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Review

Imaging fetal anatomy

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ABSTRACT

Due to advancements in ultrasound techniques, the focus of antenatal ultrasound screening is moving towards the first trimester of pregnancy. The early first trimester however remains in part, a 'black box', due to the size of the developing embryo and the limitations of contemporary scanning techniques. Therefore there is a need for images of early anatomical developmental to improve our understanding of this area. By using new imaging techniques, we can not only obtain better images to further our knowledge of early embryonic development, but clear images of embryonic and fetal development can also be used in training for e.g. sonographers and fetal surgeons, or to educate parents expecting a child with a fetal anomaly.

The aim of this review is to provide an overview of the past, present and future techniques used to capture images of the developing human embryo and fetus and provide the reader newest insights in upcoming and promising imaging techniques. The reader is taken from the earliest drawings of da Vinci, along the advancements in the fields of in utero ultrasound and MR imaging techniques towards high-resolution ex utero imaging using Micro-CT and ultra-high field MRI. Finally, a future perspective is given about the use of artificial intelligence in ultrasound and new potential imaging techniques such as synchrotron radiation-based CT to increase our knowledge regarding human development.

1. Introduction

The creation of a *3D Atlas of Human Embryology* [1] and subsequent research into human development during the fetal period made us humbly realize how little is known about our own development. Despite very promising initiatives such as the *Human Developmental Cell Atlas* [2], transcriptomic data of single-cell profiling technologies can only be fully appreciated when interpreted within the three-dimensional (3D) context of the developing embryo or fetus. 3D-imaging methods facilitate interactive study of early and late organogenesis, and enable translation of molecular and -omics research towards to clinical practice.

As the focus of pregnancy ultrasound screening shifts towards the first trimester of pregnancy due to technological advancements in ultrasound imaging, the need to comprehensively map fetal anatomical development throughout gestation increases. Furthermore, through

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; 3DISCO, three-dimensional imaging of solvent cleared organs; AC, abdominal circumference; AI, artificial intelligence; CESAs, contrast-enhancing staining agents; CRL, crown-rump-length; CT, computed tomography; diceCT, diffusible iodine-based contrast-enhanced computed tomography; DL, deep learning; DTI, diffusion tensor imaging; GB, gigabyte; HiP-CT, hierarchical phase-contrast tomography; LSFM, light sheet fluorescence microscopy; Micro-CT, microfocus computed tomography; MRI, magnetic resonance imaging; MR DTI, magnetic resonance diffusion tensor imaging; NT, nuchal translucency; sCT, synchrotron radiation-based X-ray phase-contrast tomography; SNR, signal-to-noise ratio; T, tesla; UHF-MRI, ultra-high field magnetic resonance imaging.

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state-of-the art imaging technologies it is possible to create clear and highly detailed images of the developing human, which can be used in training for students, sonographers and fetal surgeons, or to educate parents expecting a child with a birth defect.

In this review, we aim to provide an overview of the past, present and future imaging techniques through which human development can be captured. After a brief historical perspective, we will elaborate on the techniques currently used to study fetal anatomy, both in- and ex utero. In the last chapter of this review, promising future advances in the field of fetal imaging will be discussed, in particular the use of artificial intelligence in two-dimensional (2D) and 3D ultrasound [3], and a potential role for synchrotron X-ray imaging [4] in the study of fetal anatomy. If promises and predictions are met, we foresee an inspiring decade in which these new advances will bring us closer to the automatic detection of birth defects during pregnancy.

2. Imaging fetal anatomy in the past

Prior to the discovery of medical imaging techniques, such as X-rays (also referred to as Röntgen radiation) and their introduction over a century ago, knowledge of embryonic and fetal anatomy was based on dissections of cadavers acquired from miscarriages, stillbirths, or from post-mortem dissection of pregnant women [5]. The valuable and unique knowledge that could only be obtained from the examination of these developing bodies made them essential to study developmental anatomy [5]. Leonardo da Vinci was in the late 1400s and early 1500s one of the first to provide evidence that embryos change in weight, size and shape over time [6]. In one of his most famous drawings, da Vinci depicted a human fetus inside a dissected uterus (Fig. 1A). This is considered to be the first time in history that a human fetus was visualized correctly positioned within the womb [7], although this later turned out to be the uterus of an ungulate [8]. He was also the first to observe and comment on the fetal membranes – the chorion, amnion, and allantois. Ever since, our knowledge regarding developmental anatomy increased, leading to new discoveries such as the separation of maternal and feto-placental circulations theorized by William Harvey in 1651 and experimentally proven by William Hunter in 1774 [8].

With the introduction of microscopy, enabling the visualization of individual cells, and the acquisition of large-scale collections of human embryonic and fetal specimens, research on developmental anatomy accelerated. One of the first major collections was the Carnegie collection (National Museum of Health and Medicine, Silver Spring, MD), which was established by Franklin P. Mall (1862–1917) in the late 1800s. The Carnegie stages, which are currently widely used in human embryology, were defined based on the morphological characteristics of embryos from this collection [9]. Embryonic illustrations and wall charts were useful for teaching to a certain point, but they were limited in dimensional scope. Adolf Ziegler successfully drew, and hand-shaped models of embryos from two-dimensional embryo illustrations into three-dimensional wax models (Fig. 1B). Later, the development of the microtome led to a new stacked modeling technique developed by Gustav Born. Resembling almost a primitive form of 3D printing, microscopic sections of the embryo would be enlarged and projected onto sheets of wax, and the excess trimmed, before being stacked to provide an accurate 3D rendition. To appreciate developmental anatomy in a three-dimensional (3D) perspective, modeler Osborne O. Heard (1890–1983) used histological sections from the Carnegie collection to create detailed reconstructions of these embryos using this wax plate modeling principle [10]. Based on these wax models, numerous famous drawings by skillful artists, like James F. Didusch (1890–1955), have been published since [9]. In addition to direct microscopic evaluation of the serial sections themselves, such solid reconstructions were one of the first imaging methods used in classic embryology. These wax models have provided important insights into the early stages of human embryonic development. However, generating such reconstructions is laborious and time-consuming, and their fragility demands that they are handled and stored with care.

