Application of clinical indices of fetal growth and wellbeing to a novel laboratory species, the spiny mouse

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SUMMARY

Ultrasound was used to measure growth of the spiny mouse fetus throughout gestation and to record Doppler measurements of heart rate and umbilical blood flow to monitor fetal blood supply and wellbeing. Female spiny mice were anesthetized on 6 occasions throughout pregnancy. Ultrasound was performed with a Philips HDI 5000 machine using a compact linear CL15-7 transducer. Fetal heart rate and growth parameters increased across gestation. Blood flow through the umbilical artery and vein showed increasing velocity over gestation, and reduced resistance index. Blood flow through the ductus venosus also increased in velocity over gestation; however the resistance index remained constant. We have determined changes in umbilical blood flow throughout pregnancy in the spiny mouse, which resemble those seen in human pregnancy. We also confirm that ultrasound can be used as a valuable, non-invasive technique.

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INTRODUCTION

While ultrasound has long been a valuable tool in obstetrics and gynecology to monitor the growth and wellbeing of the human fetus, it is only recently that such technologies are being applied to animal based research. Higher frequency imaging has recently become available, making it possible to accurately image the small animal. In utero ultrasound technology has been utilized in a variety of animal models including, sheep [1, 29], rats [16] and dogs [9]. Further, the use of high frequency ultrasound has been reported and described in many mouse studies of pregnancy and development [3, 10-20, 23, 26-28, 31].

Given the need to understand fetal growth and placental function, and the wide range of perinatal models (non-human primates, sheep, guinea pigs, rats, mice) being used to study effects on these, it is necessary to apply clinically relevant measures of fetal growth and wellbeing to accurately assess the effects of any treatments likely to alter fetal growth and wellbeing. While measurements of fetal and placental growth have been well documented in conventional rodents, few have measured umbilical blood flow. In those studies where umbilical blood flow measurements were taken, these values do not represent that seen during human pregnancy. Therefore we proposed to measure umbilical blood flow, as well as ductus venous flow in a precocial rodent species, the spiny mouse, where development of major organ systems reflects that seen in newborn humans. We hypothesized that late gestation blood flow would be similar in spiny mouse and human pregnancy, further highlighting the appropriateness of the spiny mouse as a model for fetal and perinatal studies.

The spiny mouse is a small rodent species currently being characterized and used as a model for fetal and neonatal studies. The spiny mouse is proving to be valuable in the study of fetal and placental growth and function.
owing to its long gestation (38-40 days), few offspring (1-5, usually 2-3),
and advanced stage of development of principal organ systems such as the
kidney [11], liver [17], some brain regions [4-8], and the immune system
[Dickinson and Swann, unpublished]. The spiny mouse is currently being
used as a model to investigate the impact of birth asphyxia on the neonatal
brain [15] as well as a model of preterm delivery. Our spiny mouse model
has also contributed knowledge to the developmental origins of health and
disease research discipline [12].

In this study we report measurements of fetal size (including bi-parietal
diameter and limb lengths), umbilical blood flows and fetal heart rate. The
spiny mouse is a precocial rodent species with organ development at the time
of birth closely resembling that of the human infant, much more so than any
other known rodent species. The observation that ductus venosus and umbili-
cal blood flow are similar in the late gestation spiny mouse and human further
highlights the spiny mouse as an ideal small animal model for perinatal stud-
ies. The authors are not aware of any other literature describing the measure-
ment of fetal ductus venosus blood flow in a small rodent species.

MATERIALS AND METHODS

Animals and experimental protocols

All experiments were approved in advance by Monash University School
of Biomedical Sciences Animal Ethics Committees and conducted in
accordance with the Australian Code of Practice for the Care and Use of
Animals for Scientific Purposes. The female spiny mice (Acomys cahirinus)
used in this study were obtained from our own laboratory colony and bred
as previously described [11].

Four female spiny mice were selected and confirmed to be pregnant on the
first day of scanning at day 15 of gestation (16 days after the delivery of the
previous litter, see [11] for details of our standard mating protocol). Subse-
quent scans were performed on days 19, 23, 28, 34 and 37 of gestation. Female
spiny mice were weighed and lightly anesthetized using isoflurane with 40%
oxygen: 60% nitrogen gas mixture (4.8% induction dose, 2.0-2.2% maintenance dose). Body temperature was maintained using a heated bench pad. The dams were allowed to naturally deliver their litter, before the pups were collected and culled (within 2 h of delivery) for measurement of selected body parameters (see below). A control group of spiny mouse dams (n=4) were allowed to naturally deliver their offspring, without exposure to the ultrasound or anesthetic. Measurement of body parameters of these offspring were taken on the day of birth (control birth) and compared to the offspring exposed to the ultrasound and anesthetic throughout gestation as described above.

