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Transabdominal Chorionic Villus Sampling at 9.5–12 Weeks' Gestation

Placental Vascular Resistance and Fetal Cardiovascular Responses

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Pregnancies subjected to chorionic villus sampling (CVS) have been associated with transverse limb reduction defects. This study was designed to examine the possible fetal cardiovascular responses to transabdominal CVS. We examined 42 patients referred for CVS between 9.5 and 12 weeks' gestation. CVS was performed transabdominally under ultrasonic guidance with a 20-gauge needle. Placental vascular resistance was evaluated by means of the umbilical artery pulsatility index. Fetal heart rate was ascertained automatically from two successive flow velocity waveforms. Paired t *test, regression analysis, power analysis and normal distribution analysis were performed, and statistical significance was set at* $P \leq .05$. *Fetal heart rate increased with increasing amounts of tissue, but placental vascular resistance did not change. The earlier the gestation, the larger the amount of tissue obtained. Multiple regression analysis demonstrated that the fetal heart rate change was influenced by neither gestation nor placental vascular resistance after CVS. Analysis of the change (difference before and after CVS) in placental resistance and fetal heart rate according to gestational age and amount of tissue did not change the above findings. Although statistically significant fetal cardiovascular responses can be elicited in relation to the amount of chorionic villi obtained during transabdominal CVS, the clinical significance of these findings remains unclear, given the fact that all the fetuses in this group of patients were normal. These responses may be secondary to various degrees of placental hemorrhage and may represent part of or the total fetal response to various degrees of fetal blood loss. Although these responses do not seem to be clinically significant, it is advisable to obtain the minimum necessary amount of tissue with the smallest degree of damage to the placenta until further studies prove transabdominal CVS to be safe.* (J Reprod Med 1995;40:453–457)

Keywords: chorionic villi sampling, birth defects, prenatal diagnosis.

Introduction

Chorionic villus sampling (CVS) has been proposed as a safe alternative to amniocentesis in patients at increased risk of aneuploidy.[1] However, pregnancies that were subjected to prenatal diagnosis by CVS have been associated with an increased risk of the development of transverse limb reduction defects.[2-7] The available clinical evidence indicates that these defects occur primarily in pregnancies subjected to transcervical or transabdominal CVS prior to 9.5 weeks' (66 days') gestation. A small number of these cases, however, were performed as late as 10 weeks' (70 days') gestation. There is no clear pattern to indicate the most likely cause, but it seems that the size (diameter) of the sampling device, method of sampling and operator experience may play a role along with gestational age.

The etiology of transverse limb reduction defects is not completely understood. Two major theories prevail[8-10]: (1) amnion disruption, in which one or more fetal limbs become trapped in the space between the amnion and chorion, leading to mechanical constriction, ischemia and limb loss, and (2) placental vascular disruption. This is a more complex mechanism, involving vasospasm and possibly embolization. Vascular injury during CVS may cause a

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cascade of events that can lead to fetal limb ischemia and limb resorption.

This study was designed to evaluate the effect of CVS on placental vascular resistance and the possible fetal cardiovascular responses following the procedure.

Materials and Methods

We examined a total of 42 patients who were referred to York Hospital's fetal testing unit for prenatal diagnosis because of advanced maternal age (n = 40) or previous pregnancy with chromosomal anomalies (n = 2). At our institution, patients with the above indications for prenatal diagnosis are offered either transabdominal CVS between 9.5 and 12 weeks' gestation or amniocentesis at 13 weeks' gestation or later. As part of our routine procedure, we establish fetal well-being before as well as after CVS by umbilical artery flow velocity waveforms. For the purposes of the study, flow velocity waveforms were obtained three minutes prior to CVS and within one to two after the needle was withdrawn from the placenta. Umbilical artery flow velocity waveforms were obtained by means of real-time ultrasonography and pulsed-wave Doppler ultrasonography (Toshiba 270-30A a-Sonolayer, Toshiba America, Yonkers, New York). The umbilical cord was first identified by real-time ultrasound, and subsequently the Doppler range gate was placed at the umbilical cord segment, close to the placenta. A minimum of 10 cardiac cycles were stored on videotape for analysis after completion of the procedure.

