VISUALSONICS
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Imaging Guide

Guide to **Embryonic Mouse Imaging**
using the Vevo® Imaging Systems

System Compatibility: This guide contains instructions and suggestions for work on the Vevo2100, VevoLAZR, Vevo 3100 systems and transducers from the MS, MZ and MX series.

Objective

This guide is designed to assist the user to:

- Select and position the transducer to visualize typical imaging targets
- Stage/age embryonic mouse
- Recognize embryonic structures in typical views at various stages of the development

This *Guide to Small Animal Mouse Embryonic Imaging* includes
Images at the following stages: E10, E12, E12.5, E16, E17

Note: All images throughout the guide were acquired non-invasively through the mother's abdomen.

Selected abbreviations and acronyms:
E: embryonic gestational day
FAC: fractional area change
LV: left ventricle
RV: right ventricle

Overview of Research Areas

Rodents as a research tool have many benefits and are currently the small animal model of choice. Humans and mice share 90% of their genes, their genotypes are well known and rodents are also prolific and inexpensive to house.

The mouse heart measures about 10 to 12 mm on the long axis, whereas the human heart measures approximately 12 to 15 cm. The ratio, mouse organs to human organs, follows the same pattern. Optimal imaging of the mouse, for example, therefore, requires an approximately 10-fold improvement in resolution if the same level of structural detail is to be observed and the Vevo Imaging Systems as a non-invasive high-resolution imaging tool provides a wide range of ultra-high frequencies.

Embryology imaging could cover vast are of research from phenotyping, developmental biology, gene therapy to drug development. For example a simple B-Mode imaging session that helps identify free fluid during the embryo development could be an early indicator of many processes such as heart failure, cancer and auto-immune disease. Other examples include imaging of arteries in embryos and the maternal to determine the health and nature of tissue supplied, studies that translate very well to clinical research.

Selecting the transducer

The main criteria in selecting the correct transducer are:

- age of the embryos
- target tissue

For early stage embryos the most appropriate are the 550S and 700 series with frequencies of 40 and 50MHz.
For later stage embryos 550D, 550S and 700 are all well suited for most types of tissue.

There are no set recommended positions for the transducers as the embryos will move around and so, the most important part of this type of imaging is the ability to recognize the tissue displayed which is the aim of this document.

Animal Preparation and Injection Protocol

The animal preparation shall adhere with the Institution's Animal Care Committee approved SOP.

Reference Material:

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Ultrasound in Med. & Biol., Vol 28, No.9, pp. 1165-1172, 2002.

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Parkes, S. Lee Adamson. Am J Physiol Heart Circ Physiol 285: H1563-H1575, 2003.

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Ultrasonic detection and development changes in calcification of the placenta during normal pregnancy in mice.
Placenta (2005), 26, 129-137

Embryological Time Points and Representative Images of Various Anatomical Targets

Embryological Time Points

E0.5 is defined as 12 noon of the day a vaginal plug is found after over-night mating.

E5.5

At this stage of embryogenesis, the inner cell mass (ICM), composed of the epiblast and primitive endoderm and, the troph-ectoderm have begun to form a cylindrical embryo. The proamniotic cavity is forming. Reichert's membrane, which is non-cellular and secreted by the distal endoderm, appears. The maternal tissue is invaded by trophoblast (primary) giant cells and the ectoplacental cone is invaded by maternal blood. The implantation site is about 2X3 mm. The embryo is visible as a diffuse bright region of approximately 250 µm in diameter in the lumen of the uterus.

E6.5

In the mesoderm of the posterior amniotic fold small cavities coalesce to form a single cavity, the exocoelomic. A slightly brighter echogenic ring will be observed around the implantation site in the uterus near the trophoblast-decidual interface. At this stage, the circumference of the ring is fairly uniform in brightness although the mesometrial side

(i.e. the side where the placenta will develop) is usually brighter than the other side.

E7

Regions where the cross-section of the uterus is enlarged are clearly visible within the maternal abdomen. The enlarged regions are relatively dark. Near the center of each enlargement is a small, echo free region that is likely the proamniotic cavity of the developing embryo. It is surrounded by a relatively bright region known to be populated by embryonic trophoblast giant cells that invade the maternal deciduas during implantation in the mouse.

E7.5

Three dark regions are visible within the conceptus. They likely correspond to the ectoplacental, amniotic and exocoelomic cavities of the developing embryo. Bight echogenic foci are primarily localized near the mesometrial side of the implantation site.

E8.5

Cardiac contractions are discernable. However the heart structures are too small for reliable cardiac measurements to be made. The embryo and amniotic membrane are visible, with the embryo wrapping around the amniotic cavity. The allantois can be seen emerging from the embryo and approaching the ectoplacental cone (where the chorio-allantoic placental later develops). At this stage, a pulsatile blood velocity signal is first detectable in the heart tube, but no signal is detected within the allantois. The foci are localized in a dense cone-shaped region (V-shaped in longitudinal section, ring-shaped in cross-section) that appears to border the ectoplacental cone (a region populated by embryonic trophoblast cells).

E9.5

Atrial and ventricular contractions are identified. However the heart structures are still too small for reliable cardiac measurements to be made. Synchronous and discernable atrial and ventricular contractions are apparent, with atrial contraction occurring at end diastole. The primitive left ventricle is prominent and bulbus cordis continues into outflow tract. The U-shaped heart tube is clearly visible and blood velocity waveforms using PW Doppler Mode can be recorded separately from the inflow and outflow regions of the heart tube. The amniotic membrane, amniotic and yolk sac cavities, brain, cerebral ventricles, umbilical cord and placenta are visible. Umbilical blood velocity waveforms are detectable using PW Doppler Mode. Upon formation of the chorio-allantoic placenta, echogenic foci appeared to reside near the materno-placental interface. As gestation advances, the echogenic foci at this location become more discrete and widely spaced and are often visibly larger and/or 'brighter' than in images from earlier gestation. Perfusion begins.

E10

This stage is defined by the closure of posterior neuropore. The hind limb bud becomes visible at the level of the 23rd-28th somites (total of 30 to 34 somite pairs). The tail bud appears as a short stump and the 3rd and 4th branchial arches are distinctly concave. Rathke's pouch and the nasal processes start to form and towards the end of this stage the posterior neuropore begins to close.

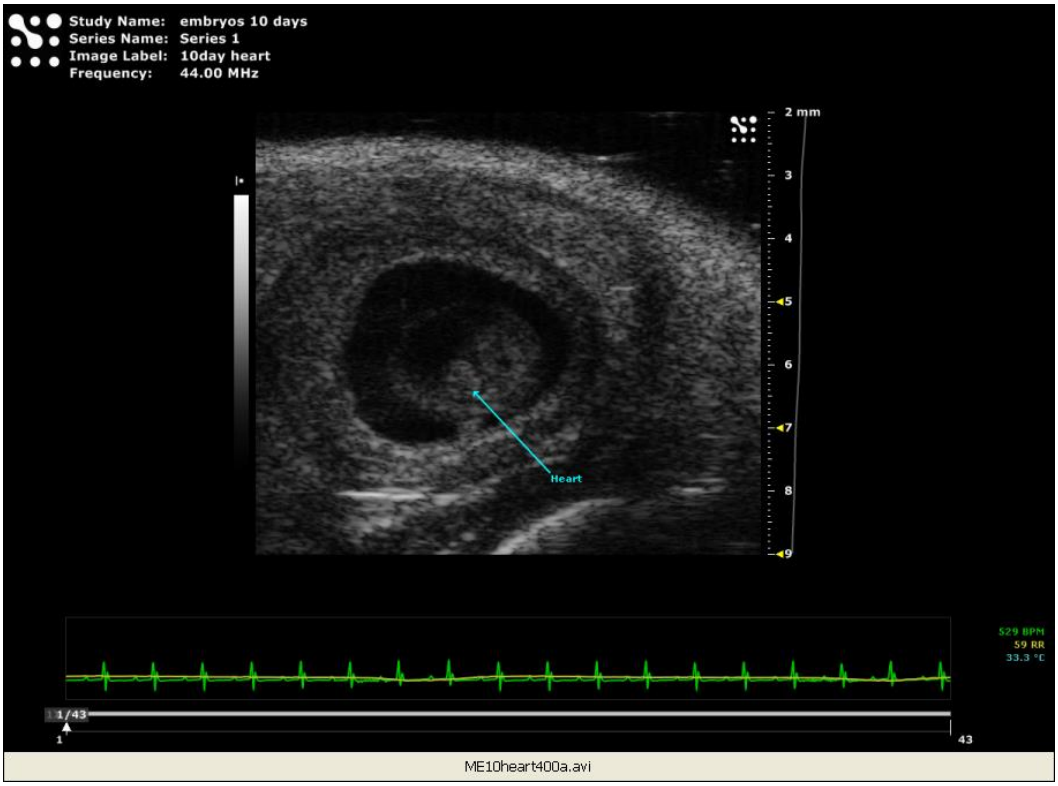

Figure 1 - B-Mode image of an embryonic mouse heart of E10 imaged at 44 MHz.

