

# Sonographic Investigation of Flow Patterns in the Perfused Human Placenta and Their Modulation by Vasoactive Agents with Enhanced Visualization by the Ultrasound Contrast Agent Albunex

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**ABSTRACT:** *Purpose.* Our objective was to demonstrate sonographically the flow distribution in the circulation of human placentae as well as the sensitivity of the human fetal capillary bed to vasoconstriction and dilatation.

*Methods.* Five human full-term placental lobules were maintained in vitro with fetal and maternal flow. Commercial ultrasound scanners were used for imaging. Albunex (1 ml bolus) was administered to the fetal "artery" to monitor patterns of flow. U46619 (1 ml,  $10^{-6}$  M; a thromboxane agonist and potent vasoconstrictor) and/or nitroglycerin (a potent vasodilator) were added to the fetal artery.

*Results.* Following the addition of U46619, mean "fetal pressures" rapidly rose from  $23.2 \pm 0.8$  to  $118 \pm 2.9$  mm Hg (mean  $\pm$  standard error of mean;  $p < 0.001$ ); venous flow rates decreased. As demonstrated by color Doppler imaging, flow markedly changed from a pattern of general distribution throughout the lobule to flow only near the chorionic plate. Color persistence was  $94.4 \pm 6.5$  seconds with Albunex after nitro-

glycerin and  $39.8 \pm 3.4$  seconds with Albunex after injection of U46619 ( $p < 0.001$ ). Nitroglycerin had no effect when injected by itself but returned "constricted" flow to a "normal" pattern when injected after U46619.

*Conclusions.* The contrast medium Albunex improved visualization of the fetal circulation throughout the lobule. Flow in the human placental capillary bed can be regionally manipulated throughout the placental lobule by vasomodulators and monitored by Albunex-enhanced sonographic examination. © 1999 John Wiley & Sons, Inc. *J Clin Ultrasound* 27:513-522, 1999.

**Keywords:** ultrasound contrast agents; human placenta; perfusion; blood flow; ultrasonography

The placenta is a complex entity fulfilling the functions of many major fetal organs and allowing for communication between 2 separate organisms (mother and embryo/fetus). In the human, the placenta is of the discoidal villous, hemomonochorial type.<sup>1</sup> In addition to the placenta's critical role in utero, the fetal circulation in

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the human placenta under in vitro perfusion can be considered a prototype for other human capillary beds. Flow distribution in the human placenta has been described in laboratory investigations and by invasive imaging methods such as scintigraphy.<sup>2</sup> Local regulation of blood flow through the human fetoplacental vascular bed is under the influence of some autacoids, particularly angiotensin II and nitric oxide.<sup>3,4</sup> Exogenous substances such as U46619, a thromboxane agonist, have also been shown to alter placental circulation.<sup>3-6</sup> The objective of this study was to demonstrate sonographically the flow distribution in the perfused human placenta in vitro. Another goal was to demonstrate that the sensitivity of the fetal capillary bed to vasoconstriction and vasodilatation can be characterized via sonographic visualization using an ultrasound contrast agent (UCA). To our knowledge, this is the first such attempt to characterize blood flow dynamics in the perfused placenta in this fashion.

**MATERIALS AND METHODS**

Five human placentae from healthy, uncomplicated, term pregnancies were obtained following delivery. A cotyledon was selected and connected (using 5-French umbilical catheters) to a maternal and a fetal pump per previously described methods.<sup>7,8</sup> The cotyledon was placed fetal side (chorionic plate) down in a water bath heated to 37°C. A catheter was introduced and sutured in place in a fetal vein and another in a fetal artery on the fetal side, and 2 catheters were introduced blindly into the maternal side (decidual plate). Figure 1 demonstrates the laboratory setup. For the present study, we used an open system (ie, without recirculation). Fetal and maternal flows were at 3 and 15 ml/minute, respectively. Placental functions that can be studied through this perfusion model include hemodynamics, transplacental transport, cellular uptake, endocrine function, and metabolism. Criteria for effective dual perfusion have been published previously<sup>8,9</sup>

