Effects of chronic reduction in uterine blood flow on fetal and placental growth in the sheep

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Lang, Uwe, R. Scott Baker, Jane Khoury, and Kenneth E. Clark. Effects of chronic reduction in uterine blood flow on fetal and placental growth in the sheep. Am J Physiol Regulatory Integrative Comp Physiol 279: R53–R59, 2000.—Pregnancy is associated with a significant increase in uteroplacental blood flow (UBF), which is responsible for delivering adequate nutrients and oxygen for fetal and placental growth. The present study was designed to determine the effects of vascular insufficiency on fetal and placental growth. Thirty-nine late-term pregnant ewes were instrumented to investigate the effects of chronic UBF reduction. Animals were split into three groups based on uterine blood flow, and all animals were killed on gestational day 138. UBF, which began at 851 ± 74 ml/min (n = 39), increased in controls (C) to 1,409 ± 98 ml/min (day 138 of gestation) and in the moderately restricted (RM) group to 986 ± 69 ml/min. In the severely restricted (RS) group, UBF was only 779 ± 79 ml/min on gestational day 138. This reduction in UBF significantly affected fetal body weight with RM fetuses weighing 3,685 ± 178 g and RS fetuses weighing 2,920 ± 164 g compared with C fetal weights of 4,318 ± 208 g. Fetal brain weight was not affected, whereas ponderal index was significantly reduced in RM (2.94 ± 0.09) and RS fetuses (2.49 ± 0.08) compared with the value of the C fetuses (3.31 ± 0.08). Placental weight was also significantly reduced in the RM group, being 302 ± 24 g, whereas the RS group placenta weighed 274 ± 61 g compared with the C values of 414 ± 57 g. Fetal heart, liver, lung, and thymus were all significantly smaller in the RS group. Thus the present study shows a clear relationship between the level of UBF and both fetal and placental size. Furthermore, the observation that fetal brain weight was not affected, whereas fetal body weight was significantly reduced suggests that this experimental preparation may provide a useful model in which to study asymmetric fetal growth restriction.

placenta; fetus; fetal growth restriction; intrauterine growth restriction

HUMAN PREGNANCY CAN COINCIDE with an array of physiological and pathological conditions that can impede fetal growth and induce intrauterine growth restriction (IUGR) or, more recently called, fetal growth restriction (FGR). Examples are genetic anomalies, multiple pregnancies, fetal and maternal cardiovascular diseases, maternal nutritional deprivation, and “placental vascular insufficiency.” Whereas smallness in itself is not pathological, growth-restricted fetuses carry an increased risk of prenatal, perinatal, and neonatal morbidity and mortality (4).

Pregnancy requires a major cardiovascular and circulatory adaptation of the maternal organism. Maternal cardiac output increases by 30–40% (22) during pregnancy, due to an increase in heart rate and stroke volume (5). A steadily growing portion of the increasing cardiac output is necessary to provide adequate perfusion of the uterus, which provides oxygen and nutrients to the growing conceptus. Thus uterine perfusion in humans increases from ~50 ml/min in week 10 of gestation to as much as 1,300 ml/min at the end of gestation (2, 16). If this physiological increase in uterine perfusion is impeded or abolished by pathological factors (16), a deficiency in the substrate and oxygen delivery to the uterus, placenta, and fetus should ensue. Inadequate uteroplacental perfusion during pregnancy therefore is considered to be one of the most likely and important causes of FGR.

Diverse animal models have been developed to experimentally investigate the problem of FGR/IUGR. In the pregnant sheep, the technique of embolizing the uteroplacental vasculature with microspheres has been employed extensively (8, 10). A main point of criticism with this method is the simultaneous necrosis of placental tissue along with the embolization-induced uterine flow restriction. Thus effects of the reduced placental exchange capacity for nutrients and substrates cannot be distinguished from the original effects of uterine blood flow restriction. Therefore, it was important to establish an animal model with uniform and generalized reduction of the uteroplacental perfusion without primary placental necrosis (3).

