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Fetal plasma erythropoietin concentration in red blood cell–isoimmunized pregnancies

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OBJECTIVE: The aim of this study was to investigate the relationship between fetal anemia, plasma erythropoietin concentration, and erythroblastosis in red blood cell–isoimmunized pregnancies.
STUDY DESIGN: Fetal plasma erythropoietin concentration in umbilical venous blood samples from 68 red blood cell–isoimmunized pregnancies at 18 to 35 weeks' gestation was measured. Measurements were compared with the appropriate reference range with gestation, and associations with blood pH, erythroblast count, and hemoglobin concentration were examined.
RESULTS: The mean fetal plasma erythropoietin concentration and erythroblast count in red blood cell–isoimmunized pregnancies were significantly increased only in severe fetal anemia (hemoglobin deficit >7 gm/dl). Furthermore, some severely anemic fetuses were hydropic and acidemic. The degree of increase in plasma erythropoietin was significantly associated with both fetal acidemia and, more strongly, fetal erythroblastosis.
CONCLUSION: These findings suggest that in fetuses from red blood cell–isoimmunized pregnancies the ability to prevent tissue hypoxia is present until anemia becomes severe, presumably by an increase in cardiac output and tissue perfusion. In severe anemia tissue hypoxia occurs, and the data indicate that fetuses respond by increasing erythropoietin production from at least 20 weeks' gestation. Furthermore, more accurate assessment of tissue oxygenation may be obtained by measuring the erythroblast count rather than the blood pH. (Am J Obstet Gynecol 1992;167:1292-7.)

Key words: Cordocentesis, red blood cell isoimmunization, erythroblastosis, erythropoietin, fetal anemia

In red blood–isoimmunized pregnancies fetal anemia results from antibody-coated red blood cell destruction in the fetal reticuloendothelial system.[1] In severe anemia there is fetal macrocytosis and erythroblastosis, which are thought to be a consequence of extramedullary hematopoiesis.[2-4] It is possible that in mild to moderate anemia the increased fetal cardiac output and hyperdynamic circulation prevent tissue hypoxia, release of erythropoietin, and therefore increased hematopoiesis.[5, 6] Alternatively, erythropoietin may be produced with small hemoglobin deficits, but recruitment of extramedullary erythropoiesis occurs only when a particular threshold is reached.[1] The aim of this study was to investigate the relationship between