All measurements, at all time points, were performed by the same operator to ensure consistency throughout the experiment. Once anesthesia was induced, the anterior abdominal region of the spiny mouse was closely shaved to reduce contact artifacts. The dam remained in the supine position with tape gently restraining the limbs to prevent movement during scanning. Warmed ultrasound gel (Aquasonic) was applied to the ultrasound probe to allow transmission and minimize reflection of sound. Ultrasound was performed with a Philips HDI 5000 machine (Philips Medical System, Bothell, Washington, USA) using a compact linear CL15-7 transducer and manufacturer ‘small parts’ pre-set. Modifications to the preset included reduced power levels and the use of image optimization tools SonoCT™ and X-Res™. The 15-7 MHz high resolution linear transducer provided an optimum field of view and resolution.

Where possible, measurements of biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), crown-rump length (CRL), fetal fat layer, tibia lengths, heart rate and umbilical cord and ductus venosus blood flows were taken at the time of each examination. At birth (ultrasound exposed and control), offspring were weighed before being euthanized by overdose of anesthetic (Isoflurane, Rhodia Australia P/L, Victoria) and BPD, AC, CRL and tibia lengths were recorded.

Fetal number: The number of fetuses and their position within the uterus were recorded. Biparietal diameter: BPD was recorded at the level of the cavum septum pellicudum (CSP), and thalamus with falx in the midline. Calipers were placed ‘leading edge to leading edge’. When fetal position made this image plane unobtainable, an axial image through the orbits and CSP was used and BPD measured at the widest diameter (fig. 1A).
Head circumference: In the same plane as the BPD, the outer table of the ossified skull was measured to obtain a circumference. This was performed using the elliptical software tool provided in the Philips 5000 (fig. 1A).

Abdominal circumference: The AC was measured at the level of the mid third of the umbilical vein, demonstrating a circular shape to reduce the possibility of an oblique plane and thus an over estimation of the AC. Fetal curling made this measurement difficult to reproduce with increasing fetal age (fig. 1B).

Crown-rump length: CRL was measured in both the coronal and mid-sagittal planes according to fetal position. The longest length was recorded not including the fetal tail (fig. 1C).

Figure 1. Growth measurements recorded in fetal spiny mice from day 19 to 37 of gestation. A: biparietal diameter (BPD) and head circumference (HC), B: abdominal circumference (AC), umbilical vein (UV), C: crown-rump length (CRL), D: subcutaneous fetal fat layer and E: tibia length.
Fetal fat layer: In the same plane as the AC, the image was magnified to enable accurate placement of the calipers over the subcutaneous fat layer (fig. 1D).

Tibia lengths: Tibia length was measured, not including the epiphysis, with the tibia perpendicular to the ultrasound beam allowing for distinct endpoints and posterior shadowing to be observed. The femur was not sufficiently ossified to be able to make accurate determinants of length until late in gestation; day 29 (fig. 1E).

Heart rate: Using M-mode the fetal heart rate was measured over three cardiac cycles using Phillips averaging software (fig. 2A).

Umbilical and ductus venosus blood flows: After locating the umbilical insertion point of the cord, Doppler traces were recorded in the cord at the greatest distance from the umbilical insertion as could be imaged, to reduce fetal influences on the waveform. Arterial and venous waveforms were recorded (fig. 2B). The ductus venosus was identified as an extension of the umbilical vein within the liver. Depending on fetal position, axial and sagittal images were used. The ductus venosus could be identified...
by the change in caliber of the vessel as it inserted into the inferior vena cava. The change in caliber resulted in altered flow patterns and color aliasing guiding the placement of the Doppler sample volume (fig. 2C). The resistance index (RI) was calculated [24] as:

\[ \text{RI} = \frac{S - D}{S} \]

where

S: peak systolic velocity and D: end diastolic velocity

**Statistical analysis**

All data are presented as means ±SEM. Values from all of the fetuses from each dam were averaged and thus n=4, representing a total of 15 fetuses. A repeated measures ANOVA was used to test for statistical differences between the measures over gestation and at birth. p<0.05 was taken to be statistically significant.