The CVS procedure was performed by the free-hand technique under continuous ultrasonic guidance. After localization of the placenta and under aseptic conditions, a 3.5-in, 20-gauge (0.9-mm external diameter) spinal needle was advanced into the placental parenchyma and away from the fetal surface. Several obese patients required a 5-in needle. All samplings were completed in one attempt. A 20-mL syringe with 2 mL of culture medium was used to aspirate the chorionic villi. Negative pressure was applied with the help of a breast needle biopsy device (Cameco Syringe Pistol, Precision Dynamics Corporation, San Fernando, California). Four strokes of the needle while 15 mL of negative pressure was applied were sufficient to yield adequate tissue in the first attempt. In the first author's experience, the posterior placental location is as accessible as all other locations and does not constitute a contraindication to the procedure.

Placental vascular resistance was evaluated by means of the pulsatility index (PI) for the umbilical artery, which reflects downstream placental vascular resistance.[11] PI is calculated automatically after manual tracing of the flow velocity waveform. An average of four cardiac cycles was considered for the analysis. Fetal heart rate was calculated automatically from two successive waveforms by means of electronic calipers. Statistical analysis was performed by means of a commercial statistical program for the Macintosh personal computer (JMP, SAS Institute, Cary, North Carolina). Comparisons of means of various parameters before and after CVS were performed by paired t test. Regression analysis was performed to explore the relationships between various variables. Power analysis was performed, when necessary, to evaluate the possibility of type II error. Normality of distribution of umbilical artery PI and fetal heart rate in this narrow gestational range was tested by the Shapiro-Wilk W test. Statistical significance was set at $P \leq .05$.

Results

The mean ± SD gestational age at the time of CVS was 10.8 ± 0.5 weeks, and the mean ± SD maternal age was 38 ± 0.0 years. No chromosomal defects were identified in this group. All infants had been born by completion of this manuscript. No anomalies were identified in any of the neonates. Umbilical artery PI and fetal heart rate are normally distributed before, as well as after, CVS in this limited period of human gestation (Shapiro-Wilk W test,

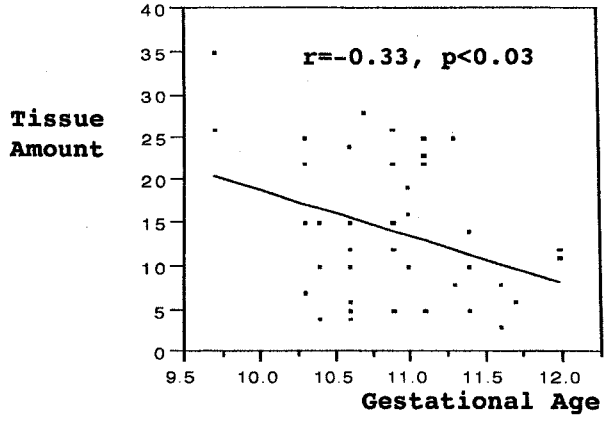

Figure 1
Amount of tissue obtained per attempt increased with decreasing gestation.

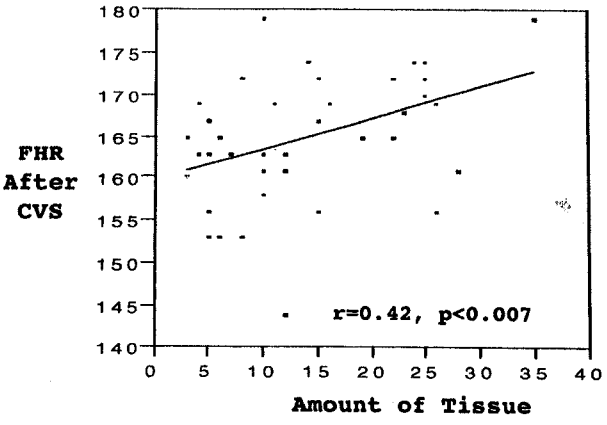

Figure 2
There was a significant relationship between fetal heart rate after CVS and the amount of tissue obtained.

$P > .05$). Umbilical artery PI did not change after CVS (2.44 ± 0.36 vs. 2.43 ± 0.40, $P = .89$). Likewise, fetal heart rate did not change significantly after CVS (165 ± 6.4 vs. 164 ± 7.4, $P = .98$). Power analysis revealed that more than 50,000 patients are required to identify a difference of 0.1 in the umbilical artery PI.

Each specimen was obtained with the first attempt. The amount of tissue obtained from each patient ranged from 3 to 35 mg (mean ± SD, 14 ± 8.2). The earlier the gestation, the larger the amount of tissue obtained per attempt ($r = .33$, $P < .03$) (Figure 1). Fetal heart rate declined with advancing gestation, but this trend was not statistically significant in this limited period ($r = .16$, $P = .03$). There was a strong positive linear correlation between fetal heart rate before and after CVS ($r = .88$, $P < .00001$). Fetal heart rate after CVS increased with increasing amounts of tissue obtained ($r = .42$, $P < .007$) (Figure 2). Multiple regression analysis documented that the fetal heart rate change was influenced by neither gestation nor placental vascular resistance after CVS.

