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● *Original Contribution*

EVALUATION OF UTERO-PLACENTAL AND FETAL HEMODYNAMIC PARAMETERS THROUGHOUT GESTATION IN PREGNANT MICE USING HIGH-FREQUENCY ULTRASOUND

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Abstract—Throughout gestation, changes in maternal and fetal Doppler parameters in pregnant mice, similar to those obtained in human fetuses, were detected using high-frequency ultrasound with a 55-MHz linear probe. In the uterine arteries (UtA), fetal umbilical artery (UA) and fetal ductus venosus (DV) peak systolic velocity increased (UtA, $p = 0.04$; UA, $p = 0.0004$; DV, $p = 0.02$), end-diastolic velocity increased (UtA, $p < 0.001$; UA, $p < 0.0001$; DV, $p = 0.01$) and resistance index decreased (UtA, $p = 0.0004$; UA, $p = 0.0001$; DV, $p = 0.04$) toward the end of pregnancy. In the middle cerebral and carotid arteries, end diastolic velocity increased ($p = 0.02$ and $p < 0.0001$) and resistance index decreased (both vessels, $p < 0.0001$). There was a reduction in the pulsatile pattern in the umbilical vein ($p < 0.05$). The increased velocities and reduced resistance index suggest a progressive increment in blood flow to the fetal mouse toward the end of pregnancy. Fetal and utero-placental vascular parameters in CD-1 mice can be reliably evaluated using high-frequency ultrasound. (E-mail: romeror@mail.nih.gov) **© 2014 Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Doppler, Experimental mouse model, Pregnancy, Ductus venosus, Resistance index.**

INTRODUCTION

Animal experimentation has been fundamental in describing the process of fetal cardiovascular development throughout pregnancy (Rudolph and Heymann 1968, 1970) and hemodynamic adaptation/deterioration in the presence of obstetrical complications (Abi-Nader et al. 2012; Bennet et al. 1999; Berman et al. 1975; Bishai et al. 2003; Block et al. 1984; Rosen et al. 1986; Sheldon et al. 1979). Indeed, the process of blood flow distribution in fetal organs was originally described in fetal sheep using radiolabeled microspheres (Heymann and Rudolph 1967; Heymann et al. 1977; Rudolph and Heymann 1967, 1968, 1972). Recently, other animal models have also been proposed; pregnant murine and rabbit models (Bassan et al. 2000; Eixarch et al. 2009, 2011) have shorter gestational periods, requiring smaller experimental areas, relatively lower costs, larger numbers of fetuses, and placental characteristics similar to those of humans (Malassine et al. 2003; Sapin et al. 2001). The use of knock-out mouse strains provides further advantages in studying the effects of specific genes during pregnancy (Pham et al. 2009; Smith et al. 2007; Tian et al. 2006; Zhang et al. 2011).

Doppler ultrasound has proven value in assessing fetal and maternal cardiovascular parameters in animal experimentation (Acharya et al. 2008; Ferrazzi et al. 2001; Gudmundsson et al. 1990; Gunnarsson et al. 1998; Hernandez-Andrade et al. 2005; Schmidt et al. 1991). However, conventional Doppler ultrasound emitting at frequencies between 2 and 16 MHz does not have the temporal resolution required to clearly display

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Doppler waveforms and color Doppler maps in murine models (such as the mouse) with a cardiac rate of 350–500 bpm (Stypmann 2007). High-frequency ultrasound enables fast signal acquisition and processing by use of linear probes emitting at 25–55 MHz (Phoon et al. 2000; Renault et al. 2006). The Doppler waveforms clearly display all velocity components, and color Doppler mapping provides reliable information about the direction of flow (Stypmann 2007; Zhang and Croy 2009).

High-frequency ultrasound has been used in the investigation of uterine and umbilical arteries in the pregnant mouse throughout gestation (Khankin et al. 2012; MacLennan and Keller 1999; Phoon et al. 2000); however, besides these two vessels, other fetal vascular territories have been scarcely evaluated. The aim of this study was to obtain Doppler recordings of fetal and placental vascular territories in CD-1 mice similar to those obtained in the human fetus; in particular, daily changes in the Doppler waveforms of the ductus venosus, which have not been described before, and to provide a better understanding of what is to be expected in normal circumstances in examining infrequently-evaluated fetal vascular territories.