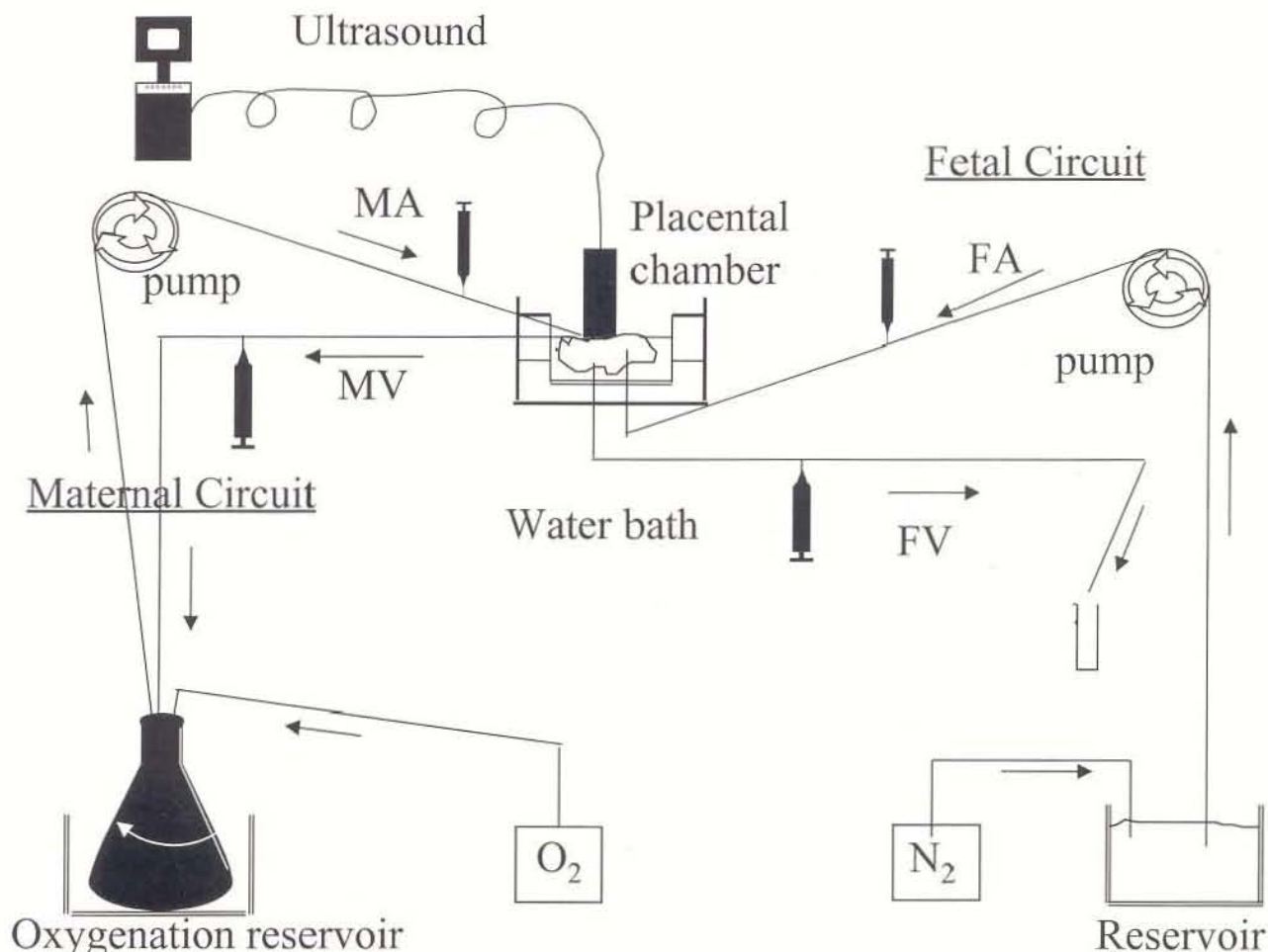


FIGURE 1. Schematic representation of the laboratory setup. FA, fetal "artery"; FV, fetal "vein"; MA, maternal "artery"; MV, maternal "vein."



those most commonly employed are oxygen consumption, glucose consumption, lactate production, human chorionic gonadotropin production, net fetal oxygen transfer, fetal pressure, and flow rates. Hemodynamic control consisted of instantaneous pressure readings with recording every 5 seconds and maintenance of fetal arterial flow at 3 ml/minute. The pressures that were obtained did not correspond to physiologic systolic and diastolic pressures in humans but were an indication of flow pressure in the system.

Commercially available APOGEE 800 or ATL-HDI 3000 ultrasound scanners (Advanced Technology Laboratories, Bothell, WA) equipped with a multi-frequency transducer were used for imaging. Optimal visualization was at a frequency of 5 MHz. Doppler settings were as follows: pulse repetition frequency, 1–2 kHz; wall filter, 50 Hz; sample volume, 1 mm. The transducer was introduced in a plastic bag containing ultrasound gel at room temperature, and the bag was applied directly to the placental surface (maternal side), being supported by a movable mechanical arm so as not to cause pressure on the placental surface. In color Doppler mode, the transducer was moved until an area of flow could be demonstrated and was then fixed in position. Manipulation of flow rates (on/off) in the maternal and fetal pumps demonstrated the presence in the same frame of both maternal and fetal flows. Color and Doppler signals were obtained but appeared weak to the

naked eye. This is probably due to the fact that the perfusate did not contain red blood cells, the scattering agent normally detected by Doppler sonography.

The ultrasound contrast medium Albunex (Molecular Biosystems, Inc., San Diego, CA) was injected into the arterial side of the fetal circuit (FA in Figure 1) over 1 minute. Albunex consists of 5% aqueous human albumin, sonicated under heat and pressure, yielding a solution of microspheres measuring 3–5 microns at a concentration of  $4 \times 10^8$ /ml when rediluted. One-milliliter boluses were injected into the fetal circuit. Fetal perfusate was collected from the fetal vein (FV in Figure 1), and flow pressure was monitored in the fetal artery. Two vasoactive substances were also employed. First, U46619, a thromboxane agonist and a potent vasoconstrictor, was injected into the fetal artery. The dosage employed (1 ml,  $10^{-6}$  M) was pharmacologic and corresponded to amounts previously used by other authors in perfusion experiments.<sup>5,6</sup> Albunex® was added after 1 minute. After 5 more minutes, 1 ml of nitroglycerin, a potent vasodilator, was injected, and again Albunex was added after 1 minute. After a “resting” period of 1 hour and with all experimental settings demonstrating “native” values, nitroglycerin was injected again (without priming by U46619), followed by Albunex.