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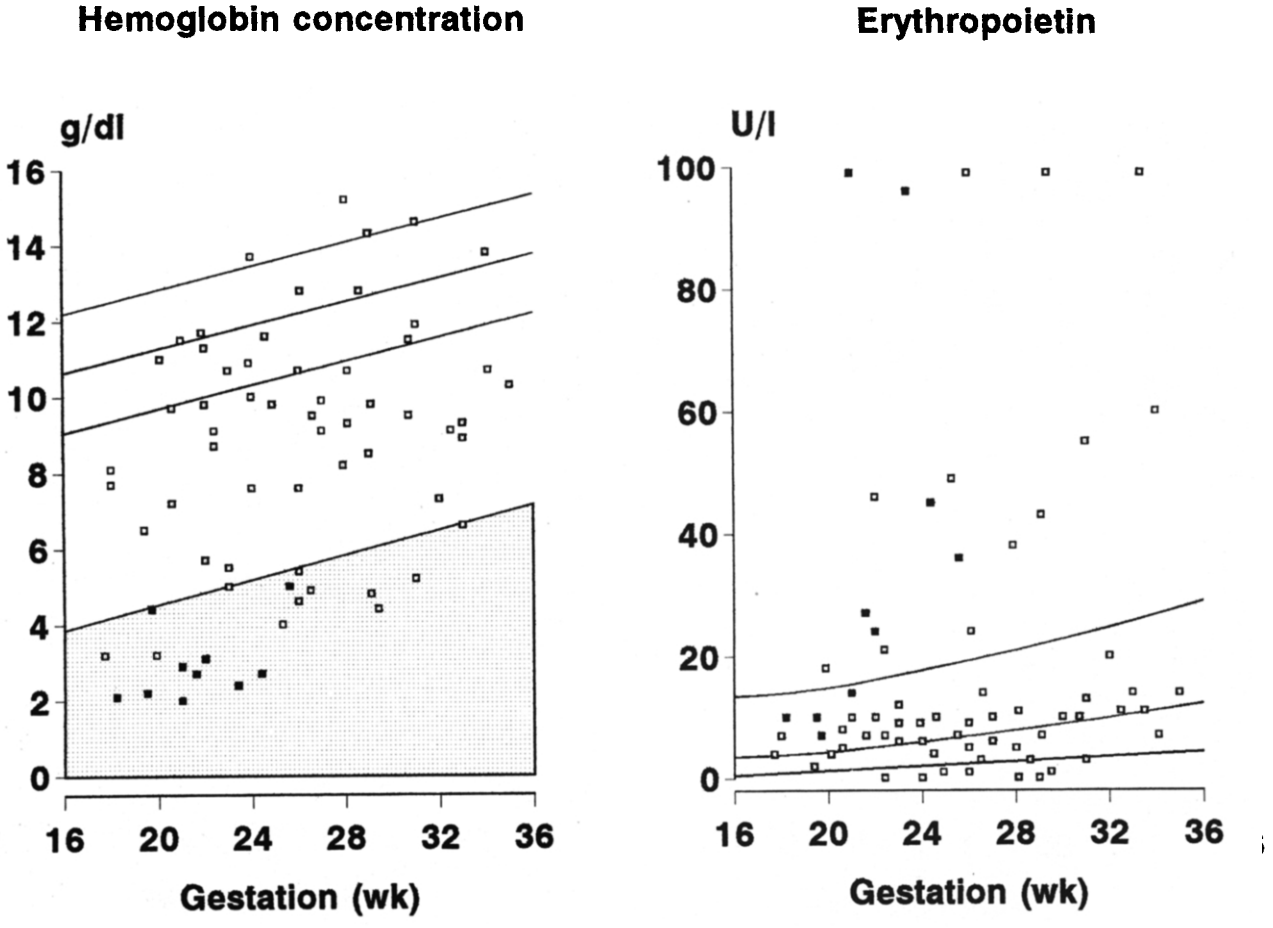

Fig. 1. Umbilical venous blood hemoglobin concentration *(left)* and plasma erythropoietin concentration *(right)* in 68 red blood cell–isoimmunized pregnancies (including 10 with hydrops fetalis, ■) plotted on appropriate reference range (mean, 5th and 95th percentiles) for gestation. Mean hemoglobin concentration was significantly decreased (mean difference −4.229 SDs, SEM 0.427, $p < 0.0001$), and mean erythropoietin concentration was increased (mean difference 0.995 SDs, SEM 0.237, $p < 0.001$).

fetal anemia, erythropoietin concentration, and erythroblastosis in red blood cell–isoimmunized pregnancies.

Patients and methods

Fetal plasma erythropoietin and hemoglobin concentrations and erythroblast count were determined in 68 red blood cell–isoimmunized patients who were referred to our unit for cordocentesis and, if necessary, intravascular blood transfusion. The selection criteria and technique for fetal transfusions have been previously described.[1-7] The antibodies involved were anti-D in 61 cases, anti-c in five, anti-E in one, and anti-Kell in one. Only data from fetuses that had not received transfusions and with positive direct Coombs' test results were considered for this study. At the time of cordocentesis the presence or absence of fetal hydrops (skin edema, ascites, pericardial and pleural effusions) was determined ultrasonographically.