Consequently, the aim of the following experimental study was to develop an animal model that allows for a chronic, uniform, and generalized restriction of utero-
placental blood flow without the disadvantage of initial placental necrosis. This animal model would allow us to determine if chronic reductions in uteroplacental blood flow significantly reduce fetal and placental growth. To conduct these studies, sheep in the last third of their gestational period were studied by instrumentation with an externally adjustable vascular occluder that allowed for chronic regulation of uteroplacental perfusion from gestational days (GD) 113-138 (0.78–0.95 of gestation). On day 138 of gestation, the effects of chronic reduction in uterine blood flow on fetal growth were determined.

MATERIAL AND METHODS

Preparation

Sheep with singleton pregnancies (Thomas Morris, Reisterstown, MD) and a body weight between 45 and 60 kg were instrumented between GD105 and GD110 (term = 145 days). Preoperatively, food and water were withdrawn for 48 and 24 h, respectively. Ewes were sedated with a 15-mg/kg bolus of pentobarbital sodium (Butler, Columbus, OH) and received a spinal anesthesia by intrathecal injection of 15 mg of Tetracaine HCL (Winthrop, New York, NY). The animals were surgically cleansed and draped and fixed on the table in a recumbent position. Maternal femoral artery and vein were dissected free through a 3- to 4-cm incision in the left groin of a recumbent position. Maternal femoral artery and vein were occluded with an arterial 15-mg/kg bolus of pentobarbital sodium (Socumb, Butler, Columbus, OH) and received a spinal anesthesia by intrathecal injection of 15 mg of Tetracaine HCL (Winthrop, New York, NY). The animals were surgically cleansed and draped and fixed on the table in a recumbent position. Maternal femoral artery and vein were dissected free through a 3- to 4-cm incision in the left groin and ligated distally. After vascular incision, 0.05 × 0.09-in. polyvinyl catheters (Tygon Microbore, Norton Plastics, Akron, OH) were inserted in the vessels so that the catheter tips reached into the vena cava and the abdominal aorta. After the procedure, each ewe received 1 liter of glucose (5%) and saline (0.9%).

After an abdominal midline incision, a previously described (9) externally adjustable vascular occluder (HMC, Dimensional Designs, Cincinnati, OH) was installed around the common internal iliac artery of the ewe. This 2- to 3-cm-long vessel is an ideal location for control of uterine perfusion, because both uterine arteries originate from it. To avoid collateral circulation, the ovarian arteries on both the right and left sides and the left, right, and middle sacral arteries were ligated. Uterine blood flow was measured by electromagnetic flow probes (Dienco, Los Angeles, CA) that were placed around both the right and left main uterine arteries (4.0–6.0 mm) before their bifurcation in the broad ligament. After palpation, a small uterine incision was made to expose the left fetal hindlimb. The hindlimb was exteriorized, and uterine and amniotic membranes were temporarily “marsupialized” to avoid excessive drainage of amniotic fluid. After local anesthesia with 2% lidocaine, indwelling vessel catheters (Tygon Microbore 0.03 × 0.05 in.) were implanted and advanced through the hindlimb artery and vein of the fetus into the descending aorta and inferior vena cava. An amniotic fluid catheter was placed into the uterus to monitor intrauterine pressure. Before closing, 1 g of Ampicillin (Apottheon-Bristol-Myers-Squibb, Princeton, NJ) was placed into the amniotic fluid and the membranes and uterus were sealed.

Maternal and fetal catheters as well as the occluder cable and the flow probe connections were then brought to the left abdominal wall and exteriorized through a subcutaneous tunnel to the sheep’s left flank. Catheters were wrapped in alcohol-soaked gauze and stored in plastic bags within a linen pouch on the sheep’s side. All catheters were flushed daily with heparin to maintain patency (1,000 units/ml maternal, and 500 units/ml fetal). Sheep received antibiotic prophylaxis, 1 g penicillin G (Hanford’s United States Veterinary Products, Syracuse, NY) 1 day before surgery, on the day of surgery, and 3 days postoperatively and also 80 mg gentamicin (Elkins-Sinn, Cherry Hill, NJ) the day of surgery. After surgery, the animals had free access to water and food (Rumilab RL5908, Ralston Purina, St. Louis, MO) at all times. To allow for recovery from surgery, measurements, and experimental studies were not begun before the fifth postoperative day. All procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee, and experiments performed in an American Association for Accreditation of Laboratory Animal Care- and United States Department of Agriculture-approved facility.

