The Mouse Embryonic Imaging Guide

I.   Introduction

The mouse has always been considered a good embryological model of mammalian development because it has good-sized litters of offspring (about 8–20 offspring per litter) and a short pregnancy (about 21 days). The introduction of transgenic and gene target methods has increased the use of mice in the study of human diseases in recent years. Alteration or misexpression of genes induces phenotypic changes at early embryonic stages in mouse fetal development and in many diseases [1]. The heart is the first organ to develop and function during the development of the mouse embryo, and many mouse models with congenital heart defects suffer early embryonic death. Therefore, assessment of the structure and function of the fetal mouse cardiac system has become an increasingly important and popular issue. In this guide, we will provide an introduction to mouse fetal heart development. Of critical importance in such studies is the availability of tools that can achieve real-time evaluation and an approximately tenfold improvement of resolution compared to clinical ultrasound machine in order to monitor in utero progress during mouse pregnancy. A high-frequency (40–50 MHz) and high-resolution (~30 μm) ultrasound imaging system, called the Prospect system, which is a useful tool for visualizing live mouse embryos in utero, was used in this guide.

Before describing the scanning process, first consider the embryonic mouse cardiovascular system and its circulation. The cardiovascular system in mammalian embryos is composed of complicated vascular networks, both within the actual embryo and in the extraembryonic tissues, which aid its development. The embryonic mouse cardiovascular system has three main components: the embryonic, yolk sac, and allantoic or umbilical circulatory systems. The embryonic circulatory system is made up of the heart and the arteries and veins; the main branches of the latter two are the aorta and the cardinal vein. The yolk sac circulatory system comprises the vitellin arteries and veins, and the main branches of the allantoic or umbilical circulation are the allantoic artery and vein. The proper development and coordination of all three circulatory systems is crucial to maintaining normal cardiovascular function and ensuring embryonic survival. Defects in any one of these systems in utero are normally fatal [2].

Rhythmic cardiac contractions can be detected as early as the 5 somite stage [~embryonic day (E) 8.25], and blood flow is detectable by Doppler ultrasound by the 7–8 somite stages. At these stages, the mouse heart approximates a straight tube. Notably, the onset of cardiac activity coincides precisely with entry of primitive erythrocytes from the hematopoietic yolk sac into the embryo proper at these early stages. These findings indicate that the establishment and early development of the initial circulation are precisely coordinated to form a functioning pump, an intact vascular circuit, and oxygen-carrying erythrocytes to support the growing embryo. The earliest origins of a functional cardiovascular system occur well before the embryo becomes dependent upon the convection of oxygen and nutrients, but are probably necessary for cardiac morphogenesis and vasculogenesis [2] [3].

The mouse embryo circulatory system gradually matures during the late embryonic stages. In brief, maternal blood enriched with oxygen and nutrients flows from the umbilical vein into the inferior vena cava via the ductus venosus, where it mixes with deoxygenized blood. It then enters the heart via the right atrium,

where it mixes with blood from the right superior vena cava. In the right atrium, the blood flow divides into two paths. In one of the paths, the largest portion of the largely oxygenated blood flows through the inferior vena cava into the left atrium via a special fetal opening between the left and right atria, called the foramen ovale. From the left atrium, it mixes with a small amount of blood returning from the undeveloped lungs through the pulmonary veins, moves into the left ventricle via the mitral valve, and is then pumped into the aorta. The ascending aorta provides blood to the head and upper extremities, and blood travels from the descending aorta to the other areas of the body, then through smaller vessels to return to the placenta via the umbilical arteries. Returning blood from the head and upper extremities travels through the right superior vena cava, where CO_2-rich and nutrient-poor blood flows, and is pumped back into the right atrium. The second path comprises about one-third of the blood; the deoxygenated blood that enters the rostral through the right superior vena cava descends into the right ventricle via the tricuspid valve and is pumped through the right-ventricle outflow tract. Most of this blood is diverted through the ductus arteriosus and into the aorta, and a small amount continues on through the pulmonary arteries to the lungs [4]. A schematic diagram of the circulatory system in the late mouse embryonic stages is shown in Figure 1.
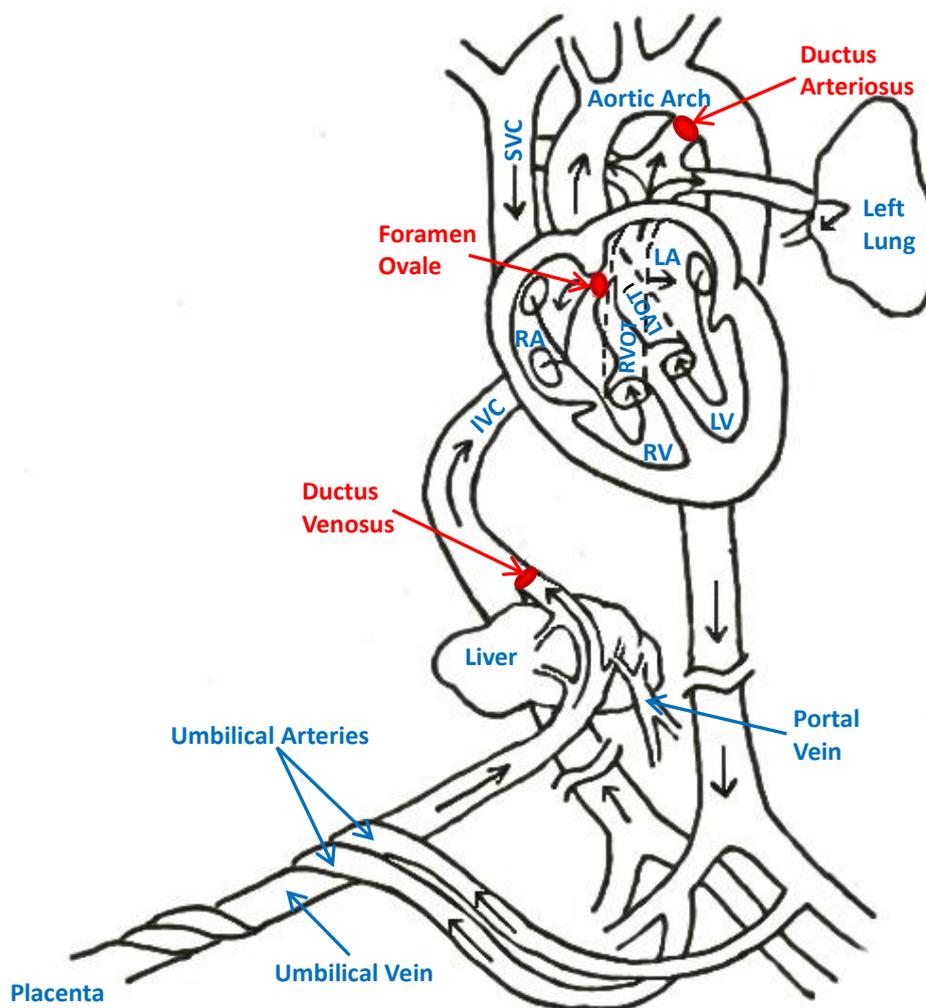

Fig. 1. Schematic diagram of the circulatory system in the late mouse embryonic stages. SVC, superior vena cava; IVC, inferior vena cava; RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; RVOT, right ventricular outflow tract; LVOT, left ventricular outflow tract.

The embryonic circulation network differs in many ways from that of adults in both humans and mice. The existence of the foramen ovale and the ductus arteriosus allows the circulatory system to run in parallel rather than in series. Most of the blood being pumped from the right side of the heart to the left thus bypasses the pulmonary circulation, which has a high resistance to blood flow, and most of the blood being pumped from the right ventricle passes through the ductus arteriosus and into the thoracic aorta. Only a small proportion of the blood will pass through the pulmonary arteries and into the lungs, and so the stroke volume is greater in the right ventricle than in the left ventricle [4].

There is a gradual increase in the embryonic heart rate and systolic cardiac work in order to meet the metabolic demands of the growing embryo. However, the continued increase in the mouse heart rate postpartum is an important part of its developmental maturation, as opposed to the postpartum relative reduction in heart rate observed in humans. Studies of myocardial diastolic function in mice have extended from the middle and late stages of pregnancy to the early postnatal and young-adult stages. The E and A waves (corresponding to the first and second peaks, respectively) across the atrioventricular valves are characterized by diastolic function, which can be measured by pulsed-wave (PW) Doppler ultrasound; diastolic function gradually matures from early gestation to the third week of postnatal life, with progressive improvements in active ventricular filling [3].

II. Animal model

Normal imprinting control region (ICR) timed pregnant dams were used in this guide. The changes in ICR mouse embryos were monitored on a daily basis, and especially their cardiac geometry and function, from E6.5 to E18.5, using in utero ultrasound with the Prospect system.

III. Embryos Scanning

The transducer is first placed above the maternal abdomen across the urethral opening to image the maternal bladder. Using the maternal bladder as a starting reference point, the uterine horns containing embryos situated on either side of the maternal bladder could be sequentially imaged. It is possible to scan adjacent embryos in order to track them; however, if there is any doubt, an embryo should be skipped to avoid double sampling, especially when spontaneous uterine movements are excessive. There are usually six to ten embryos available for imaging in each litter.

It is common to scan a fetus in the sagittal, frontal, and transverse planes when performing echocardiography (as shown in Figure 2). The sagittal and frontal planes in the embryo correspond to the parasternal long- and short-axis views in the adult mouse, respectively. The four-chamber apical view in the adult corresponds to the transverse plane through the fetal thorax. The sagittal view should be used to identify the atrioventricular valves. Using this view, the two ventricles and their synchronous contractions are readily visible. The right and left ventricles, and the interventricular septum could be displayed in the frontal plane. This is the optimal view for observing the changes in cardiac ventricular dimensions in M-mode imaging. The embryonic heart and arms can be imaged in the transverse plane. This view simultaneously reveals the left ventricle, right ventricle, and atrioventricular valves, corresponding to the four-chamber view in the adult mouse heart [4].
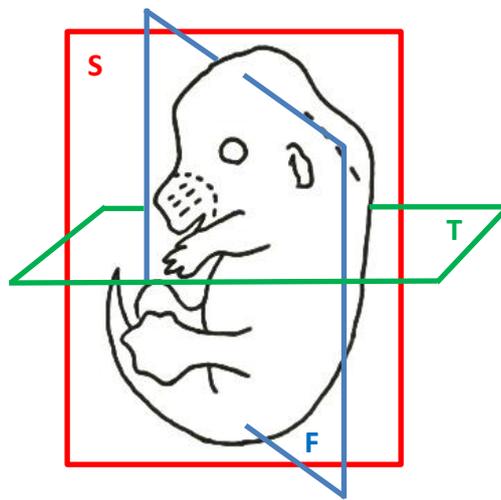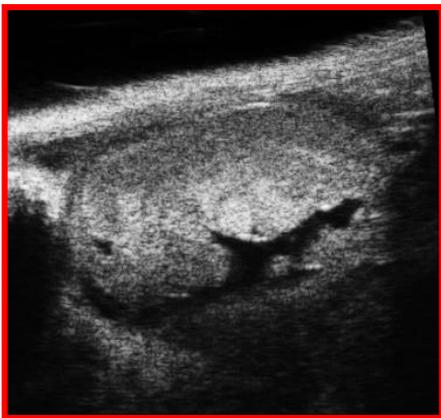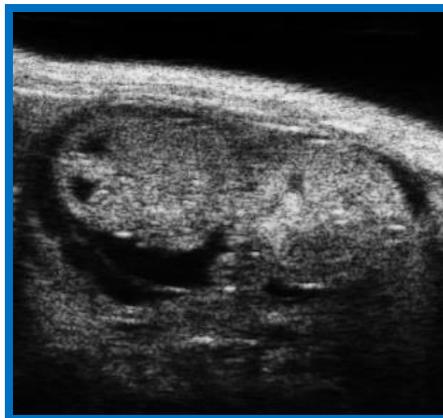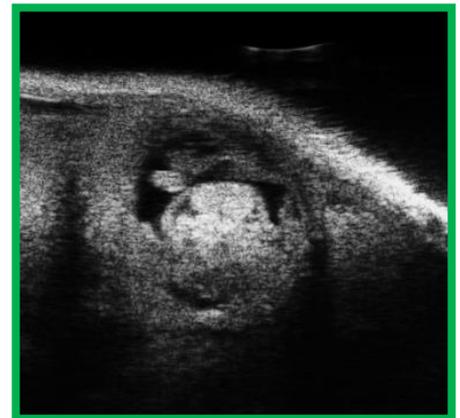
Fig. 2. (A) Schematic diagram of the fetus and the three orthogonal imaging planes. (B) Embryonic B-mode image in the sagittal plane at E13.5. (C) Embryonic B-mode image in the frontal plane at E13.5. (D) Embryonic B-mode image in the transverse plane at E12.5. S, sagittal plane; F, frontal plane; T, transverse plane.

B-mode imaging, which is a two-dimensional (2-D) imaging mode, allows observation of the target structure, positioning, and analysis of the physical parameters. For example, the changes in cardiac dimension during contraction may be used to gauge cardiac function, while the measurement of vessel size, in conjunction with Doppler flow waveforms, allows the calculation of volume flow. Use of B-mode scanning during the entire embryonic developmental period revealed that the size and structure of the embryonic left and right cardiac ventricles are similar. The volume of the left ventricle becomes significantly greater than that of the right ventricle after birth. The mouse embryonic heart can be readily identified on real-time images as a bright, highly echogenic structure with rhythmic contractions in B-mode images produced at frequencies of 40~50 MHz because the blood pool in the heart is echo-dense at these frequencies. As such, the blood vessels prominently appear in real time as dramatic speckle patterns representing blood flow. This effect is not evident on still frames since the moving speckle pattern is lost. On real-time video images, the umbilical vessels, vitelline vessels, dorsal aorta, great veins, and cerebral vessels can be all easily identified, and allow PW Doppler to yield clear blood velocity waveforms.

Doppler ultrasound is a noninvasive, in utero approach for measuring cardiovascular function. With the Prospect system, the color/power Doppler mode provides a 2-D color velocity map superimposed on the B-mode image, which facilitates the positioning of the targets of interest. PW Doppler mode, on the other hand, produces the spectrum (i.e., the velocity distribution) as a function of time. Employing the triplex function in PW Doppler mode (i.e., B-mode, color Doppler, and spectral Doppler images displayed simultaneously in the same window) makes it possible to locate the targets and conduct quantitative flow measurements. The analysis of cardiovascular function in mouse embryos has been further extended by including 40- to 50-MHz pulsed Doppler, which enables precise measurements of blood velocity waveforms. The higher-frequency Doppler transducers are also conducive to the acquisition of low-velocity blood flow signals. The PW Doppler beam should be aligned with the blood flow direction as accurately as possible so as to minimize anomalies in subsequent calculations of flow velocity, since the cosine of the angle between the flow direction and the Doppler beam can affect the calculations of the Doppler frequency shift. Furthermore, the Doppler shift frequency is proportional to the flow velocity, and so directly affects the estimation of flow velocity. It should be noted that blood is echo-dense, and the endocardial lumen cannot be visualized in the early to midgestation mouse embryo by using high center frequencies of 40~50 MHz [2].

That color Doppler and PW Doppler in combination with 2-D imaging is highly effective for determining the left-to-right orientation of the heart [4]. A great deal of information is available regarding the acquisition of images of the embryonic and extraembryonic circulatory systems using Doppler waveforms. Data are available on both the measurements of arterial and venous flow velocities and the visualization of the placenta. We have previously been able to discern clear Doppler signals from mouse embryos as early as E9, which appear as biphasic inflow patterns. Embryonic blood exhibits laminar flow with a parabolic spatial velocity, and therefore appears to behave like a Newtonian fluid. The study of major vascular development is aided by the intrinsic contrast of primitive nucleated erythroblasts at high frequencies [3]. Moreover, it is possible to obtain details of the mitral and tricuspid inflow in the frontal plane of the embryo by using PW Doppler. The shape of the Doppler waveforms across the atrioventricular valves is bicuspid. The first and second peaks (i.e., E and A waves, respectively) correspond to early ventricular filling of diastole and atrial systole, respectively. The velocity of the A wave is usually higher than that of the E wave, suggesting that atrial systole in ventricular filling is more important during the fetal period than postnatally [4]. It is important to note that the cardiac valves do not develop in the mouse embryo until E13.5. Prior to this stage, the dynamic apposition of the endocardial cushions is used to prevent the regurgitation of blood flow. Another vessel worthy of particular attention during the embryonic developmental period is the dorsal aorta. The embryonic dorsal aorta is the primary artery in the developing mouse embryo, and has a diameter of ~300 μm. Fundamental characterization of cardiac systolic function and blood flow in mouse embryos may be estimated using Doppler waveform analysis of the dorsal aorta [2].