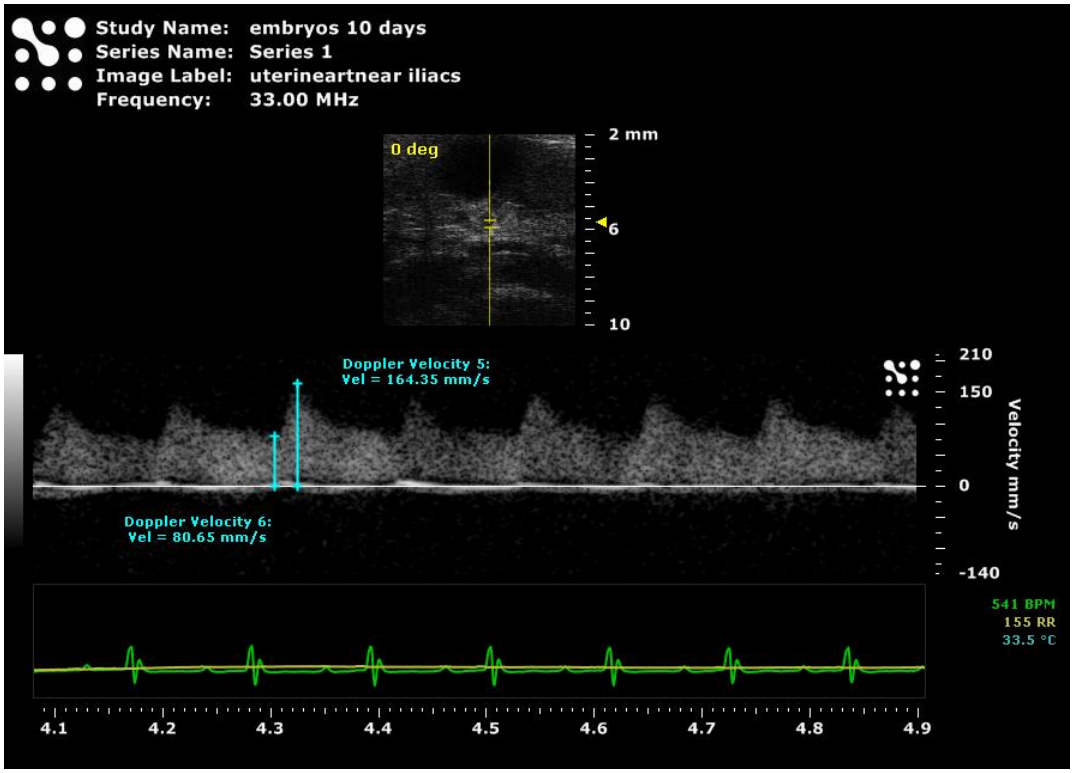

Figure 2 - Diastolic and Systolic velocity measurements on the uterine artery, E10.

Quick velocity measurements on the uterine artery at various time points could signal changes in tissue perfusion, as values of the Resistive Index change, which in turn could translate in preeclampsia development.

E10.5

Due to lack of significant development of the interventricular septum the RV and LV are analyzed together, however when imaging the distinction between the presumptive LV and RV becomes more apparent. The outflow tract is prominent and two parallel streams of blood flow are apparent. The combined ventricular area is 1.47+/- 0.33 mm2 in diastole and 0.86 +/- 0.18mm2 in systole. Given the constant remodeling of the embryonic heart during cardiac morphogenesis, calculation of ventricular volumes by use of geometric assumptions is likely to be inaccurate. The FAC as an index of contractile function FAC is 41 +/- 7%. The embryo, amniotic cavity and a chorio-allantoic placenta are visible as are the cerebral ventricles. Blood velocity waveforms from the umbilical artery and vein can be visualized and measured using PW Doppler Mode.

E11.5

The outflow tract is larger but no separation is evident. The combined ventricular area is 2.02 +/- 0.21 mm2 in diastole and 1.27 +/- 0.15 mm2 in systole. FAC is 37 +/- 5%.
A beating heart is visible as well as a neural tube and developing somites in the tail region.

Images of placental structures E12:

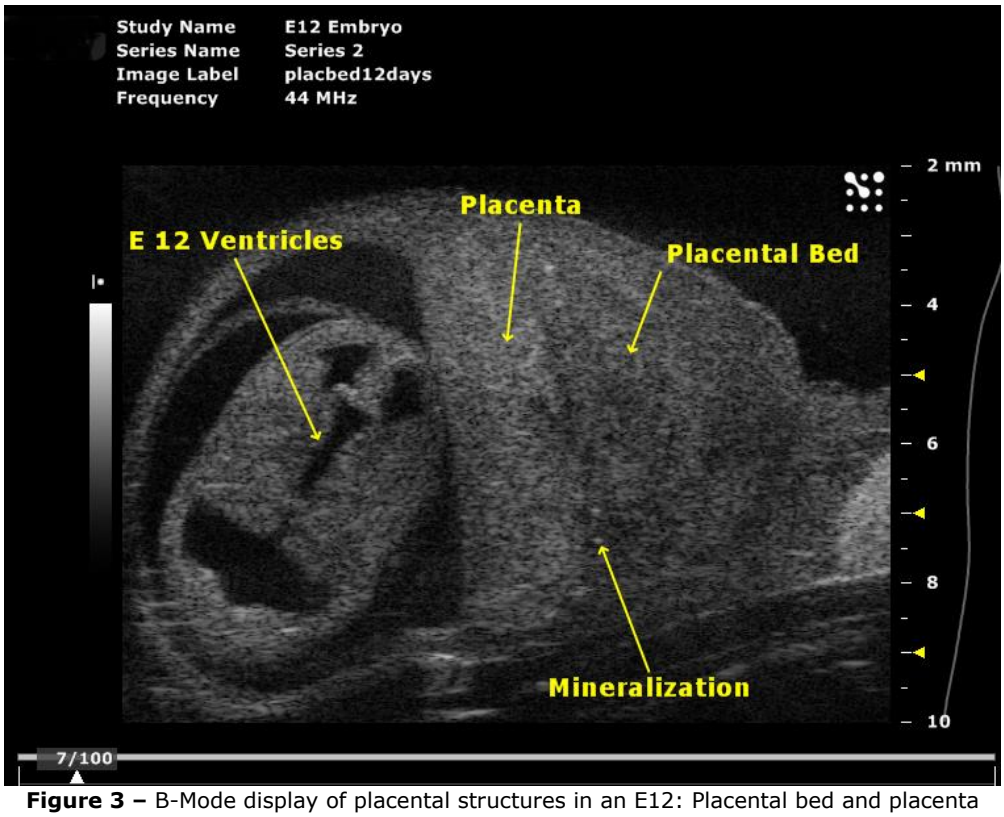

Figure 3 – B-Mode display of placental structures in an E12: Placental bed and placenta

