

Application of Ductus venosus Doppler Velocimetry for the Detection of Fetal Aneuploidy in the First Trimester of Pregnancy

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Key Words

Doppler velocimetry · Ductus venosus · Nuchal translucency · Chromosomal abnormalities

Abstract

Objective: To test the hypothesis the application of ductus venosus Doppler velocimetry may serve as a screening tool between 10 and 14 weeks' gestation for the detection of fetuses with chromosomal abnormalities.

Methods: 372 consecutive fetuses were studied. Based on prior study, a chromosomal abnormality was suspected when either the nuchal translucency was above the 95th centile, or there was reversed or absent flow in the ductus venosus during atrial contraction. Sensitivity, specificity, and the negative and positive predictive values were calculated. **Results:** There were 29 chromosomally abnormal fetuses. Of these 29 fetuses, ductus venosus blood flow during atrial contraction was either absent (n = 2) or reversed (n = 25) in 93.1%. In the chromosomally normal fetuses (n = 343), only 6 (1.7%) had abnormal Doppler profiles in the ductus venosus (specificity = 98.3%, positive and negative predictive values = 81.8% and 99.4%, respectively). **Conclusion:** The Doppler waveform of the ductus venosus was at least equal to NT thickness measurement for the detection of chromosomal abnormalities.

Introduction

There is a well-established association between chromosomal anomalies and structural cardiac diseases [1, 2]. Sixty-five percent of trisomy 21 fetuses in the first trimester have cardiac atrioventricular or ventricular septal defects [3], and 49% narrowing of the aortic isthmus [4]. In one study, all fetuses with trisomy 18 had ventricular septal defects and/or valvular abnormalities [5]. In addition, such fetuses may show heterochrony, i.e. a delay or asynchrony in chronologic development [6]. We hypothesize that chromosomally abnormal fetuses might present with delayed myocardial development, particularly in relationship to the emergence of the contractile units (sarcomeres), resulting in cardiac insufficiency. If true, early cardiac failure could be the mechanism underlying the known association between increased nuchal translucency (NT) and aneuploidy [7–10]. In addition, it would be logical to hypothesize that the venous circuit of chromosomally abnormal fetuses would show peculiarities in their Doppler flow profiles.

Recently, several investigators noted a correlation between alterations in the venous Doppler flow profiles and the presence of chromosomal abnormalities [10–16]. Some reported either reversed flow in the ductus venosus during atrial contraction [10–15] or in the umbilical vein [16] of fetuses with increased NT. These observations sug-

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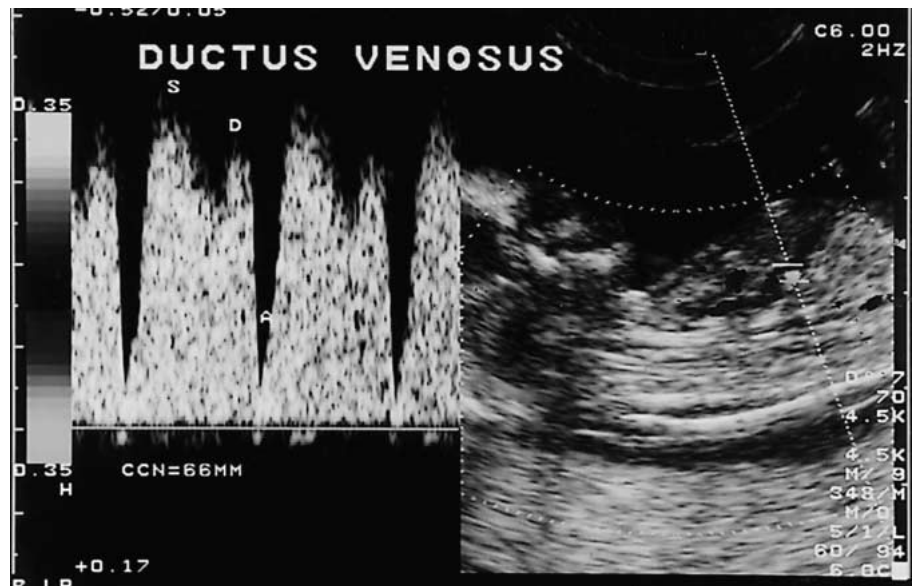


