The Value of Reference Ranges for Middle Cerebral Artery Peak Systolic Velocity in the Management of Rhesus Alloimmunized Pregnancies

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┌─ ABSTRACT ─┐

Objectives: To establish reference ranges for middle cerebral artery peak systolic velocity (MCA PSV) and to certify their value in the management of Rhesus alloimmunized pregnancies.

Material and methods: A reference range of MCA PSV with gestation was constructed by studying 342 pregnancies at 25-40 weeks. A comparison was made between the reference ranges produced in our study and those already published. Fetal MCA PSV was also measured in 30 fetuses from Rhesus alloimmunized pregnancies at 25-39 weeks. Last MCA PSV measurement was made within 7 days before measurement of umbilical cord hemoglobin at delivery. MCA PSV and hemoglobin were expressed as multiples of median (MoM).

Outcomes: In the normal pregnancies a significant increase in MCA PSV with gestation resulted. The reference ranges for MCA PSV in normal pregnancies were similar to those already in use up to 34 weeks. From 35 to 40 weeks, our values were lower. In the Rhesus alloimmunized pregnancies, MCA PSV was increased. We found a good correlation between MoM MCA PSV and MoM hemoglobin. Using a threshold of 1.29 for MoM PSV, the sensitivity and specificity of MCA PSV in predicting any degree of anemia (Hb ≤0.84 MoM) were 88.46% and 98.27%.

Conclusions: Our reference ranges for MCA PSV can perform well from 25 until 35 weeks of gestation. Based on this, measurement of the MCA PSV in fetuses at risk for anemia provides a reliable, non-invasive clinical test for the prediction of fetal anemia.

Keywords: MCA PSV, reference ranges, Rhesus alloimmunization

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INTRODUCTION

Maternal Rhesus (or red cell) iso-immunization occurs when a pregnant woman develops an immunological response to a paternally derived red blood cell antigen (D) foreign to the mother and inherited by the fetus. The antibodies may cross the placenta, bind to antigens present on the fetal erythrocytes, and cause hemolysis. Hemolysis of the erythrocytes causes anemia in the fetus, and if severe, may result in edema, hydrops fetalis, and fetal death. Hemolytic disease of the fetus/neonate can also be caused by other antigens of the Rh blood group system (the Rh blood system consists of the C, c, D, E, e, and G antigens - there is no d antigen) and by the so-called 'irregular antigens' of the non-rhesus blood group system such as Kell, MNS, and Kidd (1). Therefore, the term red cell or Rhesus alloimmunization is more commonly used. Maternal antibody titer and disease history in a previously affected pregnancy have proved poorly predictive of the onset of fetal anemia.

The aim of managing maternal Rhesus alloimmunization is to identify anemic fetuses in utero and to treat them by intrauterine transfusion or to end the pregnancy by inducing labour or cesarean section. In 1961, Liley described a method which uses the observed change in the optical density at 450 nm to predict the severity of hemolytic anemia in fetuses (2). Since then, amniocentesis has been used to indirectly diagnose fetal anemia in Rhesus alloimmunized pregnancies by assessing the amniotic fluid optical density deviation at 450 nm. Amniocentesis allows quantification of bilirubin, present in the amniotic fluid as a result of fetal hemolysis, to indicate the severity of anemia. It provides no information about the hemopoietic response of the fetus and serial procedures are usually required to monitor the pregnancy. This method, which has often been used to manage these pregnancies, showed a lack of accuracy before 27 weeks of gestation (3) and in predicting the severity of anemia (4).

Cordocentesis is the only reliable method to determine hemoglobin concentration directly, but it is an invasive procedure associated with complications such as infection, bleeding from the cord puncture site, transient bradycardia, feto-maternal hemorrhage which can worsen fetal alloimmunization and fetal demise (5).

To discover a non-invasive test for fetal anemia has been the aim of many workers in the recent years. The fetus compensates by hemodynamic adaptations that can be assessed by Doppler ultrasound (6). Mari et al. (7) suggested that by measuring the peak systolic velocity in the fetal middle cerebral artery (MCA PSV), the non-invasive prediction of fetal anemia in fetuses at risk due to maternal Rhesus alloimmunization could be significantly improved: the fetal MCA PSV correlates well with hemoglobin concentration and hematocrit. This method is based on the fact that anemic fetuses have an increased blood flow velocity (hyperdynamic circulation). The advantages of studying the MCA rather than other vessels is that it allows measurements of velocity without angle correction because, in the axial plane, the angle of insonation of the MCA is close to 0°, improving reproducibility.

