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

Junwu Mu1,2,3 and S. Lee Adamson1,2,3

1Samuel Lunenfeld Research Institute of Mount Sinai Hospital; and Departments of 2Obstetrics and Gynecology and 3Physiology, University of Toronto, Toronto, Ontario, Canada

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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 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 artery, the EDV detection rate was 0% at ≤E14.5 and 94% at E18.5, and the RI decreased from 1 to 0.82 ± 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 fetoplacentat 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 have 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 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 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
trophoblast cells derived from the embryo, and it carries the relatively echo-lucent maternal arterial blood into the placenta (Fig. 2A). 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 umbilical 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 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 very 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. 5C). 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 umbilico-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-

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

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<tr>
<th></th>
<th>Mouse</th>
<th>Human*</th>
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<tbody>
<tr>
<td></td>
<td>Not pregnant</td>
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</tr>
<tr>
<td>Peak systolic velocity, cm/s</td>
<td>23</td>
<td>52</td>
</tr>
<tr>
<td>Resistance index</td>
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<td>0.6</td>
</tr>
<tr>
<td>Diastolic notch</td>
<td>yes</td>
<td>no</td>
</tr>
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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.
namics in mice and humans during normal pregnancy suggest that abnormal uterine hemodynamics typical of severe preeclampsic and IUGR pregnancies in humans, e.g., diastolic notch and low end-diastolic velocity in midgestation, may be pheno-
copied in mouse models of preeclampsia.

We demonstrated that the peak systolic velocity of the
maternal arterial canal and maternal arterial canal branch
increased with gestational age. This is likely associated with an
increase in volume flow caused by decreases in uteroplacental
vascular resistance. A decrease in vascular resistance is con-
istent with the increased volume and surface area of the
maternal blood spaces in the placental labyrinth of the mouse
during pregnancy (13). Thus, despite the stasis in placental
weight in late gestation, increased perfusion and surface area of
the placenta, together with decreased thickness of the tissue
separating maternal and embryonic blood (12, 30), are capable
of increasing placental exchange sufficiently to support rapid
growth of the embryo in late gestation. We also showed that
peak systolic velocity decreased between the maternal arterial
canal and its branches and likely decreased further when
entering the labyrinth, given that pulsatile flows with a mater-
nal heart rate were not detectable in the labyrinth. Low velocity
in the terminal trophoblast-lined exchange spaces likely pro-
motes effective exchange of nutrients, wastes, and gases be-
tween maternal and embryonic or fetal circulations.

In the umbilical artery, end-diastolic velocity was zero in
embryos at E14.5 or younger as in prior reports (35, 45). Positive velocities were first detected at E15.5, and end-
diastolic velocity then increased progressively to term at E18.5.
The age of onset of end-diastolic velocity in our noninvasive
study contrasts with prior work on surgically exposed mouse
embryos in which end-diastolic velocities were zero in all
embryos from E10.5 to E16.5 (final day of study) (35). In
humans, umbilical arterial end-diastolic flow velocity is zero
during the first trimester, progressively appears from 13 to 17
wk, and is normally present in all fetuses after 18 wk of
gestation (21). Interestingly, in both species, the gestational
age at which positive end-diastolic velocities are first detected
(E15.5 in mice and 13 wk in humans) is shortly after the end
of organogenesis (E14.5 in mice and 10 wk in humans). Thus
the appearance of end-diastolic velocities appears to be caused
by changes associated with the maturation phase of placental
and/or cardiovascular development. Increased placental vascu-
larity (12, 13, 30), leading to a decrease in peripheral vascular
resistance, and increased elastin deposition (25), leading to
increased aortic capacitance, may both play a role (2). In the
umbilical vein, the velocity waveform is pulsatile from E9.5 to
E14.5 in the mouse (45). In the current study, we found that the
umbilical vein retains a pulsatile component to term in mice,
whereas a pulsatile component is absent in >90% of human
fetuses after 23 wk of gestation (41). The pulsatile component
in the umbilical vein is generally considered to be due to
retrograde waves caused by cardiac contractions (31). In com-
promised 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 wave-
forms 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 in-
traplacental 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
cord 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 ex-
change between the embryo and mother during early organo-
genesis in the rodent (48) and human (10). Yolk sac functions
include histotrophic nutrition, hematopoiesis, gas exchange,
protein synthesis, and primordial germ cell formation in ro-
dents (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). Neverthe-
less, umbilical perfusion began in all embryos examined by
E9.5, at which stage the peak velocities were already approxi-
mately 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 para-
mount (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

Table 2. Umbilical hemodynamics in mouse and human

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<th>Mouse</th>
<th>Human*</th>
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<tr>
<td></td>
<td>E14.5</td>
<td>E18.5</td>
</tr>
<tr>
<td>Arterial peak systolic velocity, cm/s</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Arterial resistance index</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Incidence of venous pulsation, %</td>
<td>100</td>
<td>100</td>
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</table>

*Source for data: Refs. 1 and 41.
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 utero-placental, umbilico-placental, 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 intrauterine death in genetically manipulated mouse models of human diseases.

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