Developmental changes in hemodynamics of uterine artery, utero- and umbilicoplacental, and vitelline circulations in mouse throughout gestation

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Submitted 6 January 2006; accepted in final form 22 March 2006

Mu, Junwu, and S. Lee Adamson. Developmental changes in hemodynamics of uterine artery, utero- and umbilicoplacental, and vitelline circulations in mouse throughout gestation. Am J Physiol Heart Circ Physiol 291: H1421–H1428, 2006. First published May 26, 2006; doi:10.1152/ajpheart.00031.2006.—In human pregnancy, abnormal placental hemodynamics likely contribute to the etiology of early-onset preeclampsia and fetal intrauterine growth restriction. The mouse is increasingly being deployed to study normal and abnormal mammalian placental development, yet the placental hemodynamics in normal pregnancy in mice is currently unknown. We used ultrasound biomicroscopy to noninvasively image and record Doppler blood velocity waveforms from the maternal and embryonic placental circulations in mice throughout gestation. In the uterine artery, peak systolic velocity (PSV) increased significantly from 23 ± 2 (SE) to 59 ± 3 cm/s, and end-diastolic velocity (EDV) increased from 7 ± 1 to 28 ± 2 cm/s in nonpregnant versus full-term females so that the uterine arterial resistance index (RI) decreased from 0.70 ± 0.02 to 0.53 ± 0.02. Velocities in the maternal arterial canal in the placenta were low and nearly steady and increased from 0.9 ± 0.03 cm/s at embryonic day 10.5 (E10.5) to 2.4 ± 0.07 cm/s at E18.5. PSV in the umbilical artery increased steadily from 0.8 ± 0.1 cm/s at E8.5 to 15 ± 0.6 cm/s at E18.5, whereas PSV in the vitelline artery increased from 0.6 ± 0.1 cm/s at E8.5 to 4 ± 0.2 cm/s at E13.5 and then remained stable to term. In the umbilical circulation, the EDV detection rate was 0% at E14.5 and 94% at E18.5, and the RI decreased from 1.02 ± 0.01 during this interval. We conclude that ultrasound biomicroscopy can be used to monitor placental hemodynamics during pregnancy in mice. These results provide novel information concerning the development of the vitelline and placental circulations in mice and reveal strong similarities in placental hemodynamics between mice and humans.

yolk sac; embryonic development; pregnancy; ultrasound biomicroscope; Doppler ultrasound; blood velocity; fetus

FUNCTIONAL CIRCULATORY and nutrient exchange systems are an essential early requirement for survival and growth of the postimplantation mammalian embryo. Effective maternal-embryonic/fetal exchange requires large increases in maternal perfusion of the uterus, directing blood to the implantation site via the spiral arteries and trophoblast-lined intervillous space in the placenta. Initial survival and growth of the embryo is dependent on perfusion of the vitelline circulation to the yolk sac because abnormalities in yolk sac hemodynamics and/or function can cause congenital malformations or embryonic lethality during organogenesis (10, 24). In the second half of mouse gestation, from embryonic day 10.5 (E10.5), embryonic/fetal growth is dependent on umbilical blood flow via the chorioallantoic placenta. In human pregnancy, abnormalities in uteroplacental and umbilical arterial hemodynamics are associated with placental pathology and with maternal preeclampsia and fetal intrauterine growth restriction (IUGR). These serious disorders become clinically evident in the last half of gestation but arise because of impaired placental formation during the first half of gestation (11). The etiologies of impaired uteroplacental and fetoplacental development are poorly understood. Thus effective treatments or preventive strategies are lacking.

Genetically altered mouse models hold considerable promise for enabling rapid advances in our understanding of the molecular basis of placental pathology. Mice are appropriate models because there are strong similarities in the cellular and circulatory architecture and gene expression between the mouse and human placentas (48). Furthermore, several mouse models displaying clinical signs of preeclampsia and/or IUGR have been identified (14, 47) as having a growing number of mutant mouse lines with a range of placental phenotypes (48, 51). These models provide the unique opportunity to study the etiology of pregnancy disorders in longitudinal studies throughout pregnancy. However, to effectively model the progression of human pregnancy disorders in mice, it is critical to have methods to monitor disease progression that parallel those used clinically in humans. In this regard, there are strong clinical associations between abnormalities in umbilical and/or uterine artery hemodynamics, placental pathology, and early-onset preeclampsia and/or IUGR in humans (11, 50) and suspected associations between early pregnancy failure and abnormal vitelline perfusion of the yolk sac (33, 36). It is, therefore, important to establish methods to quantify placental and yolk sac hemodynamics during in utero development in mice.