The aims of the present study were to establish reference ranges for MCA PSV for a non-selected group of pregnant women from our population and to certify their value in the management of Rhesus alloimmunized pregnancies. We used the reference ranges already published for MCA PSV and, comparatively, our reference ranges. ❑

MATERIAL AND METHODS

The study was carried out at the Department of Obstetrics and Gynecology of Elias University Hospital, Bucharest, between February 2006 and March 2011.

The first group comprised normal singleton pregnancies attending for ultrasound examination at 25-40 weeks of gestation. Pregnancies complicated by anti-D or other "irregular" antibodies, diabetes mellitus or hypertension at the time of examination, multiple pregnancies, polyhydramnios, intrauterine growth restriction and congenital malformations were excluded from the study. Although we enrolled initially 366 women in this group, the final number was 342 women, based on we formulated the final conclusions. 16 patients were lost to follow-up, 3 fetuses developed polyhydramnios and 5 fetuses were growth restricted. Gestational age was determined from the date of the last menstrual period and confirmed by first-trimester crown-rump length or early second-trimester fetal biometry. We measured MCA PSV for 3-5 times for every fetus. All deliveries were after

37 weeks of gestation. The data from this group were normalized by logarithmic transformation and regression analysis was used to construct a reference range for MCA PSV with gestational age.

The second group comprised 30 singleton pregnancies with Rhesus alloimmunization, many referred to our Department from several counties around Bucharest. Initially we enrolled 33 patients with Rhesus alloimmunization in this group, but 3 cases were lost to follow-up. We included only women with anti-D hemolytic antibody titer >1/16 (8) and we measured MCA PSV from 25 weeks of gestation until birth, following an algorithm proposed by Mari et al. (9). All Doppler results that were correlated with hemoglobin values in this group were obtained within 7 days (in 22 cases in the same day) before fetal cord blood sampling for measurement of hemoglobin concentration at delivery.

A transverse section of the fetal head was obtained by ultrasonography (2-5 MHz curvilinear Micro4D probe Voluson 730 Pro, General Electric Healthcare, Milwaukee Winsconsin, USA) and color flow mapping was used to identify the circle of Willis and the MCA. The MCA was insonated close to its origin from the internal carotid artery. The angle between the ultrasound beam and the blood flow was kept as close as possible to 0°. At least three consecutive waveforms, in the absence of fetal body or breathing movements were recorded and the highest point of the Doppler waveform was considered as the PSV (cm/s).

In order to adjust for the effect of gestational age on the measurements, we expressed the hemoglobin and the MCA PSV values in multiples of the median (MoM). This was calculated by dividing the measured value (hemoglobin or MCA PSV) by the expected value for gestational age. Because in Romania we don't have yet a reference range for fetal hemoglobin concentration and for MCA PSV, we adopted the reference ranges suggested by Mari (10). In the same time, we calculated MoM values for MCA PSV with the reference range obtained from the first group and compared the results. Fetal anemia was defined by Mari (10) as a hemoglobin value of ≤ 0.84 MoM ($\leq 5^{th}$ percentile), moderate anemia as a hemoglobin value between 0.65 and 0.55 MoM, and severe anemia as a hemoglobin concentration of 0.55 MoM.

In the Rhesus alloimmunized pregnancies we measured MCA PSV starting from 25 weeks of gestation. According to Mari's proposed algorithm (9), a value of MoM MCA PSV higher than 1.50 MoM was indication of cordocentesis, intrauterine transfusion or elective delivery, especially if the trend for MCA PSV was rising. Regression analysis was used to calculate the relation between MoM for MCA PSV and MoM for hemoglobin in the risk group. We calculated the sensitivity and specificity of the MCA