Fig. 1. Normal unidirectional and triphasic waveform in the ductus venosus of a normal fetus (46,XX) at 11 weeks. The first peak reflects ventricular systole (S) and the second reflects ventricular diastole (D). The nadir of velocities that appears between the two cycles represents the end of diastole and atrial contraction (A).

gest not only the possibility of cardiac insufficiency in fetuses with chromosomal abnormalities, but also the potential for early detection by venous Doppler.

The objective of the present study was to test the hypothesis that the application of ductus venosus Doppler velocimetry between 10 and 14 weeks' gestation could serve as a screening tool to identify fetuses with chromosomal abnormalities.

Material and Methods

Three hundred seventy-two fetuses were studied consecutively between September 1998 and October 2000, including 39 with increased NT thickness that were recruited for the present study. The NT was measured and the Doppler flow profiles of the ductus venosus were obtained in all fetuses. In 128 pregnancies, a cytogenetic study was performed on material obtained by chorionic villus sampling (CVS). Every CVS was performed after the ultrasound examination. In the remaining 244 pregnancies, newborns with a normal examination were assumed to be karyotypically normal. Six cases of fetal demise were excluded at the study.

The maximal NT thickness was measured in a sagittal section typical for a crown-rump length (CRL) measurement with the spine posterior with the fetus occupied three-quarters of the screen. To distinguish fetal skin from amnion, the measurement was obtained when the fetus 'jumped' away from the amnion. The hypoechoic space between the skin and the subcutaneous tissue that covers the cervical spine was measured in millimeters.

The ductal flow was identified in the fetal liver using color Doppler between the right and left lobes, in a midsagittal and ventral section on the right of the fetal trunk. The sample gate was positioned in the isthmus region, immediately above the umbilical vein (proximal ductus) in the region where flow acceleration occurs within its

lumen, producing aliasing effects during color flow imaging of the venous circulation. This aliasing facilitates identification of the ductus venosus during ultrasound scanning and, allows for the correct positioning of the sample volume when analyzing Doppler flow velocity patterns. Figure 1 illustrates the normal blood flow velocity waveform of the ductus venosus. Note it is unidirectional and triphasic. The first peak occurs during ventricular systole (S wave) and the second during ventricular diastole (D wave). The third waveform represents the end of diastole and the atrial contraction (A wave). We focus on the 'A' phase. Because of the short length of the ductus venosus, care must be taken to avoid contamination from the umbilical vein, the left hepatic vein or the inferior vena cava. The velocities 'S', 'D' and during atrial contraction (A wave) and the pulsatility index (PI) were measured.

The studies in São Paulo were performed transabdominally (Diassonic Synergi apparatus, with a 3.5-MHz curvilinear probe), while in Vitória, a transvaginal approach predominated (Toshiba 140 apparatus with a 6.0-MHz probe). Both ultrasound units are capable of displaying simultaneously conventional ultrasound (gray) and the Doppler (colored and pulsatile). The sample volume gate was adjusted to the minimum size to avoid interference with the adjacent vessels. It typically ranged between 1 and 2 mm. The high-pass filter was set between 50 and 70 Hz to remove low-frequency interference caused by the movement of the vessel walls. An average of three consecutive high-quality flow velocity waveforms of similar appearance with the highest velocity was used to establish the value for each parameter.