IV.   Embryonic Staging and Main Developmental Events

The mouse embryonic development stages can be roughly separated into two periods: preimplantation and postimplantation [5]. A summary of mouse embryogenesis throughout development will now be provided to enable the features of embryonic mouse development to be more easily distinguished in ultrasound images. It should be stressed that when using the Prospect system, mouse embryos can only be visualized in the postimplantation period, around 6.5 days after conception. In this guide, embryos were staged in days of gestation, where day 0.5 (i.e., E0.5) was defined as noon on the day on which a vaginal plug was detected after overnight mating [1].

A. Pre-implantation Period

The various stages of the preimplantation period are described in the following sections.

E 0-1

Fertilized one-cell-stage egg (embryo). The embryo is located in the ampullary region [5].

E 1

Two-cell-stage embryo. The embryo can be seen passing down the oviduct, beyond its ampullary region [5].

E 2

Four- to 16-cell-stage embryos. These may range from early to fully compacted morulae. Embryos can be seen passing down the oviduct toward the uterotubal junction [5].

E 3

Embryos have developed from the morula to the blastocyst stage (zona-intact) during this stage, and possess a distinct inner cell mass and an outer layer of trophectoderm cells. This is the result of the polarization process. Such embryos are usually located in the uterine lumen [5].

E4

Embryos at this stage are invariably zona-free blastocysts and are located within the uterine lumen [5].

B. Post-implantation Period

The various stages of the postimplantation period are described in the following sections.

E4.5

During this stage, the blastocyst implants. The embryonic proximal or visceral endoderm cells appear as a unique layer covering the blastocoelic surface of the inner cell mass. The endoderm cells begin to migrate to the blastocoelic surface of the mural trophectoderm at a slightly later stage, and they complete this during E5. The extraembryonic distal (or parietal) layer of the endoderm is composed of this second group of cells [5].

E5.5

The number of cells constituting the inner cell mass is substantially increased by this stage, resulting in the embryo subsequently growing toward the abembryonic pole of the implanting blastocyst, to form the egg cylinder [5].

E 6.5

The Prospect system enables the early detection of the conceptus at E6.5. At this stage the inner cell mass, which is composed of the epiblast, primitive endoderm, and trophectoderm, has begun to form a cylindrical embryo [6]. Around the implantation in the lumen of the uterus, the embryo can be seen as a slightly brighter echogenic ring near the trophoblast–decidual interface. This bright region is approximately 250 μm in diameter [6] [7]. The circumference of the ring is fairly uniform in brightness, although the mesometrial side where the placenta will develop is brighter than the antimesometrial side [7]. Figure 3 shows the earliest detection of the conceptus at day 6.5.

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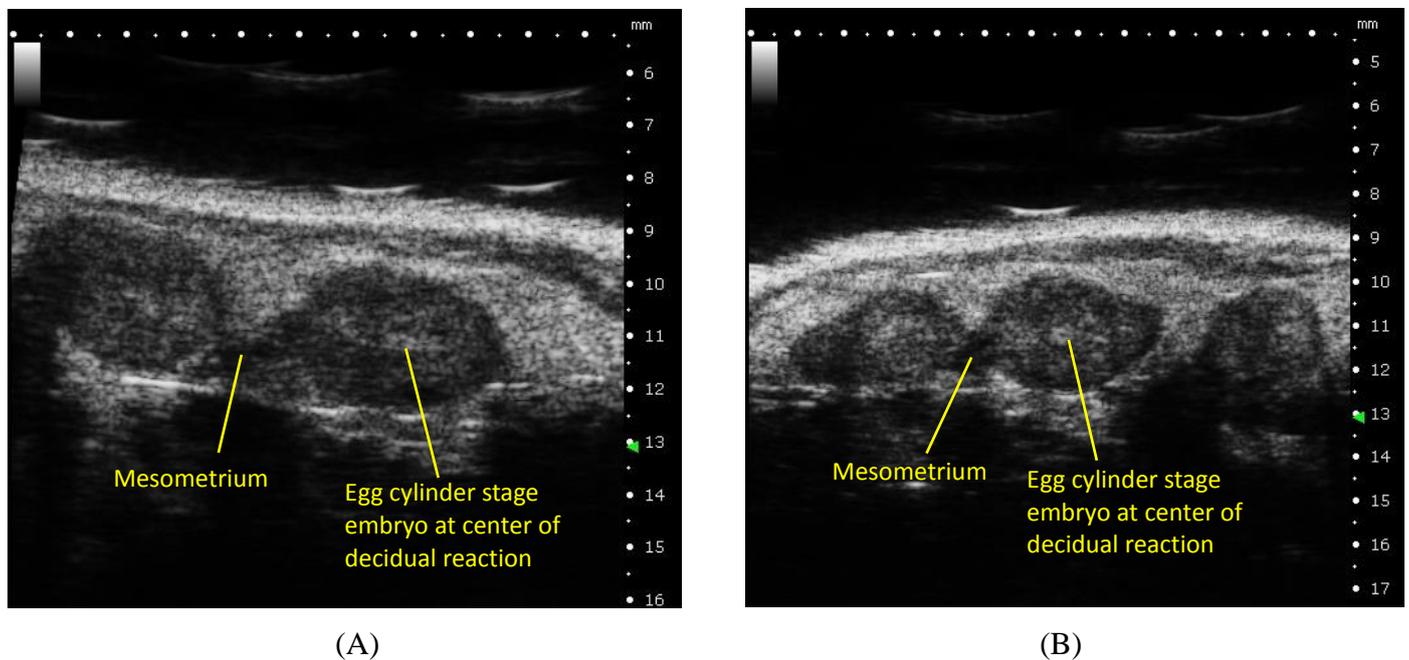
Fig. 3. Both (A) and (B) display the early development of the mouse embryo at E6.5, showing a slightly brighter echogenic ring in each embryo in B-mode imaging using the Prospect system.

E 7.5

By day 7.5 the embryo has developed three distinct cavities: the amniotic cavity (AC), the **exo**coelomic cavity (ECC), and the ectoplacental cavity (EC) [3] [6]. The bright echogenic region is localized primarily near the mesometrial side of the implantation site [7]. Shortly after the onset of this stage, the anterior and posterior amniotic folds amalgamate, resulting in the obliteration of the proamniotic canal and the formation of the ECC. This is attributable to the increased growing rate of the allantois into the cavity of the posterior amniotic fold. As a consequence of these events, the embryo becomes divided into the aforementioned EC, the ECC, and the AC, which are separated by the chorion and amnion, respectively. In the more advanced embryos observed at this stage, the expansion of the ECC and AC leads to a reduction in the EC volume [5]. B-mode images of E7.5 mouse embryos are shown in Figure 4.
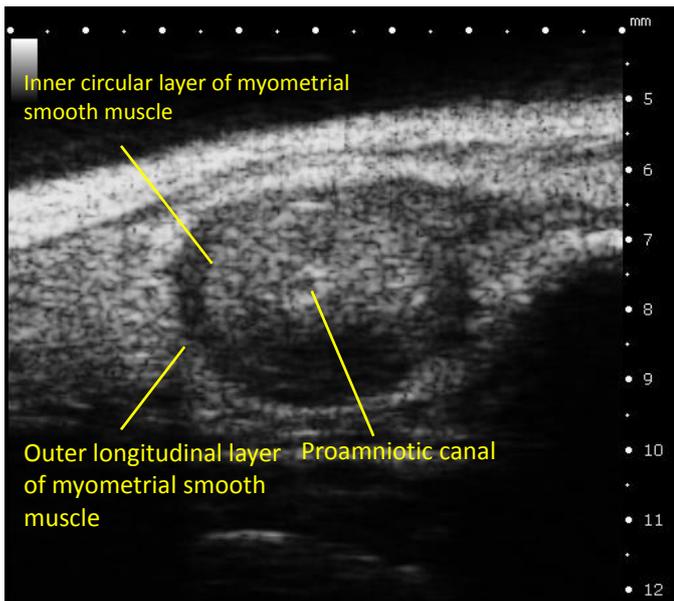
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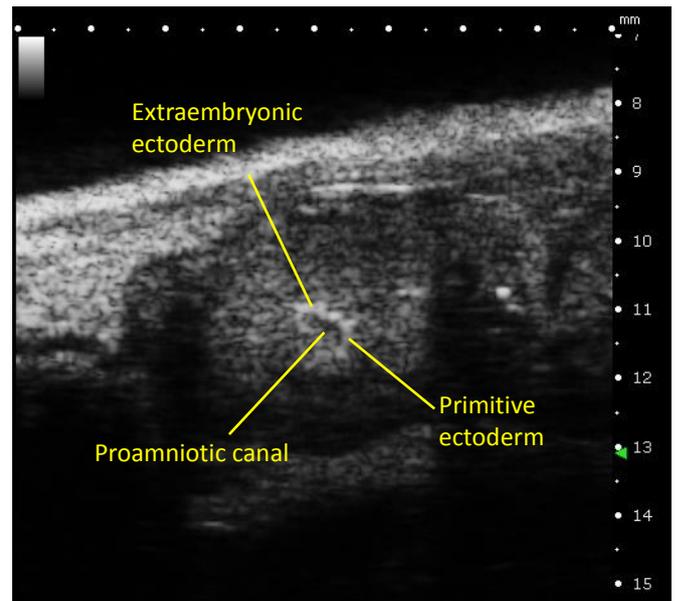
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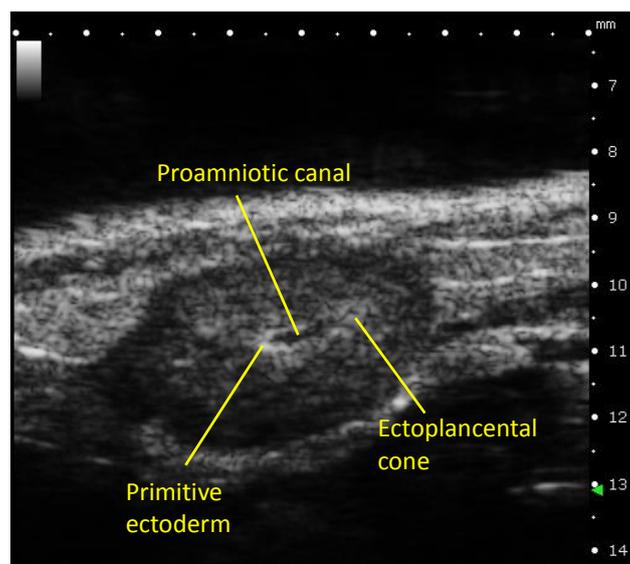
(C)

Fig. 4. B-mode images of E7.5 mouse embryos obtained using the Prospect system. (A) The proamniotic canal, inner circular layer, and the outer longitudinal layer of myometrial smooth muscle can be clearly discerned. (B) Visualization of the proamniotic canal, and the extraembryonic and primitive ectoderm. (C) The proamniotic canal, ectoplancental cone, and primitive ectoderm inside the uterus.

E 8.5

The developmental E8.5–E13.5 stages in mouse correspond to 3–6 weeks of human gestation. The earliest stage of heart and brain development in utero in a mouse embryo is at E8.5 [1] [8]. The allantois can be visualized and the embryo can be seen wrapping around the amniotic cavity [6]. The most conspicuous features evident at this stage on high-frequency ultrasound images are the open neural folds, which make it easy to distinguish between embryonic and extraembryonic tissues. Early heart activity in the primitive heart tube and the initial formation of the chorioallantoic placenta can be detected at this stage in more advanced E8.5 embryos [1] [4].

The embryo changes morphologically between E8.5 and E9.5, whereby its dorsal region changes from being the inside part of the U-shaped embryo (concave) to being the outside part (convex). A fluid-filled neural tube will be formed at E9.5, when the open cephalic neural folds start to grow and turn over at E8.5. This will eventually become the central nervous system of the adult mouse. Between E8.5 and E9.5 the primitive heart is transformed from a straight tube through a looping process to form a single atrium and ventricle. Then, at around E10.5 the initial stages of atrial and ventricular septation give rise to recognizable precursors of the final four cardiac chambers [1].

The heart is the first organ system to differentiate and to function, and is the most prominent organ present in the embryo at this stage. At E8.5 the heart is an asymmetrical globular structure in embryos with eight to ten pairs of somites, and the embryonic vasculature at this stage contains primitive nucleated red blood cells. At this stage the heart is divided into a common atrial chamber and a common ventricular chamber. The common atrial chamber receives blood from the right and left horns of the sinus venosus, and the common ventricular chamber is in direct continuity with the bulbus cordis region of the primitive heart, which in turn is in direct continuity with the outflow tract of the heart [5]. Transabdominal B-mode images of E8.5 mouse embryos are shown in Figure 5.
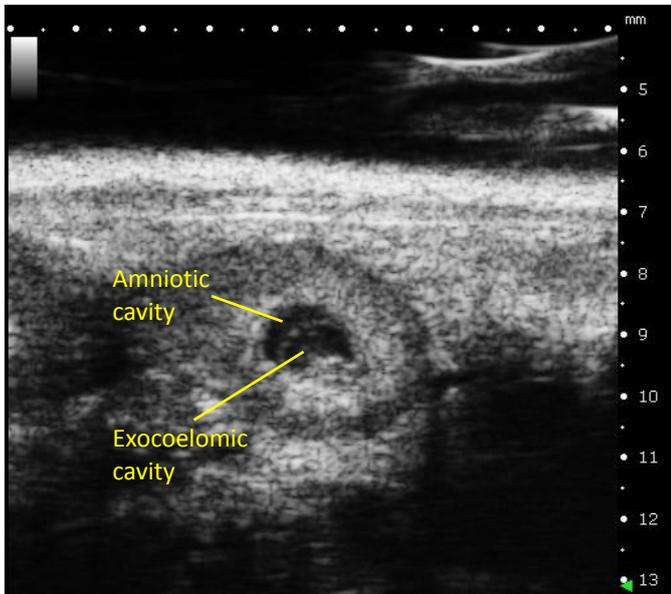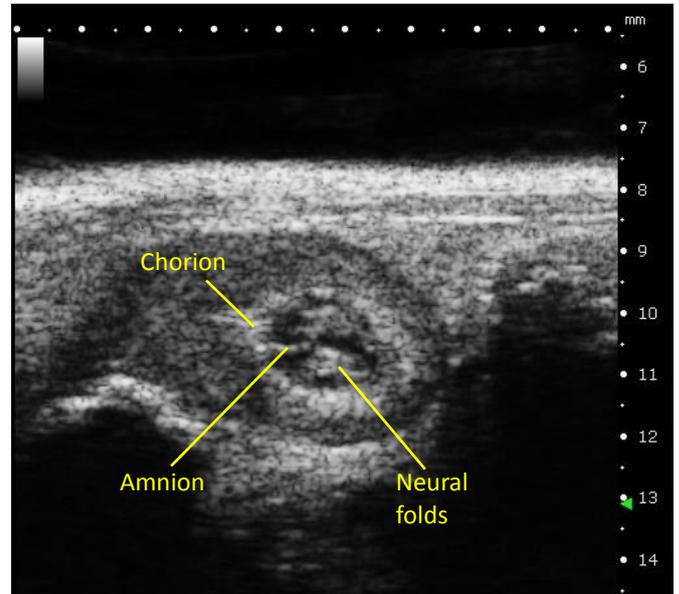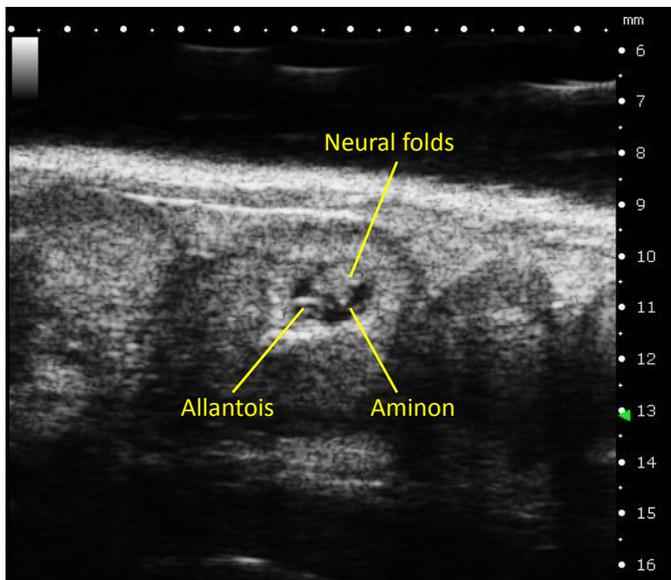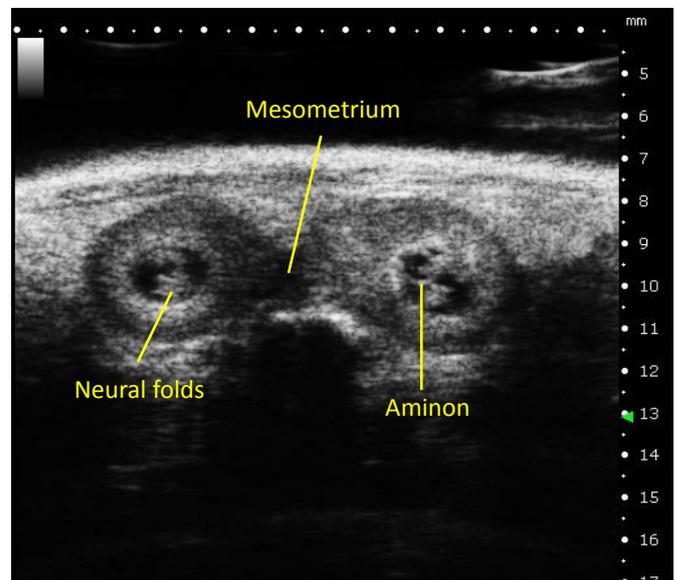
Fig. 5. B-mode images of E8.5 mouse embryos obtained using the Prospect system. (A) The amniotic and coelomic cavities of the embryo. (B) The neural folds, chorion, and amnion inside the uterus. (C) The neural folds, allantois, and amnion of the embryo are clearly visible. (D) The neural folds, amnion, and mesometrium, which connect two embryos, are displayed.