Thus the objective of the current study was to use Doppler ultrasound biomicroscopy (19, 46) to quantify blood flow velocities in the uterine artery and in the uteroplacental, umbilicoplacental, and vitelline circulations throughout gestation in the mouse. Results were compared with available results from human pregnancy to determine whether hemodynamics in the arteries supplying these critical exchange organs are similar between mice and humans.

MATERIALS AND METHODS

Mice. Experiments were approved by the Animal Care Committee of Mount Sinai Hospital (Toronto, ON, Canada) and were conducted in accord with guidelines established by the Canadian Council on Animal Care. We studied nonpregnant and pregnant outbred mice
Day 0.5 of pregnancy (E0.5) was defined as the morning on the day a vaginal plug was found after overnight mating. Mouse "embryos" become "fetuses" after the end of organogenesis at E14.5; however, we will refer to them as embryos throughout gestation as is the convention in this species. Mice were lightly anesthetized with 1.5% isoflurane in oxygen by face mask during ultrasound exams. Maternal heart rate and rectal temperature were monitored (model THM100; Indus Instruments, Houston, TX), and heating was adjusted to maintain rectal temperature between 36° and 38°C. All hair was removed from the abdomen by shaving, followed by a chemical hair remover. Pre-warmed gel was used as an ultrasound coupling medium.

Ultrasound. More than three nonpregnant or pregnant mice were imaged transcutaneously when nonpregnant or at each gestational day between E6.5 and E18.5 with the use of the ultrasound biomicroscope (UBM) and a 30- or 40-MHz transducer operating at 30 frames/s (model Vevo 660, VisualSonics, Toronto, ON, Canada). Studies were performed between 1 PM and 5 PM. In Doppler mode, the high-pass filter was set at 6 Hz, and the pulsed repetition frequency was set between 4 and 48 kHz, to detect low to high blood flow velocities, respectively. A 0.2- to 0.5-mm pulsed Doppler gate was used, and the angle between the Doppler beam and the vessel was recorded and was ≤30°. Waveforms were saved for later offline analysis. The duration of anesthesia was limited to ~1 h. During this time, either the maternal or embryonic circulation was evaluated in three to five of the ~12 implantation sites per mouse. Pregnant mice between 10.5 and 18.5 days of gestation were dissected, and embryonic body weight and placental weight were measured (n = 24–51 embryos were weighed at each day of gestation). Maternal (n = 12–22 mice/day) and embryonic (n = 20–33 embryos/day) heart rates were measured from Doppler waveforms every third day from 9.5 to 18.5 days of gestation.

Doppler recordings. Vascular corrosion casts, prepared as previously described (3), showed that the uterine artery arises as a branch from the internal iliac artery (Fig. 1A). This branch site could be visualized by UBM (Fig. 1B). Doppler waveforms were obtained in the uterine artery near the lateral-inferior margin of the uterocervical junction close to the iliac artery on each side (n = 12–16 mice/day). Doppler waveforms were also obtained from the maternal arterial canal located near the center of the chorioallantoic placenta (e.g., Fig. 2) (n = 9–18 embryos/day). The canal is a conduit created by

Fig. 1. Uteroplacental circulation and location of uterine artery (UtA) in a vascular cast from a pregnant mouse. A: uterine artery arises from common iliac artery (CIA; arrowhead). B: uterine artery (arrow) is shown where it arises from the common iliac artery as visualized with ultrasound biomicroscope (UBM). C: Doppler flow velocity waveforms were obtained in the uterine artery. Doppler flow velocity waveform had a prominent notch (arrow) and relatively low diastolic velocity at embryonic day 9.5 (E9.5). Notch was minimal or absent at E15.5, and diastolic velocities were increased. D: peak systolic velocity (PSV) and end-diastolic velocity (EDV) in the uterine artery increased with gestational age. E: resistance index of the uterine artery decreased during gestation. Different letters in D and E indicate significant changes with gestational age (P < 0.05). Ao, aortic artery; IVC, inferior vena cava; NP, not pregnant.

Fig. 2. Histological section of placenta at E15.5. A: maternal arterial canal (MAC) is located at center of placenta (arrow) and carries maternal blood to first maternal arterial canal branch (MACB; arrowhead). B: UBM image of placenta at E15.5. Arrow indicates MAC and arrowhead indicates MACB. C: Doppler velocity waveform of MAC at E17.5. Peak systolic velocities in MAC and MACB increase with gestational age. Different letters in C indicate significant changes with gestational age (P < 0.05). D, decidua; F, fetal surface; P, placenta.
trophoblast cells derived from the embryo, and it carries the relatively echo-lucent maternal arterial blood into the placenta (Fig. 2F). Doppler waveforms were also obtained from the first major branch of the maternal arterial canal (MACB) (n = 12–16 embryos/day).