Experimental Protocol

Animals underwent surgical preparation between 105 and 110 days of gestation, and long-term studies were begun 5 days postoperatively. Sheep were housed in metabolism cages with a food bowl and automatic watering system. Sheep were randomized into one of three groups that were all identically instrumented. The first adjustments of uterine blood flow in the experimental groups took place between GD111 and GD115 (mean GD113) after a 60-min baseline. After these first adjustments, both the control group (C) and the experimental groups [moderately restricted (RM) and severely restricted (RS)] were checked each day at the same time, and uteroplacental blood flow was readjusted with the occluder in the RM and RS groups. The C was allowed to undergo normal gestationally related increases in uterine blood flow up to GD138. The first experimental group of animals underwent a strict reduction (RS) in uterine blood flow with flows of ~750 ml/min (50% of term values) maintained during the experimental period. A second experimental group of animals was exposed to a moderate reduction in uterine blood flow (RM), in which flows reached a maximum of ~950 ml/min. On GD138, the experiment was terminated and morphometric data obtained. To assess relationships between perfusion and morphometric variables, the daily perfusion values of each animal were determined over the observation period and then divided by the number of days in the experimental period. This calculation yielded the “average uterine blood flow” throughout the observation period, a measure of perfusion during the experiment. All growth variables were plotted against the average flow.

Measurements

Morphometrics. To ensure the uniformity and comparability of data, the experiment was terminated on GD138, 1 wk before expected delivery. Ewes received a lethal dose of pentobarbital sodium (Socumb, Butler, Columbus, OH), and the morphometric evaluation of uterus, fetus, and placenta followed immediately.

Total uterine weight. The uterus was tied off at the cervix and taken out as a whole to determine total uterine weight in grams. After removal of placenta, fetus, and amniotic fluid, the uterus (endometrium and myometrium) were blotted dry with a towel and weighed to obtain the uterine tissue weight.

Placenta. The maternal part of the caruncle cotyledon complex (placentoma) was not separated from the fetal cotyledons to have an assessment of weight of the maternal-fetal “perfusion unit” as a whole. Each placenta was weighed...
separately, and the total number and combined weight of all placentomes was also determined.

Weight and crown-rump length. The umbilical cord was ligated within a centimeter of the fetus, and fetal body weight was obtained after washing the fetus off and drying it with a towel. Fetal crown-rump length (CRL) was measured on a flat surface with the spine flat against the wall.

Ponderal index. The fetal ponderal index (PI) was calculated using the formula of Rohrer (20)

\[ PI = \frac{\text{weight (g)}}{\text{(length (cm))^3}} \times 100 \]

Fetal organ weights. The brain, including the cerebellum, was taken out of the skull without the membranes. It was severed from the spinal cord at the level of the Atlas vertebrae, blotted dry, and then weighed. Fetal heart, liver, lung, and thymus weights were also determined as indicators of fetal growth and potential intrauterine growth restriction (8, 10) parameters.

Blood gas analysis. Fetal blood gases were taken before the experimental period to ensure the viability of the fetal preparation before assigning them to one of the three groups. To ensure the continuous viability of the fetal preparations, blood gases were checked biweekly. Samples were taken in heparinized syringes, placed on ice, and immediately analyzed using a blood gas analyzer adjusted to 39°C.

Uterine blood flow. Uterine blood flow was determined with Dienceo electromagnetic flow probes (Dienceo, Los Angeles, CA) on the right and left middle uterine arteries and measured with RF-2100 Dienceo Flowmeters (Dienceo) with an accuracy of ± 10%. After the animals were killed, zero blood flow was confirmed. All measurements were recorded using an eight-channel SensorMedic-R612 recorder (Beckman Instruments, Yorba Linda, CA).

Statistical analysis. One-way ANOVA and regression analysis were used where appropriate. Group differences were examined by post hoc Student-Newman-Keuls test after the ANOVA was performed. P values < 0.05 were considered statically significant. Linear and nonlinear regressions were determined by best fit. Data are given as means ± SE.