E 9.5

By E9.5 the neural tube is divided into three regions: forebrain, midbrain, and hindbrain. The neural tube and beating heart are the most prominent organs present in the embryo at this stage on real-time high-frequency ultrasound images [1]. In ultrasound images they appear as echogenic foci residing near the maternoplacental interface upon formation of the chorioallantoic placenta. As gestation progresses the echogenic foci will become more discrete and widely spaced at this location, and also larger and/or brighter in images than at earlier stages [7].

Cardiac looping can be visualized from E9.5, and the common atrium and ventricle can be distinguished. Simultaneously, synchronous atrial and ventricular contractions and the atrial contraction at end diastole are discernible [3] [8]. At E9.5 the overall volume of the common atrial chamber in an embryo increases substantially, and the component parts of the common atrium become more clearly delineated [5]. Between E9.5 and E14.5 the cardiac systolic work and output—such as heart rate, peak aortic flow velocity, velocity–time integral, and ejection time—increase geometrically, and become proportional to the cycle length which is defined as the total time of one complete injection cycle (in seconds) [2]. Images of E9.5 mouse embryos are presented in Figure 6.
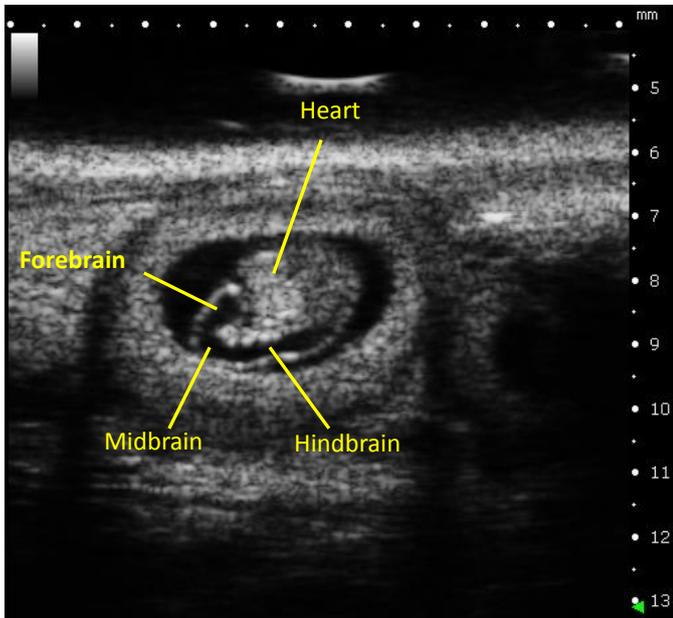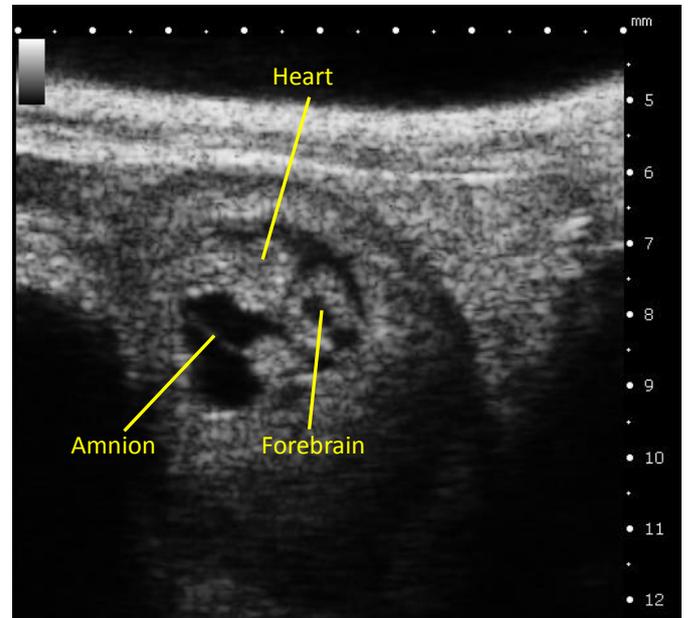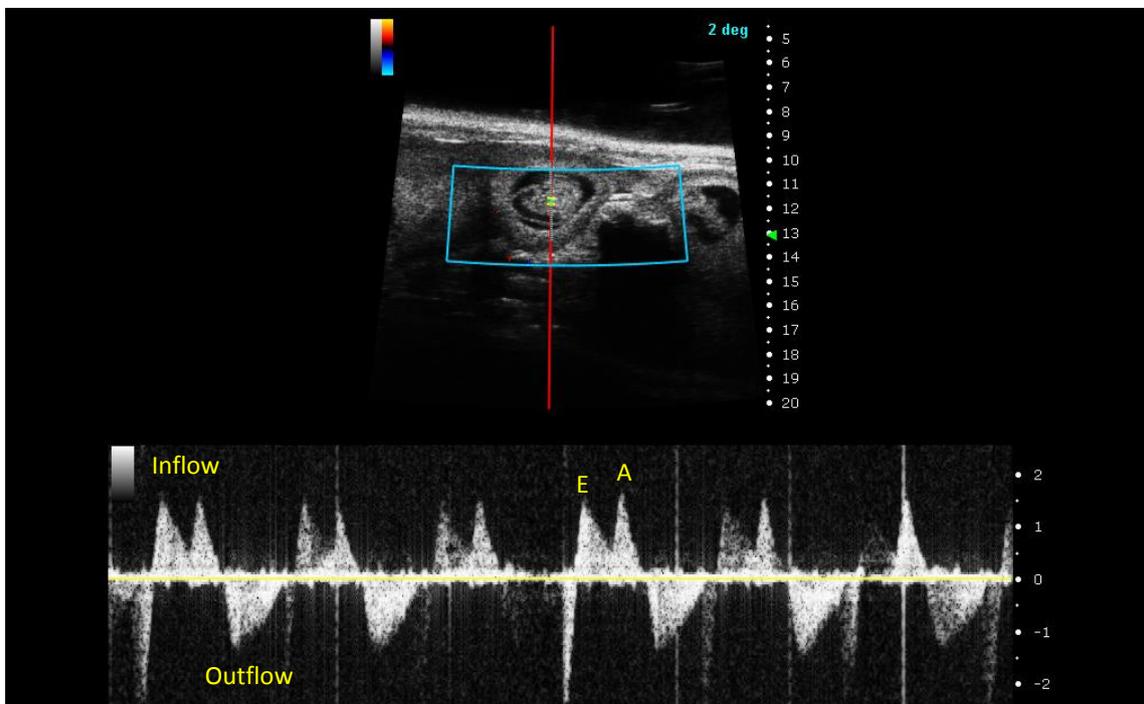
Fig. 6. High-frequency ultrasound images of E9.5 mouse embryos produced using the Prospect system. (A) The heart, forebrain, midbrain, and hindbrain of the embryo are clearly visualized on B-mode imaging. (B) The heart, amnion, and forebrain of the embryo on B-mode imaging. (C) At earlier developmental stages of the mouse embryo, the cardiac inflow and outflow signals are generated in opposite directions in the PW Doppler mode, since the cardiac inflow and outflow axis are at almost 180° from one another. The inflow Doppler waveform demonstrates the presence of separate E and A waves, with A-wave dominance.

E 10.5

At E10.5 the shape and subdivisions of the neural-tube cavity are easily identified on high-frequency ultrasound images. Sagittal views of the embryonic cardiac image demonstrate the allantois, pericardial effusion, ventricle, and the neural tube (forebrain, midbrain, and hindbrain).

The most obvious events that occur at this stage are the changes taking place in the embryonic heart and the vessels that emerge from the heart and drain into it. The most important changes within the heart are the earliest events associated with the process of septation, and a relatively broad and thickened region may be observed in the midline in the dorsal part of the wall of the common atrial chamber [5]. The atria and ventricles of the embryonic heart achieve a sufficiently well-developed state at E10.5 such that they can be readily visualized (Fig. 7A). By E10.5–E11.5 the distinction between the presumptive left and right ventricles becomes more apparent, the outflow tract is prominent, and two parallel streams of blood flow can be displayed in real time [1] [8]. It is worth noting that the major blood vessels, such as the aorta, bulbus cordis, or outflow tract, and the umbilical vessels can be distinguished due to the high-frequency ultrasound signals from the blood and the resulting moving speckle patterns on real-time images [1].

In addition, the limb buds become increasingly prominent at this stage, and their sharp apical ectodermal ridges are more clearly seen in the forelimb buds than in the hindlimb buds [5]. Furthermore, the liver primordium can be identified lying posterior to the heart; it undergoes its first expansion at E10.5. Between E10.5 and E13.5 the placenta changes from a discoid structure to a larger planoconvex structure, with prominent sinusoids on its outer surface [8]. Figure 7 shows images of the E10.5 mouse embryo.
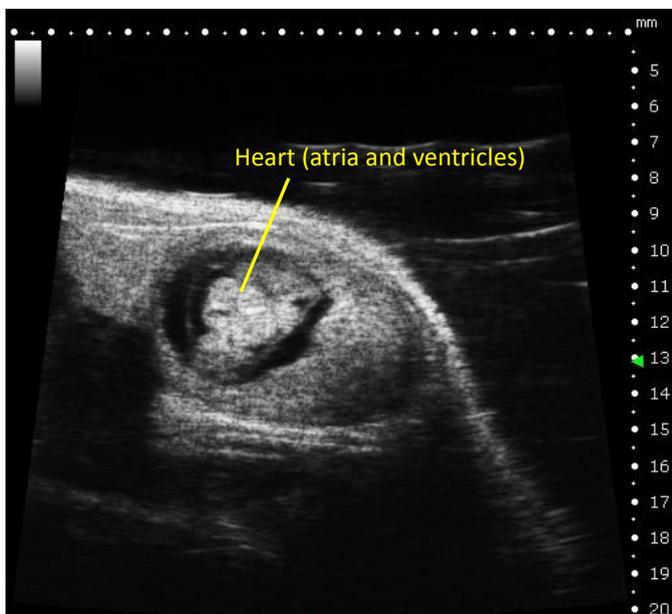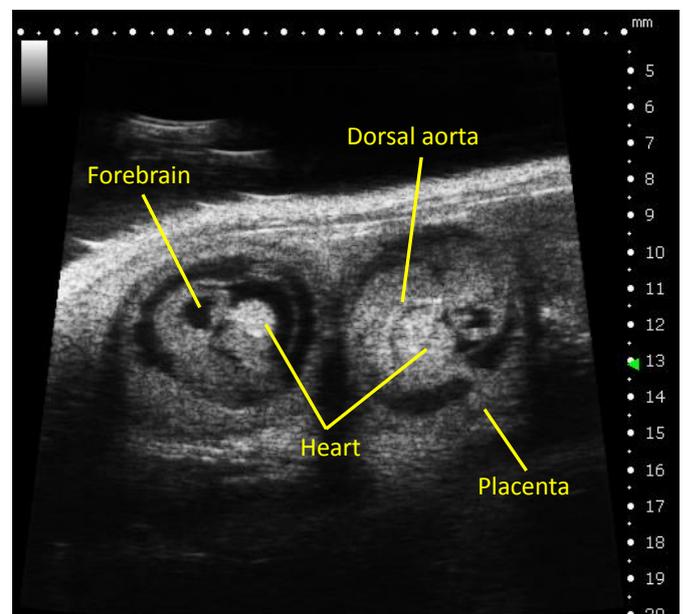
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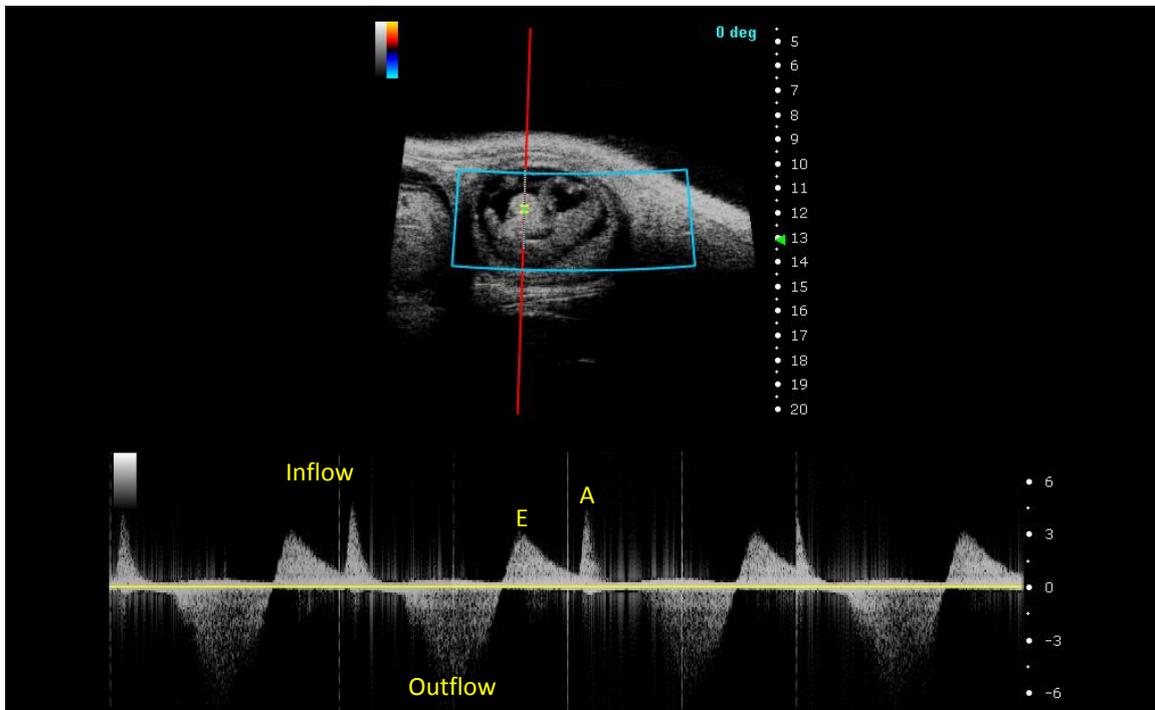
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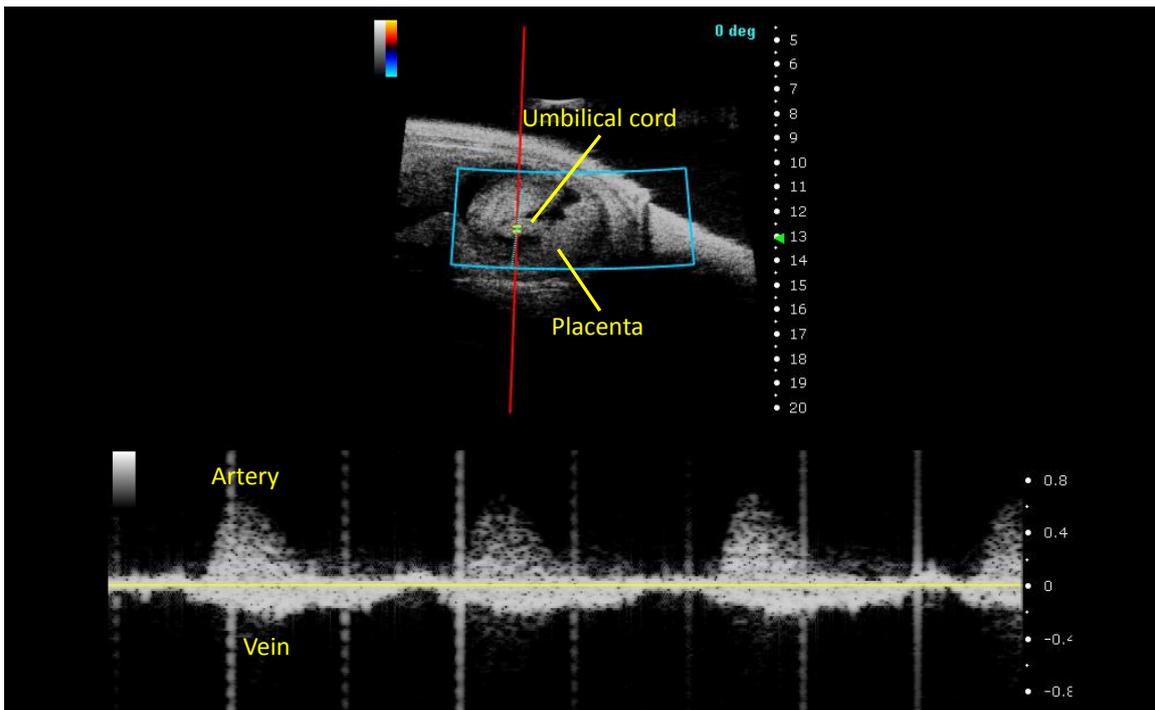
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Fig. 7. High-frequency ultrasound images of E10.5 mouse embryos obtained using the Prospect system. (A) The heart of the embryo, including the developing atria and ventricles, can be identified on B-mode imaging. (B) The heart, dorsal aorta, and forebrain of the embryo are clearly evident on B-mode imaging. (C) PW Doppler waveforms of mouse embryonic cardiac inflow and outflow. Increased inflow and outflow velocities can normally only be observed in older embryos. (D) PW Doppler image of the umbilical cord. The vessels of the umbilical circulation can be distinguished by PW Doppler imaging. Since the respective arteries and veins flow in opposite directions, their Doppler waveforms are distinct and appear on either side of the velocity baseline.

