LA TESIS

El objetivo principal de esta tesis es estudiar si la valoración rutinaria del flujo en el ductos venoso en la ecografía de las 11-13 semanas mejora la predicción de cromosomopatías, cardiopatías congénitas y resultados adversos del embarazo.

Los objetivos específicos de cada estudio son:

Objetivos del primer estudio

- Determinar el número de ecografías necesarias en el entrenamiento de los ecografistas para que éstos sean capaces valorar con precisión el flujo en el ductos venoso a las 11-13 semanas.

Objetivos del segundo estudio

- Valorar la contribución independiente del flujo en el ductus venoso en el screening de cardiopatías congénitas en fetos cromosómicamente normales con TN aumentada a las 11-13 semanas.

Objetivos del tercer estudio

- Valorar la eficacia de de un algoritmo específico que combine el flujo en el ductus venoso con la edad materna, el grosor de la TN, la frecuencia cardiaca fetal, y los marcadores bioquímicos de suero materno, la ß-hCG libre y la PAPP-A en el screening de trisomías 21, 18, 13 y síndrome de Turner.

- Valorar la eficacia de dos estrategias de screening, la primera en la que se valora el flujo en el ductus venoso en todos los pacientes y la segunda en la que solo se valora en aquellas con un riesgo intermedio, desde 1 entre 51 a uno entre 1000, tras la primera fase del screening.

Objetivos del cuarto estudio

- Estimar la contribución independiente del flujo anormal en el ductos venoso a las 11-13 semanas de gestación en la predicción de anomalías estructurales mayores y muerte fetal.

Objetivos del quinto estudio

- Valorar la prevalencia del flujo anormal en el ductus venoso en gestaciones gemelares monocoriales y bicoriales.

- Valorar la asociación del flujo anormal en el ductus venoso con el resultado adverso del embarazo en gestaciones gemelares.

4. PACIENTES Y MÉTODOS

4.1. Primer estudio.

Learning curve for Doppler assessment of ductus venosus flow at 11-13 weeks.

Diez ecografistas con dilatada experiencia en medición del grosor de la translucencia nucal fueron entrenados para valorar con precisión el flujo en el ductos venoso. Se les pidió que examinaran el ductos venoso durante la ecografía rutinaria de las 11-13 semanas. Cada ecografía fue valorada por un ecografista experto, y clasificada como aprobada o fallida (fallo para obtener la onda de flujo, calidad de imagen pobre con contaminación o clasificación anómala de la onda-a). Cada ecografista realizó un total de 300 ecografías. Los datos se analizaron en grupos de 20 y en cada grupo se calculó el porcentaje de exámenes fallidos.

4.2. Segundo estudio.

Ductus venosus Doppler in fetuses with cardiac defects.

Se obtuvo la onda de velocidad de flujo inmediatamente antes de la biopsia corial para estudio de cariotipo en fetos con TN de 3,5 mm o más a las 11-13 semanas de gestación. Al grupo de fetos cromosómicamente normales también se les practicó una ecocardiografía por un cardiólogo pediátrico a las 11-13 semanas y/o a las 18-22 semanas.

4.3. Tercer estudio.

Ductus venosus Doppler in screening for chromosomal abnormalities.

Se realizó un screening combinado en embarazos únicos a 19.614 fetos euploides, 122 con trisomía 21, 36 con trisomía 18, 20 con trisomía 13 y 8 con síndrome de Turner. En todos los casos se valoró la onda-a en el ductos venoso. Se valoró la

eficacia de dos estrategias de screening: en primer lugar valorando la onda-a a todas las pacientes, y en segundo lugar realizando en una primera fase un screening combinado a todas las pacientes y en una segunda fase valorando la onda-a solo a aquellas pacientes que tras la primera fase tuvieron un riesgo intermedio entre 1 en 51 y 1 en 1.000.

4.4. Cuarto estudio.

Ductus venosus in screening for adverse pregnancy outcome.