In the beginning of the 1900s, radiography was the first technique employed to image pregnancy and the developing fetus. It was used to confirm pregnancy by visualizing fetal osseous structures, assess fetal position, estimate gestational age, and diagnose fetal bone anomalies such as achrondroplasia (Fig. 1C). At this point, the harmful effects of X-rays to the fetus were still unknown. By 1975, strong evidence had been compiled proving that radiation exposure during pregnancy can cause a miscarriage, or may seriously damage the fetus including increased risk of leukemia and other malignancies [11]. Until the development of medical ultrasonography (US) in the early 1960s and magnetic resonance imaging (MRI) in the 1980s, imaging pregnancy and the developing fetus remained harmful and undesirable.

3. New advances in imaging fetal anatomy

Since the introduction of non-invasive and safe imaging modalities such as ultrasound and magnetic resonance imaging (MRI), innovative research and technological evolution have led to numerous rapidly evolving advancements in medical imaging. Their applications in fetal

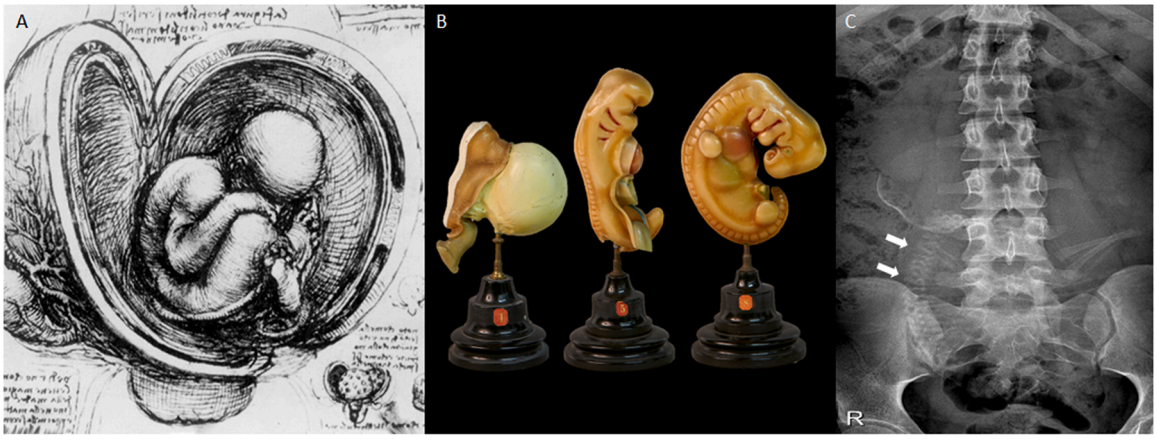

Fig. 1. Imaging fetal anatomy in the past. (A) da Vinci's drawing of a fetus correctly positioned in the womb, circa 1505. (B) Historic embryonic and fetal wax models from the Ziegler collection from museum Vrolik, Amsterdam UMC, Amsterdam, the Netherlands. Photography by Sanne Mos. (C) Radiograph in pregnant woman demonstrates the fetus in breech presentation, with its spine (arrows) in on the right. Image on courtesy of the Department of Radiology of the Borsod County Hospital, dr. I.L. Lakatos.
Adapted from Dunn et al., 1997 with permission [95].

imaging have expanded dramatically over the past decades. Today, ultrasound with real-time two- and three-dimensional scanning provides detailed in utero images of the fetus, yolk sac and placenta, as well as dynamic visualization of the fetal heart, breathing patterns and fetal movements. In addition, as MR imaging acquisition techniques have improved, MRI is gaining a more prominent role for imaging fetal anatomy in utero.

Here, we will discuss recent advances of the aforementioned imaging techniques by first focussing on in utero applications, after which we will provide an overview of the imaging modalities currently available for ex utero fetal imaging which can generate an unprecedented level of detail without the need of invasive procedures. As these imaging modalities have become increasingly available, the detailed study of fetal anatomy post-mortem (ex utero) is no longer solely limited to autopsy and cadaveric dissection studies, but can be complemented by the study of fetal anatomy in intact specimens, enhancing our knowledge of human developmental anatomy.

3.1. In utero

3.1.1. Ultrasound

Ultrasound uses high-frequency soundwaves to create real-time images of the fetus within the uterus. It has revolutionized obstetric practice, allowing detailed visualization of fetal anatomy and monitoring of fetal well-being during pregnancy [12]. Despite other emerging technologies for fetal imaging, ultrasound remains the main imaging modality to study fetal development in utero because of its safety, affordability and portability, and it is expected to keep playing a central role in prenatal imaging [12,13]. Worldwide, ultrasound imaging is offered to pregnant women to determine pregnancy viability, gestational age, detect multiple pregnancy, screen for structural abnormalities, and monitor growth and fetal well-being [14]. Initially, the fine balance between optimizing penetration, depth and ultrasound frequency (one of the main determinants of ultrasound resolution) formed a major limitation to visualize fetal anatomy at early gestation [13]. Advances in ultrasound technology and specifically, the transvaginal approach, have greatly improved image resolution and now allow detailed visualization of fetal anatomy as early as the first trimester of pregnancy. This led to the emergence of a novel field of research, coined “sonoembryology” by Timor-Tritsch and colleagues in 1990 [15] and has played a key role in moving human embryology from post-mortem to in vivo studies [16].

The main strengths of ultrasound include the detailed structural information that can be obtained from the first trimester onwards, its low cost, widespread availability, and real-time nature of the examination. Traditionally, structural information is primarily obtained through conventional B-mode or 2D ultrasound imaging. However, 3D ultrasound is increasingly used for evaluating anomalies [17], to perform volumetric measurements [18] and to visualize surface anatomy. It provides the benefit of unlimited reassessment of the stored 3D ultrasound volumes [19]. Moreover, major recent advances in 3D ultrasound technology have been made over the recent years. Novel 3D visualization softwares have resulted in life-like visualization of early human development (see Fig. 2), making ultrasound images more easily discernible for clinicians, students and parents alike [20]. In addition, these novel rendering technologies can be combined with so-called see-through 3D ultrasound rendering applications, which allow visualization of internal structures through contrast enhancement. These rendering technologies are available on advanced ultrasound systems of most major manufacturers as proprietary software; Samsung’s CrystalVue™ (Samsung Medison co. Ltd., Seoul, Korea), GE’s HD Live Silhouette (GE Healthcare, Massachusetts, Illinois, USA) and Philips’ GlassVue (Philips Healthcare, Eindhoven, the Netherlands). Although differences between these applications exist, all rely on specialized volume rendering algorithms that create gradients at tissue boundaries where there is a marked difference in acoustic impedance, resulting in 3D visualization of, for example, organ boundaries, fluid-filled cavities such as the cerebral ventricles, and skeletal elements (see Fig. 3). Although these see-through technologies are currently not part of routine fetal anatomical evaluations, successful visualization of several internal structures such as the fetal kidneys and adrenal glands, cerebral ventricles, esophagus, hard and soft palate and the vertebral column and ribs have been published [21–27].