Gestation at cordocentesis was 17 to 35 weeks, as determined from the menstrual history or an ultrasonographic scan performed in early pregnancy. Umbilical cord blood samples were obtained by cordocentesis, which was performed without maternal sedation or fetal paralysis. Fetal blood gases were measured in samples (250 μl) collected into heparinized syringes (Radiometer ABL 330, Copenhagen). Fetal blood (180 μl) was also collected into 20 μl of isotonic edetic acid solution (0.5 mmol/L in 0.15 mmol/L sodium chloride) to exclude maternal contamination by the acid-elution (Kleihauer) method and to determine the hemoglobin concentration and red blood cell and total nucleated cell counts (Coulter Stacker Automated Cytometer, Coulter Electronics, Luton, England). Blood films were stained by the May-Gruwald-Giemsa method, and the number of erythroblasts was counted.

A further 0.5 ml of fetal blood was collected in heparinized syringes, and the plasma was separated and stored at −20° C until thawed for assay. Erythropoietin concentration was measured with an enzyme immunoassay kit (Clinigen TM, Amgen Diagnostics, Thousand Oaks, Calif.). The reference curve was standard-

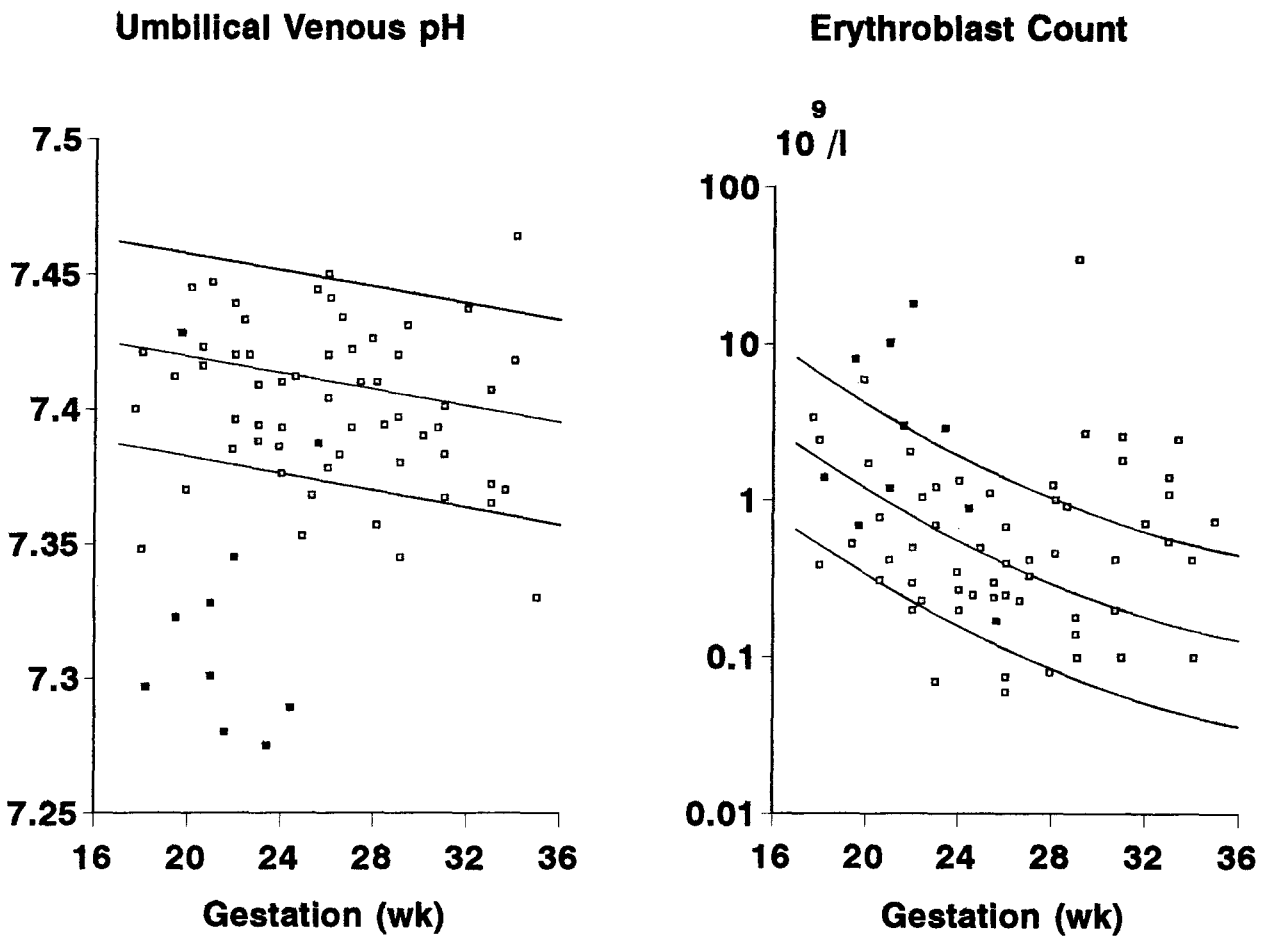

Fig. 2. Umbilical venous blood pH *(left)* and erythroblast count *(right)* in 68 red blood cell–isoimmunized pregnancies (including 10 with hydrops fetalis, ■) plotted on appropriate reference range (mean, 5th and 95th percentiles) for gestation. Mean pH was significantly decreased (mean difference −0.715 SDs, SEM 0.205, $p < 0.001$), and mean erythroblast count was significantly increased (mean difference 0.436 SDs, SEM 0.217, $p < 0.05$).

ized against World Health Organization, second international reference preparation of human urinary erythropoietin. The limit of sensitivity of the assay was determined to be 2 mU/ml (limit at 3 SDs from the zero erythropoietin standard).

Statistical analysis. Because all parameters measured change with gestation, individual values were expressed as the number of standard deviations by which the measurements differed from the appropriate normal mean for gestation (Δ values).[1,3,8,9] For hemoglobin 1 SD is equivalent to 1 gm/dl.[3]