RESULTS

Thirty-nine animals completed the study, and their morphometrical data were obtained on GD138. Thirty-three of these yielded complete morphometrical data sets. In six animals, not all morphometric variables could be determined. Total uterine weight, for example, could not be obtained when amniotic fluid leakage had occurred. Sixteen ewes were in the C, 10 ewes were in the R M group, and 13 animals were in the R S group.

Uterine Blood Flow

Before uterine flow restriction on GD113, C animals had an average uterine blood flow of 872 ± 84 ml/min. Ewes randomized to the R M group had a uterine blood flow of 812 ± 79 ml/min, and those that were randomized for R S had a uterine blood flow of 854 ± 58 ml/min. There was no significant difference in uterine perfusion between groups.

On GD138, uterine blood flow in C animals had increased to 1,409 ± 98 ml/min. The R M group had a uterine blood flow of 986 ± 69 ml/min on GD138 (P = 0.002 vs. C), and the R S ewes had a blood flow of 779 ± 79 ml/min (P = 0.00001 from C and P = 0.05 from R M; Fig. 1), reductions of 30% and 45%, respectively. When the “average daily uterine blood flow” was calculated for each group, the C group had an average blood flow of 1,261 ± 75 ml/min, the R M group 978 ± 108 ml/min, and the R S group 744 ± 89 ml/min. Uterine blood flow in the experimental groups R M and R S had to be adjusted daily for the first 7–10 days because uterine blood flow tended to rise overnight. After 7–10 days, this apparent autoregulatory mechanism ceased to occur, and adjustments were rarely required.

Morphometric Data

Total uterine weight varied between the three groups. C animals had a total uterine weight of 7,382 ± 456 g, whereas R M and R S had significantly lower uterine weights, 6,483 ± 409 g and 5,989 ± 347 g, respectively. Fetal body weight was 4,318 ± 208 g for C fetuses. Fetuses of R M ewes were lighter (3,685 ± 178 g) than C fetuses, and those of the R S group were significantly smaller than R M and C fetuses (2,920 ± 164 g, a 32% reduction, P < 0.01; Table 1). Average fetal CRL did not differ significantly among groups (Table 1).

Because the fetal length did not change, whereas fetal body weight significantly decreased, PI of the flow-restricted fetuses was significantly different. The PI of fetuses exposed to uterine blood flow of ~750 ml/min (R S) was 2.49 ± 0.08. This was lower than the PI of the R M group at 2.94 ± 0.09 and significantly lower than the PI in fetuses of C animals (3.31 ± 0.08, P < 0.01).

Placental weight (Table 2) was also significantly reduced from a C values, being reduced by 27% in the R M group and 34% in the R S group. The placenta weights in R S and R M groups were significantly lower than in C (P < 0.05). The number of cotyledons did not differ among groups, nor was there any gross evidence of necrosis observed in the tissue. Finally, fetal organ weights were also compared among the three different groups (Table 2). Heart, liver, thymus, and lung weights in RS and RM groups were significantly lower than in C (P < 0.05).
weights (although to different degrees) were reduced in the RM and RS groups compared with C. Brain weight, however, was not significantly affected in the flow-restricted groups.

### Perfusion-Morphometry

Data on total uterine weight were available for 33 animals and showed a positive, significant correlation with average uterine perfusion in the observation period ($r^2 = 0.53; P < 0.01$). Endometrium and myometrium, however, showed no relationship to uterine blood flow (data not shown).

Fetal body weight (Fig. 2A) and placental weight (Fig. 2B) were positively correlated to average uterine blood flow (fetus $r^2 = 0.58, P < 0.01$; placenta $r^2 = 0.56, P < 0.01$), as was PI ($r^2 = 0.48, P < 0.01$; Fig. 2C). Fetal heart, liver, lung, and thymus weights all show the impact of restricted uterine blood flow on the growth of these organs (Fig. 3, A and B, Table 2).