To evaluate the efficacy of the addition of the UCA in assessing placental perfusion patterns,

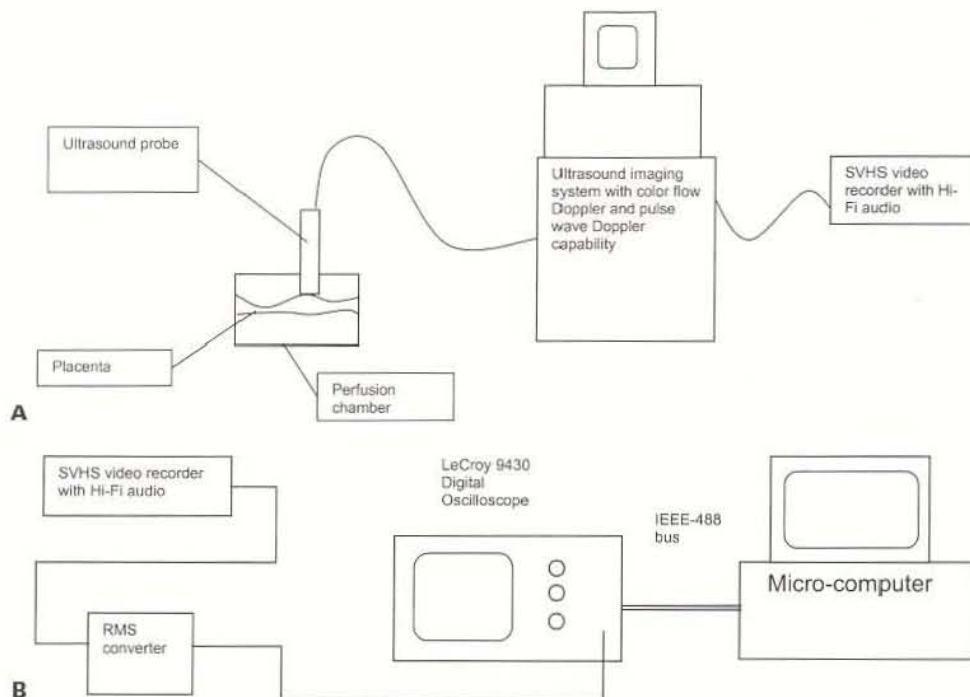


FIGURE 2. Schematic representation of the Doppler acquisition (A) and processing (B) setup.



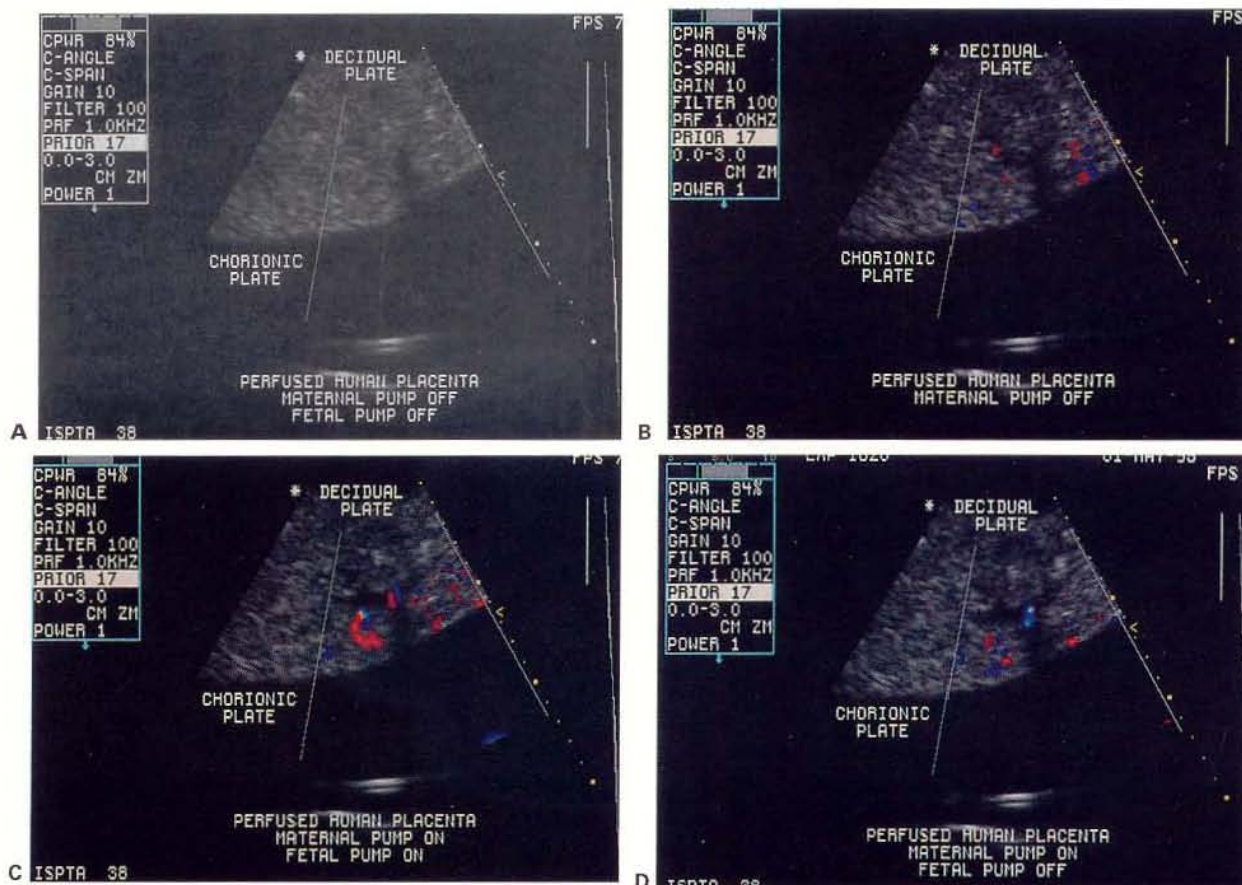


FIGURE 3. Imaging with no contrast medium to establish anatomy and location of the decidua and chorionic plates. (A) Both maternal and fetal pumps off. (B) Maternal pump off; fetal pump on. (C) Maternal pump on; fetal pump off. (D) Both maternal and fetal pumps on.