Microcomputed tomography was used to visualize the arteries and arterioles (>20 μm diameter) in the umbilicoplacental circulation (Fig. 3A) by M. Rennie and Dr. J. G. Sled at the Mouse Imaging Centre (The Hospital for Sick Children, Toronto, ON, Canada). A radio-opaque contrast medium (Microfil) (38) was infused into the umbilical artery using published microinjection methods (3) (Fig. 3A). Sites of motion of highly echogenic embryonic blood in ultrasound images corresponded to the anatomic location of the umbilical vessels, chorionic vessels, and the embryonic intraplacental arteries visualized by microcomputed tomography (Fig. 3C; see also Movie 1 in the supplemental data, available online at http://ajpheart.physiology.org).

Doppler velocity waveforms were obtained in the umbilical artery in the umbilical cord near the placental surface, in the chorionic plate arteries at the surface of the chorionic plate near the midpoint between the cord insertion and the outer edge of the placenta, and in the embryonic intraplacental arteries of the labyrinth near the midpoint between the chorionic and basal plates (Fig. 3B) (n = 12–36 embryos/day). The maternal and embryonic circulations interdigitate in the labyrinth region of the placenta; however, embryonic vessels could be differentiated because of the much greater echogenicity of the embryonic blood they carried and by the much lower heart rate of the embryo (Fig. 4A) as observed on the Doppler waveforms.

Doppler blood velocity waveforms were also obtained in the vitelline artery to the yolk sac (n = 10–30 embryos/day). The vessels within the yolk sac membrane (Fig. 5A) are supplied by the vitelline vessels, which are visible by UBM where they traverse the amniotic cavity between the umbilicus and the wall of yolk sac (Fig. 5B; see also Movie 2 in supplemental data).

Peak systolic velocity (PSV) and end-diastolic velocity (EDV) were measured from three consecutive cardiac cycles that were not affected by motion caused by maternal breathing, and the results were averaged. The resistance index [RI = (PSV – EDV)/PSV] was calculated when EDV > 0 to quantify the pulsatility of arterial blood velocity waveforms. The pulsatility of arterial velocity waveforms tends to increase when downstream resistance increases, although it is also dependent on other factors, including the pulsatility of the arterial pressure (2).

Reproducibility. Intraobserver variability was determined by repeating offline analysis of waveforms obtained twice from 12 embryos by one of the authors (J. Mu). The coefficients of variability for repeated measurements of peak systolic velocities from the umbilical artery, vitelline artery, chorionic plate arteries, and embryonic intraplacental arteries were 1.5 to 5.3% from the uterine artery, maternal arterial canal, and first major branch of the maternal arterial canal, the coefficients of variability were 2.7 to 7.3%.

Statistical analysis. All data are expressed as means ± SE. For uterine artery measurements, n is the number of nonpregnant or pregnant mice. For all other variables, n is the number of embryos studied. Comparisons were made by Student’s t-test or one-way ANOVA where appropriate. If statistical significance was shown by ANOVA, then a Student-Newman-Keuls test was used for post hoc analysis. A P value of <0.05 was considered statistically significant.
RESULTS

Maternal and embryonic heart rates increased significantly with gestational age, and at each age, maternal heart rate was more than twice that of the embryo (Fig. 4A). Placental weight increased rapidly until E14.5 and then more slowly to term. By contrast, embryonic body weight increased exponentially with gestational age (Fig. 4, B and C). Embryonic body weight increased >75-fold between E10.5 and term.

In nonpregnant and early pregnant mice, the uterine artery blood velocity waveform was characterized by a low end-diastolic velocity with a prominent early diastolic notch (Fig. 1C). As gestation advanced, peak systolic and end-diastolic velocities increased significantly, and the calculated resistance index decreased significantly (Fig. 1, D and E). There was no significant difference in the peak systolic velocity or the resistance index between the left and right uterine artery. The
early diastolic notch was minimal or absent in uterine artery waveforms past E14.5 of gestation.

Blood velocity in the maternal arterial canal (Fig. 2) was detectable from E10.5 and in the arterial canal branches from E12.5 to term (see Movie 1 in supplemental data). The velocity waveform was characterized by a low peak systolic velocity with a high end-diastolic component. The small-amplitude pulsatile component of the waveform was not always perceptible. Peak systolic velocity increased in the maternal arterial canal (0.9 ± 0.03 cm/s at E10.5 to 2.4 ± 0.07 cm/s at E18.5) and in the first maternal arterial canal branch (0.6 ± 0.04 cm/s at E12.5 to 1.5 ± 0.06 cm/s at E18.5). Blood velocity in the spiral arteries of the decidua was not consistently detectable.