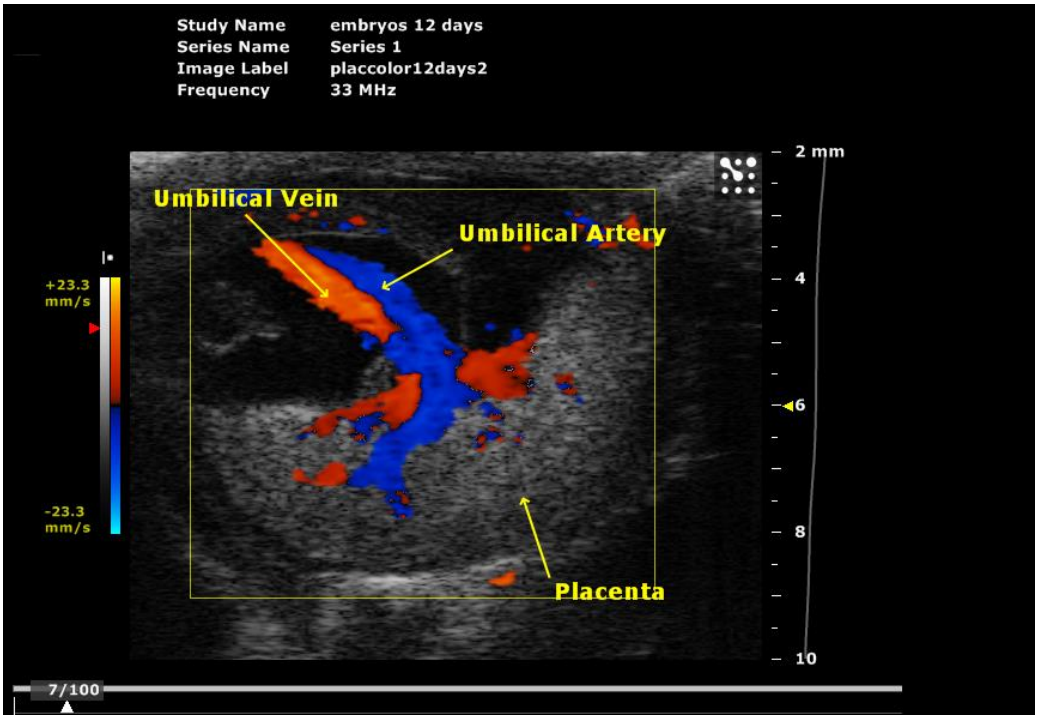

Figure 4 - Color Doppler Mode display of embryonic placental flow through the umbilical vein and artery.

Images of brain development E12:

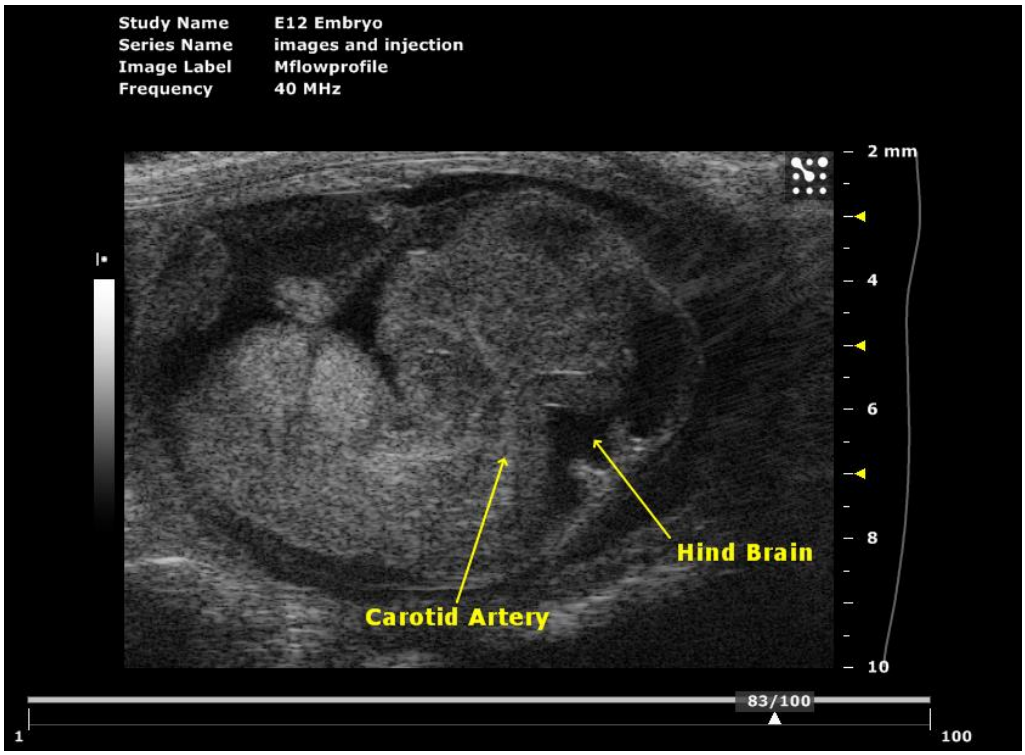

Figure 5 –B-Mode display of E12 profile with fourth ventricle

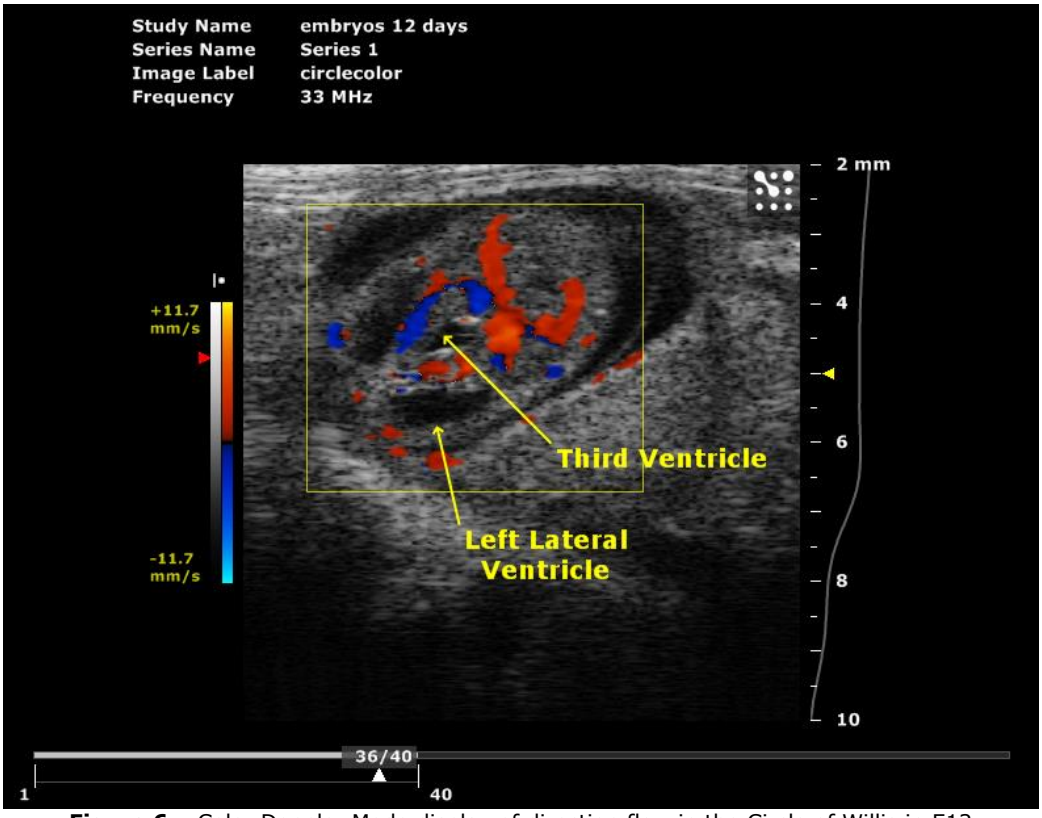

Figure 6 – Color Doppler Mode display of direction flow in the Circle of Willis in E12