E 11.5

At E11.5 the beating heart, the neural tube, and developing somites in the tail region are visible in longitudinal views of the embryo; the crown–rump length can be measured in this view [6]. The most obvious events that occur at this stage are septation of the outflow tract, atria, and ventricles. The details of these events are described below.

The undivided outflow tract can be divided into three parts: the aortic sac, truncus (anteriosus), and conus. These different parts contribute to the outlets, valves, and bases of the aortic and pulmonary trunks, respectively, during septation of the common outflow tract, and are formed by E13.5 [9]. Atrial septation occurs at this stage, which involves growth of the septum primum toward the atrioventricular bulbar cushion tissue within the common atrial chamber of the heart. The peripheral margin of the septum primum appears to be quite bulbous at E11.5 [5]. The interventricular septum is derived from the region of ventricular wall adjacent to the bulboventricular groove, which is apparent at this stage, although the connection between the future ventricles is still open through the bulboventricular canal. This process is known as ventricular septation. The interventricular septum will be produced between the future ventricles at later stages of development [9]. Images of the E11.5 mouse embryo are shown in Figure 8.
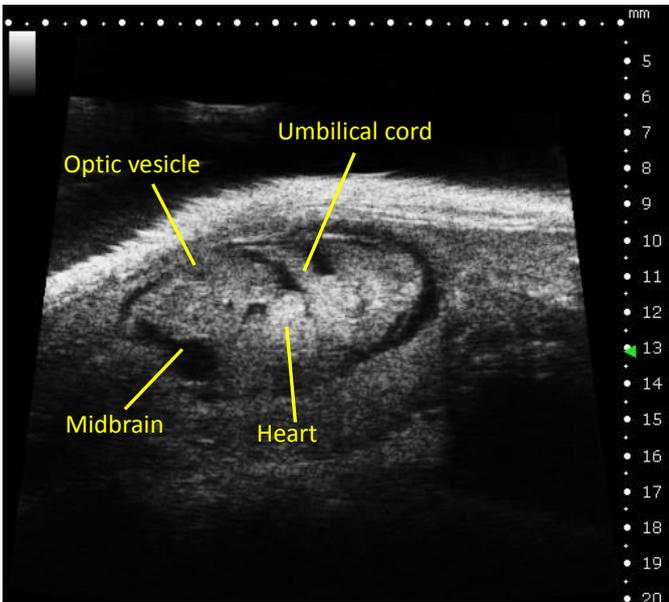
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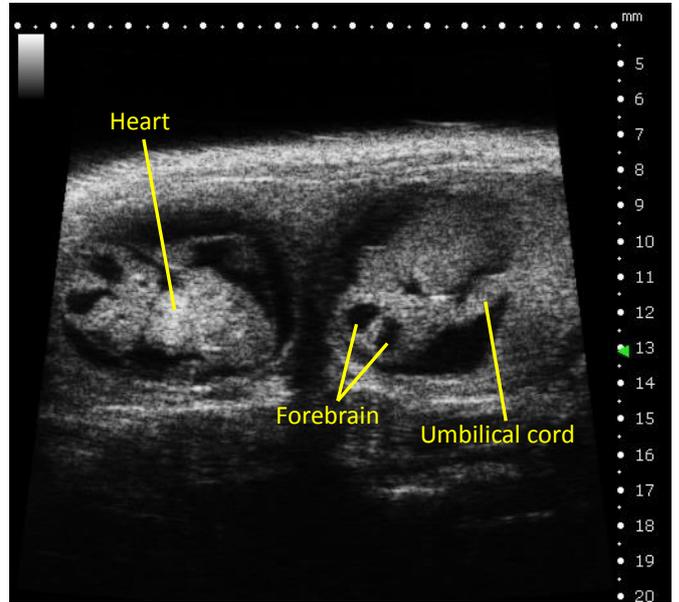
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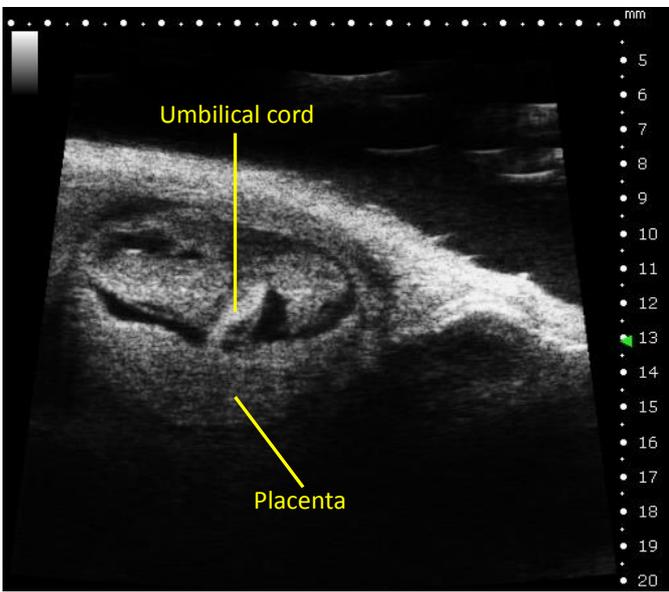
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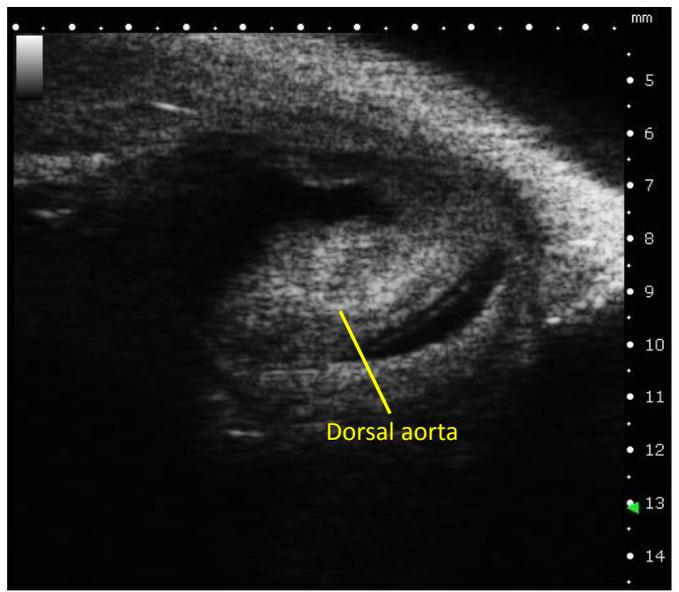
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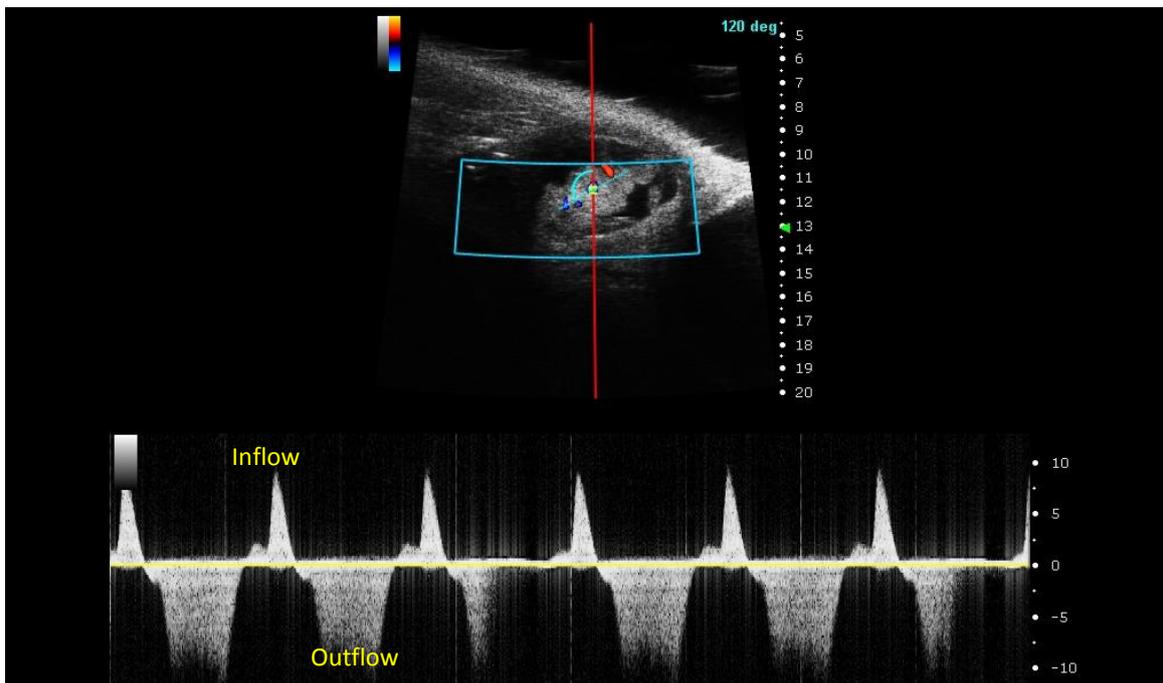
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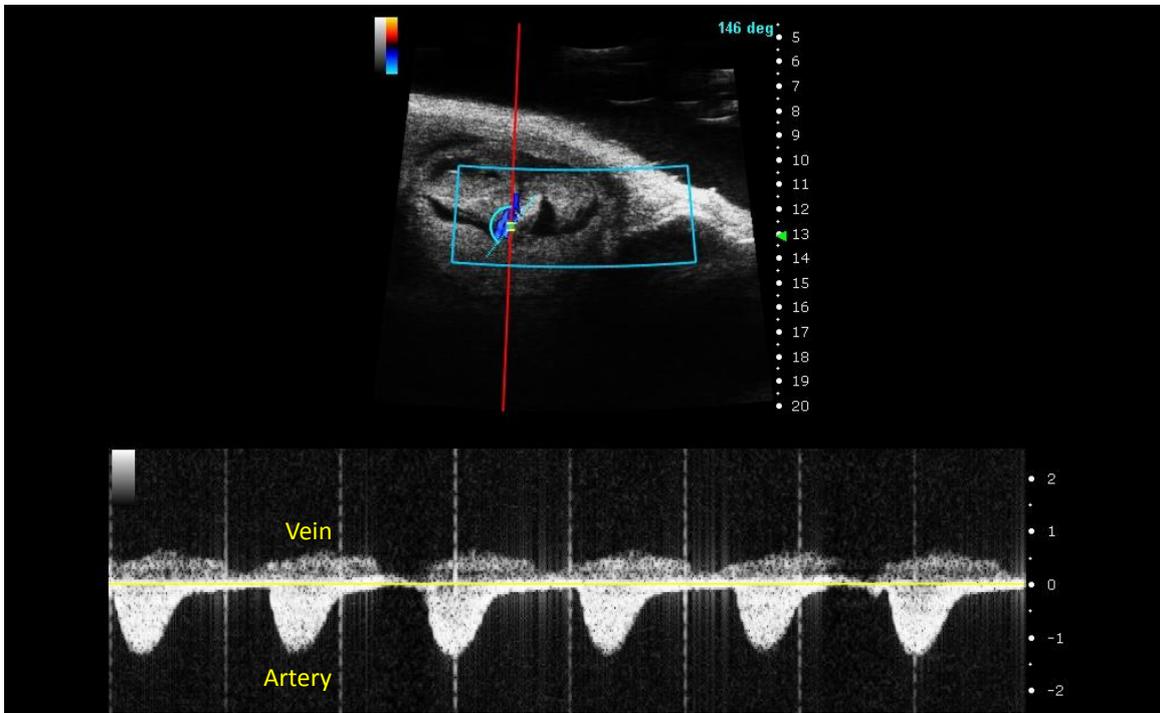
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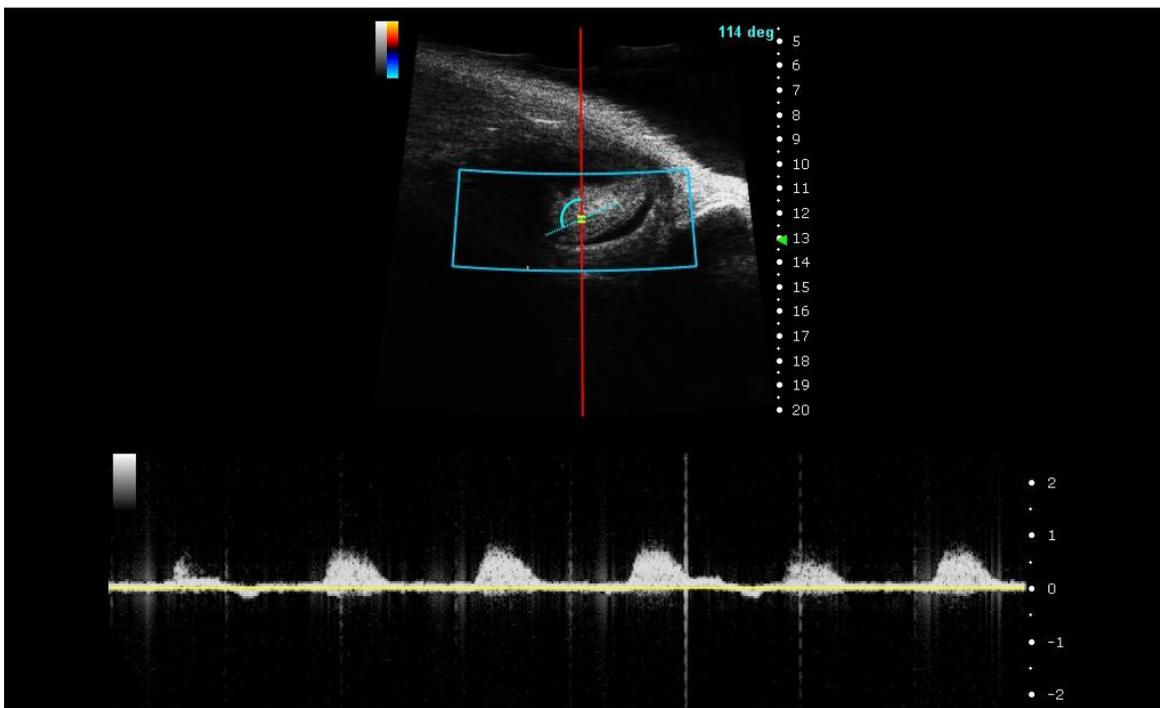
(G)

*Fig. 8. High-frequency ultrasound images of E11.5 mouse embryos obtained using the Prospect system. (A) The heart, midbrain, optic vesicle, and umbilical cord of the embryo can be identified on B-mode imaging. (B) The heart, forebrain, and umbilical cord of the embryo are clearly evident on B-mode imaging. (C) The umbilical cord and placenta are observed on B-mode imaging. (D) B-mode image of the embryonic dorsal aorta. (E) PW Doppler waveform of mouse embryonic cardiac inflow and outflow. (F) PW Doppler waveform of the umbilical vessels obtained from the B-mode image shown in panel C. (G) PW Doppler waveform of the mouse embryonic dorsal aorta obtained from the B-mode image shown in panel D.*

E 12.5

The main developmental events that occur at E12.5 are the progressive septation of the common outflow tract, atria, and ventricles, and the initiation of atrioventricular canal septation [3] [9]. By E12.5 the septation of the distal portion of the outflow tract, including the semilunar outflow tract valves, is complete. The proximal part of the outflow tract septum in the conus starts to close in a zipper-like fashion from distal to proximal toward the ventricles. In order to divide the atrioventricular canal into separate right and left canals, the superior and inferior atrioventricular cushions fuse to form an atrioventricular septum or septum intermedium. The atrioventricular septum shifts over the interventricular septum, which allows the right and left atrioventricular canals to be aligned with the corresponding ventricles. At this stage there is still communication between the two ventricles, but by E13.5–14.0 the most proximal part of the conal ridges together with the atrioventricular cushions will contribute to the membranous component of the interventricular septum and will close the connection between the ventricles [9].