The results were separated in three groups. The first consisted of all fetuses ($n = 372$), the second group of fetuses ($n = 30$) with either an abnormal karyotype ($n = 29$) or a cardiac defect ($n = 1$), and the third group of fetuses either with a known, normal karyotype or phenotypically normal ($n = 342$). Based on prior study, a chromosomal abnormality was suspected when the NT was above than 95th centile, or when there was either reversed or absent flow during atrial contraction in the ductus venosus [13].

We investigated the relationship between the NT measurement and the 'A' wave velocity using linear regression. The normal and aneuploid fetuses were analyzed first together and then as separate groups. Sensitivity, specificity and the negative and positive predictive value were calculated. The χ^2 test (Fisher exact test) and the Mann-Whitney U test were used to determine the significance of the differences found in the variables analyzed in the normal group (n = 343, in this group we included the case of cardiac defect) and chromosomally abnormal group (n = 29). SPSS software was used for calculation.

Table 1. Mean (range) values for maternal age, ultrasound and ductus venosus Doppler findings in normal and karyotypically abnormal fetuses (mean \pm SD)

Variable	Normal group (n = 343)	Abnormal group (n = 29)	p value ¹
Maternal age	30.9 \pm 5.9	35 \pm 7.1	<0.001
CRL, mm	59.1 \pm 11.7	60 \pm 11.8	NS
FHR	159 \pm 11	158.3 \pm 18.9	NS
NT, mm	1.7 \pm 0.7	4.4 \pm 2	<0.001
Velocity S	30.5 \pm 10.5	32.5 \pm 14.3	NS
Velocity D	25.1 \pm 9.1	23.9 \pm 9.6	NS
Velocity A	6.4 \pm 3.1	-6.0 \pm 6.1	<0.001
PI	1.1 \pm 0.4	2.1 \pm 0.7	<0.001

¹ Mann-Whitney test.

Results

The median maternal age for all women was 32 years (range 17–47). The median age of the women with a chromosomally abnormal fetus was 34 years (range 17–45) compared to 31 years for women with chromosomally normal fetuses (range from 17 to 47 years) (p < 0.001). The mean CRL was 59 mm (range 38–84) and the mean gestational age was 12 weeks. Highly significant differences were observed between normal and chromosomally abnormal groups in their mean NT thickness, in the frequency of absent or reversed 'A' waves, and in the PI of the ductus venosus. No characteristic of the 'S' and 'D' waves was identified to distinguish between normal and chromosomally abnormal fetuses (table 1).

There were 29 chromosomally abnormal fetuses. The karyotype was abnormal in 23 of the 39 fetuses (58.9%) with a NT measurement above the 95th centile (16 cases of trisomy 21, 2 of trisomy 13, 1 of trisomy 9, 1 triploidy, 2 monosomy of chromosome X and 1 case of triple X) (table 2). Of the 333 fetuses with an NT below the 95th centile, 6 had a chromosomal abnormality (2 trisomy 21, 1 trisomy 22, 1 triple X, 1 trisomy 18 and 1 case of Klinefelters). Thus, the sensitivity for the detection of a karyotypic abnormality by NT in our referral population was 79.3%, the positive predictive value 59%, the negative predictive value 98.2% and the false-positive rate 4.7%.

Table 2. Blood flow pattern in the ductus venosus of normal and karyotypically abnormal fetuses in relation to their NT at 10–14 weeks of gestation: reversed or absent velocity during atrial contraction was considered abnormal; the type of aneuploidy is specified

Nuchal translucency	Normal fetuses (n = 343)		Abnormal karyotype (n = 29)		
	normal flow	abnormal flow	normal flow	abnormal flow	type of aneuploidy
<95 percentile	321	6	2	4	Trisomy 21 = 2 Trisomy 22 = 1 Trisomy 18 = 1 Triple X = 1 Klinefelters = 1
>95 percentile	15	1 ¹	0	23	Trisomy 21 = 16 Trisomy 9 = 1 Trisomy 13 = 2 Triple X = 1 Turner's syndrome = 2 Triploidy = 1

¹ One fetus with a hypoplastic left heart malformation.