Despite the many advantages of ultrasound imaging for the real-time visualization of fetal anatomy and early human development in utero, there are some important limitations to this technology. The main limitation of ultrasound imaging is that the quality of the images can be highly influenced by both fetal and maternal factors. For example, ultrasound imaging relies on the presence of amniotic fluid between the ultrasound transducer and the fetus to adequately visualize fetal anatomy. When the amount of amniotic fluid is decreased or completely absent, ultrasound imaging quality will inevitably be greatly impaired. When a birth defect is suspected to be the underlying cause, reaching a definitive diagnosis is complicated by the poor imaging quality. Similarly, in the case of maternal obesity, the signal-to-noise ratio and as a consequence imaging quality, is negatively impacted as the ultrasound signal is attenuated by adipose tissue, reducing the strength of the ultrasound beam that reaches the fetus. Moreover, increased adipose tissue increases distance that the ultrasound beam needs to cover, necessitating the use of lower frequency sound waves, which in turn results in lower resolution of the ultrasound image. Furthermore, an unfavorable fetal position, together with increasing ossification of the maturing fetal skeleton, can cause major challenges in achieving adequate visualization of fetal anatomy. Especially in the late second and third trimester, when fetal movements are reduced, ossification of the fetal skull and thorax can greatly hinder obtaining adequate ultrasound imaging [28]. In addition to being subjective to fetal and maternal factors, ultrasound imaging is highly dependent on the skill of the

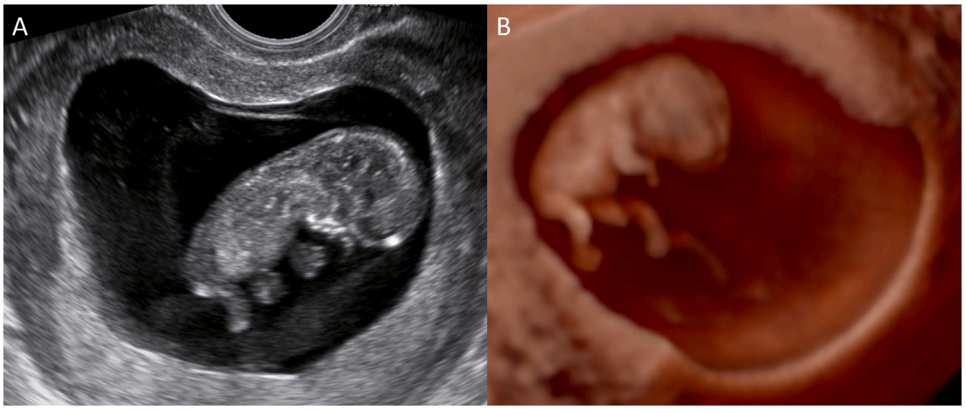

Fig. 2. 2D and 3D ultrasound imaging of a 9 weeks gestation fetus in utero. First trimester transvaginal ultrasound imaging enables visualization of the entire fetus and uterine cavity. (A) 2D ultrasound image of the developing first trimester fetus using a transvaginal probe (5–9 MHz); crown-rump length 28 mm. (B) 3D ultrasound volume acquired using a transvaginal probe (5–9 MHz) and interrogated using Crystal Vue™ and Realistic Vue™ rendering software (WS80A Elite, Samsung Medison Ltd, Seoul, Republic of Korea) enabling visualization of fetal limbs and umbilical cord.

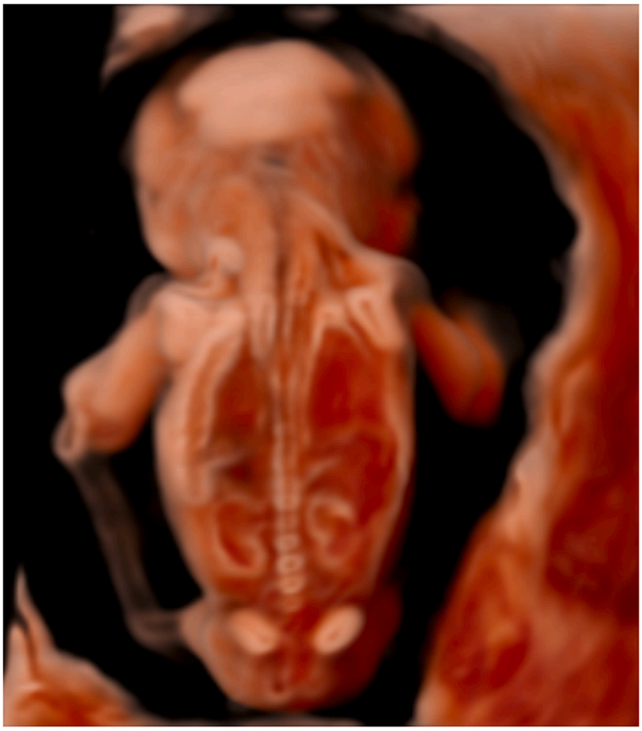

Fig. 3. Imaging internal fetal anatomy using 3D ultrasound in a 12 weeks gestation fetus. A 3D ultrasound image of a 12 week fetus is demonstrated; crown-rump length 66 mm. The volume was acquired using a transabdominal probe (1–8 MHz) and interrogated using CrystalVue™ and RealisticVue™ rendering software (WS80A Elite, Samsung Medison Ltd, Seoul, Republic of Korea). The fetal kidneys, adrenal glands, stomach and spine can be clearly identified by adjusting the transparency, complexity and light direction settings.
Figure adapted from Shah et al., 2019 with permission [96].

operator and requires extensive training and experience [29]. Finally, some current limitations specific to 3D/4D ultrasound are a lack of standardization, potential for motion artifacts during volume acquisition [30], and resolution which is currently not at the same level as conventional 2D ultrasound [14]. However, further technological developments resulting in faster acquisition and improved resolution may improve or resolve these issues in the future.