Valoración prospectiva de embarazos únicos mediante historia materna, ß-hCG libre, PAPP-A, TN y Doppler del ductos venoso. Se dividió a las mujeres en cinco grupos según el resultado perinatal: resultado normal (n=10.120), aborto o pérdida fetal (n=185), cariotipo anormal (n=95), defecto cardiaco (n=20) o defecto no cardiaco (n=70). Se realizó un análisis de regresión para determinar la significancia de la contribución al resultado adverso de la onda-a reversa en el ductos venoso, las características maternas, el delta de la TN y la log MoM PAPP-A y log MoM ß- hCG libre del suero materno

4.5. Quinto estudio.

Ductus venosus Doppler at 11-13 weeks in the prediction of outcome in twin pregnancies.

Este fue un estudio prospectivo en 516 embarazos gemelares bicoriales y 179 monocoriales en los que el ductos venoso se valoró a las 11-13 semanas de gestación. La prevalencia de la onda-a reversa en el ductos venoso fue comparada entre monocoriales y bicoriales y entre aquellos con y sin complicaciones en el embarazo.

5. RESULTADOS

5.1 Learning curve for Doppler assessment of ductus venosus flow at 11-13 weeks.

En los 3.000 casos examinados por 10 ecografistas hubo 2.849 (95,0%) exámenes satisfactorios y 151 fallidos, incluyendo 104 fallos para obtener la onda, 32 casos

donde la calidad de la imagen se consideró inadecuada y 15 casos en los que la clasificación de la onda-a era errónea. La frecuencia global de exámenes fallidos disminuyó significativamente con el número de ecografías (r=0,982, p<0,0001). El análisis de regresión demostró que el examen satisfactorio del ductos venoso dependía de la experiencia del ecografista (definido como el número acumulado de ecografías), y no de la longitud cráneo-caudal (LCC), el origen étnico materno o el índice de masa corporal (IMC). Los ecografistas requirieron una media de 80 exámenes antes de que pudieran examinar el ductos satisfactoriamente en el grupo de 20 ecografías. Aunque uno de los 10 ecografistas consiguió pasar sin ningún fallo el primer bloque de 20 ecografías, algunos de los ecografistas requirieron un entrenamiento de 100 ecografías.

5.2 Ductus venosus Doppler in fetuses with cardiac defects.

Se diagnosticó una cardiopatía mayor en 16 (8,4%) de los 191 fetos. Se observó un flujo ausente o reverso durante la contracción atrial en 11 de los 16 (68,8%) de los fetos con cardiopatías y en 40 de los 175 (22,9%) sin cardiopatías. El análisis multivariante demostró que la prevalencia de la onda-a reversa en el ductos venoso en fetos sin cardiopatía mayor aumentó con el grosor de la TN (OR, 1,463; 95% CI, 1,183-1,809; p<0,0001) pero no con la LCC (OR, 0,962, 95% CI, 0,925-1,0; p=0.053). En aquellos fetos con defectos cardiacos la prevalencia de la onda-a anormal no cambió significativamente con el grosor de la TN (OR, 2,054; 95% CI, 0,573-7,360; p=0,269) ni con la LCC (OR, 0,930, 95%CI, 0,834-1,038; p=0,196). El cociente de probabilidades para cardiopatías mayores cuando el ductos venoso era anormal disminuyó con el grosor de la TN desde 4,58 para una TN de 3,5 mm hasta 2,47 para una TN de 5,5 mm, y el cociente de probabilidades cuando el flujo en el ductos venoso era normal aumentó desde 0,37 para una TN de 3,5 mm hasta 0,43 para una TN de 5,5 mm.

5.3 Ductus venosus Doppler in screening for chromosomal abnormalities.

El análisis de regresión logística demostró que la prevalencia de la onda-a reversa aumentó significativamente con la TN fetal (p<0,0001), disminuyó con la LCC fetal y la PAPP-A sérica materna, y era más común en mujeres de raza negra. El flujo reverso se observó en 3,2% de los fetos euploides y en 66,4%, 58,3%, 55,0% y 75,0% de los fetos con trisomía 21, 18 y 13 y síndrome de Turner, respectivamente. La inclusión del

flujo de ductos venoso en todos los embarazos detectaría un 96%, 92%, 100% y 100% de las trisomías 21, 18 y 13 y síndrome de Turner , respectivamente, para una tasa de falsos positivos de un 3%. La tasa de detección fue la misma mediante la estrategia de dos fases, para una tasa de falsos positivos de 2,6%, en la tan sólo fue necesario valorar el ductos venoso a un 15% de la población.