At E12.5–E13.5 the size of all four chambers of the heart has increased markedly, and the configuration of the outflow tract has changed significantly. In addition, a distinct C-shaped aorta can be traced, arising from the left ventricle and passing to the right of the pulmonary artery before looping to the left [8]. Images of the E12.5 mouse embryo are shown in Figure 9.

(A)

(B)

(C)

(D)
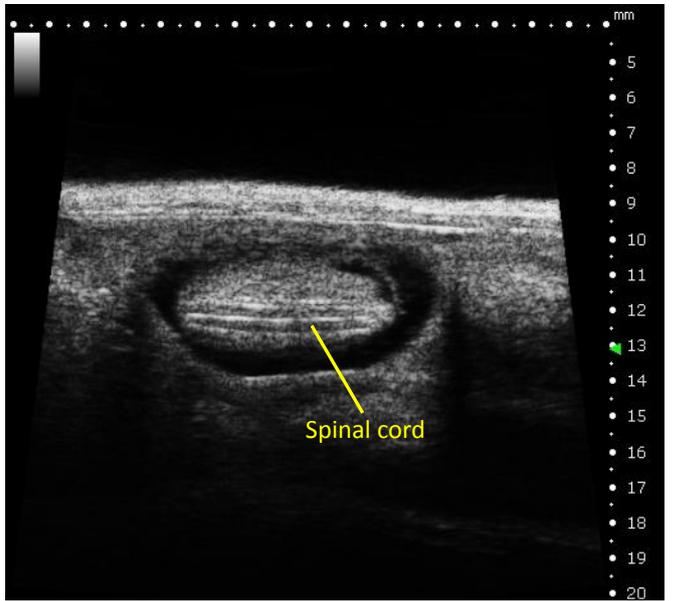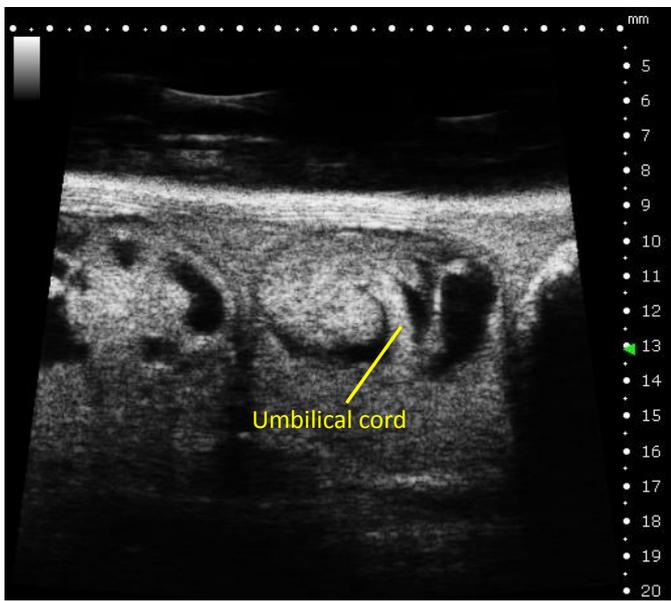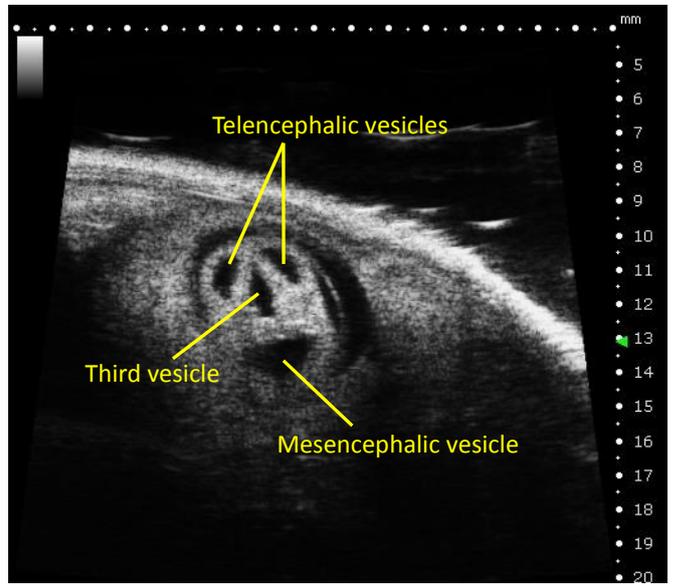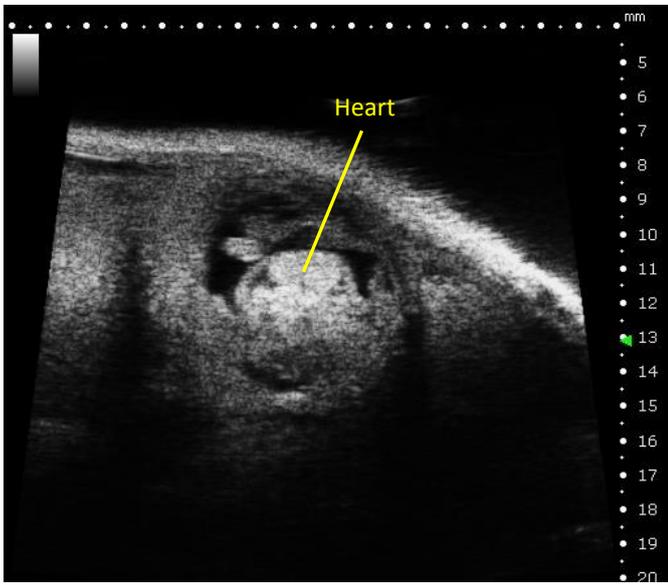
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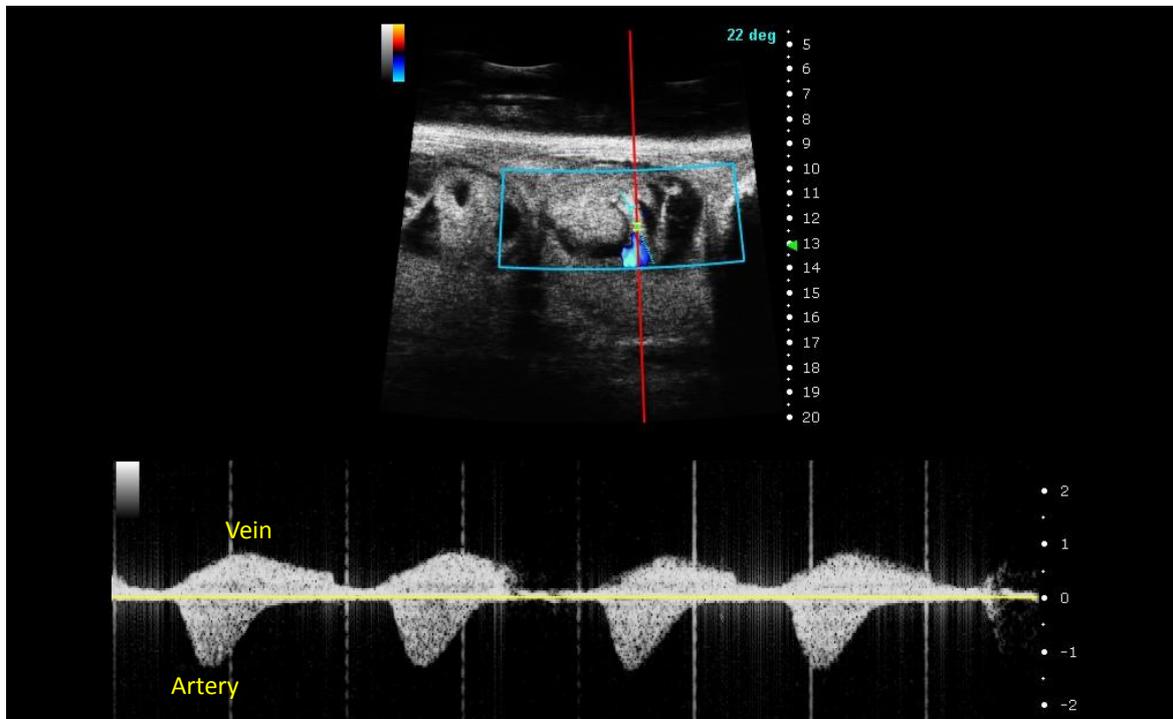
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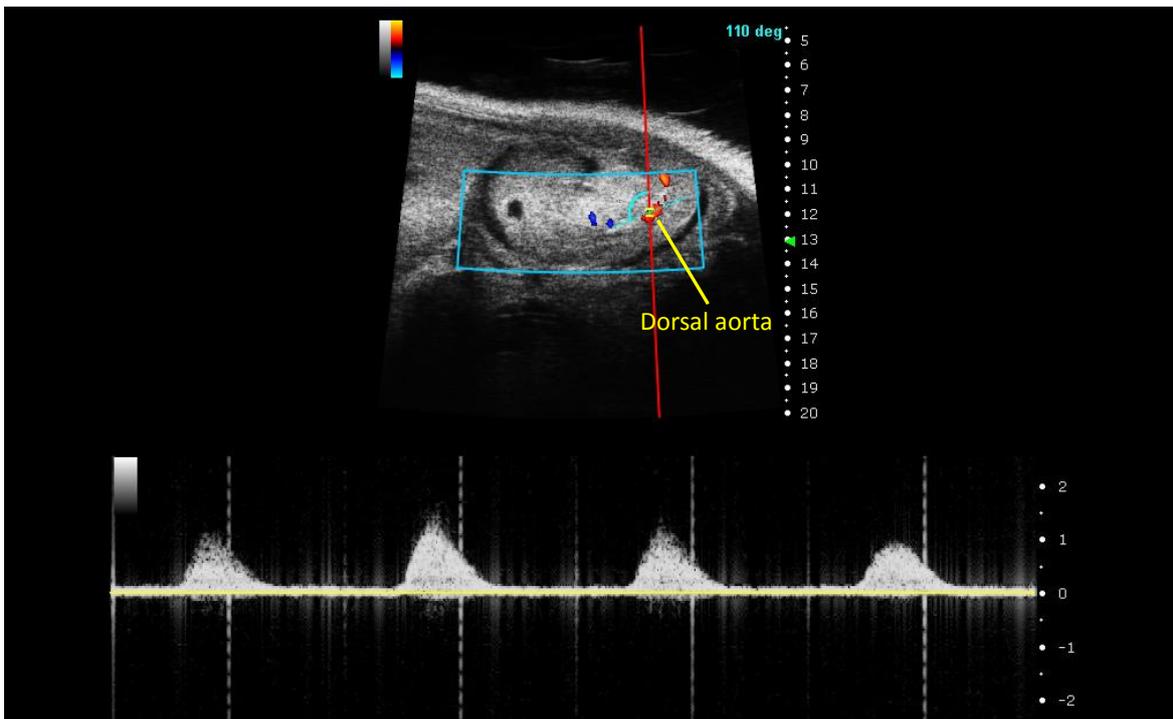
(G)

Fig. 9. High-frequency ultrasound images of E12.5 mouse embryos obtained using the Prospect system. (A) B-mode image of the embryonic heart, in which the four chambers can be identified. (B) Embryonic B-mode image of the brain. The telencephalic vesicles (future lateral ventricles), third vesicle, and mesencephalic vesicle (future aqueduct) inside the brain can be visualized clearly. (C) B-mode image of the umbilical cord. (D) B-mode image of the embryonic spinal cord. (E) PW Doppler waveform of mouse embryonic cardiac blood flow through the mitral orifice. (F) PW Doppler waveform of the umbilical vessels obtained from the B-mode image shown in panel C. (G) PW Doppler waveform of the mouse embryonic dorsal aorta.

E 13.5

At E13.5 several embryonic structures become visible, such as the vertebra and ribs, and the size of the embryo begins to increase dramatically [4]. The other main event that occurs at this stage is separation of the common outflow tract and ventricles, although the atrial and atrioventricular septa are still under formation [3] [9]. At the site of fusion of the conal ridges, there is a fibrous raphe in the wall between the pulmonary and aortic roots, which will be transformed into a muscularized, adult-type septum.

At this stage the various types of valve leaflet have not yet fully developed. The leaflets of both the tricuspid and mitral valves appear to be elongated and press closely against each other. A process of myocardial delamination separates the lower part of the atrioventricular valve leaflets from the myocardial wall and the interventricular septum, resulting in freely moveable, bilayered leaflets. The final shaping and composition of the atrioventricular valve leaflets occur as a result of an apoptotic process called excavation, which takes place in the atrial face of the leaflets during E12.5–15.5. The maturation of the semilunar valve leaflet is also progressing at this stage, and is the last major morphogenetic event pertaining to the outflow tract [9].Images of the E13.5 mouse embryo are shown in Figure 10.
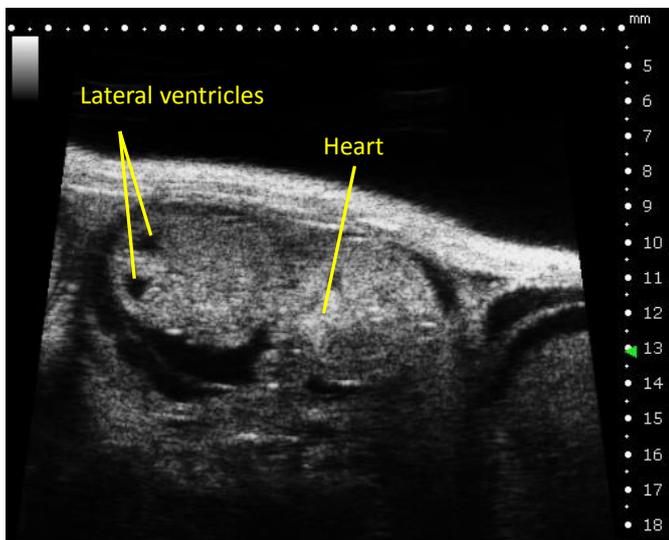