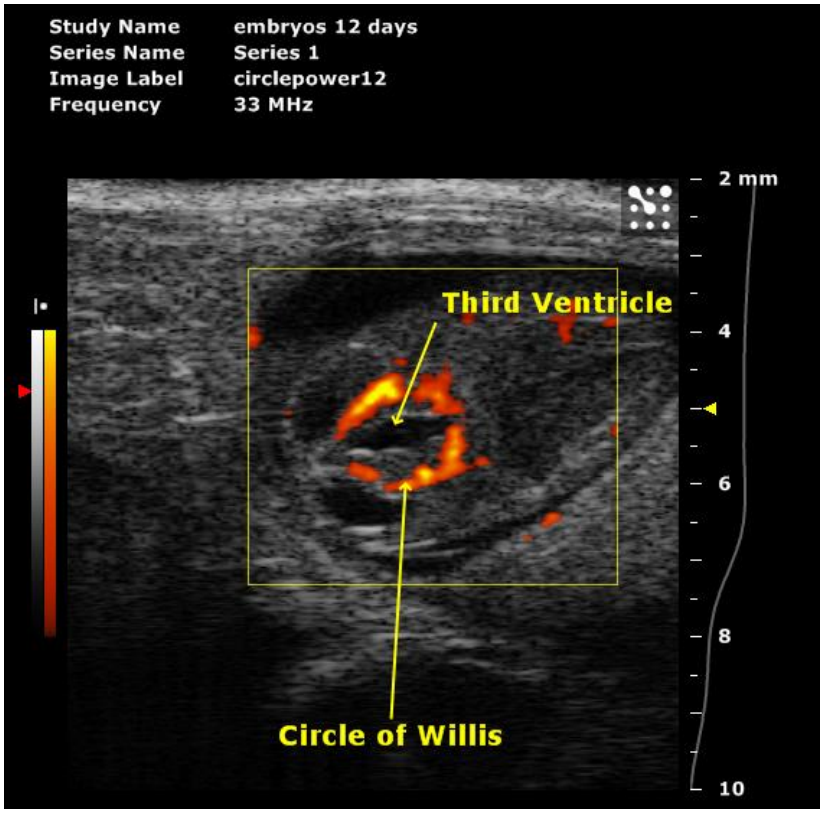

Figure 7 - Power Doppler Mode display of flow around the third ventricle in E12 brain

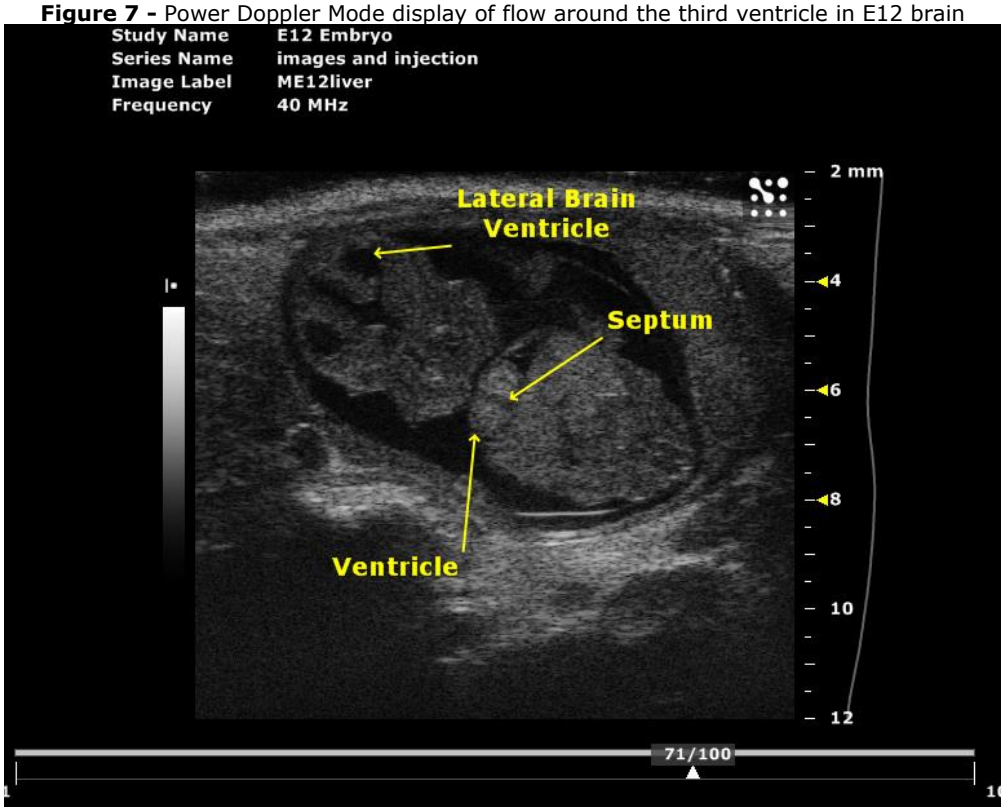

Figure 8 – B-Mode display of heart and brain structures in E12

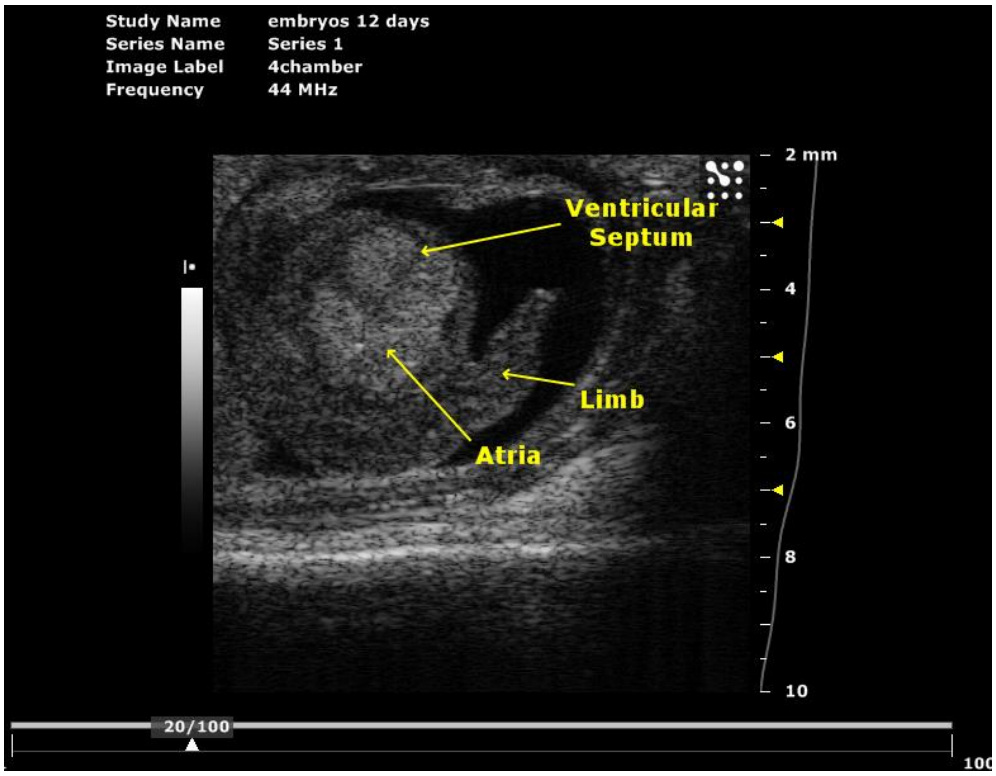

Figure 9 – B-Mode display of heart structures for E12 heart: with both ventricles and atria and the dark interventricular septum with the whiter, stronger echo from the blood.

E12.5

The difference in shape of the two ventricles is apparent; the right ventricle appearing more triangular compared to the left ventricle, which is more ellipsoid. At this stage, the left ventricle and right ventricle are well balanced with no significant difference in size. FAC is similar for both ventricles RV, 32 +/- 7%, LV, 34 +/- 5%. The separation of the aorta and main pulmonary artery appeared complete, but the interventricular septum is visibly incomplete and flow streams from both ventricles can be seen entering the aorta. A distinct 'C'-shaped aorta can be traced, arising from the LV and passing to the right of the pulmonary artery before looping to the left.