pulsed Doppler measurements were made at chosen sites. A quantitative measure of flow rate was provided by observing the root-mean-square (rms) value of the spectral Doppler audio output. The addition of the UCA provided the scatterers necessary for Doppler velocity detection. For relatively dilute concentrations of such scatterers, the scattered intensity is roughly proportional to their concentration; this permits evaluation of flow in a region of interest when a given concentration of the UCA is being delivered at a constant rate of infusion. The change in Doppler intensity can be related to the change in volume flow rate.<sup>10,11</sup> The precise relationship depends on a number of factors, particularly the stability of the scattering characteristics of the UCA over time in response to parameters such as the infusion pressure and the qualities of the infusate itself such as gas solubilities and surfactant properties. Nonetheless, for a constant infusion of UCA, a change in volume flow will be reflected in a change in the observed Doppler signal intensity.

A region of active flow was determined by positioning both the transducer and Doppler gate in

a region of observable Doppler signal intensity as observed on the Doppler spectral display that corresponded to starting and stopping of the fetal perfusion pump. The Doppler spectral display and audio signal were recorded on an S-VHS video recorder with the automatic gain control disabled and with constant gain setting. Epochs of flow measurements were recorded in this manner as injections of the UCA were applied with and without prior injection of the vasoactive agents of interest. The audio signals were then analyzed off-line by measuring the variation of the rms value of the audio signal level over time. The Doppler gate included a region of vasculature of varying orientation such that the left and right channels of the Doppler signal provided signals of equivalent amplitude. The rms value was measured and converted into a decibel value (10 times the common logarithm of the voltage squared) utilizing a circuit based on an AD637 monolithic rms-to-dB converter (Analog Devices, Norwood, MA). The output voltage of the circuit was recorded with a 9430 Digital Oscilloscope (LeCroy, Chestnut Ridge, NY), and the data were trans-



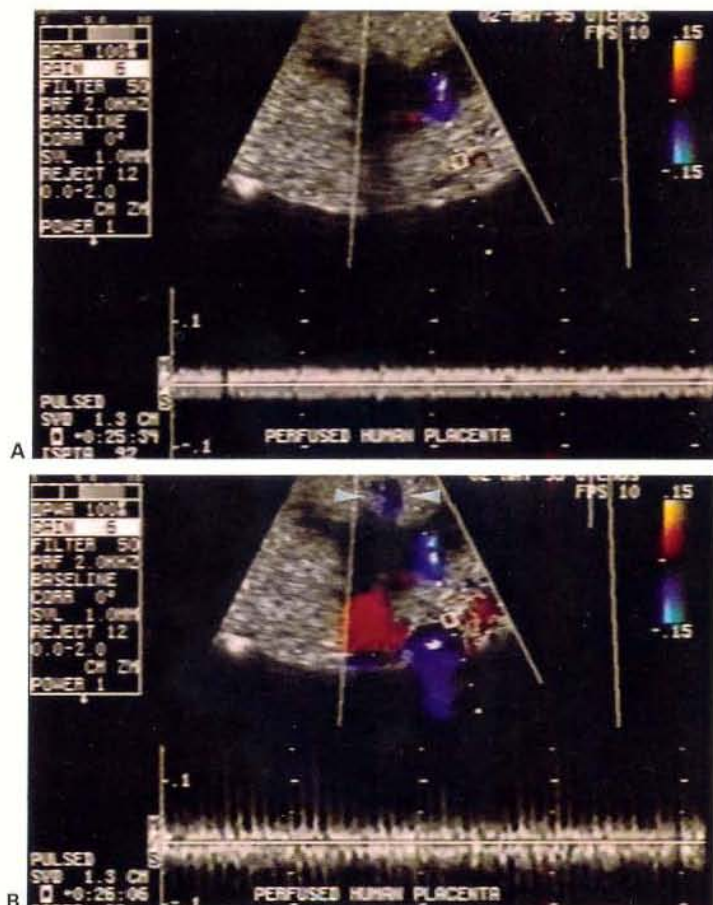


FIGURE 4. Imaging before (A) and after (B) injection of Albunex. Flow not discernible without Albunex is demonstrated near the decidual plate (arrowheads) after injection of Albunex. Note also enhanced Doppler signals.

ferred to a personal computer for storage and further analysis. A diagram of the Doppler acquisition and analysis process is shown in Figure 2. The duration of color persistence was determined by observing the appearance of color and measuring the amount of time that color was present.

Paired *t*-tests were used to evaluate pressure changes and color persistence with or without the addition of vasoactive substances. A *p* value below 0.05 was considered statistically significant.