GA (weeks)	FETAL MCA PSV (cm/s)							
	Present study				Mari's study			
	Median	1.29 MoM	1.50 MoM	1.55 MoM	Median	1.29 MoM	1.50 MoM	1.55 MoM
25	32.05	41.3	48	49.6	32.1	41.4	48.2	49.7
26	33.11	42.7	49.6	51.3	33.6	43.3	50.4	52.1
27	35.19	45.3	52.8	54.5	35.2	45.4	52.8	54.5
28	37.19	48	55.8	57.6	36.9	47.6	55.4	57.2
29	38.74	50	58.1	60	38.7	49.9	58	60
30	40.05	51.6	60	62	40.5	52.2	60.7	62.8
31	42.13	54.3	63.2	65.3	42.4	54.7	63.6	65.7
32	44.87	57.8	67.3	69.5	44.4	57.3	66.6	68.9
33	46.59	60.1	69.9	72.2	46.5	60	69.7	72
34	48.25	62.2	72.4	74.8	48.7	62.9	73.1	75.6
35	50.35	64.9	75.5	78	51.1	65.9	76.6	79.2
36	52.15	67.3	78.2	80.8	53.5	69	80.2	82.9
37	54.80	70.7	82.2	84.9	56	72.2	84	86.8
38	57.40	74	86.1	89	58.7	75.7	88	91
39	60.45	78	90.7	93.7	61.5	79.3	92.2	95.3
40	62.05	80	93	96.2	64.4	83	96.6	99.8

TABLE 1. The median, 1.29 MoM, 1.50 MoM and 1.55 MoM values for fetal MCA PSV with gestation calculated in our study, compared to those published by Mari

PSV of ≥1.29 MoM, the treshhold proposed by Mari (10) in detecting hemoglobin values ≤0.84 MoM in fetuses at risk of fetal anemia.

Statistical analysys was performed with the SPSS 16.0 (Statistical Package for Social Science, Chicago, IL, USA) and KyPlot 2.0 (Kyens-Lab Inc., Tokyo, Japan) statistical software programs. P-values <0.05 were considered statistically significant. ❑

RESULTS

In the normal pregnancies there was a significant association between fetal MCA PSV and gestation MCA PSV = 2.04872*GA - 20.535, where GA is gestational age in weeks (Figure 1). The median, 1.29 MoM, 1.50 MoM and 1.55 MoM values in this study are similar to the median, 1.29 MoM, 1.50 MoM and 1.55 MoM of those reported by Mari et al (10) from 25 to 34 weeks of gestation (Table 1). However, between 35 and 40 weeks of gestation, the median MCA PSV values in this study were lower compared to those reported by Mari, not much, but highly significant (p <0.001).

In the Rhesus alloimmunized pregnancies, there were 4 cases of non-anemic fetuses at birth, 20 cases with mild anemia, 4 cases with moderate anemia and 2 cases with severe anemia (Figure 2). All the fetuses, except one, were born at gestational ages between 35 and 39 weeks.

The fetal MCA PSV was increased (Figure 3) and there was a significant association between MoM MCA PSV and MoM hemoglobin (MoM MCA PSV= 2.0973 – 0.9555 × MoM Hb; R2 = 0.8354; p <0.0001; Figure 4): the MoM MCA PSV increased significantly with decreasing MoM hemoglobin.

Using the reference ranges for MCA PSV proposed by Mari (10), 5 fetuses had the last values of MoM MCA PSV before birth below 1.29 MoM, 19 fetuses between 1.29 and 1.50 MoM, 4 fetuses between 1.50 MoM and 1.55 MoM and 2 fetuses above 1.55 MoM. In all cases with MCA PSV above 1.50 MoM elective delivery was decided. All the cases of moderate and severe anemia were diagnosed.

Comparatively, using our reference ranges for MCA PSV calculated in the first group, 1 fetus had the last values of MoM MCA PSV before birth smaller than 1.29 MoM, 21 fetuses between 1.29 and 1.50 MoM, 6 fetuses between 1.50 MoM and 1.55 MoM and 2 fetuses above 1.55 MoM. There were 2 cases false

positive for moderate anemia, at birth they presented mild anemia.

One case of severe anemia was diagnosed at 26 weeks, with MoM MCA PSV 1.85. The fetus presented hydrops at ultrasound scan. Because there wasn't time to send the pregnant women to a specialized center for intrauterine transfusion, we performed cesarean section. The fetus had 4.3 g/dl hemoglobin (0.34 MoM). The other case of severe anemia was diagnosed at 35 weeks, with MoM MCA PSV 1.76. The fetus presented ascites at ultrasound scan. We decided for elective delivery and performed cesarean section. The fetus had 4.2 g/dl hemoglobin (0.32 MoM). Both newborns needed multiple transfusions.