Fetal body weight and fetal liver weight are similarly affected by uterine flow restriction. Therefore, the quotient of liver weight to body weight did not change with

### Table 1. Fetal morphometric parameters

<table>
<thead>
<tr>
<th></th>
<th>Control ($n = 16$)</th>
<th>RM ($n = 10$)</th>
<th>RS ($n = 13$)</th>
<th>Significance ($P \leq 0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight, g</td>
<td>4,318 ± 208</td>
<td>3,685 ± 178</td>
<td>2,920 ± 164</td>
<td>RM vs. C</td>
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<td></td>
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<td>RS vs. C</td>
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<td></td>
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<td></td>
<td>RS vs. RM</td>
</tr>
<tr>
<td>Fetal length, cm</td>
<td>51 ± 2</td>
<td>50 ± 1</td>
<td>49 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Ponderal index</td>
<td>3.31 ± 0.08</td>
<td>2.94 ± 0.09</td>
<td>2.49 ± 0.08</td>
<td>RM vs. C</td>
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<td>RS vs. C</td>
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<td>RS vs. RM</td>
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</table>

Values are means ± SE. Fetal weight, fetal crown-rump length, and ponderal index in control (C) and flow-restricted (moderately restricted (RM); severely restricted (RS)) animals. NS, nonsignificant.

### Table 2. Fetal organ morphometrics

<table>
<thead>
<tr>
<th></th>
<th>Control ($n = 16$)</th>
<th>RM ($n = 10$)</th>
<th>RS ($n = 13$)</th>
<th>Significance ($P \leq 0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental weight, g</td>
<td>414 ± 57</td>
<td>302 ± 24</td>
<td>274 ± 61</td>
<td>RM vs. C</td>
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<td></td>
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<td></td>
<td>RS vs. C</td>
</tr>
<tr>
<td>Brain weight, g</td>
<td>53 ± 1.9</td>
<td>52 ± 1.2</td>
<td>50 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>32 ± 1.3</td>
<td>27 ± 1.0</td>
<td>22 ± 1.2</td>
<td>RM vs. C</td>
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<td>RS vs. C</td>
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<tr>
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<td></td>
<td>RS vs. RM</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>145 ± 7.5</td>
<td>105 ± 6.2</td>
<td>93 ± 6.5</td>
<td>RM vs. C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RS vs. C</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>131 ± 8.4</td>
<td>116 ± 9.5</td>
<td>95 ± 9.6</td>
<td>RS vs. C</td>
</tr>
<tr>
<td>Thymus weight, g</td>
<td>19 ± 2.4</td>
<td>16 ± 2.1</td>
<td>10 ± 1.2</td>
<td>RS vs. C</td>
</tr>
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<td></td>
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<td>RS vs. RM</td>
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</table>

Values are means ± SE. Fetal organ weight comparison among C, RM, and RS animals.

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Fig. 2. Fetal body weight (A) on gestational day (GD) 138 vs. average uterine blood flow (ml/min) over the gestational period from days 115 to 138. Placental weight (B) on gestational day 138 vs. average uterine blood flow over the gestational period from day 115 to 138. Ponderal index (PI, C) on GD138 vs. average uterine blood flow over the gestational period from days 115 to 138. R, $r^2$; N, no. of ewes.
increasing or decreasing uterine blood flow, but stayed within a narrow range without correlation to perfusion (data not shown). This does not hold true for the brain-to-body weight quotient. With negligible differences in brain weight but flow-dependent changes in body weight, the brain-to-body weight quotient had a significant negative correlation to uterine blood flow ($r^2 = -0.49$, $P < 0.05$, Fig. 4A). Fetal body weight was closely related to placental weight as shown in Fig. 4B, $r^2 = 0.77$, $P < 0.001$.

**DISCUSSION**

In the human (12) as well as in the ewe (18), cardiac output increases throughout pregnancy in response to decreasing systemic and uteroplacental vascular resistance. In the ewe, Rosenfeld (21) demonstrated that in addition to an $\sim 70\%$ increase in cardiac output, a redistribution of the percentage of cardiac output going to various organs occurs. Maternal brain perfusion, which represents 1% of cardiac output in the nonpregnant sheep, decreases to 0.5% at the end of gestation (actual flow doesn’t change). On the other hand, uterine blood flow, which accounts for only 0.5% in the nonpregnant animal, accounts for $\sim 20\%$ of cardiac output in the pregnant animal at term. In absolute numbers, this is an increase from $\sim 25$ (nonpregnant sheep) to $\sim 1,500$ ml/min at the end of gestation. The enormous increase in uterine perfusion occurs in response to vessel growth and vasodilation and is required to provide adequate nutrients and oxygen to the growing conceptus.