The NT thickness distribution of the 372 fetuses is shown in figure 2.

The velocity waveform of the ductus venosus blood flow was successfully obtained in 372 of the 373 subjects increasing the length of the examination by an average of 5 min. In one instance, we failed both transabdominally and transvaginally to obtain a ductal waveform. The examination was repeated on four different occasions without success. An appropriately grown fetus was assumed (but not examined postnatally) to have agenesis of the ductus venosus.

Absent (n = 2) or reversed (n = 25) flow during atrial contraction in the ductus venosus (fig. 3) was observed in 93.1% (27/29) of chromosomally abnormal fetuses and in only 1.7% (7/343) normal fetuses (table 2). Ninety-three percent of embryos with chromosomal abnormalities would have been detected in this referral population with a specificity of 98.3% using only the flow profile of the ductus venosus during atrial contraction. The positive predictive value was 81.8%, the negative predictive value 99.4% and the false-positive rate was 1.7%. Concerning the 'A' wave velocity in the ductus venosus, the difference between the two groups (normal fetuses and abnormal karyotype fetuses) was highly significant by the Fisher exact test (p < 0.001).

The 'A' wave distribution of the 372 fetuses is shown in figure 4. Six aneuploidy fetuses had a NT measurement <95th centile; 4 of these 6 had absent or reversal of the 'A' wave velocity. The figure 5 illustrates the distribution of

values of the 'A' wave of the ductus venosus and the NT measurements of the normal fetuses (n = 343) and those with an abnormal karyotype group (n = 29). In one embryo with a hypoplastic left heart malformation, the NT measured 6.5 mm and the 'A' velocity was -11 cm/s.

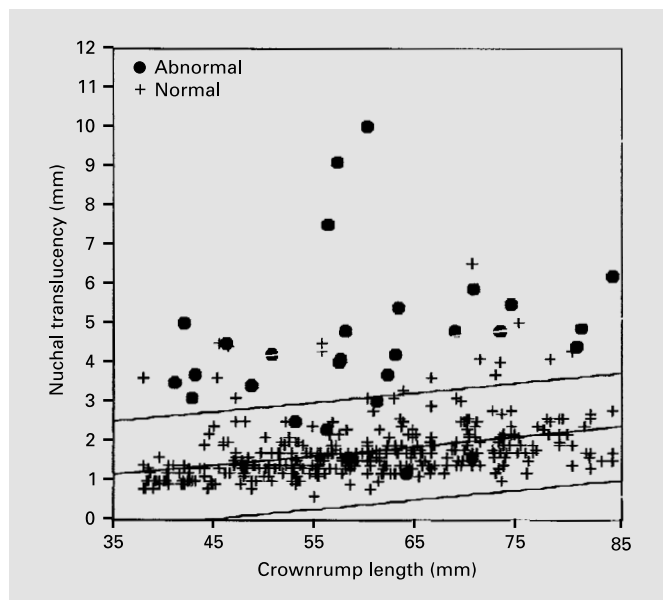


Fig. 2. Relationship between the NT thickness distribution and CRL value at 10–14 weeks in normal fetuses (n = 343) and abnormal karyotype fetuses (n = 29).