Systolic blood velocity was detected in the umbilical artery of 19 of 25 embryos examined at E8.5, whereas systolic blood velocity was detected in all 19 embryos studied at E9.5. End-diastolic blood velocity was near zero in the umbilical artery at E14.5 (Fig. 3E). The detection rate of positive end-diastolic velocity in umbilical arteries was 38% at E15.5 and 94% at E18.5 (Fig. 3F). The resistance index in the umbilical artery was 1 at E14.5 and decreased significantly to 0.82 ± 0.01 at E18.5 (Fig. 3F). Peak systolic blood velocity in the umbilical artery increased significantly and linearly with gestational age, and there was a consistent decrement between the three levels of this circulation; peak systolic velocity in the chorionic arteries was ~38%, and in the embryonic intraplacental arteries it was ~17% that of the umbilical artery throughout gestation (Fig. 3D). Umbilical venous velocity waveforms were pulsatile throughout gestation in the mouse (Fig. 6).

Blood velocity was detected in the vitelline artery supplying the yolk sac placenta in all embryos studied at E8.5. Peak velocity increased almost sevenfold between E8.5 and E13.5 and then remained relatively stable until term (Fig. 3D). Positive end-diastolic velocity was only detected in the vitelline artery at E18.5, and at this stage it was observed in most cases (11 of 17 examined) (Fig. 5D).

### DISCUSSION

The present study is the first to noninvasively quantify developmental changes in the uterine artery, in the umbilical-placental circulation, and in the vitelline artery during gestation in the mouse and is the first detailed study of vitelline artery and intraplacental hemodynamics during organogenesis in any species to our knowledge. We showed that blood flow velocity increased and resistance index decreased with advancing gestational age in the uterine and umbilical circulations in a manner similar to that described during human pregnancy [Table 1 (7, 20, 27, 29, 34, 49) and Table 2 (1, 41)], whereas the vitelline artery velocity waveforms increased during embryonic organogenesis and then remained constant to term, which contrasts with the transient appearance of this circulation during the first trimester in human pregnancy.

The characteristics of the uterine artery waveform in the mouse were similar to that of the human, despite their much higher heart rate (~500 beats/min in mice vs. ~75 beats/min in humans) and their much smaller uterine artery diameter (0.46 mm in mice vs. 3.4 mm in humans) (23, 43). In both species, the nonpregnant uterine artery waveform has a prominent diastolic notch and a high resistance index of 0.7, although the peak systolic velocity of 23 cm/s in the mouse tends to be lower than the 32 cm/s velocity observed in humans (Table 1). During pregnancy, the diastolic notch in the uterine artery waveform is normally not observed past day 15.5 in the mouse and 26 wk in the human (49), and in both species, the end-diastolic blood velocity increases more rapidly with gestational age than systolic blood velocity so that the resistance index decreases progressively, reaching ~0.5 in both species at term (8) (Table 1). The similarities in uterine artery hemody-

### Table 1. Uterine artery hemodynamics in mouse and human

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<th>Mouse</th>
<th>Human*</th>
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<td>Diastolic notch</td>
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N/A, not available. *Source for data: Refs. 7, 20, 27, 29, 34, and 49. E15.5, embryonic day 15.5.
developmental changes in hemodynamics in mouse placenta

Table 2. Umbilical hemodynamics in mouse and human

<table>
<thead>
<tr>
<th></th>
<th>Mouse</th>
<th>Human*</th>
</tr>
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<tr>
<td>Arterial peak systolic velocity, cm/s</td>
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<td>E18.5</td>
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<tr>
<td>Arterial resistance index</td>
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<td>Incidence of venous pulsation, %</td>
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*Source for data: Refs. 1 and 41.

in the umbilical vein is generally considered to be due to retrograde waves caused by cardiac contractions (31). In compromised human fetuses, venous pulsations are elevated (31), apparently due to increases in the force of cardiac contraction, and enhanced propagation of the retrograde wave caused by increased venous stiffness and dilatation of the ductus venosus (6). Thus we speculate that, in IUGR mouse models, as in human fetuses with IUGR (17), the normal late-gestational increase in end-diastolic blood velocity in the umbilical artery may be absent or delayed, and umbilical venous pulsations may be increased.