METHODS

Animals and husbandry

The experiments described herein were conducted as part of an ongoing *in vivo* study under an animal protocol approved by the Institutional Animal Care and Use Committee of Wayne State University, Detroit, Michigan, USA (IACUC Protocol A-11-03-11). Animal handling and care followed all the standards of the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health 2011).

Thirteen pregnant CD-1 mice (Charles River Laboratories, Wilmington, MA, USA) were evaluated. Mice were kept in separate filtered-top rodent cages with *ad libitum* food and water. Constant temperature (24 ± 1°C) and humidity (50 ± 5%) were maintained in the room, with a daily regular 12-h:12-h light/dark schedule.

Ultrasound recordings were performed daily from gestational day (GD) 8 to GD 18 in three mice; at GD 8, 13 and 18 in seven mice; and at GD 18 in three mice. The mice evaluated every day constituted the group in which we studied daily hemodynamic changes; mice evaluated on GD 8, 13 and 18 constituted the pilot group designed to optimize the main operative protocol; and the mice evaluated on GD 18 formed the control group necessary to assess differences in neonatal characteristics among fetuses exposed to different periods of ultrasound insonation and anesthesia. No other experimental procedures were performed. A total of 26 fetuses (2 per pregnant mouse) were evaluated. Fifty-seven scanning sessions were performed: 33 for the daily group; 21 for the group evaluated at GD 8, 13 and 18; and 3 for the control group.

Ultrasound assessments of maternal, fetal and placental blood flow

General anesthesia was induced by inhalation of 4%–5% isoflurane (Aerrane, Baxter Healthcare, Deerfield, IL, USA) and 1 L/min O_2. Anesthesia was maintained by inhalation with a mixture of 1–2% isoflurane and 1 L/min O_2. Leakage of anesthesia gas was scavenged using a ventilation system equipped with a charcoal filter canister. Mice were positioned on a heating pad (Vevo Imaging Station, VisualSonics, Toronto, ON, Canada) and gently stabilized with adhesive tape (Fig. 1). Abdominal and chest hair was shaved with a clipper and was further cleaned with a chemical hair remover (Nair cream, Church & Dwight Canada, Mississaugua, ON, Canada) to minimize ultrasound attenuation. Nair cream was wiped off 15–20 s after its application with alternating wet and dry gauzes to prevent damage to the skin. Mouse cardiac and respiratory rates were monitored during the entire ultrasound procedure, and body temperature was maintained in the range 37 ± 1°C.

The first fetus in each uterine horn was evaluated with a high-frequency linear 55-MHz ultrasound probe (Vevo 2010, VisualSonics). The ultrasound probe was fixed and mobilized with a mechanical holder. Crown-rump length was first measured in all fetuses. The uterine arteries were located in the maternal pelvis on each side of the maternal bladder; the fetal carotid artery was visualized in a lateral sagittal view of the fetal thorax and neck; the fetal middle cerebral artery was localized in a cross-sectional view of the fetal head at the level of the intracranial arterial circle; the ductus venosus was

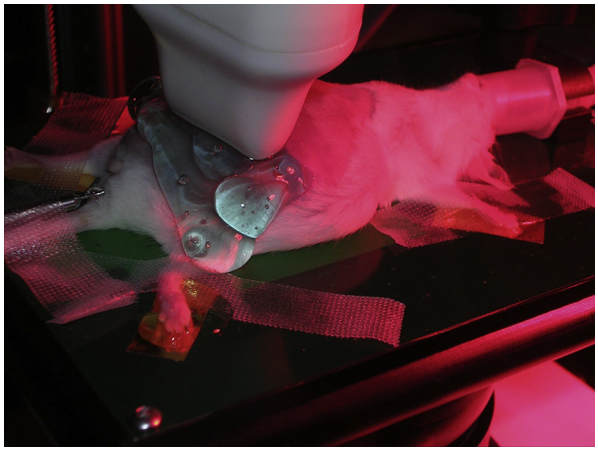

Fig. 1. Position of a pregnant mouse in the ultrasound scanning platform.