## RESULTS

Before beginning the ultrasound component of this study, the placental perfusions met all of the previously established physiologic criteria.<sup>8</sup> For the Albunex portion of the experiment, an open circuit was used on the fetal side. Thus, not all physiologic parameters could be documented, but this was of no relevance for the present experiments. Those documented during the perfusion were fetal venous flow rate, system pressure,  $PO_2$ ,  $PCO_2$ , and pH.

### Imaging with No Contrast Medium

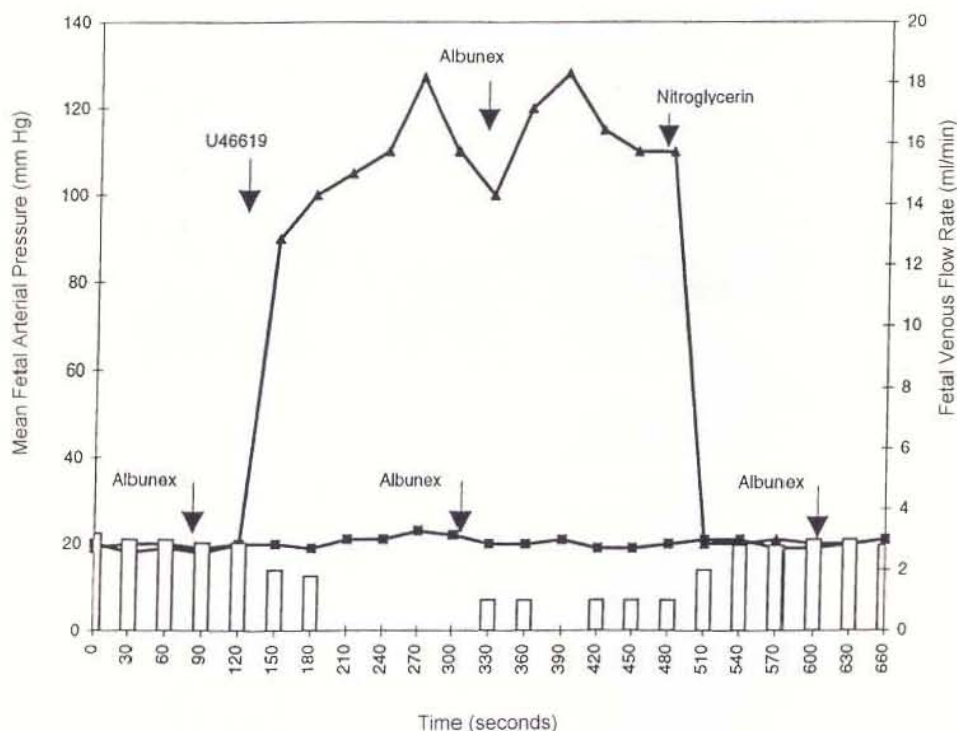
Intermittently stopping the maternal and/or fetal pumps permitted demonstration of the 2 circulations. Both the spectral and color Doppler signals, as previously mentioned, were demonstrable but weak. Figure 3 shows the presence or absence of flow with either 1 or both pumps on or off.

### Imaging with Contrast Medium

Injection of Albunex produced a notable increase in the color signals. Because of the high intensity, "spillage" of the color occurred. This was not corrected because of our desire not to change any settings such as sensitivity and pulse repetition frequency. Flow was demonstrated in areas where it was not previously visualized (Figure 4). There was no noticeable change in pressure or in flow. Doppler signals were greatly enhanced (Figure 4).

### Modulation of Flow

After the addition of U46619, the mean fetal pressure abruptly rose to  $118 \pm 2.9$  mm Hg (standard



**FIGURE 5.** Fetal arterial pressure and fetal venous flow rate with injection of vasomodulators and Albunex for a sample experiment. Control represents injection of Albunex only (flow rates in the control experiments did not change). White boxes, fetal venous flow rate; line with squares, control; line with triangles, vasomodulators.

error of mean) from a baseline of 20 mm Hg; the mean pressure was  $23.2 \pm 0.8$  mm Hg after Albunex alone ( $p < 0.0001$ ). The addition of Albunex after U46619 caused a small increase in pressure, presumably because of some washout effect of the U46619 still in the tubing. Within 1 minute of U46619 injection, fetal flow, as demonstrated by the amount of fetal perfusate collected at the fetal venous side, began to decrease and was absent after 2 minutes. The addition of nitroglycerin rapidly returned the pressure to baseline. Figure 5 is the graphic representation of 1 experiment. When Albunex was added to the system after injection of U46619, imaging (Figure 6A) demonstrated flow only in the area of the chorionic plate (ie, larger vessels), as opposed to the generalized flow (ie, chorionic and decidual plates as well as placental substance) seen with no vasomodulator (Figure 3B). After the addition of nitroglycerin, flow was again demonstrated in all placental regions (Figure 6B). No changes in pressure or flow were noticeable when nitroglycerin was injected alone. By qualitative evaluation, the Doppler signals appeared weaker with Albunex after U46619 (Figure 6A) and stronger after Albunex alone (Figure 4B) or Albunex after nitroglycerin (Figure 6B).