Clapp and co-workers (7) estimated the increase in ovine uterine perfusion per day at $\sim 20$ ml/min in the last 30 days of gestation. This is in agreement with the increase observed in our C animals, in which blood flow rose from 872 to 1,409 ml/min in 25 days, averaging 22 ml·min$^{-1}$·day$^{-1}$. In the studies by Creasy et al. (10), in which ovine FGR was produced by microsphere embolization, fetal body weight was reduced by 30%, fetal CRL was decreased by 18%, and changes in PIs were not reported. In contrast, Clapp and co-workers (7) reported a 40% reduction in fetal body weight with no change in fetal CRL, resulting in a significant
change in the PI (4.09 ± 0.24 vs. 3.12 ± 0.05). The present model of a uniform reduction of uterine blood flow exhibited a 32% decrease of fetal body weight in the RS group. Fetal CRL was unchanged so that a significant decrease in the PI occurred, (3.31 ± 0.08 to 2.49 ± 0.08). The reduction in uterine blood flow that causes these differences between RS and C fetuses is ~45% when calculated using GD138 blood flows or ~40% when the average uterine blood flow during the observation period is taken as a calculatory basis.

The reduction of 40–45% is well within the margin of safety for fetal oxygenation in the sheep that Wilk- ening and Meschia (23) described. They found that in the control state, uterine oxygen supply to the uterus ranged between 2.8 and 1.6 umol · min⁻¹ · kg fetal body wt⁻¹. They concluded that a relatively large portion of the normal uterine perfusion in the sheep exceeds the minimal requirements for fetal oxygenation, providing a margin of safety of ~50% (17). This agrees with our observation of fetal blood gases obtained from C and FGR fetuses in both the RM and RS groups to determine viability of the preparation were almost identical and did not indicate any lack of oxygen in the flow-restricted groups. Whether this is also true for fetal oxygen consumption and extraction could not be determined in the current animal preparation.

The morphometric effects of this mechanical reduction of flow (without causing hypoxia) in the last one-third of gestation seem to be more pronounced than the ones reported for chronic hypoxia by Jacobs et al. (13). In that study, fetal sheep exposed to hypobaric oxygen from GD30 to GD135 experienced a 20% weight reduction, whereas fetuses exposed from GD120 to GD141 experienced a 15% reduction. Long-term exposure to hypoxia also caused a significant reduction in fetal weight. The fetal brain-to-body weight quotients were 1.3 (GD30–GD135) and 1.4 (GD120–GD141) for hypoxic animals and 1.2 in C animals. In our model, the average brain-to-body weight quotient in the fetuses changed significantly from 1.2 for C to 1.7 in RS ewes. With negligible differences in brain weight but flow-dependent changes in body weight, the brain-to-body quotient has a significant negative correlation to uterine blood flow ($r^2 = 0.49; P < 0.01$; Fig. 4A). The cause of this relative fetal "brain sparing" is most likely a redistribution of blood flow in the fetal circulation. This has been shown in acute experiments (14) and also in other chronic preparations (6, 11).

The morphometric comparison of the three different groups of ewes shows significant differences between the RS and the C groups. RM animals clearly form an intermediate group between C and RS ewes. This fact together with the variability of uterine blood flow measurements in the C group and, to an obviously much lesser extent, the flow-restricted groups gives hint of a continuous relationship between uterine blood flow and morphometric data (Figs. 2, 3, and 4).