The Student *t* test was used to examine whether mean Δ values for measurements in the red blood cell–isoimmunized group differed significantly from 0 (the appropriate normal mean for gestation) and to examine the significance of any differences in these values in the severely anemic fetuses. Regression analysis was then applied to examine whether Δ pH, Δ hemoglobin concentration, and Δ erythroblast count were significantly related to Δ erythropoietin.

Results

In the 68 red blood cell–isoimmunized pregnancies the mean fetal hemoglobin concentration was significantly lower and the plasma erythropoietin concentration significantly higher than the appropriate normal mean for gestation (Fig. 1). Furthermore, the mean umbilical venous blood pH was significantly reduced and the erythroblast count was significantly increased (Fig. 2).

The Δ hemoglobin concentration was significantly associated with Δ erythropoietin concentration (Fig. 3; $r = -0.601$, $p < 0.0001$), Δ pH ($r = 0.708$, $p < 0.0001$), and Δ erythroblast count ($r = 0.436$, $p < 0.05$). The associations with hemoglobin concentration were best described by quadratic equations, such that the

mean erythropoietin concentration and erythroblast count exceeded the 97.5th percentile and the pH was <2.5th percentile of the reference range when the hemoglobin deficit was >7 SDs. Multiple regression analysis showed that gestational age did not contribute significantly to the association between Δ erythropoietin and Δ hemoglobin concentration (F to remove gestation = 0.48, t = 0.69, p = 0.490).

Hydrops was present in 10 of the 21 fetuses that had a hemoglobin deficit >7 SDs. There was no significant difference in the Δ erythropoietin concentration and Δ erythroblast count between the hydropic and nonhydropic fetuses (Δ erythropoietin, t = 0.67; Δ erythroblast count, t = −0.14), but the Δ pH was significantly lower in the hydropic group (Δ pH, mean difference −2.762; SEM 0.660; t = −4.19; $p < 0.001$).