**RESULTS**

**Anesthesia, birth dates and offspring outcome**

Mean anesthesia time increased with gestational age (range 20-45 mins/dam), a reflection of increased scanning time that was required as more measurements were recorded as the fetuses matured (data not shown). A total of 15 fetuses were scanned from 4 mothers, with 3 dams carrying 4 pups, and 1 dam carrying 3 pups. Gestation lengths following the ultrasound exposure (1 dam each at 37 and 39 days and 2 dams at 38 days) were within the normal range of gestational lengths seen within our colony of spiny mice [10]. Data for day 37 of gestation (n=11 fetuses) does not include that of the dam that delivered on this day and data for the day of birth (n=12 pups) does not include 3 of 15 pups which were cannibalised by adult spiny mice in the cage (this rate of cannibalism has occurred previously in our colony). The mean birth weight (5.26±0.07g), cannibalism rate (3/15) and sex ratio (5 males, 7 females) in this cohort of animals subjected to
the ultrasound procedure were not different when compared to control, untreated offspring (5.24±0.08g; 1/12; 6 males, 5 females).

**Fetal growth**

As expected, all measures of fetal biometry increased throughout the second half of pregnancy in the spiny mouse. Abdominal circumference (fig. 3A), biparietal diameter (fig. 3B) and head circumference (fig. 3D) showed slowing of growth from day 34 of gestation to birth (p<0.001). Crown-rump length could not be measured after day 19 of gestation due to the curled posture of the fetuses, but was increased at the time of birth (p<0.001; fig. 3C). Tibia length (fig. 3E) increased steadily throughout late gestation to birth (p<0.001). On the day of birth, abdominal circumference, biparietal diameter, crown-rump length and tibia lengths of offspring exposed to ultrasound throughout gestation were not different when compared to control offspring (control birth, fig. 3A-C, E). At day 34 of gestation, a subdermal fat layer was detectable and its thickness appeared to increase from this time onwards, although not significantly (fig. 3F).

**Fetal heart rate and umbilical and ductus venosus blood flows**

Measurement of fetal heart rate was possible from day 19 of gestation, although fetal heart movements could be detected from day 15 of gestation. Fetal heart rate increased across gestation with the peak fetal heart rate reaching 300 bpm just before term (p<0.001; fig. 4F). Measurement of flow velocities and resistance waveforms within umbilical vessels was possible from day 23 of gestation (fig. 4A-E). Umbilical vessel velocities increased throughout late gestation, whereas resistance index in the umbilical artery decreased (p<0.001; fig. 4C-E). Ductus venosus flow was not measurable until day 29 of gestation. Ductus venosus velocity increased throughout late gestation (p<0.001) whereas the resistance index remained consistent at 0.51 throughout gestation (fig. 4A-B).
Figure 3. Fetal growth parameters increased throughout the second half of gestation in the fetal spiny mouse (p<0.001). Abdominal circumference (A), biparietal diameter (B), crown-rump length (C), head circumference (D), tibia lengths (E), and subcutaneous fetal fat layer (F). Control birth represents offspring born to normal, untreated spiny mouse dams. Values are expressed as means±SEM and differences tested by repeated measures ANOVA, n=15 for day 15-34 measures, n=11 for day 37 measures, n=12 for day of birth measures, n=11 for control birth measures.
Figure 4. Fetal heart rate and umbilical flows were measured from day 19-23 of gestation in the fetal spiny mouse. Ductus venosus velocity (B) increased throughout late gestation (p<0.001), while the resistance index (A) did not change. Umbilical vessels (C and E) increased in velocity over the second half of gestation (p<0.001) and umbilical artery resistance index (D) decreased (p<0.001). Values are expressed as means±SEM and differences tested by repeated measures ANOVA, n=15 for day 15-34 measures, n=11 for day 37 measures, n=12 for day of birth measures.
Other observations

At day 19 of gestation the fetal spine was completely enclosed, limb buds were clearly visible and blood flow could be detected in the maternal side of the placenta. We were able to observe fetal swallowing from Day 23 of gestation, which correlated with the presence of a fluid-filled stomach and bladder at this age. Ossification of the mandible, ribs and digits was sufficiently at this age to allow them to be identified. Renal blood flow and fetal breathing movements were detectable from day 29 of gestation.