3.1.2. MRI

Fetal MRI is an increasingly used modality for imaging fetal anatomy in utero. Since the initial use of MRI for fetal imaging, image acquisition has dramatically improved in speed and efficiency, allowing diagnostic images to be obtained without the need to use sedation to reduce fetal movements. Typically, a field strength of 1.5 Tesla (T) is used, which yields acceptable resolution from 18 weeks' gestation onwards [31], although the use of 3.0T for improved resolution and signal-to-noise ratio is being investigated [32].

MRI is generally regarded as a valuable adjunct to ultrasound imaging in clinical practice, especially for further characterization of anomalies detected on ultrasound imaging, in cases of diagnostic uncertainty, or when ultrasound is technically limited. The main technical advantages of MRI over ultrasound imaging are that MRI quality is less affected by conditions that can greatly impair ultrasound quality such as reduced amniotic fluid, poor fetal position, maternal obesity, and ossification of the fetal skeleton [33]. Moreover, MRI can generate superior soft-tissue contrast compared to ultrasound, and provide highly detailed structural information. This makes MRI especially suitable for fetal brain imaging for both clinical and basic research. Furthermore, the complementary use of in-utero MRI and ultrasound has advanced the field of fetal ultrasonography by alerting researchers and clinicians to anatomical details visualized on ultrasound imaging, which had previously not been recognized [34]. For instance, a characteristic histological feature of the developing fetal brain at mid-gestation is its laminar pattern. Traditionally, evaluation of this characteristic developmental pattern has been restricted to histological examinations, and thus post-mortem studies. However, following identification of this characteristic pattern on both ex and in utero fetal MRI, Pugash and colleagues [35] were able to subsequently also reliably demonstrate the same pattern on ultrasound imaging, further pushing the boundaries of what can be achieved in ultrasound assessment of fetal anatomy [34]. Further significant leaps forward in visualizing fetal brain development in-utero have been established using Diffusion Tensor Imaging (DTI), which uses specific MRI sequences to qualitatively and quantitatively characterize microstructure, white matter tract anatomy and structural connectivity of the evolving brain in utero, leading to novel insights into normal and abnormal brain development [36,37]. Emergence of such novel imaging modalities create the need for new specific reference atlases to evaluate the quality of image acquisition, reconstruction, and analysis, such as the recently published spatiotemporal diffusion tensor MRI atlas by Khan and colleagues [37].

Despite the advantages that MRI provides over ultrasound imaging in specific conditions, there are important limitations to this modality. Contrary to ultrasound, MRI is less readily available, associated with high costs, and there are some absolute contra-indications to MRI such as the presence of a maternal pacemaker or claustrophobia, whereas there are no contra-indications to ultrasound imaging [38]. Another important limitation to MRI for in utero fetal imaging is the poor signal-to-noise ratio in fetuses below 18 weeks' gestation due to the small fetal size and excessive movements, making it less useful for early pregnancy diagnostic imaging [31,38]. Finally, although much progress has been made, MRI remains highly susceptible to motion artifacts, which can be detrimental to image quality [39]. Nevertheless, MRI plays an important role in fetal imaging and has the potential to improve accuracy of prenatal diagnosis, as well as advance the field of ultrasound imaging by providing more detailed images which can be used to facilitate better understanding of ultrasound imaging and in utero fetal development.

3.2. Ex utero

3.2.1. Histology and whole-mount immunostaining

Histology has been used for centuries to study the microscopic structure of tissues and the relation between tissues [40]. In the 1800s, Franklin Mall and colleagues at the Carnegie Institute collected, sectioned, and stained thousands of human embryos and fetuses. Wax models were generated to illustrate the various stages of human development [41]. A major issue with these wax models is that bias can be introduced by the artist creating the models, as he/she needed to interpret the information conveyed by the scientist (Figs 4 and 5).

More recently, the sections of various histological collections were re-used utilizing modern digitalization and reconstruction techniques to create unbiased 3D reconstructions and thereby revisiting our current knowledge on human embryology [1], [42], [43]. Using this technique a 3D atlas and quantitative database of human embryonic development was created for clinical, research and educational purposes [1]. From this study it became evident that figures in most human embryology textbooks, regarding the development of various organs, such as the kidneys, pharyngeal arch cartilages, and notochord, are based on animal models rather than on human specimens (Fig. 6) [1]. Reconstructions prepared from human specimens have led to new insights, one of these new insights is on the development of the human kidney. It is general contention that the developing human kidney passes through three morphological stages: pronephros, mesonephros, and metanephros. De Bakker et al. [44] showed that animals of which the embryos have a comparatively small yolk and hence have a free-swimming larval stage,

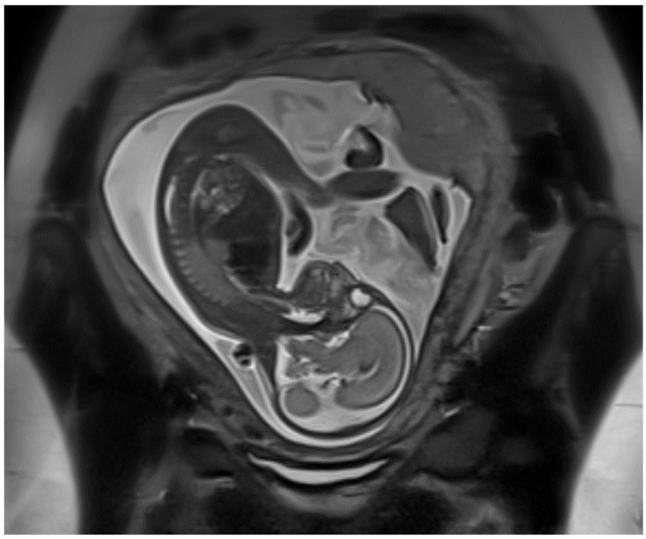

Fig. 4. In utero fetal MRI. A T2-weighted MRI of a 20 weeks gestation fetus, scanned at 1.5 tesla is demonstrated. A coronal plane through the mother's abdomen shows the full gravid uterus, placenta and surrounding maternal structures. This plane also provides a parasagittal view through the fetus, clearly visualizing the midbrain, fourth ventricle and cerebellum. Image on courtesy of the Department of Radiology and Nuclear Medicine of the Amsterdam UMC, dr. N. Ahmadi.