Δ Erythropoietin concentration was significantly associated with both Δ erythroblast count (Fig. 4; r = 0.422, $p < 0.001$) and Δ pH (Fig. 4; r = −0.314, $p < 0.001$). Multiple regression with both Δ erythroblast count and Δ pH did not provide a significantly better prediction of delta erythropoietin than that obtained from Δ erythroblast count alone (pH, F to remove 5.55; t = −2.36; p = 0.215).

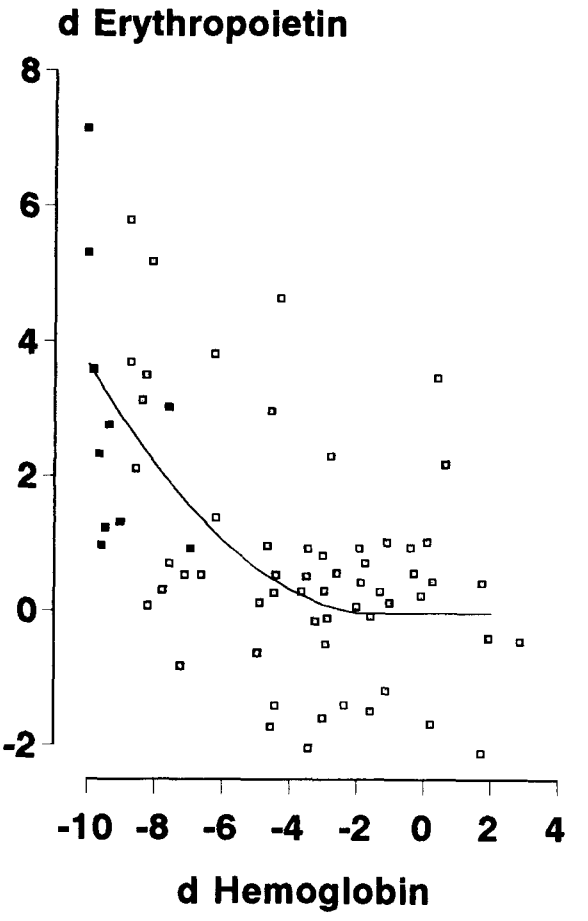

Fig. 3. Relationship between Δ *(d)* hemoglobin concentration and Δ erythropoietin concentration (constant 0.036, linear constant 0.098, quadratic constant 0.046, residual SD 1.586, r = −0.601, $p < 0.0001$) in 68 red blood cell–isoimmunized pregnancies, including 10 with hydrops fetalis (■). Units are in SDs from appropriate normal mean for gestation.

Comment

The data of this study demonstrate that the human fetus is capable of responding to anemic hypoxia by increasing erythropoietin production from at least 20 weeks' gestation, but this response does not occur until the hemoglobin deficit is >7 gm/dl. In a previous study Millard et al.[10] measured fetal plasma erythropoietin in 11 red blood cell–isoimmunized pregnancies at 22 to 31 weeks' gestation and reported increased erythropoietin concentration, compared with adult values, in only one of the fetuses. It was suggested that in fetal life the erythropoietin response to anemic hypoxia is suppressed because of reduced fetal oxygen requirement, decreased hepatic responsiveness to hypoxia, the presence of other unspecified erythrogenic factors, or an erythropoietin inhibitory substance.

The finding that the mean erythropoietin concentration only exceeds the normal range when the anemia is severe suggests that in mild to moderate anemia oxygenation of the liver, which is the main site of erythropoietin production in the fetus, is maintained.[11] This may be a consequence of the increased cardiac output and peripheral perfusion that has previously been demonstrated in Doppler studies of anemic fetuses.[5,6] These findings are compatible with data from studies by Finne,[12,13] who measured erythropoietin in amniotic fluid from pregnancies complicated by red blood cell isoimmunization and showed that the erythropoietin concentration was increased when the hemoglobin concentration, which was measured in cord blood at delivery, was <11 gm/dl, which corresponds to a deficit of 6 to 7 SDs.