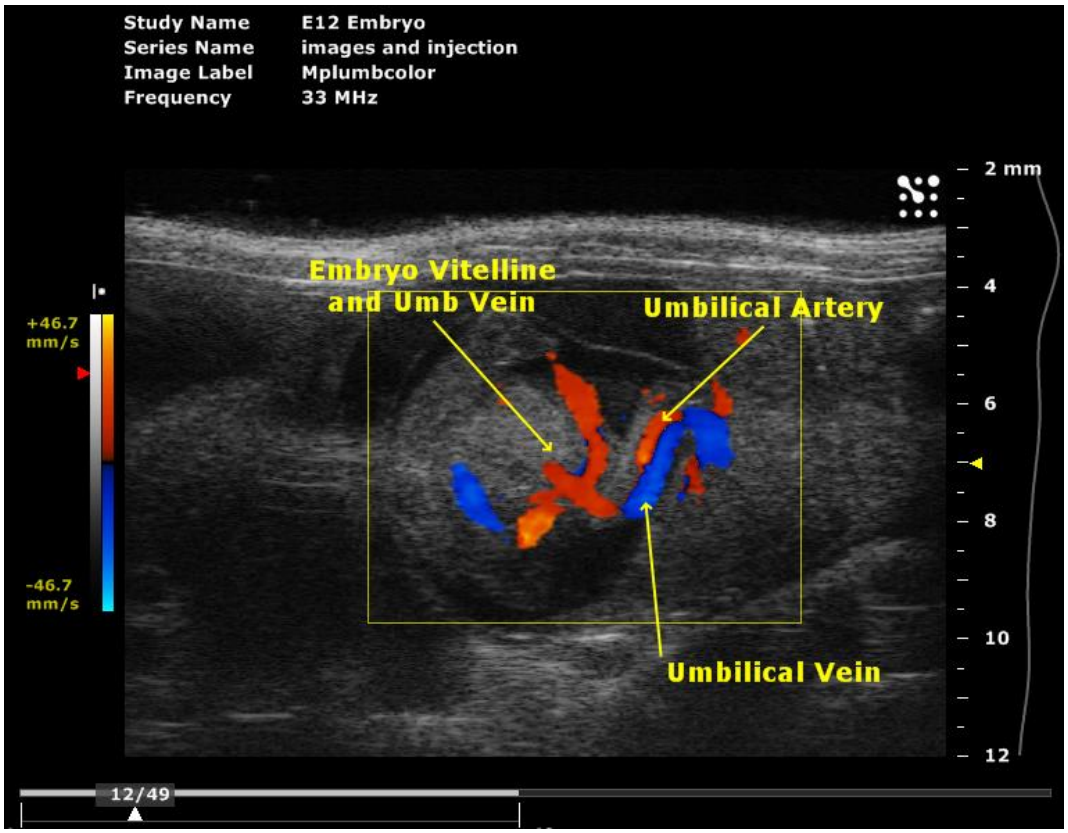

Figure 10 - Umbilical vein and artery flow at E12 as it flows into the liver.

E13.5

The placenta changed from a discoid structure to a larger planoconvex structure with prominent sinusoids on its outer surface. The embryonic ventricles are fully septated, the atrioventricular valves are visible and the heart has a mature fetal form with a heart rate of about 196 +/-27 bpm. The pulmonary artery and interwining aorta are visible. FAC for the RV is 34 +/- 6% while for the LV is of 34 +/- 6%.

E14

Fingers separate distally. Individual 'fingers' are visible in the anterior footplate and there are deep indentations between the 'toes' which are not yet separated. The long bones of the limbs are present are there are hair follicles in the pectoral, pelvic and trunk regions. The pinna is turned forwards and the umbilical hernia is conspicuous. Absent: hair follicles in the cephalic region and 56-60 somite pairs.

E 14.5

The forelimb buds and the spine are visible as well as early eye development, the third ventricle and the superior horns of the lateral ventricles. The interventricular septum fully separates the ventricular inflow tracts of the embryonic heart. Both ventricles of the embryonic heart at this stage are similar in shape and size. The heart rate is about 180 beats/min. The width of the mitral and tricuspid orifices is about 300-350 µm.

Table 1: Diastolic function of both ventricles

Mitral flow	
Heart rate, beats/min	180+/-5
Peak E, cm/s	9.8+/-0.6
Peak A, cm/s	34.4+/-1.3
Peak E/A ration	0.28+/-0.02
Total VTI, cm	1.4+/-0.1
Peak E/total TVI ratio, s-1	7.0+/-0.3
Tricuspid flow	
Heart rate, beats/min	174+/-5
Peak E, cm/s	8.9+/--0.6
Peak A, cm/s	33.4+/-0.9
Peak E/A ratio	0.27+/-0.02
Total VTI, cm	1.5+/-0.1
Peak E/tital TVI ratio, s-1	5.9+/-0.3
LV IVRT, ms	47.4+/-2.4
LV %IVRT	14.3+/-0.6

E15.5

The heart chambers begin to darken in the ultrasound image and the ventricular wall, endocardium and septum become easier to discern. The improved contrast after this stage means that ventricular chamber dimensions and wall thickness measurements become feasible. Echogenic foci are sometimes visible scattered throughout the labyrinth region of the placenta.

The space within the rodent embryonic heart and blood vessels viewed at ≤ 14.5 days gestation appears brighter on ultrasound images, whereas this space generally appears darker in postnatal subjects. This is likely due to higher blood echogenicity caused by the short wavelength of high frequency ultrasound and the relatively large size embryonic blood cells. Red cell nucleation may also be a factor, because blood appears less echogenic in embryos near term (E16.5), when red cells are still large but are no longer nucleated. Embryonic heart rates are sensitive to the level of sedation and temperature and it is important to minimize heat loss.

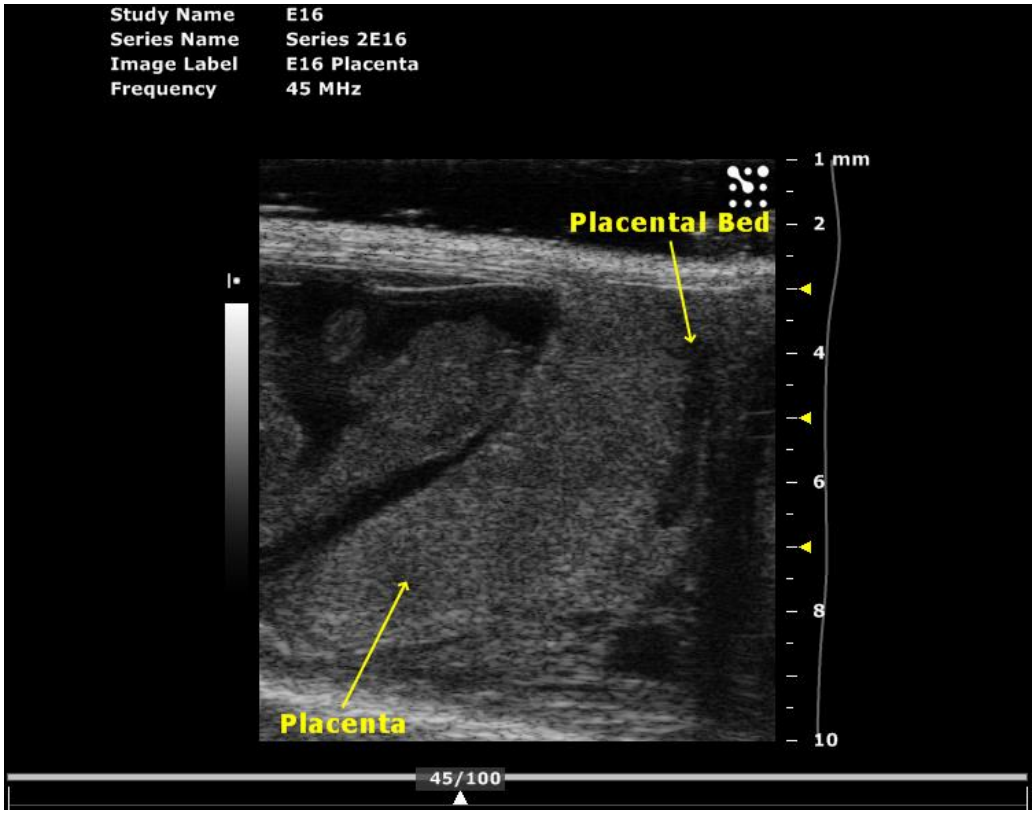

Figure 11 - Placenta at stage E16.