do develop a pronephros that is dedicated to survival in the aquatic environment. In amniote embryos that obviously do not have a free-swimming larval stage, have a large yolk or develop within the body of the parent (i.e., elasmobranchii, reptilia, aves, and mammalia), the pronephros is usually not present or incompletely developed and functionless. Furthermore, with the use of histological sections, sometimes in combination with immunohistochemistry, new observations with respect to the development of the neural tube [45], the hyoid bone [46], the heart [47], the dorsal mesentery [48], the omental bursa [49] and the skeletal musculature [50] have been made. An important strength of digitalizing histological sections and their reconstruction into 3D models is that the (primary) data are easily available for re-analyses and re-evaluation through the internet. Limitations of sectioning, aligning and reconstructing specimens is that it is a destructive, costly and time consuming imaging method which is often accompanied by shrinkage of the tissue and sectioning artifacts [1]. Furthermore, it is essentially only applicable for smaller specimens (i.e., embryos) and not for larger specimens (i.e. fetuses), because of technical limitations. With the constantly increasing size of the developing fetus, the number of histological sections increases dramatically, making it extremely time consuming and labor intensive.

Although the above-discussed technique provides a description of growth and organ topography, molecular information is often lacking or at best very limited. Therefore, researchers sought to create 3D images of human embryos including molecular information by combining solvent-based clearing methods. One of these techniques is three-dimensional imaging of solvent cleared organs (3DISCO), which combines whole-mount immunostaining with light sheet fluorescence microscopy (LSFM) [51]. Using this technique on whole-body and whole-organs

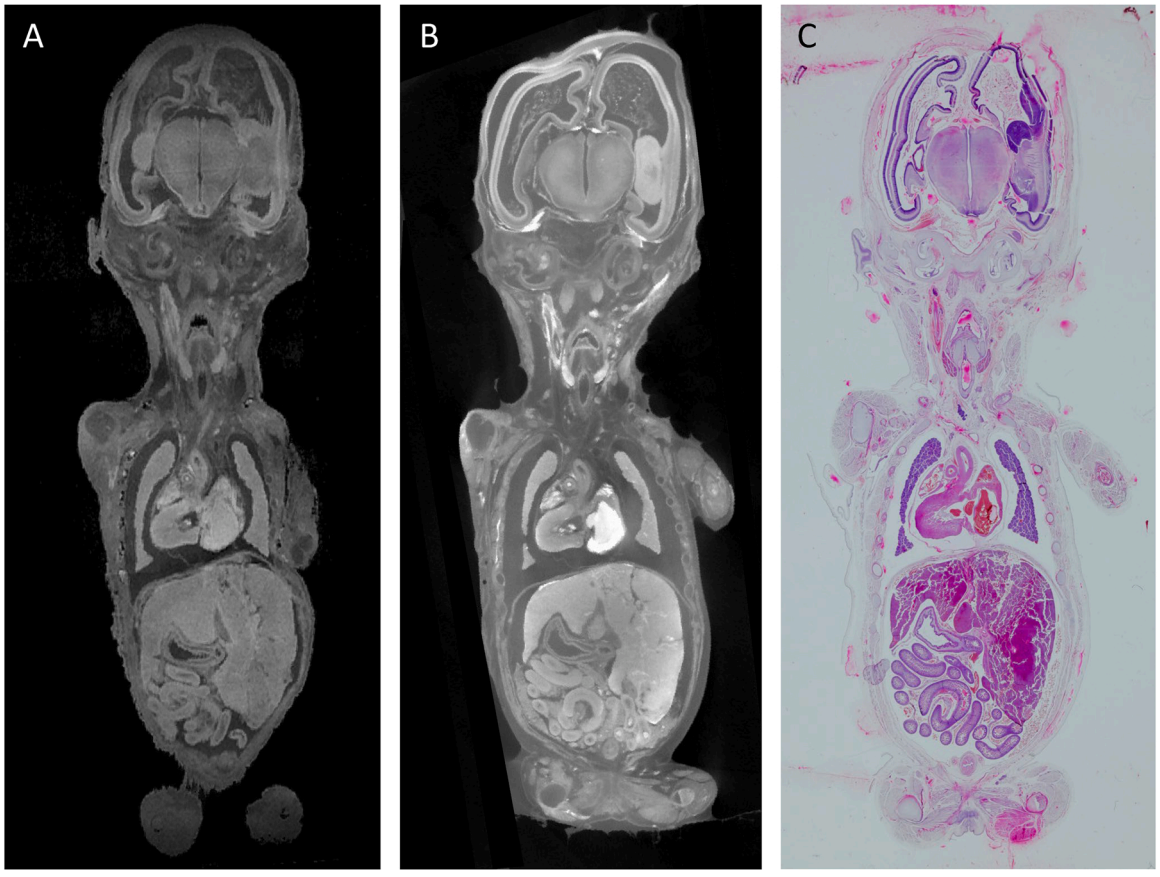

Fig. 5. Ex utero fetal imaging – 13 weeks gestation. Fetus donated to the Dutch Fetal Biobank after termination of pregnancy due to trisomy 21; total length = 9.5 cm, weight = 15 g. (A) First scanned using ultra-high field MRI (UHF-MRI) at a resolution of 100 μm. (B) Subsequently stained using 3.75% Lugol's solution and scanned using a micro-CT at resolution of 40 μm. (C) Thereafter the Lugol's solution was washed away using 4% sodium thiosulfate and the specimen was prepared for conventional histology using a haematoxylin and eosin stain.