Severe anemia was accompanied by increased erythropoietin concentration and erythroblastosis. Additionally, some severely anemic fetuses were hydropic and acidemic. These findings indicate that when the hemoglobin deficit is >7 gm/dl compensatory cardiovascular adjustments are not sufficient to prevent tissue hypoxia.[14] Similar to findings in anemic fetuses, some growth-retarded fetuses have an increased fetal plasma erythropoietin concentration with reduced venous blood pH and increased erythroblast count.[15] In these cases tissue hypoxia presumably results from a combination of hypoxemic hypoxia, caused by reduced uteroplacental perfusion and oxygen transfer, and isch-

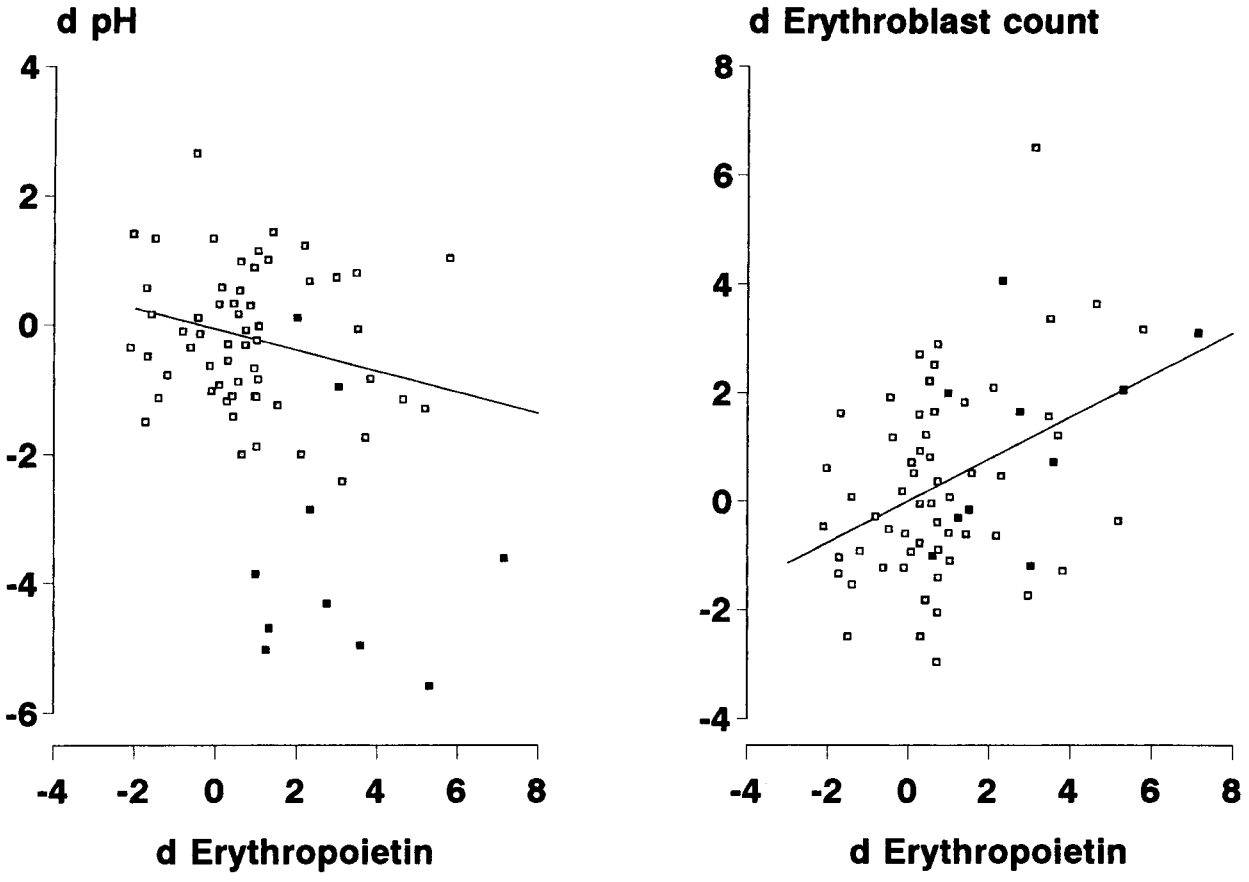

Fig. 4. Relationship between Δ *(d)* erythropoietin concentration and Δ pH (*left*, $r = -0.314$, $p < 0.001$) and Δ erythroblast count (*right*, $r = 0.422$, $p < 0.001$) in 68 red blood cell–isoimmunized pregnancies, including 10 with hydrops fetalis (■). Units are in standard deviations from appropriate normal mean for gestation.

emic hypoxia, caused by redistribution of fetal blood to the brain at the expense of the viscera.[8, 16]

Blood pH is widely accepted as an index of fetal oxygenation. Although in both the anemic and hypoxic-ischemic hypoxia models of human fetal hypoxia an increase in erythropoietin was associated with acidemia and erythroblastosis, better prediction of erythropoietin concentration was obtained from the degree of erythroblastosis. Therefore impaired fetal oxygenation may be more accurately predicted by the presence of erythroblastosis than by a low blood pH.[15, 17]

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Doppler velocimetry and fetal heart rate studies in nephropathic diabetics

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OBJECTIVES: Our objectives were to determine in pregnancies complicated by diabetic nephropathy (1) if impedance to flow in the uterine and umbilical arteries is normal and (2) if these fetuses are hypoxemic and acidemic and if they have decreased fetal heart rate variation and Doppler blood flow redistribution.