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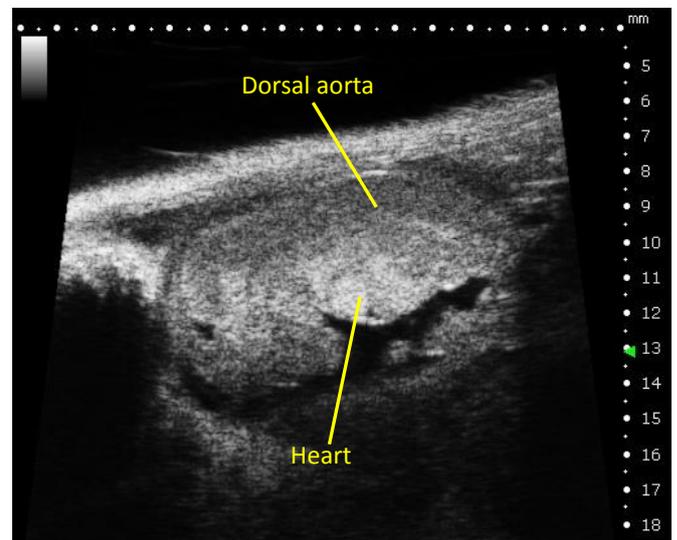
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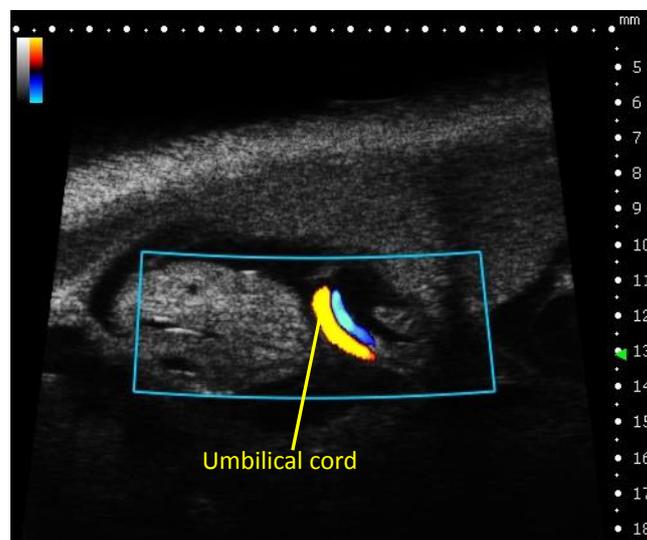
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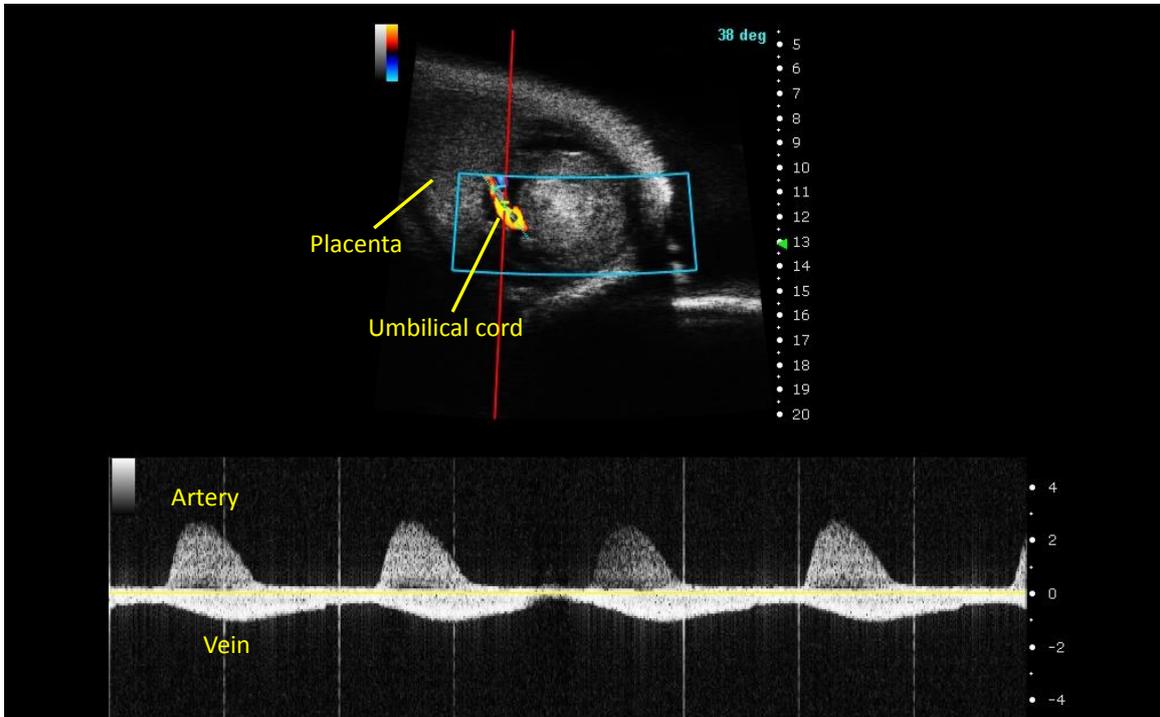
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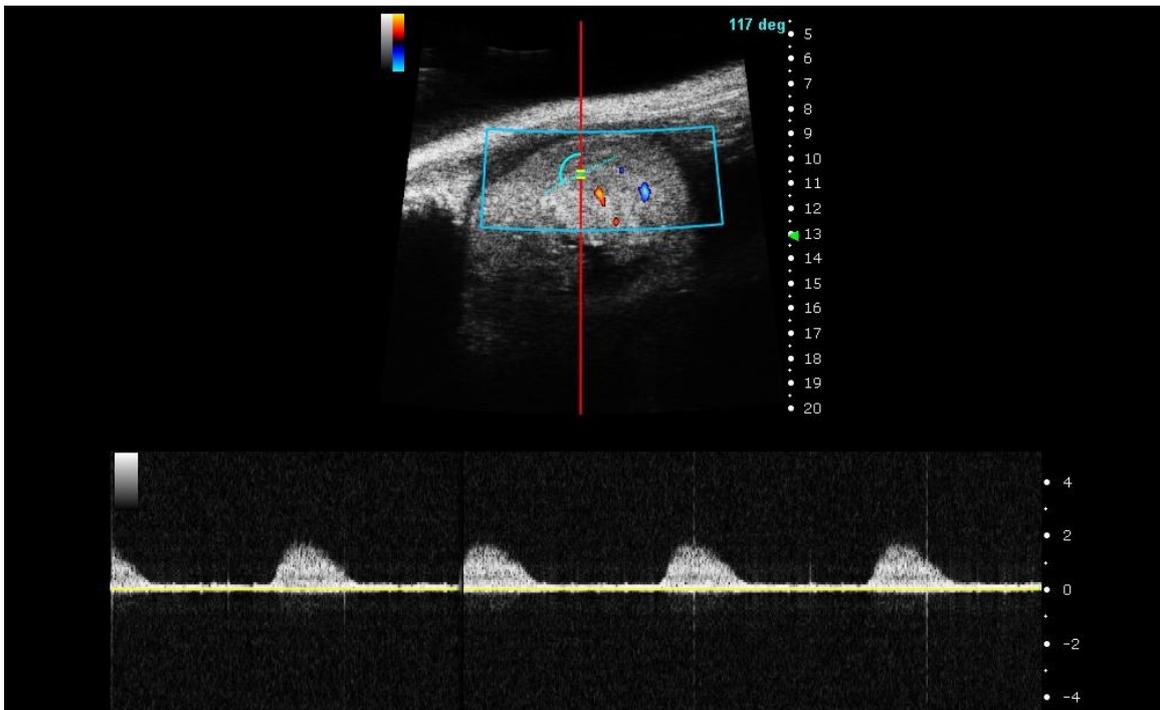
(E)

Fig. 10. High-frequency ultrasound images of E13.5 mouse embryos obtained using the Prospect system. (A) B-mode image of the embryo in the frontal plane; the lateral ventricle and heart can be visualized. (B) B-mode image of the embryo in the sagittal plane; the heart and dorsal aorta can be identified. (C) Color Doppler image of the umbilical cord. The opposing blood flow directions of the umbilical arteries and veins are clearly evident. (D) PW Doppler image and waveform of the embryonic umbilical cord. (E) PW Doppler waveform and image of the mouse embryonic dorsal aorta.

E14.5

At E14.5 the different components of the central cushion tissue contribute to the atrial, ventricular, and atrioventricular septa, and hence a normal ventricular septal defect can be seen at this stage [3]. The common atrial chamber is divided into two by E14.0, but the lower border of the septum secundum never completely fuses with the atrioventricular cushion, leaving an opening. There is considerable blood flow from the right to the left atrium during fetal development via this opening and the ostium secundum. This interatrial channel is called the foramen ovale, and its function is to allow the largely oxygenated blood to enter the right atrium through the inferior vena cava, which is directed across the midline into the cavity of the left atrium. The deoxygenated blood, which enters the rostral part of the right atrium through the right superior vena cava, is directed toward the right ventricle. The foramen ovale is normally closed at birth by the fusion of the two atrial septa [9]. In addition, the chest in cross-section with the heart, forelimb buds, and the spine can be depicted in the transverse images at E14.5. At this stage early eye development, the third ventricle, and the superior horns of the lateral ventricles can also be distinguished in oblique sections through the brain and torso [6]. Images of the mouse embryo at E14.5 are shown in Figure 11.
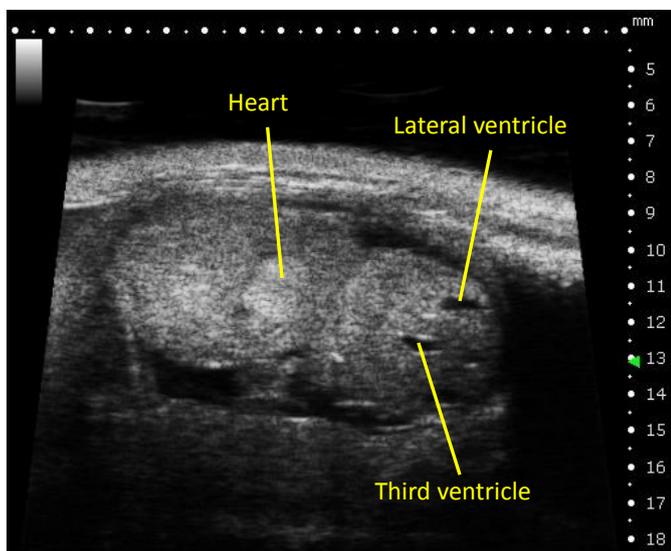

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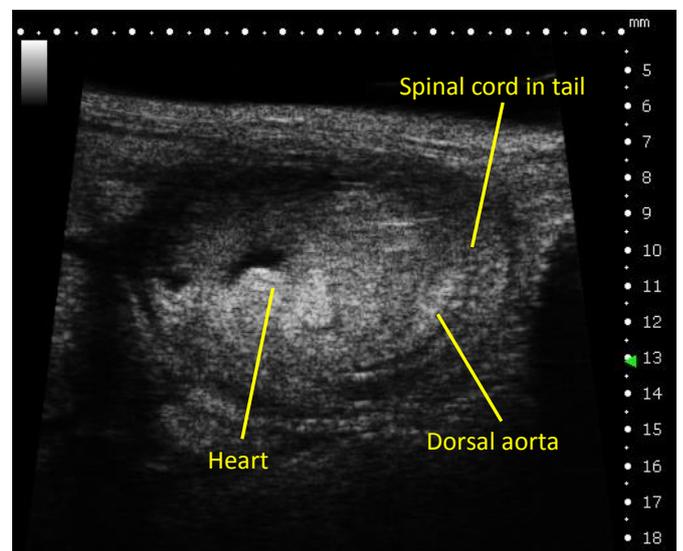
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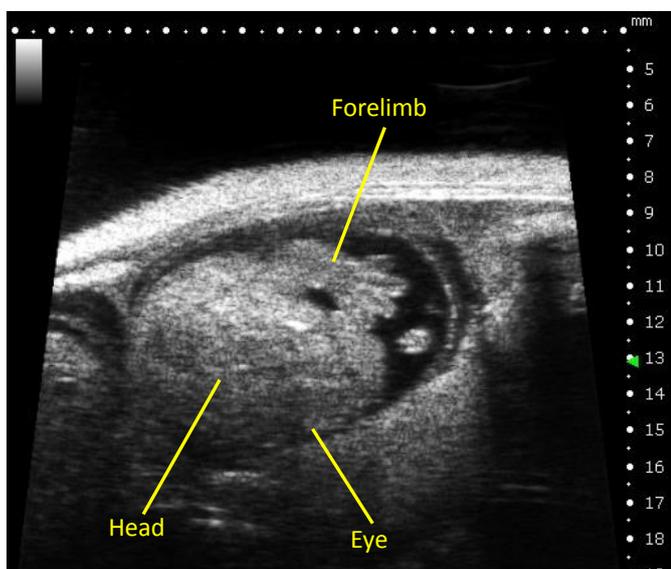
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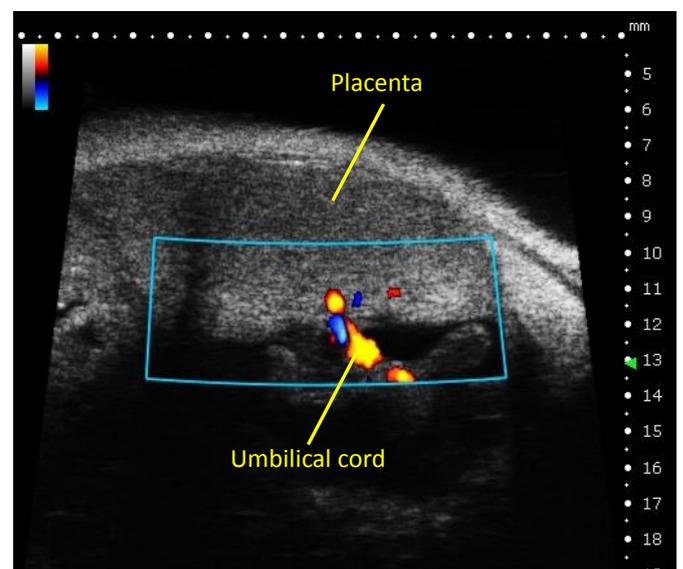
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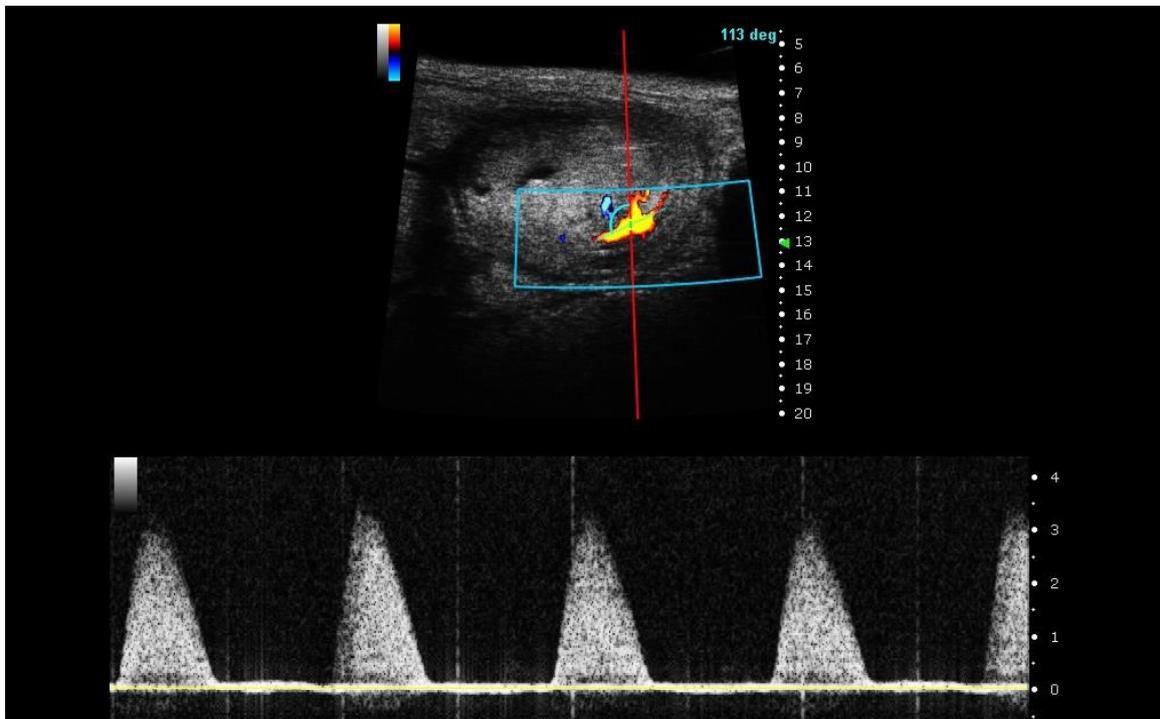
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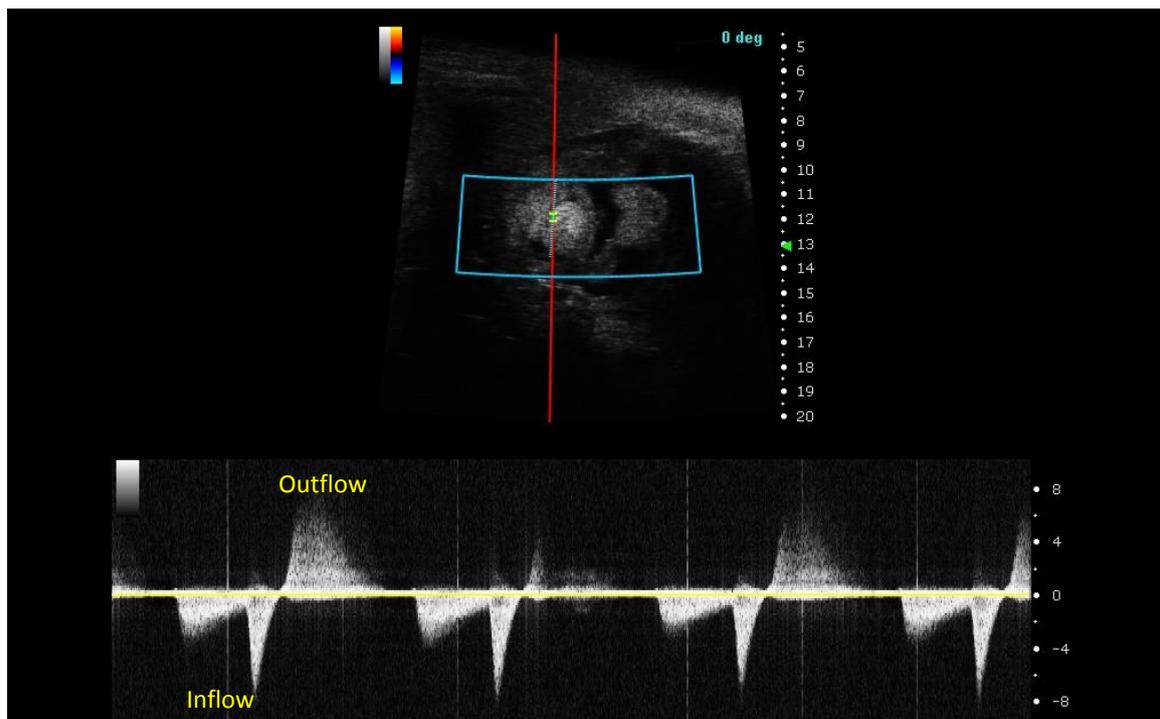
(F)

Fig. 11. High-frequency ultrasound images of E13.5 mouse embryos obtained using the Prospect system. (A) B-mode image of the embryo in the frontal plane; the lateral ventricle, third ventricle, and heart can be visualized. (B) B-mode image of the embryo in the sagittal plane showing the heart, dorsal aorta, and the spinal cord in the tail. (C) The head, eyes, and forelimb can be observed in B-mode in the transverse plane. (D) Color Doppler image of the umbilical cord, and the interlaced blood flow reveal the spiral structure of the umbilical arteries and vein. (D) PW Doppler waveform of the mouse embryonic dorsal aorta. (E) PW Doppler waveform of the mouse embryonic cardiac blood flow through the mitral orifice.