### Flow Analysis

Our semiquantitative estimation of the presence of color demonstrated the shortest persistence ( $39.8 \pm 3.4$  seconds) when Albunex was added after injection of U46619 and the longest ( $94 \pm 6.5$  seconds) when Albunex was added after injection of nitroglycerin ( $p < 0.0001$ ). The persistences for Albunex alone and Albunex after nitroglycerin were not significantly different. A plot of a typical UCA injection study is shown in Figure 7A. The abscissa is time, and the ordinate indicates Doppler signal intensity in decibels. A plot of a UCA flow study after injection of the vasoactive modulator U46619 is shown in Figure 7B. A plot of a UCA flow study following the injection of the vasodilator nitroglycerin is shown in Figure 7C. The change in the time course and decrease in magnitude of the measured Doppler signals are evident after injection of the vasoconstrictor U46619. After injection of the vasodilator nitroglycerin, the time course and magnitude of the Doppler signals are similar to those before U46619 was injected, indicating restoration of the initial flow rates.



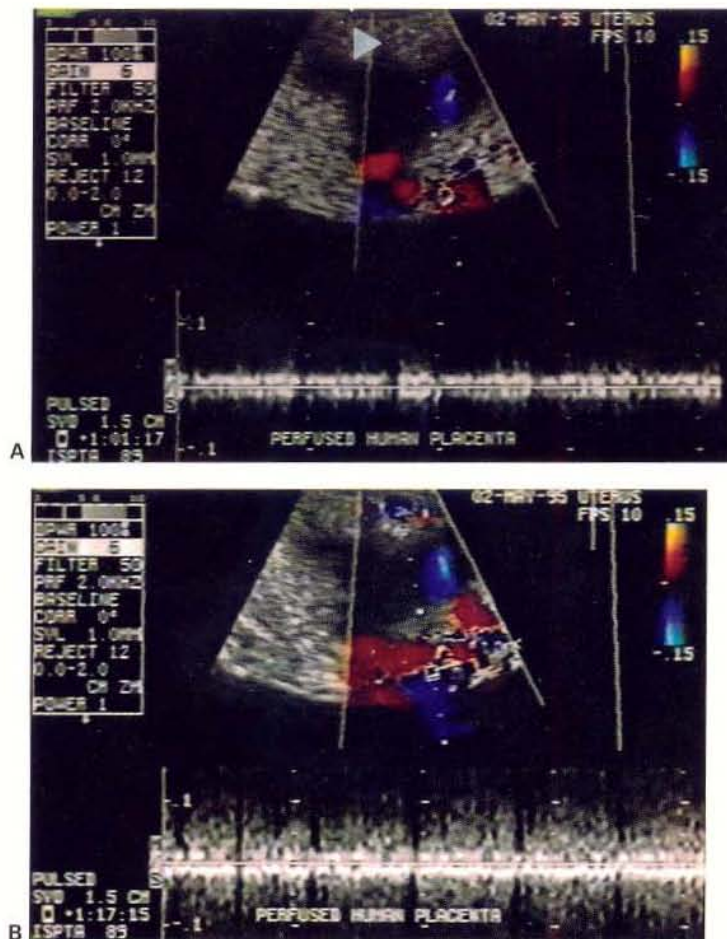


FIGURE 6. Modulation of flow. (A) Injection of Albunex after addition of U46619. Note absence of flow in the decidual plate region (arrowhead). (B) After addition of nitroglycerin. Flow is generalized.

## DISCUSSION

One of the major functions of the placenta is substance transfer from the maternal circulation to the fetus. Placental morphology can be examined today by several imaging techniques such as radiography,<sup>12</sup> MRI,<sup>13</sup> and sonography.<sup>2</sup> Placental function is more difficult to evaluate directly. One can examine fetal well-being and growth as an assumption of a properly functioning placenta, but the reverse is not necessarily so: poor fetal growth may have its origin in maternal or intrinsic fetal disorders, as well as "placental insufficiency."

Doppler imaging is a technique allowing evaluation of uterine and umbilical circulation but is less effective in evaluation of the placental circulation per se. Alterations in downstream hemodynamics are reflected by changes in Doppler velocity waveforms, as demonstrated in laboratory studies.<sup>14,15</sup> Abnormal Doppler tracings have been correlated with placental vascular pathology

in morphologic studies.<sup>16-18</sup> This is particularly obvious in cases with absent end-diastolic flow, when severe placental morphologic changes occur, such as decreased weight, decreased basal area, perivillous microfibrinous deposits, and villous fibrosis.<sup>19,20</sup> Changes in the Doppler characterization of umbilical artery blood flow velocity have been described in multiple publications, particularly in cases of intrauterine growth restriction.<sup>21-24</sup> Alterations in vessel tone or actual changes in the placental vascular tree have been correlated with Doppler tracings.<sup>25,26</sup> Placental vessels lack local innervation but are clearly responsive to autacoids,<sup>4,5,27</sup> particularly U46619, apparently via thromboxane A<sub>2</sub> receptor activation.<sup>6,28,29</sup> New ultrasound techniques such as color and amplitude-based Doppler (color Doppler imaging and power Doppler imaging) as well as the use of UCAs may allow visualization of intraplacental circulation and verify previously published studies on placental vascularization.<sup>2,30,31</sup>