Umbilical artery (UA) PI did not correlate with gestational age between 9.5 and 12 weeks' gestation ($r = .03$, $P > .9$). There was a significant negative linear correlation between UA PI and fetal heart rate prior to CVS ($r = .44$, $P < .007$), and this relationship ended after CVS ($r = .25$, $P > .10$) (Figure 3). UA PI after CVS did not correlate with the amount of tissue obtained ($r = .02$, $P > .70$) (Figure 4). A moderate positive linear correlation was noted between UA PI prior to and after CVS ($r = .56$, $P < .0003$). Analysis of the change (difference before and after CVS) of placental resistance and fetal heart rate after CVS according to gestational age and amount of tissue did not change the above findings.

Discussion

So far the majority of lesions reported to have been caused by CVS have been bilateral and rather symmetrical, and in some of the unilateral lesions there has been evidence of vascular pathology, including

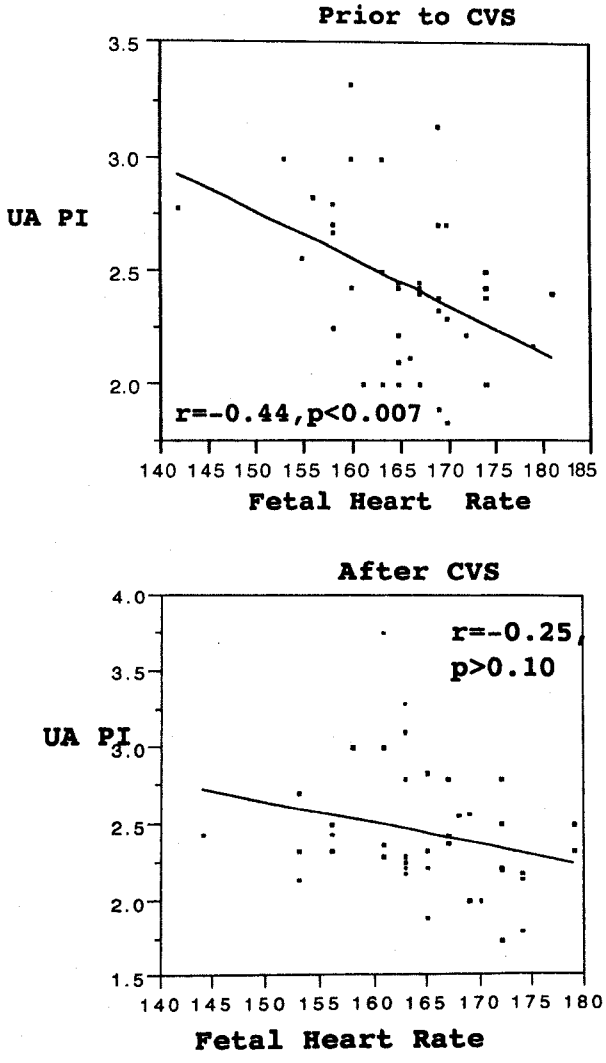

Figure 3
Note the change in the relationship between UA PI and the fetal heart rate after CVS in comparison to that relationship prior to CVS.

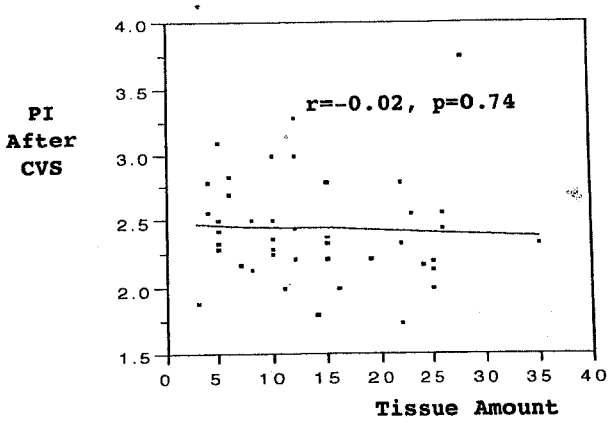

Figure 4
There was no correlation between UA PI after CVS and the amount of tissue obtained.