E 15.5

At E15.5 all of the components of the heart are readily recognized. Both the heart and the vascular system have achieved their definitive external prenatal configuration, but the atrioventricular valve leaflets and coronary arteries continue to be modified after birth. Thus, the heart size only increases after E15.5 [5] [9]. The thicknesses of the two ventricular walls are approximately equal. The volume of the left ventricular cavity is slightly greater than that of the right side. The two atrial chambers of the heart are in continuity through the foramen ovale [5].

With regard to the brain, the cerebellar primordium and the olfactory lobes are the two regions of the brain that exhibit the greatest degree of differentiation between E13.5–14 and E14.5–15. As the rhombic lip was previously the first site of differentiation of the intraventricular septum, the size of this region has increased considerably by this stage, and begins to encroach upon the lumen of the fourth ventricular lateral recess [5].

Furthermore, the eyes, which are distinguishable from the rest of the structures as large hypoechoic masses located cranially to the heart, can be identified at this stage. Echogenic foci are now sometimes visible scattered throughout the labyrinth region of the placenta [4] [7]. Images of the E15.5 mouse embryo are shown in Figure 12.
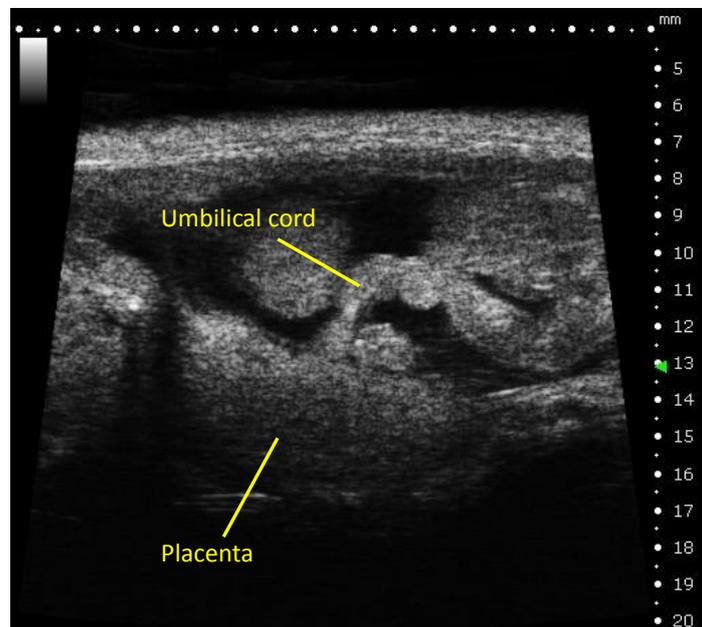

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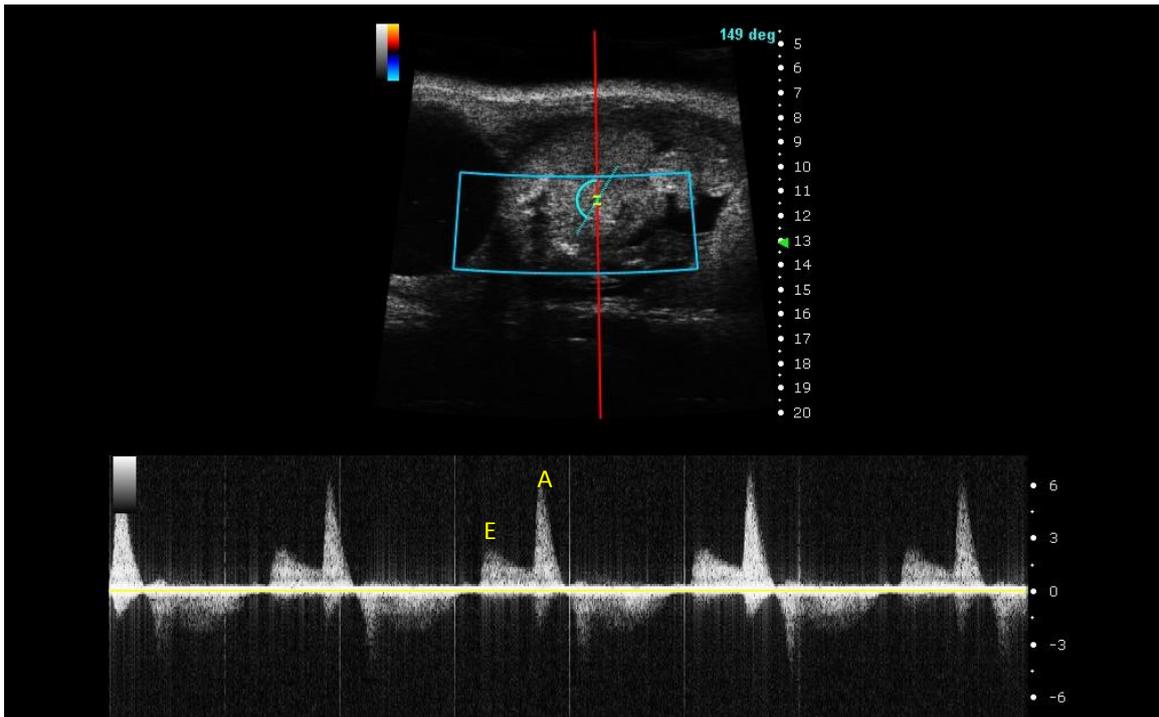
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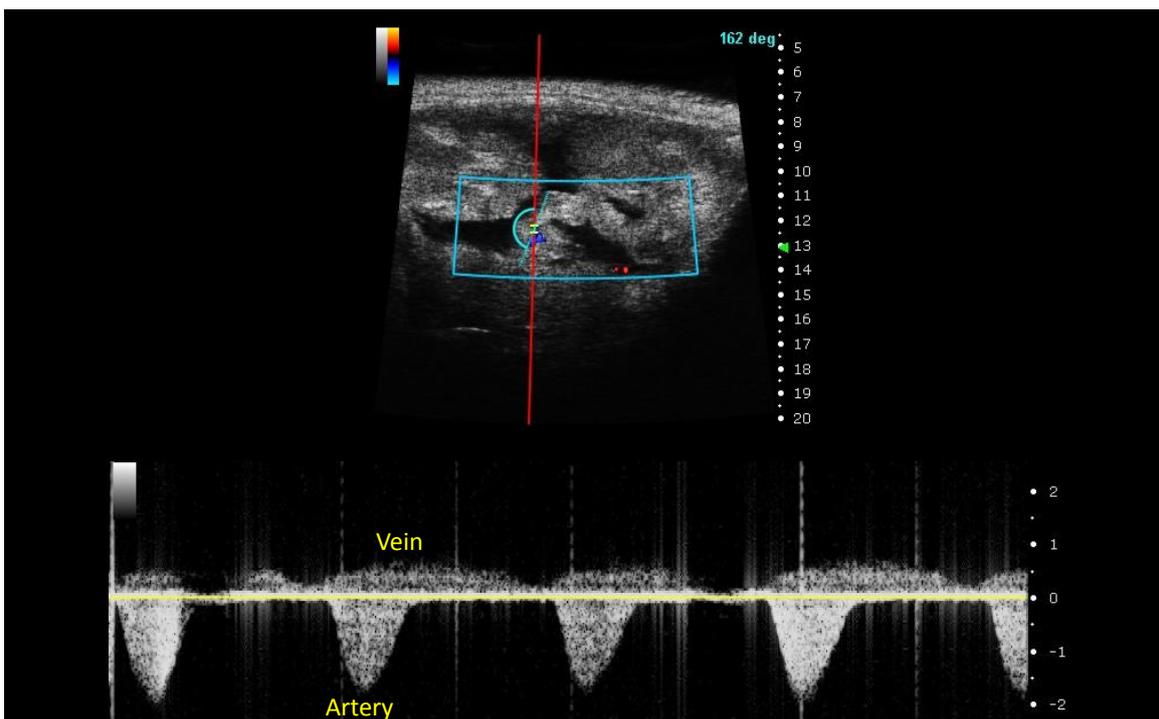
(C)

Fig. 12. High-frequency ultrasound images of E15.5 mouse embryos obtained using the Prospect system. (A) B-mode image of the umbilical cord. (B) PW Doppler waveform of mouse embryonic cardiac blood flow through the mitral orifice. The Doppler data of the inflow through the mitral orifice demonstrate the presence of separate E and A waves, with A-wave dominance. (C) PW Doppler waveform of the embryonic umbilical cord obtained from the B-mode image in panel A.

E 16.5

    The most significant change in the heart at this stage is an alteration in its axis. Of particular interest is the right atrium now being located considerably more caudally than the left atrium. The interventricular groove is now only evident toward the apical region of the heart, and is clearly directed toward the left anterolaterally. The axis of the heart appears to have changed from a principally anteroposterior direction to a more oblique orientation. The main axis of the heart is oriented from the upper right to the lower left part of the thoracic cavity. The principal axis of the heart has continued to grow, and is clearly visible in embryos at E16.5, and at subsequent stages of development. Furthermore, the increase in the volume of the right atrial chamber compared to that of the left is now more marked. The volume of the left ventricle continues to be slightly greater than that of the right ventricle. In addition, the leaflets of the pulmonary and aortic valves are considerably more differentiated than before. This equally applies to the atrioventricular valves [5]. Images of E16.5 mouse embryos are shown in Figure 13.
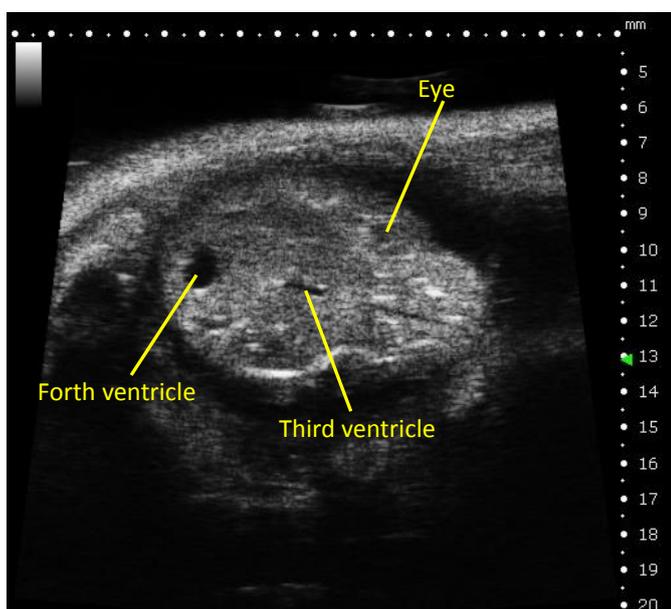

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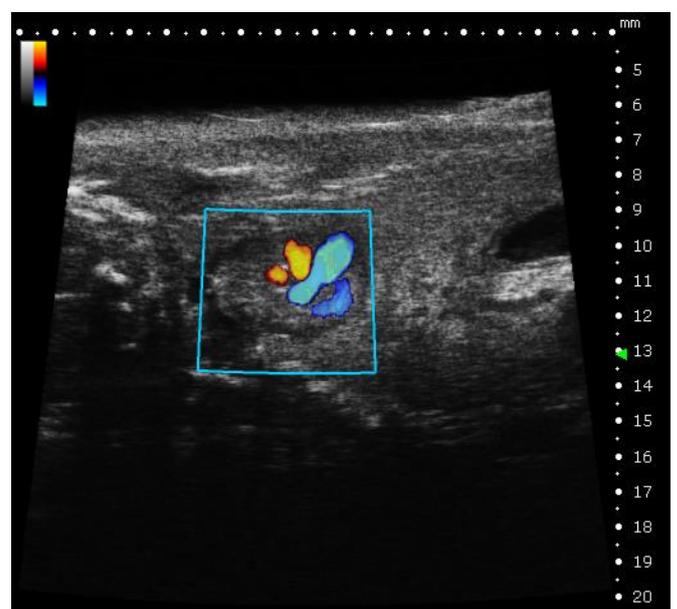
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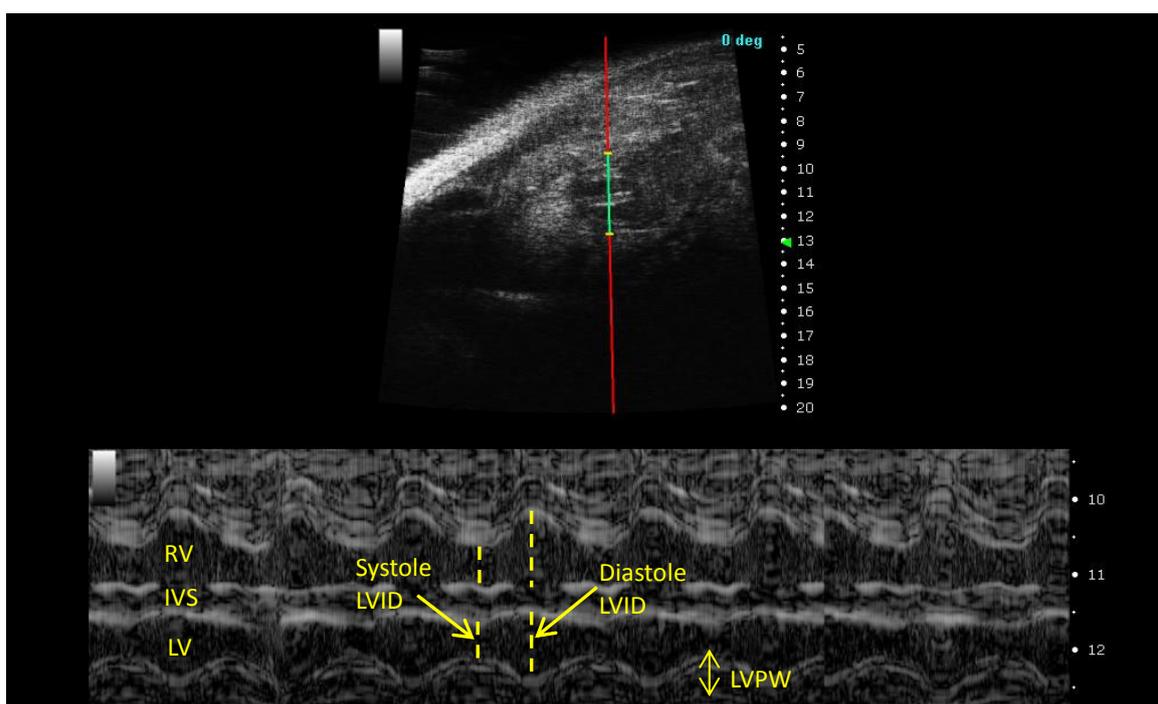
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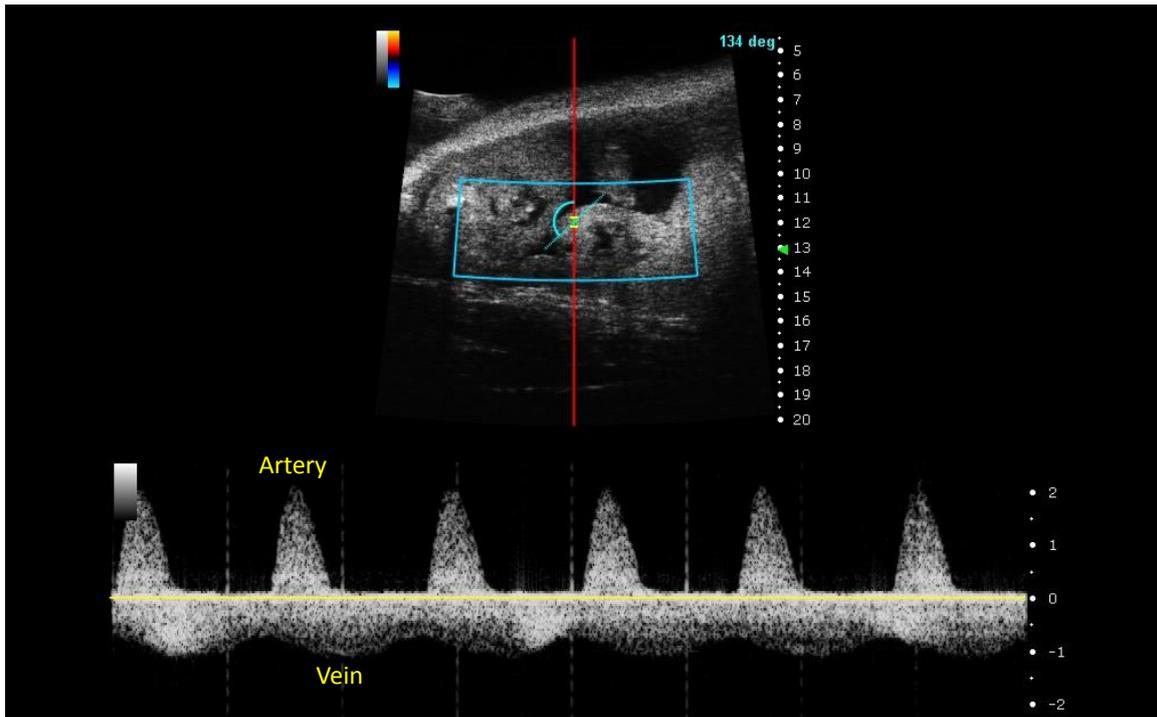
(D)

Fig. 13. High-frequency ultrasound images of E16.5 mouse embryos obtained using the Prospect system. (A) B-mode image of the embryonic head, revealing the fourth ventricle, third ventricle, and eyes. (B) Color Doppler image of the embryonic heart (C) M-mode tracing of the right and left ventricles. IVS, interventricular septal wall; LVID, left ventricular internal dimension; LVPW, left ventricular posterior wall thickness. (D) PW Doppler image of the embryonic umbilical cord.