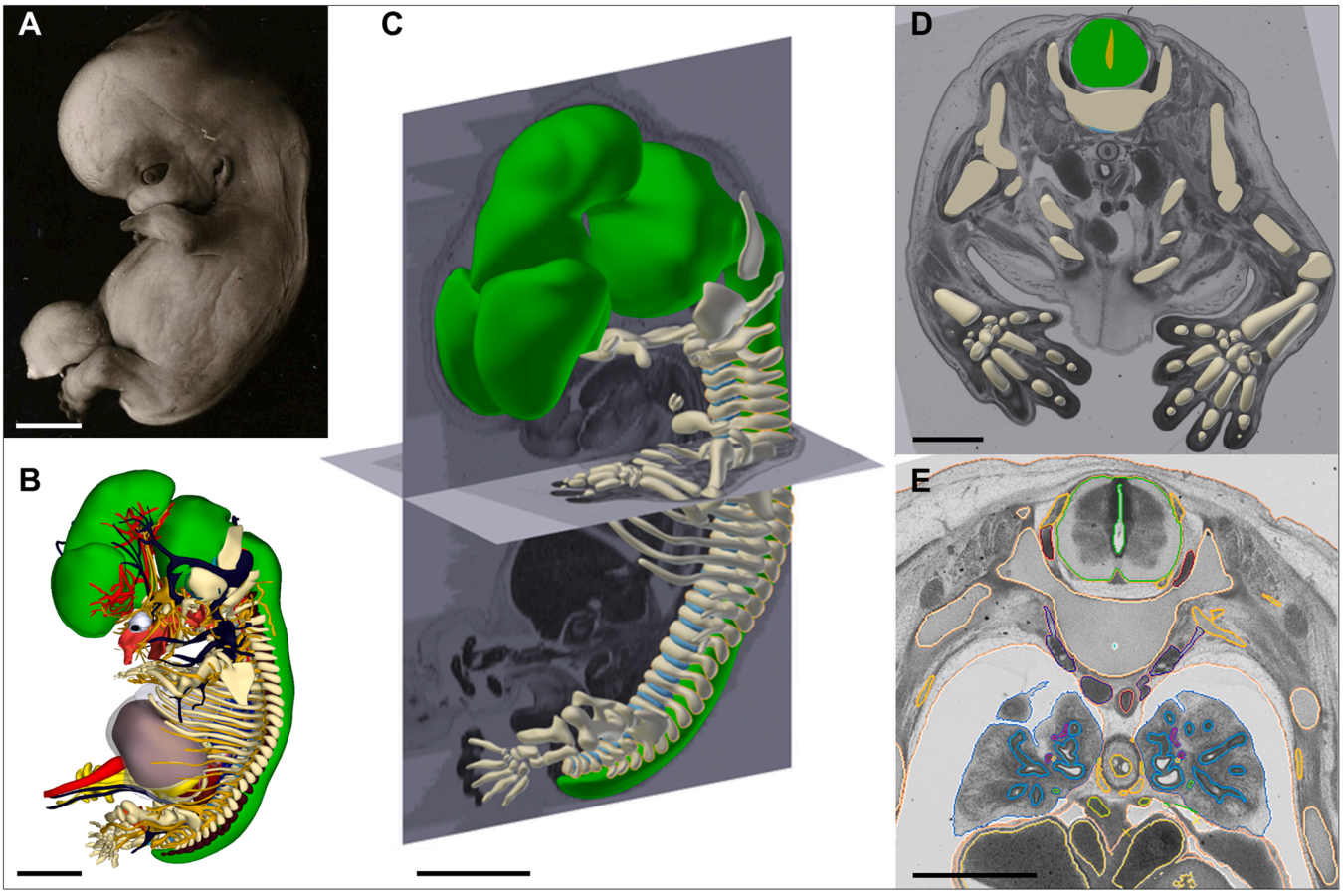

Fig. 6. Three-dimensional model of a Carnegie stage 20 human embryo (~9 weeks of gestation). (A) Lateral view of the original embryo before sectioning. (B) Lateral view of all reconstructed organs and structures, except for the skin. (C) Three-dimensional view of the reconstructed embryo highlighting the skeleton and neural tube. The sagittal plane cuts through the digitized image stack. (D) Cranial view on the transverse section from (C) through the shoulder region. (E) A detail of a transverse section through the lungs, as presented in Amira. Note the colored outline of each annotated structure. The neural tube is represented in green, the skeleton in off-white; the transparent body cavities enable inspection of the liver (brown). Scale bars, 2.5 mm [(A) to (C)], 1 mm [(D) and (E)]. Figure adapted from de Bakker et al. 2016 with permission [1].

provided insight into the contribution and distribution of different cell types within the peripheral nervous-, muscle-, vasculature-, cardiopulmonary- and urogenital system [51] (Fig. 7). Further research using similar techniques provided detailed insight into the developing human upper and lower limb muscles between 8 and 13 gestational weeks [52] and the internal- and external urogenital system between 9 and 16 weeks of gestation [53].

A major strength of this technique is that specimens can be imaged non-destructively in 3D, while achieving rapidly and highly reproducible their cellular resolution [51]. A further extension of this technique is provided by the use of multiple different fluorochromes in a single specimen, allowing the assessment of different structures or cell types within their 3D context [51], [53]. Moreover, it is possible to clear specimens of previous immunolabels and re-stain them with another set of antibodies. Currently, the main limitations of this imaging method are tissue size with respect to the imaging chamber and the diffusion of the antibody into the tissue, the availability of specific antibodies, the number of fluorochromes that can be combined, insufficient tissue clearing, and the number of consecutive staining procedures [51], [54]. We expect that most of these methodological limitations will be addressed to forward this technique [54], [55].

3.2.2. *UHF-MRI*

Ultra-high field (UHF-MRI) is a technique in which increased magnetic field strength (7.0 T and higher), this results in a higher signal-to-noise ratio (SNR), better spatial resolution and thereby more detailed imaging compared to lower field MRI (Fig. 8). So far, it has been used to study human developmental anatomy of the brain, [56], [57], face [58], and ear [59],.