STUDY DESIGN: In a cross-sectional study at the Harris Birthright Research Centre for Fetal Medicine, London, serial assessment of fetal heart rate variation and Doppler velocimetry of the placental and fetal circulations was undertaken in six pregnancies complicated by diabetic nephropathy. In all cases cordocentesis was performed within 24 hours before delivery for the measurement of umbilical venous blood gases.
RESULTS: Cordocentesis demonstrated these fetuses to be hypoxemic and acidemic. The fetal heart rate variation was decreased; however, impedance to flow in the uterine artery was normal, and increased impedance to flow in the umbilical artery with evidence of blood flow redistribution was observed in only one case.
CONCLUSIONS: Fetal hypoxemia and acidemia in pregnancies complicated by diabetic nephropathy is not a consequence of impaired placental perfusion, and the degree of metabolic derangement may be obscured by the apparent normal growth and failure of these fetuses to demonstrate blood flow redistribution. (AM J OBSTET GYNECOL 1992;167:1297-303.)

Key words: Diabetic nephropathy, cordocentesis, fetal Doppler studies, blood gases, fetal heart rate patterns

In pregnancies complicated by preeclampsia or intrauterine growth retardation (IUGR) there is histologic evidence of abnormal trophoblastic invasion of the maternal spiral arteries.[1-3] Doppler studies in such cases have documented increased impedance to flow in the uterine arteries, which may precede the development of growth retardation or pregnancy-induced hypertension.[4-6] Furthermore, hypoxemic IUGR is associated with pathologic fetal heart rate (FHR) patterns and Doppler evidence of redistribution of the fetal circulation in favor of the brain and at the expense of the viscera.[7-10]

In women with diabetic nephropathy the incidence of both proteinuric hypertension and IUGR is increased.[11, 12] Previous Doppler studies in pregnancies complicated by maternal diabetes are limited to investigation of the umbilical artery; these studies have provided conflicting results.[13-15] The aim of the current study was to determine (1) if impedance to flow in the uterine artery and or umbilical artery of pregnant diabetics with nephropathy is increased and (2) if these fetuses are hypoxemic and acidemic and have appropriate changes in FHR variation and Doppler evidence of redistribution in their circulation.

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