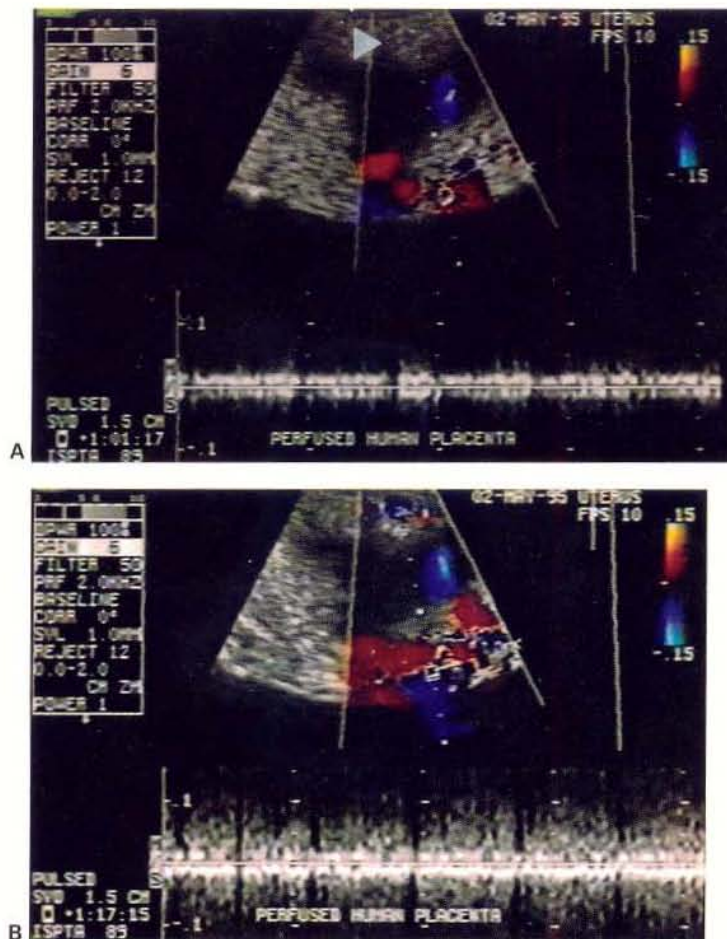


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We have demonstrated in a laboratory setting that maternal and fetal circulation can be examined by color Doppler imaging. The addition of a UCA allows noninvasive Doppler measurements of flow modulation due to vasoactive agents in the perfused placenta in a quantitative manner. Use of pulsed Doppler imaging permits evaluation of flow changes at specified sites within the placental tissue. By utilizing available ultrasound scanning systems that provide simultaneous B-mode imaging, color Doppler imaging, and pulsed Doppler velocity measurement, it is possible to assess the regional distribution of vasoactive agents' effects in the perfusion model. In particular, color Doppler imaging can be used to guide the placement of the pulsed Doppler sampling gate for quantitative measurements, and placement is facilitated by the use of a UCA. The backscatter enhancement provided by the UCA can also be used to determine the distribution of the flow modulation by analysis of the real-time B-mode images. Areas of enhanced contrast appear as areas of increased brightness over time as the injection of the UCA proceeds. Measuring the brightness (or echogenicity) variation over time can provide another assessment of flow distribution and time course. This can be used to study flow variations at specific locations or over a specified region of the placental tissue. As described by Schwarz et al,<sup>32</sup> video-densitometric analysis of contrast-enhanced B-mode images requires consideration of the imaging system's video-processing parameters as well as the attenuation properties of both the tissue of interest and the UCA agent, including the effect of its concentration. The addition of a UCA may allow documentation of flow in areas that are beyond the sensitivity of the usual instruments because of low velocity and low signal-to-noise ratios in non-contrast-enhanced situations. Whether a UCA was used should be indicated when describing "absent" flow by color Doppler imaging. In addition, quantification of placental insufficiency secondary to vascular changes<sup>33</sup> may be possible in the future by measuring the amount of contrast medium taken up or cleared.

Even though our investigations were done in vitro, they demonstrate that the placental bed circulation can be monitored to identify not only circulatory patterns but, more important, continuing changes in circulatory patterns as a first step to assessing pathophysiology that may be associated with fetal compromise. Quantification of these changes will be critical to evaluating such pathophysiology. We have recently begun using a more quantitative method of estimation of color

intensity and duration using a frame grabber and color analysis software.

In conclusion, it is possible to characterize changes in the human placental blood flow using color Doppler imaging, spectral analysis, and UCAs. The next step is to characterize the pathophysiology leading to fetal compromise.

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#### REFERENCES

1. Benirschke K, Kaufmann P. Pathology of the human placenta. 3rd ed. New York: Springer-Verlag; 1995.
2. Panigel M, Abramowicz JS, Miller RK. Techniques: biophysical methods for assessment of placental function. In: Sastry BVR, editor. Placental pharmacology. Boca Raton, FL: CRC Press; 1996. p. 1-22.
3. Clifton VL, Read MA, Leitch IM, et al. Corticotropin-releasing hormone-induced vasodilation in the human fetal-placental circulation: involvement of the nitric oxide-cyclic guanosine 3',5'-monophosphate-mediated pathway. *J Clin Endocrinol Metab* 1995;80:2888.
4. King RG, Gude NM, Dilulio JL, et al. Regulation of human placental fetal vessel tone: role of nitric oxide. *Reprod Fertil Dev* 1995;7:1407.
5. Maguire MH, Howard RB, Hosokawa T, et al. Effects of some autacoids and humoral agents on human fetoplacental vascular resistance: candidates for local regulation of fetoplacental blood flow. *Trophoblast Research* 1988;3:203.
6. Myatt L, Eis AL, Kossejans W, et al. Autocoid synthesis and action in abnormal placental flows reviewed: causative vs. compensatory roles. *Trophoblast Research* 1998;11:315.
7. Miller RK, Wier PJ, Maulik D, et al. Human placenta in vitro: characterization during 12 hours of dual perfusion. *Contrib Gynecol Obstet* 1985;13:77.
8. Miller RK, Wier PJ, Perez-D'Gregorio R, et al. Human dual placental perfusions—criteria for toxicity evaluations. *Methods in Toxicology* 1993;3B:246.
9. di Sant'Agnese PA, DeMesy Jensen K, Miller RK, et al. Long term human placental lobule perfusion—an ultrastructural study. *Trophoblast Research* 1987;2:549.
10. Schwarz KQ, Bezante GP, Chen X, et al. Quanti-