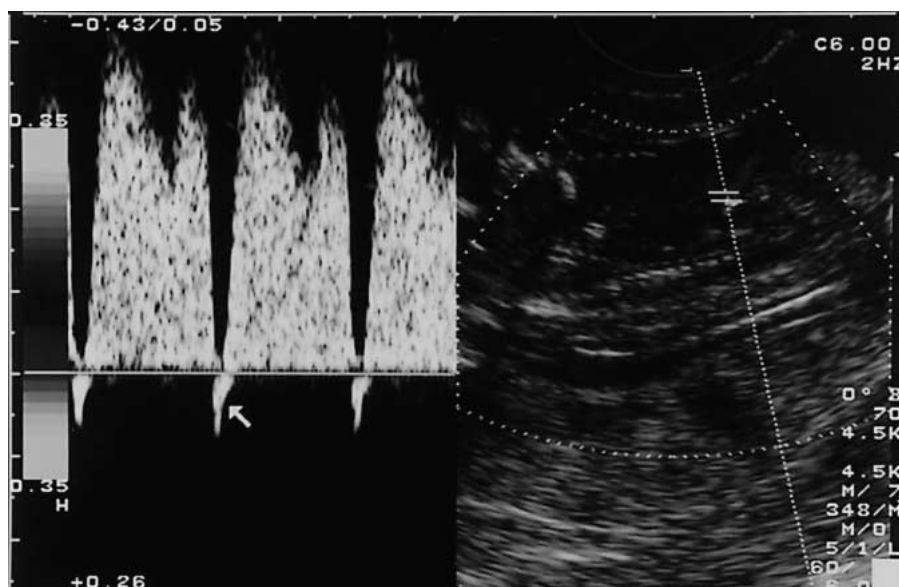


Fig. 3. An example of a ductus venosus blood flow velocity waveform demonstrating reversed blood flow velocity during atrial contraction in a fetus of 12 weeks of gestation. Cytogenetic study revealed trisomy 21.

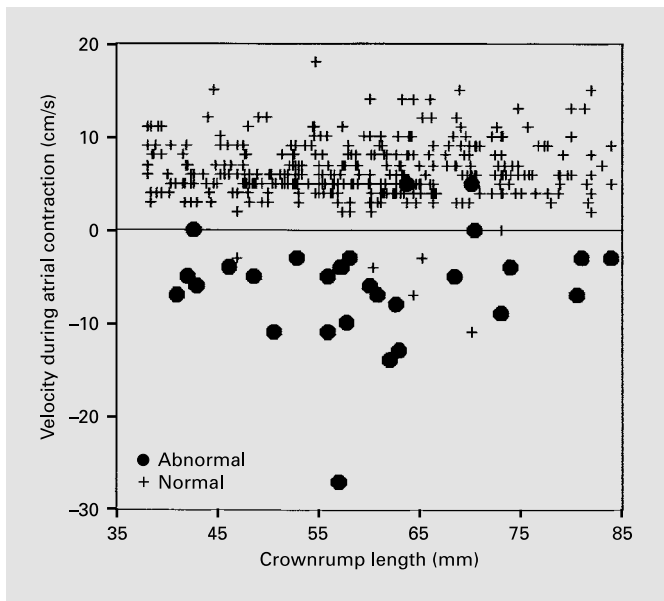


Fig. 4. Relationship between the velocity of the ductus venosus during atrial contraction and CRL value at 10–14 weeks of gestation in normal fetuses (n = 343) and abnormal karyotype fetuses (n = 29).

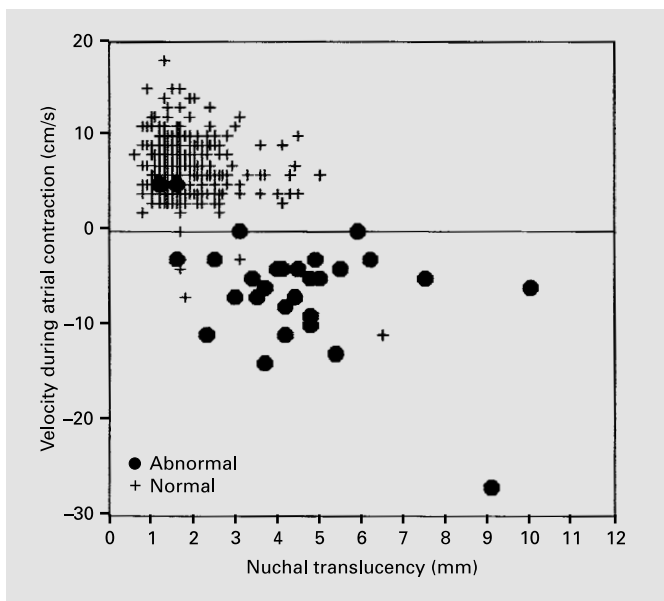


Fig. 5. Relationship between the waveform 'A' obtained in the ductus venosus and the NT thickness value at 10–14 weeks of gestation in normal fetuses (n = 343) and abnormal karyotype fetuses (n = 29).