An important factor is the different fate of the placenta in different models. The sheep placenta reaches its final weight at a gestational age of 90–95 days (1, 15). This has been confirmed in a group of animals that were not instrumented but were killed in our laboratory on either GD105 ($n = 7$) or GD138 ($n = 10$), in which the placental weights were 379 ± 24 and 403 ± 27 g, respectively (unpublished observation). Thus the placental mass appears to be unchanged despite the rapid increase in fetal mass. Kulhanek et al. (15) showed that instead of a further increase in weight, an increase in functional capacity (i.e., functional maturation) takes place, so that the placenta can meet the increasing oxygen and substrate requirements of the fetuses. Owens et al. (19), in the caruncle-ectomized sheep, and Clapp et al. (7), in the embolization model, demonstrated a reduction in placental weight. In the caruncle-ectomy model, the destruction of implantation sites before pregnancy limits the number of fully functional placentomes and thus interferes with placental development in approximately one-half of the animals. The other half of the animals compensate by increasing their placental mass of the remaining cotyledons. In contrast, the embolization model, which is initiated around GD110, occurs after placenta is complete and causes placental necrosis due to vascular embolization. Whereas the essence of the carunculcectomy model is abnormal placentation, the embolization model modifies both uterine perfusion and placental transfer. The present study, in contrast, permits us to look at the effects of reduced blood flow only, whereas, at least theoretically, retaining the previous placental surface area that allows for nutrient and oxygen transfer. So, in the present model, although placental mass was reduced, the number of cotyledons was almost identical in the three groups and no visible placental necrosis was observed. It seems likely that reduction in uteroplacental blood flow would first affect the placenta, because this organ is the first to be exposed to the reduced delivery of nutrients and oxygen. Furthermore, it clearly undergoes adaptation by decreasing its size. This decrease in placental size may ultimately lead to reduced transfer of oxygen and nutrients and reduced production of growth factors by the ovine placenta. Because the fetuses in the restricted groups do not seem to be hypoxic, intraterine growth restriction does not necessarily seem to have to be caused by hypoxia, which appears to be a less potent growth-restricting stimulus (13). Diminished nutrient supplies or diminished synthesis of growth factors in response to flow restriction may also be responsible.

In the occlusion model, there is no primary insult to the placenta, but rather an exposure to the general restriction of uterine perfusion. The loss of placental mass that can be observed seems to be some sort of adaptive regression of the organ. Average uterine perfusion in the RS animals is decreased by ~40% versus C animals. Placental mass in these growth-restricted pregnancies is diminished by 34% versus C. The RM animals had reduced body weight of 25% versus C; their placental mass was reduced by 27%. The magnitude of placental mass reduction seems to parallel the magnitude of uterine flow reduction, which lends support to the idea of a flow-dependent placental regression. Placental weight and fetal weight, both affected
by the restriction of uterine flow, were correlated with each other. This feature of the occlusion model reflects the well-known corresponding occurrence of small placentas and small fetus in human IUGR/FGR.

In summary, uniform restriction of uterine blood flow without primary placental insults in the last one-third of gestation in the pregnant ewe leads to changes in the growth pattern of the affected fetuses and placentas. Fetal growth and placental development appear to be dependent on the progressive rise in uterine perfusion during this period. Restriction of uterine blood flow reproducibly generates a general restriction in fetal development that is accompanied by sparing of the brain and the axial skeleton. Thus the present experimental animal preparation appears to provide a useful model of both late-gestational vascular insufficiency and asymmetric intrauterine FGR in the fetal lamb.

Perspectives

Pregnancy is associated with remarkable adaptations of the maternal cardiovascular system. To meet the metabolic needs of the fetus, uterine blood flow rises from 10–20 ml/min to as high as 1,500 ml/min as gestation progresses. Although the margin of safety is large, vascular diseases, such as preeclampsia, can significantly reduce uteroplacental blood flow, leading to altered fetal growth. The goal of the present study was, therefore, to develop a new model of chronic uteroplacental blood flow reduction, which would be associated with FGR, so that the mechanism of fetal adaptation to this altered environment can be investigated under controlled conditions. In this model, both the placenta and fetus are affected by the altered environment. It is not clear which is affected first, but the placenta slowly undergoes regression in size, whereas the fetus fails to grow normally. The present study cannot answer the question, but future studies designed to evaluate umbilical vascular resistance and determine placental and fetal delivery and uptake of glucose and oxygen as well as determine release of fetal growth factors from the placenta may help to solve this mystery. However, the present ovine model shows that significant reductions in uteroplacental blood flow results in significant reductions in fetal body and placental weight.

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