Peak systolic velocity decreased progressively from the umbilical to the intraplacental arteries. Thus, as on the maternal side of the placental circulation, blood velocity decreased as it approached the exchange surfaces within the placenta. The ability to monitor velocity in the intraplacental arteries is valuable because this site is closer to known sites of vascular pathology in human IUGR placentas (22) and because waveforms in intraplacental fetal arteries may be abnormal before waveforms in the umbilical artery are affected in human fetuses with IUGR (52). The number of detectable fetal intraplacental arteries in human fetuses with intrauterine growth retardation is also reduced (40). Thus, in mouse models of IUGR, we speculate that abnormalities may be more apparent in the intraplacental than umbilical arterial waveforms.

Peak systolic velocity in the vitelline artery to the yolk sac was first detected at E8.5, coincident with the onset of the beating primordial heart tube and immediately after embryonic inversion, whereon the mouse embryo becomes enclosed within the yolk sac membrane. The onset of the embryonic heart beat begins perfusion of the primitive capillary plexus of the mesodermal layer of the yolk sac, which brings red blood cells into the embryonic circulatory system (39). Thus blood velocities were detectable from the onset of perfusion of the yolk sac, a structure that serves as an important site of exchange between the embryo and mother during early organogenesis in the rodent (48) and human (10). Yolk sac functions include histotrophic nutrition, hematopoiesis, gas exchange, protein synthesis, and primordial germ cell formation in rodents (15). The yolk sac performs similar functions in humans before ~10 wk of gestation (16). Yolk sac dysfunction has been associated with congenital malformations and embryonic death in rats (10), chicks (24) and humans (9, 26). Nevertheless, umbilical perfusion began in all embryos examined by E9.5, at which stage the peak velocities were already approximately twofold higher than in the vitelline artery, and they remained approximately twofold higher until E13.5. Despite the higher peak velocities in the umbilical arteries, severe yolk sac defects are lethal to the embryo by E10.5 (18, 48). Indeed, the yolk sac circulation is believed to be the most important source of nutrition for the embryo until E13.5 of gestation, at which stage the umbilicoplacental circulation becomes paramount (5, 48) and, as we have shown, the peak systolic velocity in the vitelline artery reaches a plateau. Similarly, in humans, yolk sac and umbilical blood velocities are detectable by ultrasound as early as 5 wk of gestation. In humans, as in mice, blood velocities in the umbilical artery are approximately twofold higher than in the vitelline artery, and both circulations are perfused from the onset of the heart beat until the end of organogenesis (~9 wk in humans) (33, 37). The necessity for simultaneous perfusion of these two organs during the period...
of organogenesis suggests that, in both species, their functions are not redundant. After organogenesis, the external, pouch-shaped yolk sac of the human embryo regresses and blood velocities become undetectable (33, 37), which contrasts with the continued perfusion and presumed importance of the yolk sac to full term in mice.

Peak systolic velocity in the vitelline artery remained constant, and end-diastolic velocity remained zero from E13.5 to E17.5, suggesting that the yolk sac had a stable perfusion requirement over this interval, despite further growth in the number and length of the yolk sac villi (28) and the large increase in yolk sac area that must have occurred, given that the yolk sac encloses the mouse embryo and the embryonic body weight increased by 75-fold. The yolk sac of the mouse in late gestation appears to play an exclusive role in calcium transfer (32) and immunoglobulin G and anionic amino acid transport (42, 44), whereas both the placenta and yolk sac exhibit hematopoietic potential until at least E17 (4).

In conclusion, blood flow velocity waveforms in the uterine artery and the uteroplacental, umbilicoplacental, and yolk sac circulations were recorded noninvasively by using high-resolution ultrasound during pregnancy in the mouse. Our results show that waveforms are similar in shape and show similar changes during gestation to those of human pregnancy. The ability to use a noninvasive tool, particularly a tool that parallels that used clinically, will facilitate longitudinal studies of placental and yolk sac hemodynamics and will maximize the clinical relevance of mutant mice as models for human diseases of pregnancy. Our study of normal cardiovascular development provides the basis for future studies of the developmental origins of preeclampsia, IUGR, and intraterine death in genetically manipulated mouse models of human diseases.

ACKNOWLEDGMENTS

The authors thank Dr. Dawei Qu, Dr. John Slevin, and Kathie Whiteley for technical assistance, and Monique Rennie and Dr. John G. Sled at the Mouse Imaging Centre (The Hospital for Sick Children, Toronto, ON, Canada) for microcomputed tomography imaging. We also thank Dr. John Kingdom for helpful discussions and critical comments.

GRANTS

This work was funded by operating grants from the Canadian Institutes for Health Research. The purchase of the ultrasound biomicroscope was funded by helpful discussions and critical comments.

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