DISCUSSION

We have shown for the first time that in late gestation the characteristics of ductus venosus blood flow in the spiny mouse are similar to that observed in the human fetus. Further we have shown that fetal growth, as well as changes in fetal heart rate and umbilical blood flow can be documented throughout the second half of pregnancy in this species using conventional ultrasound technology.

Measurement of ductus venosus and umbilical blood flow in human pregnancies allows early detection of fetal compromise and are considered the gold standard in detecting fetal wellbeing [14]. The velocity and resistance index of the umbilical artery measured in the spiny mouse was similar to that of human fetuses. Velocity increased across gestation in the spiny mouse ranging from 10 to 25 cm/sec, and in human pregnancy, blood flow velocity in the umbilical artery also increases (range 27 to 36 cm/sec; [2]). Blood flow resistance in the umbilical artery was identical in the fetal spiny mouse and the human, with the resistance index decreasing from 0.8 to 0.6 by the end of term [2]. The resistance index of ductus venosus blood flow (0.51) was similar at mid-gestation as at late gestation in the spiny mouse, and was also similar to that measured in the human fetus 0.57 at <22 weeks and 0.51 at <28 weeks gestational age, in normal, non-compromised human pregnancies [30]. The absence of reverse flow in the ductus venosus blood flow profile is also consistent with that observed in
A decrease in ductus flow to zero, or a reversal of flow has been associated with intrauterine death, emergency pre-term delivery, and neonatal death [13]. Within the clinical setting, measurement of changes in ductus venosus, and umbilical artery flow are used to reflect fetal compromise. Other measures of fetal compromise, such as growth parameters are possible in utero, however these changes are downstream from the initial change in blood flow and often only detectable well after the fetus has been compromised.

An extensive randomized controlled trial of pregnant women exposed to either multiple bouts of ultrasound imaging and Doppler blood flow studies from 18 to 36 weeks gestational age, or to only a single imaging scan at 18 weeks (with further scans only as indicated on clinical grounds) found a slight increase in the proportion of growth restricted offspring in the repeated ultrasound group [22]. Longer term follow-up of these infants up to 8 years of age found no differences in growth and other measures of development between the two groups [21]. Despite this recent evidence from animal studies has suggested that ultrasound exposure at critical periods of gestation can induce harmful effects on growth and development. Pregnant mice exposed to a single 30 minute bout of diagnostic levels (3.5 MHz) of ultrasound on days 11, 12, 14 and 16 gave birth to offspring of a significantly lower birth weight when compared to non-exposed control offspring [25]. In the current study, female spiny mice were exposed to 6 × 20-40 minute bouts of ultrasound, but this was without apparent effect on gestation length, or birth weight compared to untreated controls. The British Medical Ultrasound Society guidelines were followed in the current study to ensure the best ultrasound practices were employed: specifically, low power levels were maintained throughout the insonation time; mechanical and thermal index were always maintained below 0.5 and insonation time was kept to a minimum following the ALARA (as low as reasonably achievable) principle.

As with any technique there are a number of short-comings of ultrasound. Late in gestation, the fetus was curled and often mobile within the amniotic sac, making it difficult to orientate the fetus for accurate biometric measurements. This was overcome by taking a repeat measurement later
in the insonation time. The ultrasound used in the current study was designed for human clinical work and, as such, was used at the limits of its capacity. For example, time gain control (TGC) was rendered inoperable due to the small field of view and therefore only overall gain was available to manipulate the images. Furthermore, there is an inherent ring-down artifact within the transducer used in this study (Phillips, Inc.) which tends to reduce the image quality. Despite these short-comings, we have been able to demonstrate that this technique is consistent and highly valuable for small animal studies and provides much of the same valuable information of fetal growth and wellbeing as required for human fetal studies.

Overall this study shows the feasibility of tracking fetal growth and obtaining measures of umbilical blood flows in fetuses of the spiny mouse. Further, it has highlighted the similarity in blood flow velocity and resistance in the umbilical artery and resistance in the ductus venosus throughout the later third of gestation between the spiny mouse and the human. This is of particular relevance for fetal and perinatal studies in a small animal species where pregnancy and fetal development are comparable to that of human patients.

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REFERENCES