infarcts of the extremities and brain.[2,9,10] A small number of cases, however, could be attributed to amnion disruption,[4,6] and for some cases there was not enough evidence to associate them with either one of the etiologic mechanisms.[5] Despite the above reports, there is not enough evidence in the literature to indicate a causative relationship between CVS and limb reduction defects. The Workshop on Chorionic Villus Sampling and Limb and Other Defects, convened by the National Institute of Child Health and Human Development and the American College of Obstetricians and Gynecologists, October 20, 1992, did not reach a consensus and concluded, "Further studies are needed to determine whether there are factors that correlate with CVS-associated limb defects."[12]

In this study there was no evidence of any significant increase in peripheral placental vascular resistance, as indicated by the lack of any change in the UA PI. It is not known whether placental vasospasm could cause secondary vasospasm of the fetal extremities, although it is very unlikely since there exists no evidence of fetal-placental sympathetic neuronal communication. Placental vasospasm could cause fetal vasospasm only by means of some humoral response involving the production of prostaglandins, bradykinins or other vasoactive peptides that may be released into the fetal circulation as a result of local placental trauma.

Our data suggest that the earlier the gestational age, the larger the amount of tissue obtained and thus the higher the degree of placental injury and fetal insult. In fact, the effect may even be synergistic since larger insults are applied to younger and more vulnerable fetuses. From the existing evidence so far, it seems that the great majority of fetuses with the defect were subjected to CVS either by transcervical catheter or transabdominal procedure with an 18-gauge spinal needle.[2-4] One may be tempted to speculate that the larger size of the sampling device leads to a greater placental insult and subsequent fetal defect. By the same token, more than one attempt may lead to greater placental damage whether or not larger amounts of tissue are obtained. In our study all procedures were completed with one attempt, and the amount of tissue was small to moderate.

Although comparison of the means of fetal heart rate before and after CVS failed to reveal any significant difference, regression analysis revealed a significant correlation between the amount of tissue obtained and the fetal heart rate. The larger the amount of tissue, the faster the heart rate. Because of the existing relationship between the fetal heart rate and gestational age as well as UA PI, we performed a multiple regression analysis, which clearly demonstrated that a relationship exists only between the amount of tissue and fetal heart rate, without any significant contribution by either gestational age or UA resistance after CVS. In addition, we analyzed the data by multiple regression analysis, evaluating the degree of change in the fetal heart rate and UA PI in relation to the amount of tissue obtained. Again, the amount of tissue was the only significant contributor. The mechanism of this increase in heart rate with increasing amounts of tissue obtained is not clear. To the best of our knowledge there have been no human or animal studies to evaluate fetal cardiovascular responses to hemorrhage or placental injury in early gestation. Whether the central cardiovascular chemoreceptors and baroreceptors are operable at 9.5–12 weeks' gestation is open to speculation.

Our data tempt us to speculate that a small amount of hemorrhage caused by CVS may be responsible for the increase in the heart rate. This is more likely in view of the fact that there was no appreciable change in placental vascular resistance. This is further supported by the effect of CVS on the relationship between UA PI and the fetal heart rate. From studies on animals at more advanced gestational ages it is known that small degrees of hemorrhage (5%) cause the fetus to respond with mild heart rate elevation but that larger degrees (≥ 15%) lead to bradycardia, which may be the result of pe-

ripheral vasoconstriction and baroreceptor stimulation.[13]

The incidence of limb reduction defects in patients with CVS ranges from 0.5%[3] to 1%.[2,5] One may argue that although none of our fetuses had evidence of the specific anomalies, our sample size was small enough to make the study subject to a type II error. We argue, however, that our study was not meant to investigate whether our procedure caused any defects but only to evaluate the possible placental vascular and fetal cardiovascular effects that might be caused by the procedure. In this respect, our study sample was judged sufficient since significant changes were identified. For the absence of change in placental vascular resistance, we determined that more than 50,000 patients would be necessary to achieve statistical significance. Since the cardiovascular changes noted in these fetuses were not associated with any developmental disruption phenomena, one may hypothesize that the degree of damage to the placenta was not high enough to cause fetal vascular disruption. It seems to be a degree of damage–related response, and the point at which the degree of damage is large enough to cause fetal vascular disruption is not clear. A similar, prospective study with a large number of abnormal fetuses could shed light and allow us to determine the maximum degree of damage that a fetus can tolerate safely.

We conclude that with a 20-gauge needle, statistically significant fetal cardiovascular responses can be elicited in relation to the amount of tissue obtained. These responses may be secondary to hemorrhage and may represent only part of or the total fetal response to various degrees of hypovolemia. Although these responses do not seem to be clinically significant, it may be advisable to obtain the absolute minimum necessary amount of tissue with the smallest degree of damage to the placenta until more information becomes available.

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