Linear regression analysis revealed a significant association between the 'A' wave velocity in the ductus venosus and the NT measurement in all fetuses ($p < 0.001$) and in the abnormal fetuses ($p < 0.001$). In contrast, there was not a significant correlation in normal fetuses between the increased NT and the 'A' wave velocity in the ductus venosus.

Discussion

At the beginning of the last decade (1991), Kiserud et al. [17] performed the first study on the ductus venosus in the human fetus and established the normal pattern for the ductus venosus velocimetry in the second and third trimester.

The findings of the present study support the hypothesis that cardiac dysfunction or dysynchronous maturation is a mechanism leading to increased NT measurements in chromosomally abnormal fetuses [7–10]. We failed to demonstrate a significant association between the reduction of blood flow velocity in the ductus venosus during atrial contraction with increased NT in normal fetuses group (fig. 5). This suggests that, at least in our normal fetuses, the increased NT was not related to an increase in the cardiac pre-load.

The ductal waveform reflects the pressure gradient between the right atrium and the umbilical vein. Its alteration in karyotypically abnormal fetuses may be an indirect sign of a defective fetal heart. Recent studies suggest just such a correlation in the second [18, 19] and first trimesters [10–15, 20, 21]. The alterations in blood flow in the ductus venosus documented in the present study are consistent with increased ventricular pressure during the final diastolic phase and atrial contraction. The reduction of blood flow velocity in the ductus venosus during atrial contraction is also associated with fetal growth restriction [19], cardiac malformation [18, 20, 21] and chromosome abnormalities [10–15]. Analysis of the ductus venosus waveform, particularly during atrial contraction, though technically more challenging, may prove either a useful adjunct or even superior to the measurement of NT thickness for the detection of fetuses with chromosomal abnormalities since it is closer to the pathophysiologic cause.

Montenegro et al. [10] compared the Doppler waveforms of the ductus venosus to the NT measurement of 65 fetuses between 10 and 13 weeks' gestational age. Of these, 17 had a NT of > 3 mm. All 5 chromosomally abnormal fetuses also showed abnormal flow during atrial contraction, which was < 2 cm/s ($p < 0.001$). The authors

suggested that both the increased NT and the velocimetric alteration in the ductus venosus reflected cardiac dysfunction, and that the detection of abnormal ductal waveforms could reduce the false-positive rate of NT measurement.

In a larger study, Matias et al. [13] screened 486 pregnancies between 10 and 14 weeks. Sixty-three fetuses had a chromosomal abnormality (38 cases of trisomy 21, 12 of trisomy 18, 7 of trisomy 13, 3 cases of Turner's syndrome and 3 cases of triploidy). In 57 (90.5%), absent or reversed flow was also seen in the ductus venosus during atrial contraction. Abnormal waveforms were also seen in 13 (3.1%) of the 423 chromosomally normal fetuses, of which 7 had a major cardiac defect.