- tative echo contrast measurement by Doppler sonography. *Ultrasound Med Biol* 1993;4:289.
11. Chen X, Schwarz KQ, Phillips D, et al. A mathematical model for the assessment of hemodynamic parameters using quantitative contrast echocardiography. *IEEE Trans Biomed Eng* 1998;6:754.
  12. Freese UE. The fetal maternal circulation of the placenta. I. Histomorphologic plastoid injection and x-ray cinematographic studies on human placentas. *Am J Obstet Gynecol* 1966;94:354.
  13. Panigel M, Dixon T, Constantidinis I, et al. Fast scan magnetic resonance imaging and Doppler ultrasonography of uteroplacental hemodynamics in the rhesus monkey. *J Med Primatol* 1993;22:393.
  14. Maulik D, Saini VD, Wanda NC, et al. Doppler evaluation of fetal hemodynamics. *Ultrasound Med Biol* 1982;8:705.
  15. Surat DR, Adamson SL. Downstream determinants of pulsatility of the mean velocity waveform in the umbilical arteries as predicted by a computer model. *Ultrasound Med Biol* 1996;22:707.
  16. Giles WB, Trudinger BJ, Baird PJ. Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlation. *Br J Obstet Gynaecol* 1985;92:31.
  17. Kingdom JC, Burrell SJ, Kaufmann P. Pathology and clinical implications of abnormal umbilical artery Doppler waveforms. *Ultrasound Obstet Gynecol* 1997;9:271.
  18. Bruch JF, Sibony O, Benali K, et al. Computerized morphometry of umbilical vessels from pregnancies with intrauterine growth retardation and abnormal umbilical artery Doppler. *Hum Pathol* 1997;28:1139.
  19. Jimenez E, Vogel M, Arabin B, et al. Correlation of ultrasonographic measurement of the utero-placental and fetal blood flow with the morphological diagnosis of placental function. *Trophoblast Research* 1988;3:325.
  20. Mitra SC, Venkataseshan VS, von Hagen S, et al. Morphometric study of the placental vessels and its correlation with umbilical artery Doppler flow. *Obstet Gynecol* 1997;89:238.
  21. Bracero LA, Beneck D, Kirshenbaum N, et al. Doppler velocimetry and placental disease. *Am J Obstet Gynecol* 1989;161:388.
  22. Joern H, Klein A, Kuehlwein H, et al. Critical comparison of indices and threshold values for assessing placenta performance using Doppler ultrasound. *Ultrasound Med Biol* 1997;23:1179.
  23. Laurin J, Lingman G, Marsál K, et al. Fetal blood flow in pregnancies complicated by intrauterine growth retardation. *Obstet Gynecol* 1987;69:895.
  24. Salafia CM, Pezzullo JC, Minior VK, et al. Placental pathology of absent and reversed end diastolic flow in growth-restricted fetuses. *Obstet Gynecol* 1997;90:830.
  25. Fok RY, Pavlova Z, Benirschke K, et al. The correlation of arterial lesions with umbilical artery Doppler velocimetry in the placentas of small-for-gestational-age pregnancies. *Obstet Gynecol* 1990;75:578.
  26. Jackson MR, Walsh AJ, Morrow RJ, et al. Reduced placental villous tree elaboration in small-for-gestational-age pregnancies: relationship with umbilical artery Doppler waveforms. *Am J Obstet Gynecol* 1995;172:518.
  27. Myatt L, Brewer AS, Langdon G, et al. Attenuation of the vasoconstrictor effects of thromboxane and endothelin by nitric oxide in the human fetal-placental circulation. *Am J Obstet Gynecol* 1992;166:244.
  28. Boura ALA, Gute NM, King RG, et al. Characterization of thromboxane A<sub>2</sub> receptors in the human fetal placental vessel and umbilical veins. *Clin Exp Pharmacol Physiol* 1986;13:83.
  29. Adamson SL, Whiteley KJ, Langille BL. Endothelin-1 constricts fetoplacental microcirculation and decreases fetal O<sub>2</sub> consumption in sheep. *Am J Physiol* 1996;270:H16.
  30. Abramowicz JS. Ultrasound contrast media and their use in obstetrics and gynecology. *Ultrasound Med Biol* 1997;23:1287.
  31. Kaufmann P, Miller RK, editors. Placental vascularization and blood flow—basic research and clinical application. New York: Plenum Medical; 1988.
  32. Schwarz KQ, Chen X, Steinmetz S. Methods for quantifying ultrasound backscatter and two-dimensional video intensity: implications for contrast-enhanced sonography. *J Am Soc Echocardiogr* 1998;2:155.
  33. Sheppard BL, Bonnar J. The maternal blood supply to the placenta in pregnancy complicated by intrauterine fetal growth retardation. *Trophoblast Research* 1988;3:69.