The screening characteristics of cut-off value ≥1.29 MoM MCA PSV in the prediction of fetal anemia are shown in Table 2. Using the cut-off value ≥1.29 MoM MCA PSV in the diagnosis of at least mild anemia (hemoglobin ≤0.84 MoM) the sensitivity and the specificity were 88.46% and 98.27%. ❑

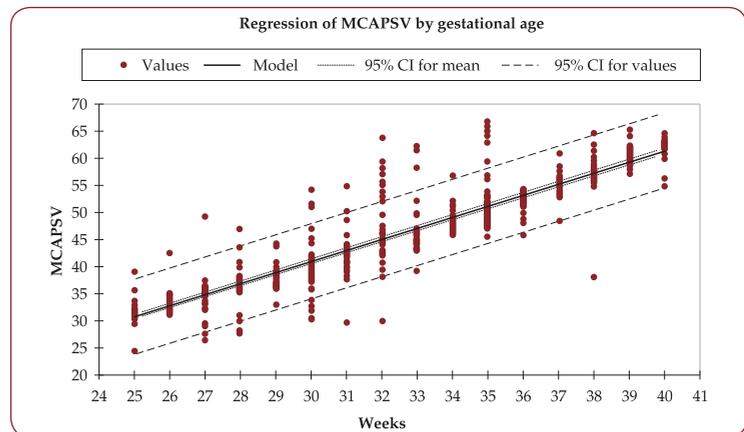**FIGURE 1.** MCA PSV regression function of gestational age
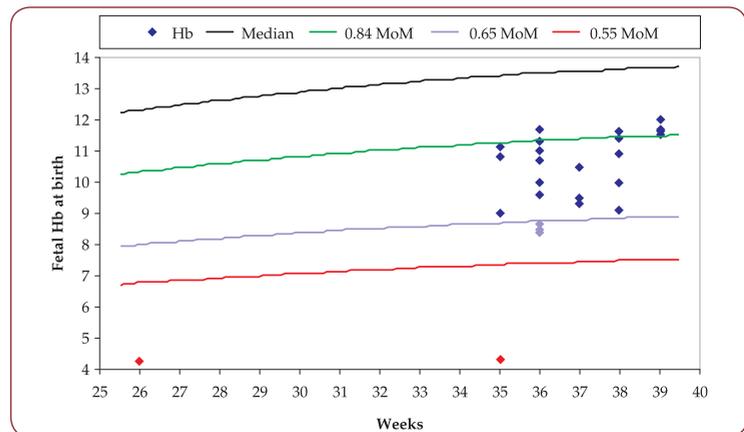

FIGURE 2. Hemoglobin concentration at birth at 30 fetuses at risk for anemia

DISCUSSION

The goals of our study were to develop reference ranges for MCA PSV for a group of non-selected normal singleton pregnancies, to compare with those already published and to certify their value in the management of Rhesus alloimmunized pregnancies. The reference ranges for MCA PSV established in the group of normal singleton pregnancies were similar to those proposed by Mari up to 34 weeks of gestation and lower between 35 and 40 weeks of

FIGURE 3. MCA PSV in 30 fetuses at risk for anemia. Each fetus had multiple measurements and the values were plotted for each week

FIGURE 4. Correlation between MoM MCA PSV measured within 7 days before birth and MoM for hemoglobin measured from umbilical cord at birth

Parameter	Value
Sensitivity (%)	88.46%
Specificity (%)	98.27%
Positive predictive value (%)	79.31%
Negative predictive value (%)	99.13%
False positive rate (%)	1.73%
False negative rate (%)	11.54%

TABLE 2. The accuracy of data for a cut-off value ≥1,29 MoM MCA PSV in prediction of any degree of fetal anemia (hemoglobin ≤0,84 MoM).

gestation. The difference after 35 weeks may be generated by multiple factors. After 35 weeks, the measurement of MCA PSV may be altered by fetal body movements and fetal breathing. We also considered the possibility of a human error in taking the measurements, but when the conditions for measuring the MCA PSV are respected, the intra- and interoperator variability are between 2.3% and 4% (11). In the group of 342 singleton pregnancies, the median value for hemoglobin at 40 weeks was 14.7 g/dl, compared to those used by Mari 13.7 g/dl. This could be an explanation for lower values of MCA PSV closer to 40 weeks in our study compared to Mari's values. Of course, in the absence of a reference range for fetal hemoglobin for our population, this is only a supposition.