These alterations, like many markers for aneuploidy, are gestationally dependent. In 1 Down syndrome fetus described by Matias et al. [13], the abnormal NT measurement and its associated abnormal ductus venosus blood flow normalized between the 13th and 15th weeks of gestation. Huisman and Bilardo [21] reported a discordant set of dizygotic twins. One fetus with trisomy 18 had reversed flow in the ductus venosus and an increased NT at 13 weeks while its co-twin was normal. By the 20th week, both the ductal velocity and the NT returned to normal. We too observed a fetus with trisomy 18 whose abnormal, ductus venosus blood flow during the 12th week normalized by the 14th week. The parents decided to continue the pregnancy. Fetal echocardiography and Doppler of the ductus venosus were normal at 16 and 20 weeks. These findings support the concept of delayed maturation as the cause of the myocardial dysfunction and reveal that the timing of the examination may be an important key variable. In the present study, 27 of the 29 fetuses with aneuploidy, or 93.1%, exhibited altered blood flow during atrial contraction. In two aneuploid fetuses with normal flow, the NT measurement was also normal and there was no ultrasonographic evidence congenital malformation. One of these fetuses had triple X and the other Klinefelters. They were discovered after CVS for advanced maternal age (37 and 40 years old respectively). The pregnancies were ended at 14 weeks, but no postmortem was performed.

The possibility that the ductus venosus flow abnormality reflects in some instances precocious cardiac failure is supported by our findings in 1 fetus with a hypoplastic left heart and a NT thickness of 6.5 mm. In this instance, examination of the ductus venosus revealed a negative velocity during atrial contraction, but the subsequent cytogenetic study was a normal. Our findings are quite similar to those of Matias et al. [13], with a detection rate

of approximately 90% and a false-positive rate of less than 4%. We detected 4 aneuploid fetuses (2 each of trisomy 21, 22 and 18) in the group with a normal NT ($n = 333$) whose Doppler examination of the ductus venosus demonstrated absent or reversed flow (table 2).

Though it is becoming clear that embryonic cardiac function is altered in chromosomally abnormal fetuses, the best way to identify it is controversial. Montenegro et al. [10] found that the only change in ductus venosus waveform in chromosomally abnormal fetuses was that the minimum velocity during atrial contraction was reduced or absent. Matias et al. [13] conducted a multivariate analysis, and concluded that only the velocity during atrial contraction contributed to the discrimination of chromosomally normal and abnormal fetuses independently despite the fact the PI was higher in the aneuploidy group. In contrast, Borrell et al. [14] concluded after studying 534 fetuses between 10 and 18 weeks that the increase in the PI (above the 95th centile) was more important in the detection of Down syndrome than the minimum 'A' wave velocity. A substantial proportion of the fetuses with trisomy 21, 73% (8/11), had an increase in PI, while the detection rate using the velocity during atrial contraction was only 27% (3/11). Our detection rate using the PI was 70.6% (12/17). A screening tool must be easily deployed with experience. In the present investigation and that of Matias et al. [13], the waveform of the ductus venosus was measurable in 99.7 and 100% of cases, respectively. In just one instance, it was not possible to identify the ductus venosus waveform despite several attempts in a fetus with probable ductal agenesis. This fetus was otherwise normal. We speculate that the ductus venosus is a reserve vessel, and that the hepatoportal system may take its place efficiently transporting well-oxygenated blood to the fetal heart when necessary.

We recognize that the frequency of aneuploidy in the study group was artificially high. Thus, the predictive values we achieved may or may not be reproducible in a screening environment. However, the consistency of the finding in the chromosomally abnormal fetuses coupled with its rarity in chromosomally normal, structurally normal fetuses strongly suggests this technique will be an excellent screening tool.

Although there is a rare contradictory result in the literature [22], NT measurement during the late first trimester has proven an excellent screening tool for the detection of chromosomally abnormal fetuses [23–25]. The method is now embraced worldwide. The present study supports an extension of that screening examination to include Doppler interrogation of the ductus venosus. In light of

the extremely high sensitivity and specificity documented here and elsewhere, fetuses with absence or reversal of the 'A' wave should be offered a karyotype even if the NT measurement is normal. Analogous to an increased NT associated with a normal karyotype, an abnormal velocity

waveform in the ductus venosus during the first trimester of gestation may be a signal for further investigation of cardiac defects later in pregnancy if the karyotype proves to be normal.

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