E 17.5

At E17.5 the orientation of the heart is essentially identical to that evident in the previous stage, although the interventricular groove is less evident. The interventricular groove only exhibits a shallow depression just proximal to the apex of the heart, and this is a relatively insignificant feature of the anterior surface of the heart compared to what is observed in previous stages. The walls of the ventricles, as well as the muscular part of the interventricular septum, are more consolidated than in the previous stage, but the muscular walls of the atria are still extremely thin compared to those of the ventricles. The volumes of these chambers of the heart are far greater at this stage than those of the ventricles [5]. Figure 14 shows images of E17.5 mouse embryos.
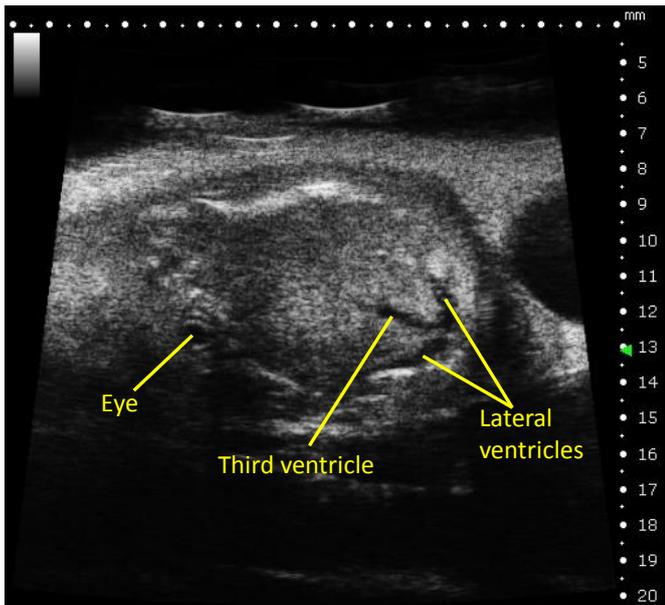

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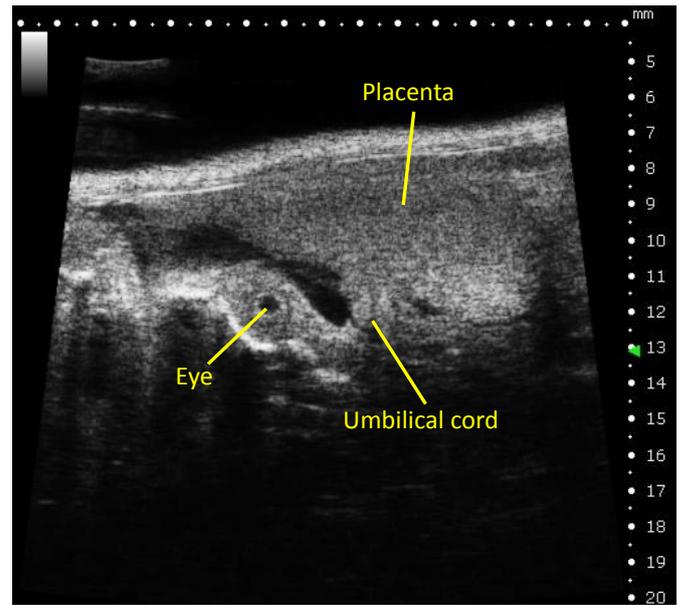
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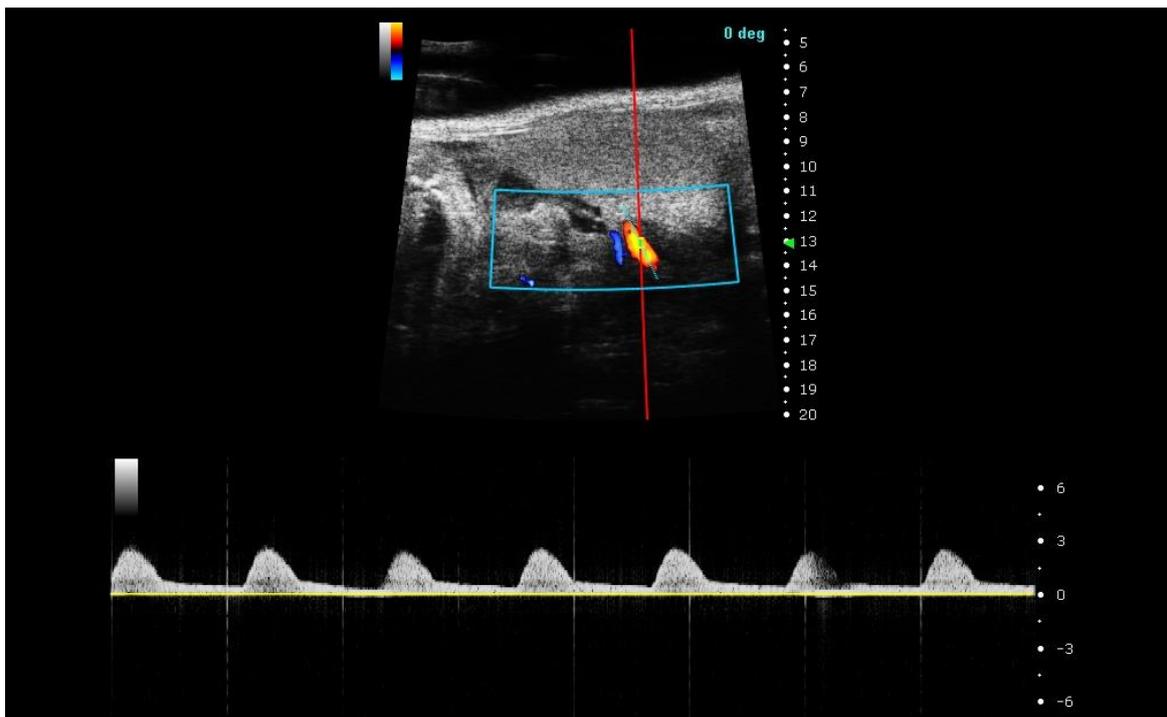
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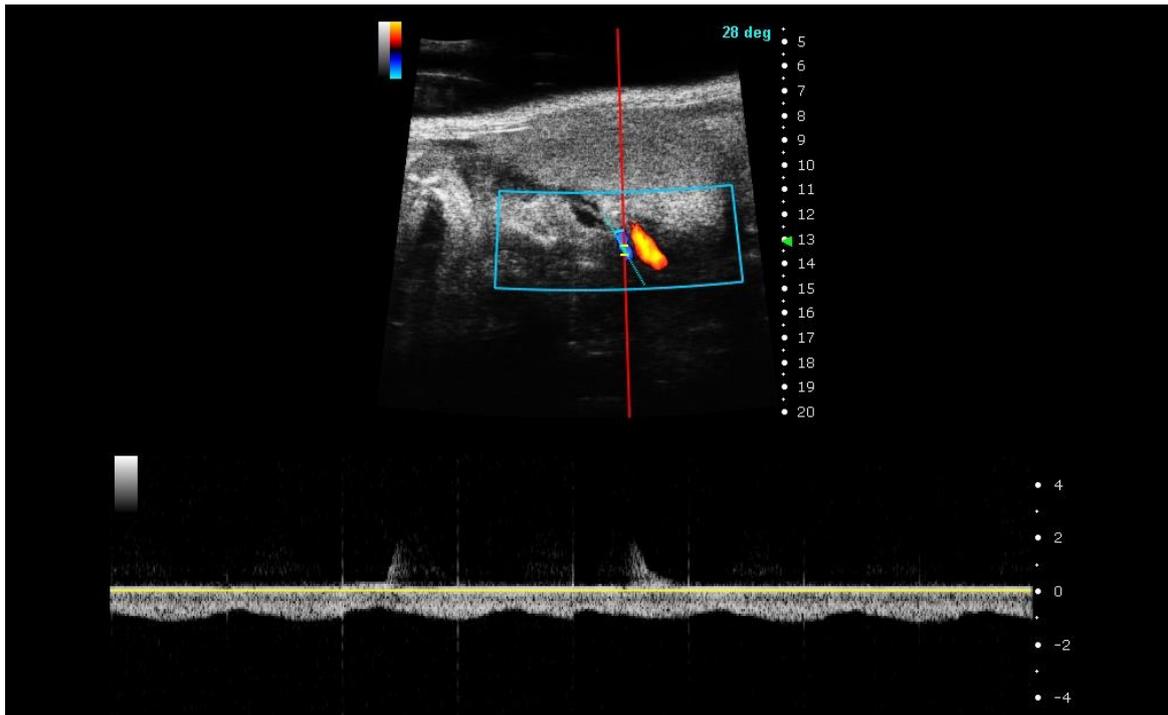
(D)

Fig. 14. High-frequency ultrasound images of E17.5 mouse embryos obtained using the Prospect system. (A) B-mode image of the embryonic head; the lateral ventricles, third ventricle, and an eye can be visualized clearly. (B) B-mode image of the embryonic umbilical cord. The placenta and an embryonic eye are also displayed in this view. (C) PW Doppler waveform of the umbilical artery. (D) PW Doppler waveform of the umbilical vein.

E 18.5

At E18.5 the heart has reached its definitive prenatal state, and exhibits only a few changes in its gross morphology compared to that evident in the previous stage. The difference in thickness between the walls of the atria and ventricles is essentially the same as indicated above, as is the substantial difference in the volumes of the atria and ventricles. The thickness appears to be substantially less than that observed previously in certain parts of the wall of the ventricles, and this is particularly noticeable in the region subjacent to and just to the right of the interventricular groove. The ventricular wall is now substantially less consolidated than observed previously, which may be due to the significant increase in the degree of trabeculation at this time. This change may indicate the differentiation of the ventricular muscle that occurs at this time, in preparation for sustaining the increased functional load after birth [5]. Images of E18.5 mouse embryos are shown in Figure 15.
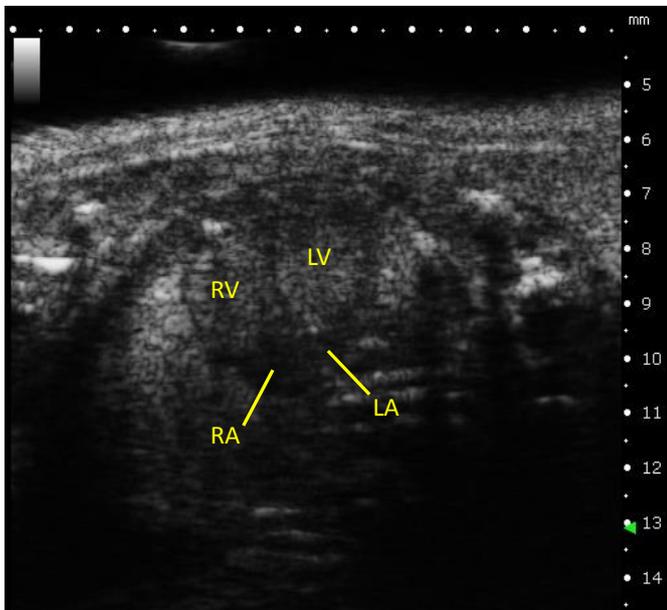
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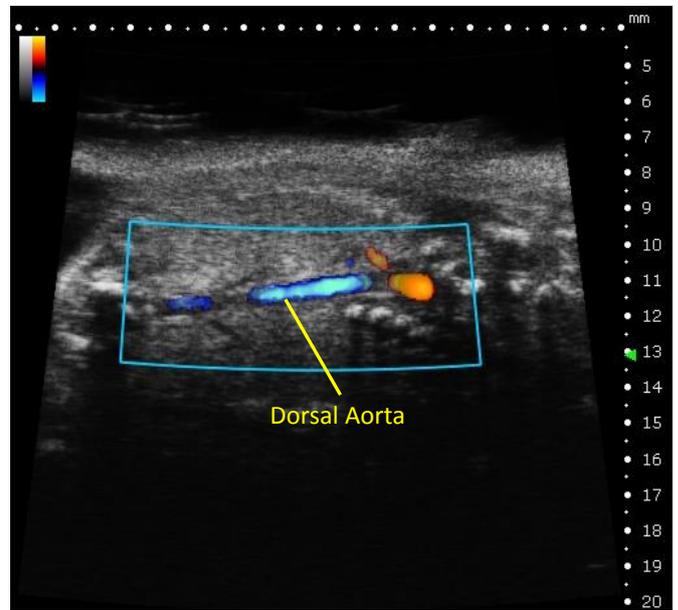
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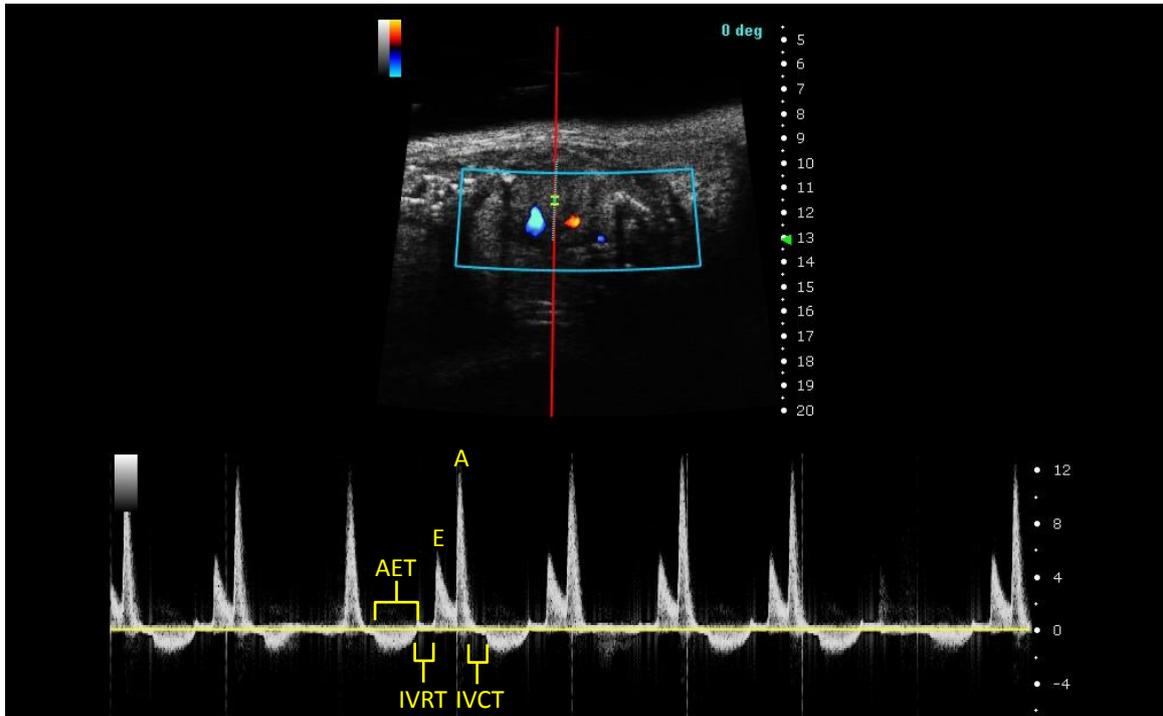
(C)

Fig. 15. High-frequency ultrasound images of E18.5 mouse embryos obtained using the Prospect system. (A) Four-chamber view of the embryonic heart in B-mode. (B) Color Doppler image of the embryonic dorsal aorta. (C) PW Doppler waveform of the mouse embryonic cardiac blood flow through the mitral orifice. The Doppler data of the inflow through the mitral orifice demonstrate the presence of separate E and A waves, with A-wave dominance. AET, aortic ejection time; IVRT, isovolumic relaxation time; IVCT, isovolumic contraction time.

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