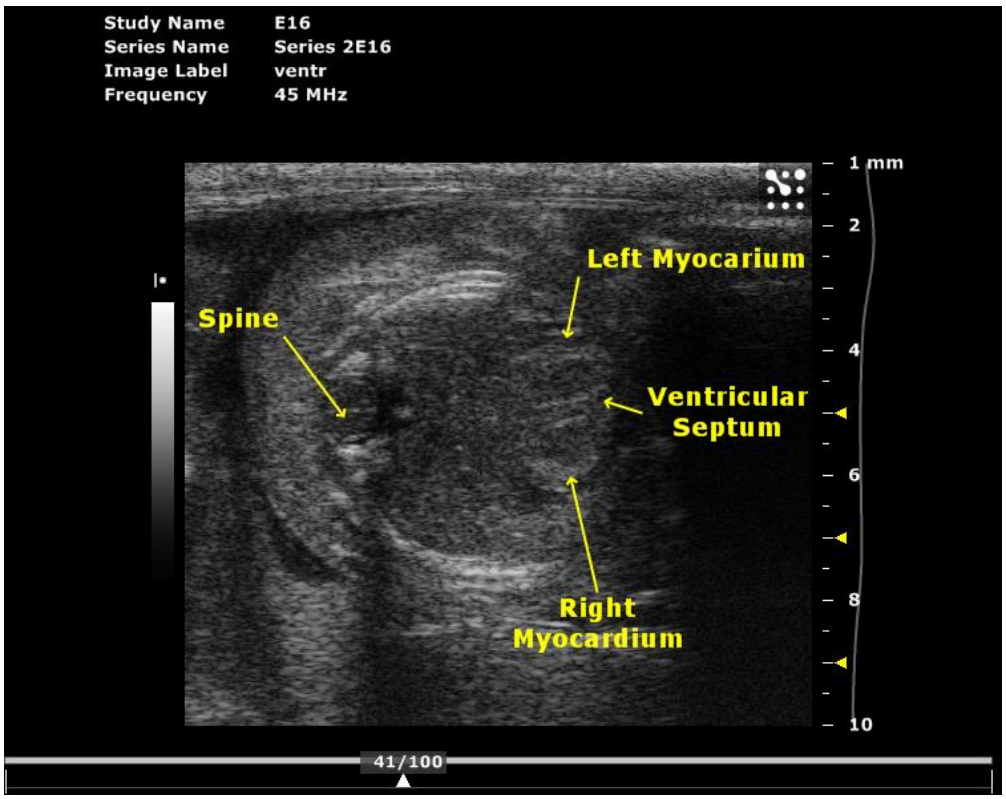

Figure 12 - Cardiac structures for E16: display of myocardium in the transverse view.

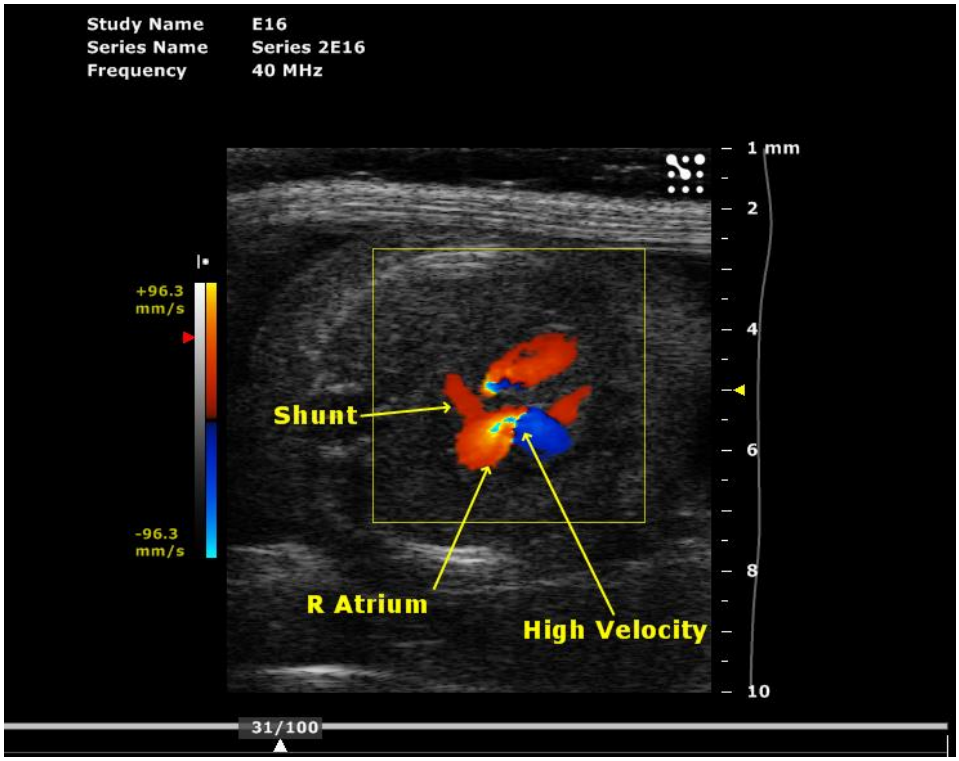

Figure 13 – Color Doppler Mode display of four chamber view shows right/left atrial shunting.

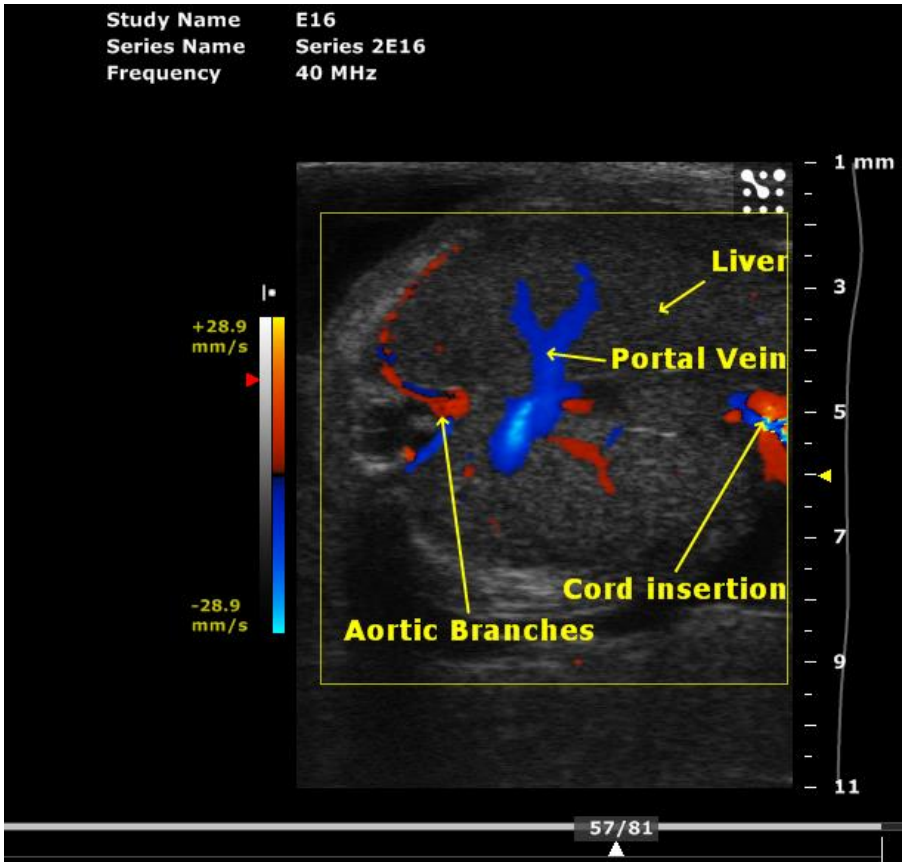

Figure 14 - Color Doppler Mode display of directional blood flow in the liver – E16.