5.4 Ductus venosus in screening for adverse pregnancy outcome.

La prevalencia de la onda-a reversa fue significativamente mayor en los grupos con aborto o muerte fetal (10,8%), cariotipo anormal (62,1%) y cardiopatías (25,0%), que en el grupo con un resultado gestacional normal (3,7%), pero no en las malformaciones no cardiacas (4,3%). El análisis de regresión logística demostró que la onda-a reversa, la edad materna, el delta de la TN, y la PAPP-A sérica materna contribuyeron significativamente a la predicción de las anomalías cromosómicas. La onda-a reversa en el ductos venoso y el delta de la TN contribuyeron significativamente a la predicción de cardiopatías. La onda-a reversa, junto con la etnicidad negra, el IMC y la PAPP-A (log MoM) sérica materna contribuyeron significativamente a la predicción de muerte fetal. Se observó un resultado gestacional adverso en 2,7% de los fetos con la TN en el 95º percentil o por debajo de éste (2,6% en aquellos con onda-a normal, y en un 7,0% de aquellos con onda-a reversa) y en 19,3% de aquellos fetos con la TN por encima del 95º percentil (en 8,9% de aquellos con una onda-a normal y en un 70,9% de aquellos con onda-a reversa).

5.5 Ductus venosus Doppler at 11-13 weeks in the prediction of outcome in twin pregnancies.

La prevalencia de una onda-a reversa en al menos uno de los fetos era significativamente más alta en embarazos monocoriales que en bicorales (18,4% vs 8,3%, p<0,001) y en gestaciones complicadas con un aborto (28,6%, p=0,005), aneuploidía fetal (70,0%, 0<0,001) y en el síndrome de transfusión feto-fetal (38,5%, p<0,001) en comparación a los embarazos que resultaron en dos recién nacidos sanos (7,7%).

En embarazos bicoriales la prevalencia de la onda-a reversa en el ductos venoso era significativamente mayor en los embarazos con anomalías cromosómicas que en aquellos con un resultado normal (66,7% vs 6,8%). En gestaciones monocoriales la prevalencia de la onda-a reversa era significativamente mayor en gestaciones que

desarrollaron un síndrome de transfusión feto-fetal que en aquellos con un resultado normal (38,5% vs 10,9%). El análisis de regresión logística del total de 695 gestaciones gemelares demostró que además de la corionicidad, la discordancia en la LCC y en la TN, la aneuploidía fetal y el síndrome de transfusión feto-fetal contribuyeron significativamente a la aparición de una onda-a reversa. El resultado perinatal fue normal en 33 de los 43 (76,7%) de los embarazos bicoriales y en 14 de los 33 (42,4%) monocoriales con onda-a reversa en al menos uno de los fetos.

6. CONCLUSIONES

6.1 Conclusiones del primer estudio

Tras un entrenamiento intenso se puede alcanzar la competencia en la valoración del flujo sanguíneo en el ductos venoso.

6.2 Conclusiones del segundo estudio

En fetos cromosómicamente normales con TN aumentada el hallazgo de un flujo ausente o reverso en la onda-a del ductos venoso aumenta tres veces la probabilidad de una cardiopatía mayor, mientras que el hallazgo de un flujo normal reduce a la mitad el riesgo de dichas anomalías.

6.3 Conclusiones del tercer estudio

La valoración del flujo en el ductos venoso mejora la eficacia del screening de anomalías cromosómicas de primer trimestre, tanto si se valora en todos los fetos como si se valora en una estrategia de dos fases, tan sólo en aquellos con un riesgo intermedio entre uno en 51 y uno en 1000 en la primera fase.

6.4 Conclusiones del cuarto estudio

El flujo reverso en el ductos venoso se asocia a un resultado adverso del embarazo, tal como anomalías cromosómicas, cardiopatías y muerte fetal.

6.5 Conclusiones del quinto estudio

El flujo reverso en la onda-a del ductos venoso es más común en gemelos monocoriales que en bicoriales.