This study confirms the observations made before that in Rhesus alloimmunization fetal anemia is associated with a hyperdynamic circulation. The most probable explanation for the observed increase in MCA PSV is that fetal anemia is associated with decreased blood viscosity leading to increased venous return and preload with consequent increase in cardiac output, the faster the velocity, the higher risk of fetal anemia. An interpretation of our data is that as the reference ranges for MCA PSV are similar up to 34 weeks, any reference range of two can potentially be used for pregnancies before 35 weeks. After 35 weeks it is wiser to use Mari's range because the rate of false positive cases is lower and the accuracy of data was verified by multiple trials. An improvement of our reference range could be enrollment in the study starting from 18 weeks of gestation.

As proposed by Mari et al. (10) a treshhold of 1.29 MoM for MCA PSV in detecting any degree of anemia provided a good sensitivity (88.46%) and specificity (98.27%). These results are similar to those found by other authors (12,13). Except one case of mild anemia, all cases of moderate and severe anemia could be diagnosed by this method. In the clinical management of the Rhesus alloimmunized pregnancies, the aim is to predict whether the fetus has moderate or severe anemia and to perform intrauterine blood transfusion in these cases. The mild anemia requires no intervention, only follow-up.

Although cordocentesis can provide the exact hemoglobin status, it is an invasive procedure associated with complications including
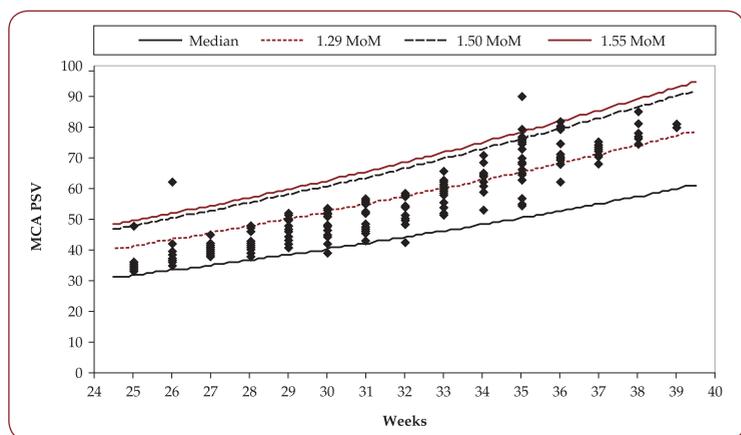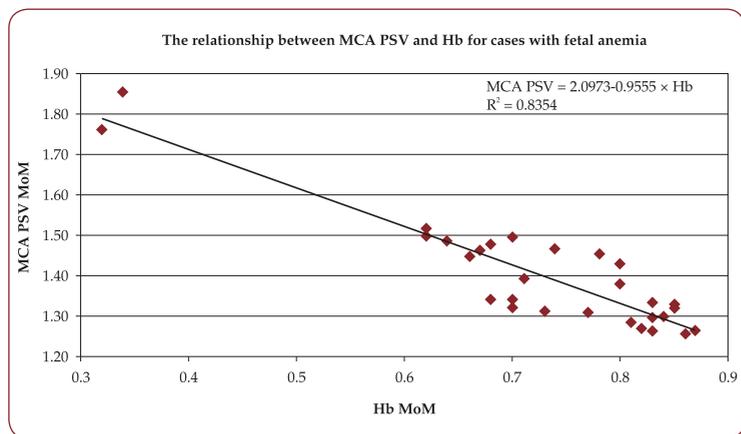
hemorrhage at puncture site, infection, intrauterine fetal demise and the worsening of maternal alloimmunization.

The findings of this study are in accord with those found in other studies (14,15) and demonstrate that cordocentesis, intrauterine transfusions and termination of pregnancy can safely be reserved only for those pregnancies with increased MCA PSV.