The main strength of UHF-MRI compared to other imaging modalities is that it renders great soft tissue contrast without any necessity of contrast enhancement or tissue preparation [60]. Furthermore, MRI offers a variety of sequences to address specific clinical or research questions. Diffusion Tensor Imaging (DTI), for example, could be of value to study the development of white matter [61] or fiber orientation and pattern of the myocardium (Fig. 9) [62]. UHF-MRI has some important limitations. Firstly, capturing high-resolution images with high SNR, even with increased field strengths, requires long scanning times (>15 h), especially for small samples [60], and is thereby not suitable for in vivo fetal studies. Secondly, the most commonly available UHF-MRI scanners are preclinical machines that are only suitable for small animal experiments, which hampers scanning of whole fetuses older than 20 weeks of gestation [60]. Although the bore diameter of these scanners is between 16 and 30 cm, the use of a radiofrequency (RF) coil limits the inner diameter of the scanning plane to typically less than 10–20 cm. Thirdly, the number UHF-MRI machines is limited, which hampers their use in research. However, it is anticipated that these machines will become increasingly available in the near future, [63].

3.2.3. *Micro-CT*

Microfocus computed tomography (Micro-CT) is an emerging

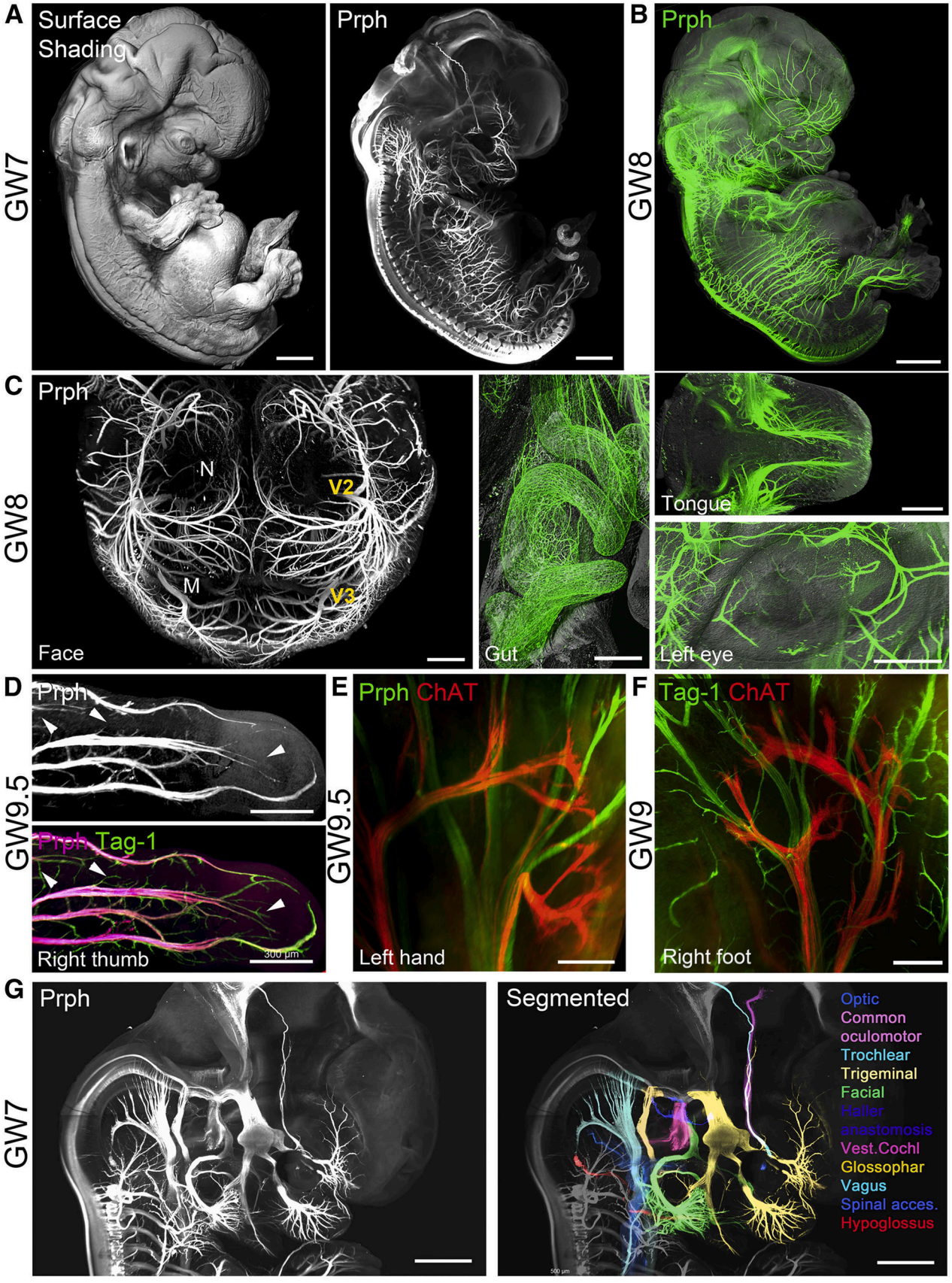

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Fig. 7. 3D analysis of peripheral nervous system development using 3DISCO. All panels are LSFM images of solvent-cleared embryos and fetuses. (A) Surface shading image (left) and Peripherin (Prph) labeling of peripheral nerves (right) at 7 weeks of gestation. (B) Overlay of the surface shading image (gray) and Prph labeling (green) at 8 weeks of gestation. (C) High-magnification images of Prph+ innervation at 8 weeks gestation. The middle and right panels are overlays of the surface contrast image (gray) and Prph labeling (green). (D) 9.5 weeks gestation thumb labeled for Prph and Tag-1. The two markers co-localize but the thinnest branches (arrowheads) are better labeled with Tag-1. (E) Dorsal view of a 9.5 weeks gestation hand double labeled for choline acetyltransferase (ChAT) (motor axons) and Prph (sensory axons), showing the lack of co-localization. (F) Dorsal view of a 9 weeks gestation foot labeled for ChAT and Tag-1. The two markers are not co-expressed. (G) Right view of the head and cranial nerves at 7 weeks of gestation (Prph staining). On the right panel, cranial nerves are segmented and highlighted with specific pseudo-colors. Abbreviations: GW, weeks of gestation; N, nostrils; M, mouth; V2 (maxillary) and V3 (mandibular), second and third branches of the trigeminal nerve. Scale bars, 1000 μm in (A) and (G), 2000 μm in (B), 500 μm in (C), 300 μm in (D), 200 μm in (E), and 150 μm in (F). Figure adapted from Belle et al. 2017 with permission [51].