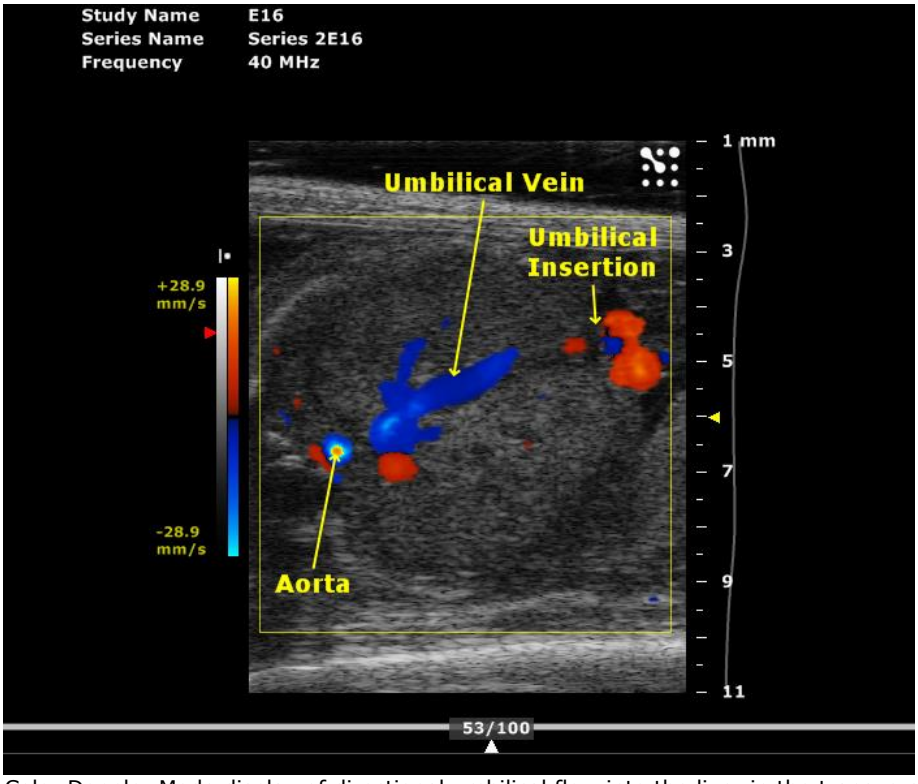

Figure 15 – Color Doppler Mode display of directional umbilical flow into the liver in the transverse view – E16

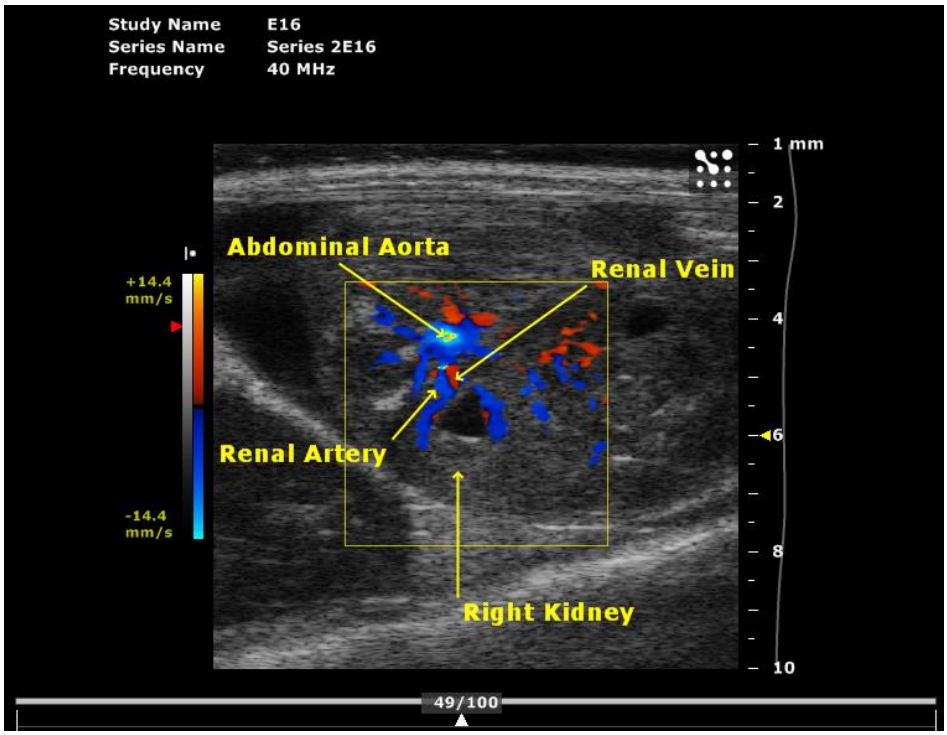

Figure 16 – Color Doppler Mode display of directional umbilical flow into the abdomen – E16

E17

The skin has thickened and formed wrinkles and the subcutaneous veins are less visible. The 'fingers' and 'toes' have become parallel and the umbilical hernia has disappeared. The eyelids have fused. Whiskers are just visible.

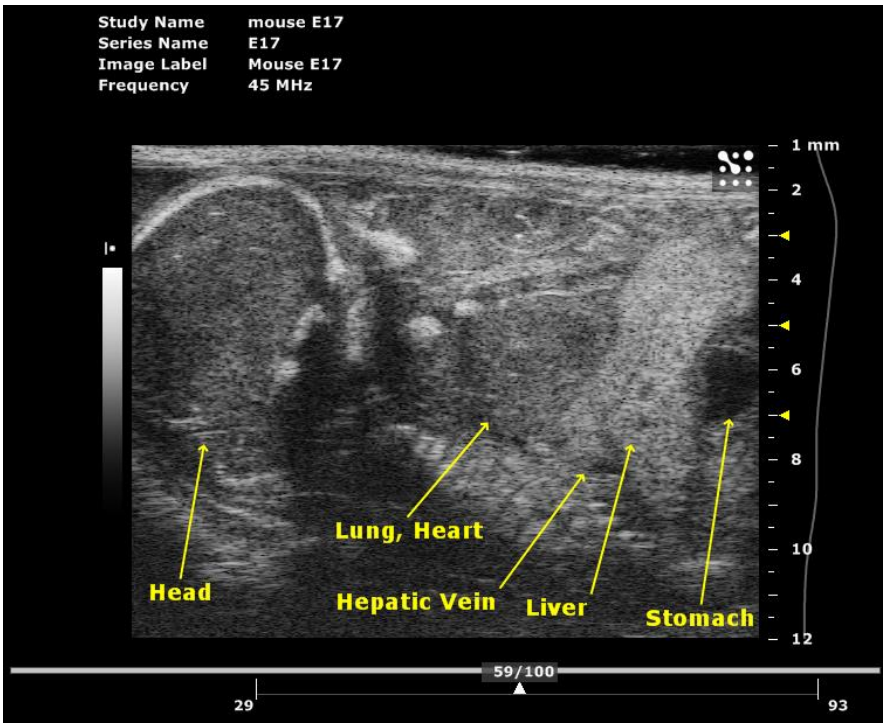

Figure 17 - B-Mode display of various major organs in E17.

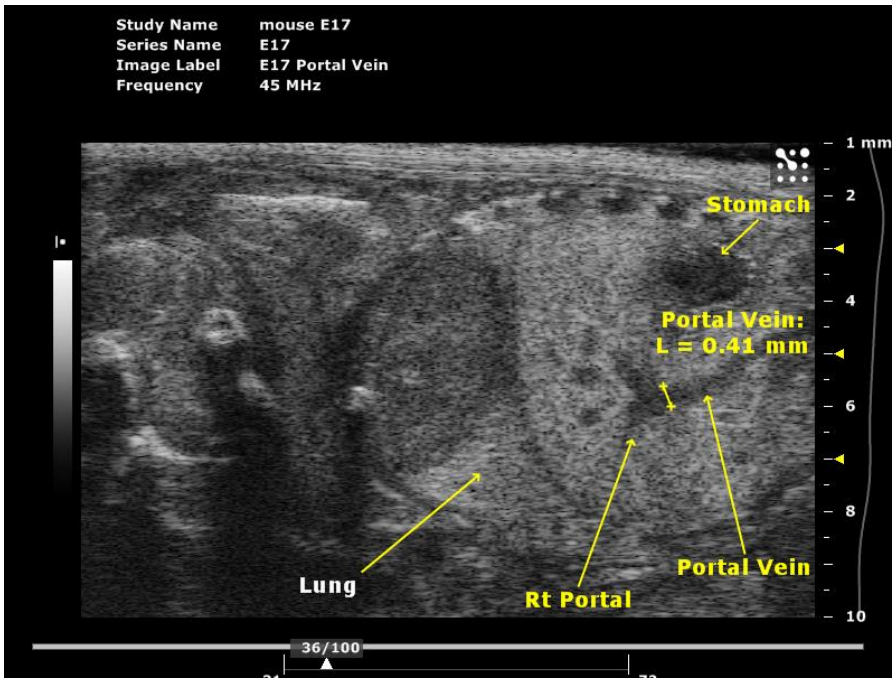

Figure 18 - B-Mode display of hepatic vasculature in E17.