En embarazos gemelares, la onda-a reversa se asocia a un riesgo aumentado de cromosomopatías, aborto y síndrome de transfusión feto-fetal.

6.6 Concusión final

Tras un intenso entrenamiento la valoración del flujo sanguíneo en el ductos venoso puede incorporarse a la ecografía del primer trimestre, donde mejora la eficacia del screening de cromosomopatías y cardiopatías, y ayuda a identificar a aquellos fetos con mayor riesgo de muerte fetal. De forma similar, en embarazos gemelares, la valoración del ductos venoso identifica los embarazos con mayor riesgo de aneuploidía en alguno de los fetos, aquellos con riesgo de aborto, y aquellos que posteriormente desarrollarán un síndrome de transfusión feto-fetal.

APPENDIX

Letters from Professor Kypros H Nicolaides, supervisor of this thesis:

1. Contribution of Dr Nerea Maiz Elizaran to the studies in the thesis

2. Impact factor of journals which published the papers in the thesis of Dr Nerea Maiz Elizaran

Contribution of Dr Nerea Maiz Elizaran to the studies in the thesis

STUDY 1 **Maiz N**, Kagan KO, Milovanovic Z, Celik E, Nicolaides KH. Learning curve for Doppler assessment of ductus venosus flow at 11-13 weeks. *Ultrasound Obstet Gynecol* 2008;31:503-6.

Dr Maiz Elizaran planned the study, trained the doctors involved, conducted the study, analysed the data and wrote the first and final draft of the paper.

STUDY 2 **Maiz N**, Plasencia W, Dagklis T, Faros E, Nicolaides KH. Ductus venosus Doppler in fetuses with cardiac defects and high nuchal translucency thickness. *Ultrasound Obstet Gynecol* 2008;31:256-60.

This a retrospective study. Dr Maiz Elizaran planned the study, carried out a search of the database, analysed the data and wrote the first and final draft of the paper.

STUDY 3 **Maiz N**, Valencia C, Kagan KO, Wright D, Nicolaides KH. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. Ultrasound Obstet Gynecol 2009;33:512-7.

This a prospective screening study. Dr Maiz Elizaran planned the study, trained the doctors involved, co-ordinated the study, analysed the data and wrote the first and final draft of the paper. In the analysis of data she was supported by Professor David Write of the Department of Mathematics and Statistics, University of Plymouth, Plymouth, UK

STUDY 4 **Maiz N**, Valencia C, Emmanuel EE, Staboulidou I, Nicolaides KH. Screening for adverse pregnancy outcome by Ductus venosus Doppler at 11-13 weeks. *Obstet Gynecol* 2008;112:598-605.

This a prospective screening study. Dr Maiz Elizaran planned the study, trained the doctors involved, co-ordinated the study, analysed the data and wrote the first and final draft of the paper.

STUDY 5 **Maiz N**, Staboulidou I, Leal AM, Minekawa R, Nicolaides KH. Ductus venosus Doppler at 11-13 weeks in the prediction of outcome in twin pregnancies. *Obstet Gynecol* 2009;113:860-5.

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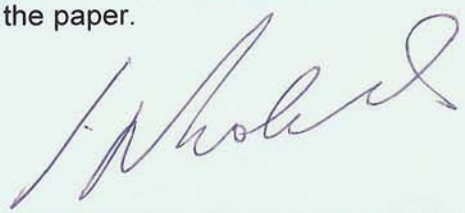

Professor Kypros Herodotou Nicolaides
King's College Hospital School of Medicine, London, England
London October 2009

Impact factor of journals which published the papers in the thesis of Dr Nerea Maiz Elizaran

STUDY 1 **Maiz N**, Kagan KO, Milovanovic Z, Celik E, Nicolaides KH. Learning curve for Doppler assessment of ductus venosus flow at 11-13 weeks. *Ultrasound Obstet Gynecol* 2008;31:503-6.

Impact factor in 2008 2.69
Category ranking in Obstetrics and Gynecology 15/61
Category ranking in Acoustics 2/26

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Impact factor in 2008 4.397
Category ranking in Obstetrics and Gynecology 2/61

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Impact factor in 2008 4.397
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