The assessment of the severity of fetal anemia should be based on the history of previous affected pregnancies, the titer of maternal hemolytic antibodies, ultrasonographic examination for the detection of ascites or hydrops and Doppler studies for diagnosis of a hyperdynamic circulation (measurement of MCA PSV) (16). In order successfully to apply the test, the population must be at risk for fetal anemia. ❑

CONCLUSIONS

Our reference ranges for MCA PSV can perform well from 25 until 35 weeks of gestation. Based on this, measurement of the MCA PSV in fetuses at risk for anemia due to Rhesus alloimmunization provides an accurate, non-invasive clinical test for the prediction of fetal anemia.

REFERENCES

1. **Carlton JP –** Defining the Rh blood group antigens. Biochemistry and molecular genetics. *Blood Rev* 1994;8:199-212.
2. **Liley AW –** Liquor amnil analysis in the management of the pregnancy complicated by rhesus sensitization. *Am J Obstet Gynecol* 1961;82:1359-1370
3. **Rahman F, Detti L, Ozcan T, et al.** – Can a single measurement of amniotic fluid delta optical density be safely used in the clinical management of Rhesus-alloimmunized pregnancies before 27 weeks' gestation? *Acta Obstet Gynecol Scand* 1988;77:804-7
4. **Nicolaides KH, Rodeck CH, Mibashan RS, et al. –** Have Liley charts outlived their usefulness? *Am J Obstet Gynecol* 1986;155:90-4
5. **Ludomirsky A, Weiner S, Aslmead GG, et al. –** Percutaneous fetal umbilical blood sampling. Procedure safety and normal fetal hematologic indices. *Am J Perinatol* 1988;5:264-6
6. **Hecher K, Snijders R, Campbell S, et al. –** Fetal venous, arterial and intracardiac blood flows in red blood-cell isoimmunization. *Obstet Gynecol* 1995; 85:122-128
7. **Mari G, Adrignolo A, Abuhamad AZ, et al. –** Diagnosis of fetal anemia with Doppler ultrasound in the pregnancy complicated by maternal blood group immunization. *Ultrasound Obstet Gynecol* 1995; 5:400-405
8. **Gooch A, Parker J, Wray J, et al. –** Guideline for blood grouping and antibody testing in pregnancy. London: British Committee for Standards in Haematology (BCSH) 2006
9. **Mari G –** Middle cerebral artery peak systolic velocity for the diagnosis of fetal anemia: the untold story. *Ultrasound Obstet Gynecol* 2005; 25:323-330
10. **Mari G, Deter RL, Carpenter RL, et al.** for Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. Non-invasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. *N Engl J Med* 2000; 342:9-14
11. **Mari G, Abuhamad A, Brumfield J, et al. –** Doppler ultrasonography of the middle cerebral artery peak systolic velocity in the fetus: reproducibility of measurement. Presented at the Twenty-second Annual Meeting of the Society for Maternal-Fetal Medicine, New Orleans, LA, January 14, 2002:669.
12. **Delle Chiaie L, Buck G, Grab D, et al.** – Prediction of fetal anemia with Doppler measurements of the middle cerebral artery peak systolic velocity in pregnancies complicated by maternal blood group alloimmunization or parvovirus B19 infection. *Ultrasound Obstet Gynecol* 2001; 18:232-6
13. **Teixeira JMA, Duncan K, Letsky E, et al. –** Middle cerebral artery peak systolic velocity in the prediction of fetal anemia. *Ultrasound Obstet Gynecol* 2000; 15:205-8
14. **Pereira L, Jenkins TM, Berghella V** – Conventional management of maternal red cell alloimmunization compared with management by Doppler assessment of middle cerebral artery peak systolic velocity. *Am J Obstet Gynecol* 2003; 189:1002-1006
15. **Oepkes D, Seaward G, Vandenbussche F, et al. –** Doppler Ultrasonography versus Amniocentesis to Predict Fetal Anemia. *N Engl J Med* 2006; 355:156-164
16. **Scheier M, Hernandez-Andrade E, Carmo A, et al. –** Prediction of fetal anemia in rhesus disease by measurement of fetal middle cerebral artery peak systolic velocity. *Ultrasound Obstet Gynecol* 2004; 23:432-436.