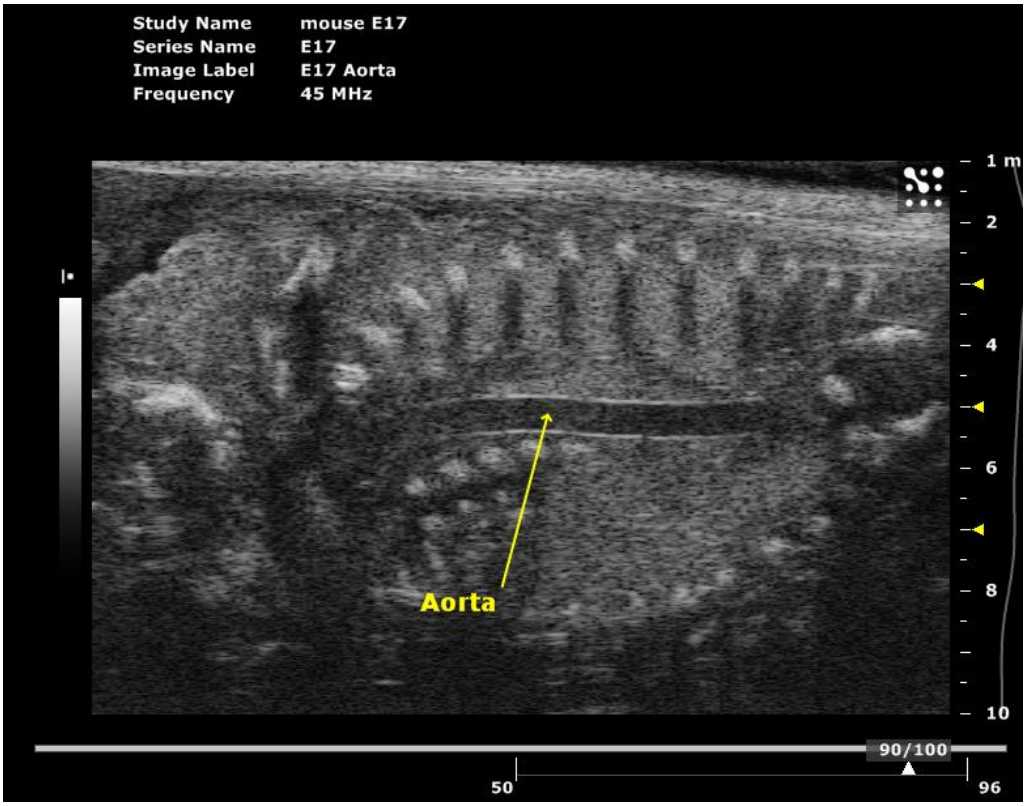

Figure 19 – B-Mode display of the abdominal aorta in E17.

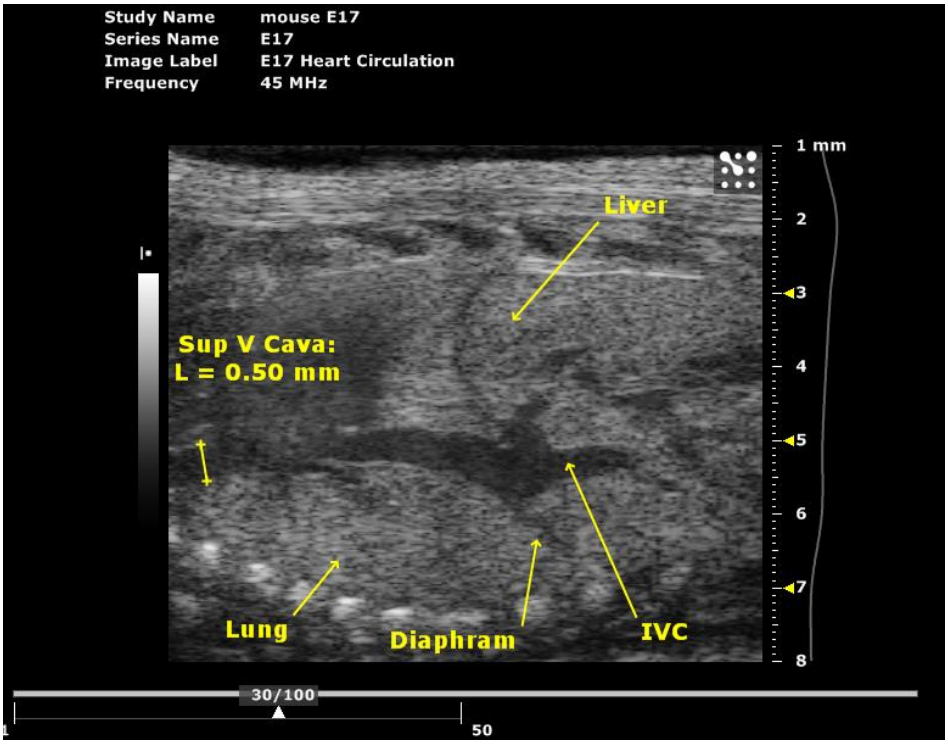

Figure 20 – B-Mode display of various structures E17

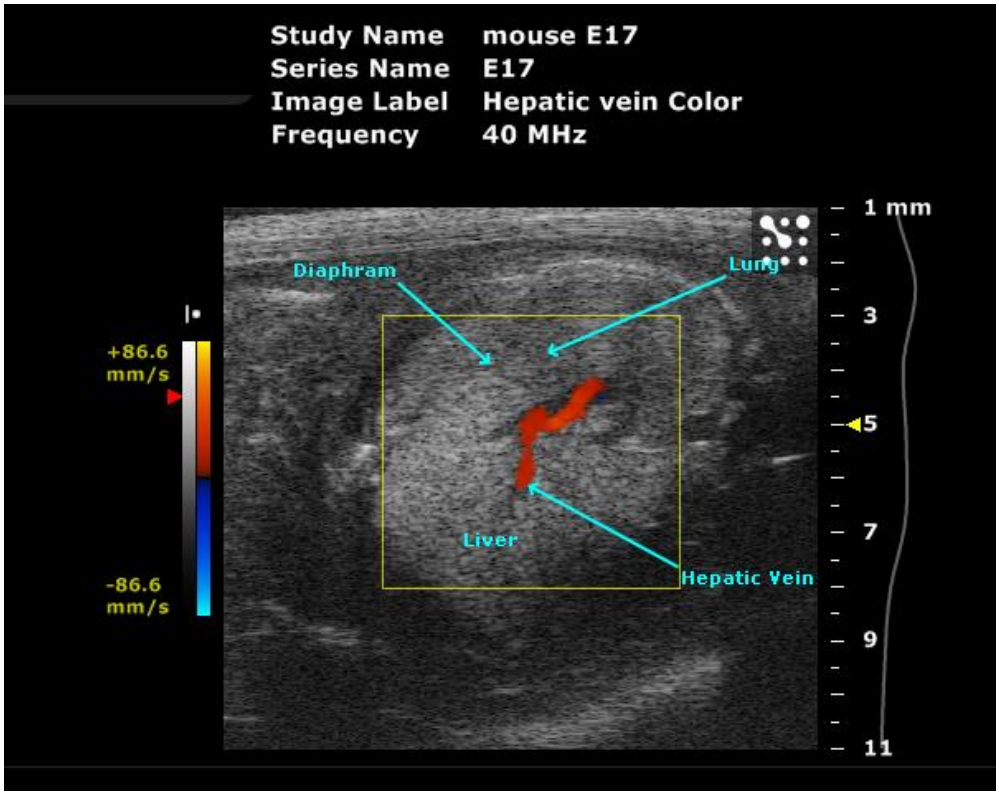

Figure 21 - Color Doppler Mode display various structures in the abdomen of an E17

E17.5

The width of the mitral and tricuspid orifices is about 450-500 µm.
Diastolic function of both ventricles:
Peak E velocity: 18 cm/s
Peak A velocity: 38 cm/s
Peak E/A ratio: 0.4

E18

The whiskers that were present at E17 are definitely longer and the skin has thickened. The pinna is larger and such that virtually none of the lumen of the auditory meatus is visible. The eyes are barely visible through the closed eyelids.

E18.5

Foci are visible throughout the labyrinth region of the placenta.

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