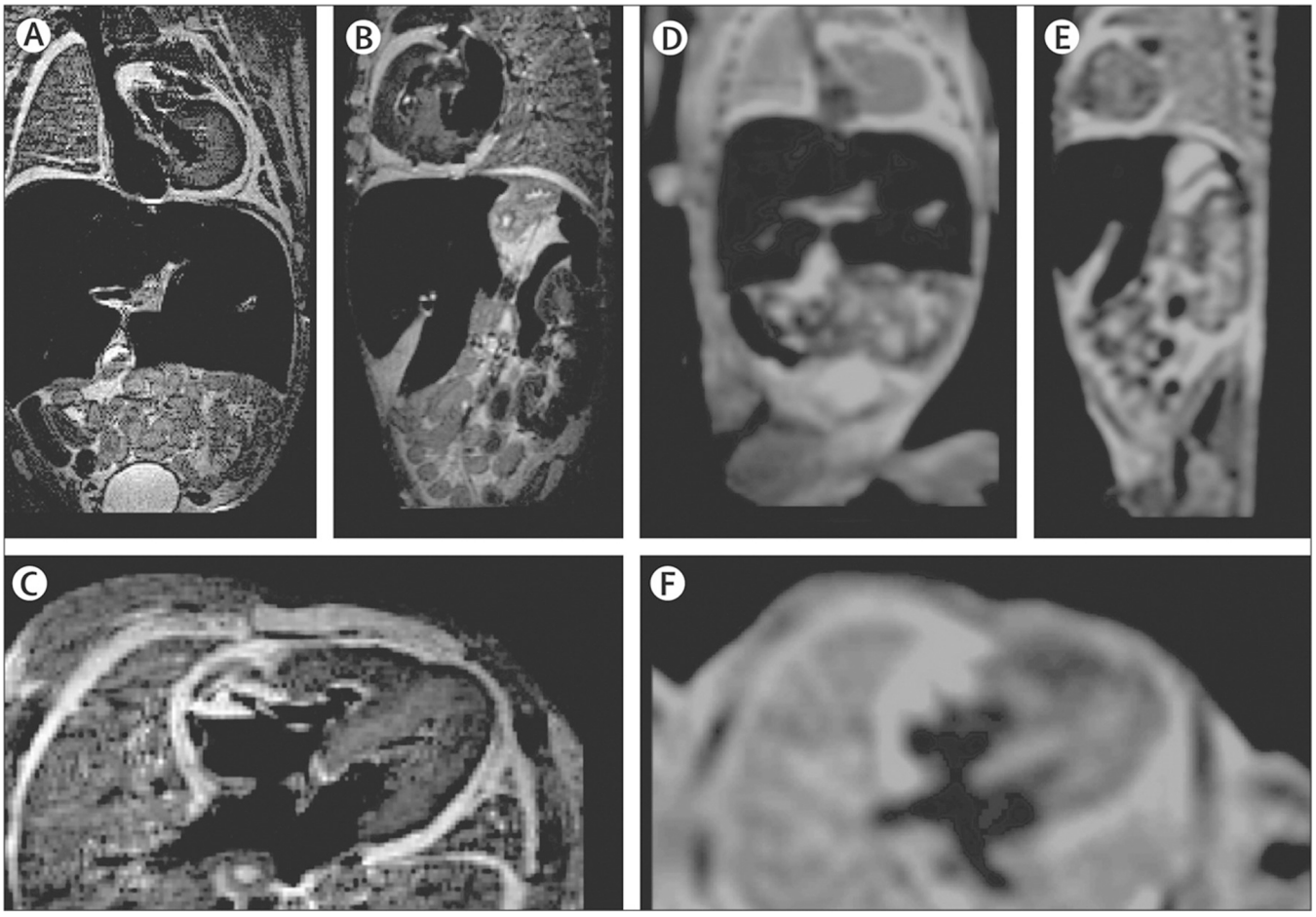

Fig. 8. Postmortem fetal imaging using MRI. Fetus obtained after termination of pregnancy at 22 weeks of gestation for skeletal dysplasia. (A) Coronal, (B) sagittal, and (C) axial images obtained with 3D T2-weighted MRI at 9.4 tesla (T). (D) Coronal, (E) sagittal, and (F) axial images obtained with 3D T2-weighted MRI at 1.5 T. Images at 9.4 T showed healthy cartilage and internal organs. Compared to the 1.5T images, the 9.4T images have a higher signal-to-noise ratio (SNR), better spatial resolution and thereby enable more detailed anatomical imaging. Figure adapted from Tayyil et al. 2009 with permission [97].

imaging tool within the biomedical field. Because micro-CT offers high-resolution images without disruption of the material, it has been used in a wide variety of non-medical industries, from non-destructive precision engineering, environmental and ecology studies to geosciences. Its technology is based on X-ray attenuation, just like conventional CT, although with some construction differences. In most micro-CT systems, the radiation source is fixed and the sample is mounted on an adjustable and rotating platform. This type of construction allows for the adjustment of the "radiation source-to-sample" and "sample-to-detector" distance yielding improved resolution (Fig. 10) [64]. However, due to low intrinsic X-ray absorption of non-mineralized embryonic and fetal tissue, this technique was ineffective to study developmental anatomy, until the identification and development of various contrast agents [65]. The addition of a contrast agent, often referred to as *staining*, enables high-resolution imaging of soft-tissue. This technique is referred to as contrast-enhanced micro-CT. For human specimens the most commonly used method to enhance contrast of soft tissue is staining with a iodine based solution [60,66]. This technique, also known as diceCT (diffusible iodine-based contrast-enhanced CT), has already been extensively used in animal research [67] and is becoming a powerful tool to study human embryonic and fetal anatomy (Fig. 11) [60], [68], [66]. The water-based iodine solutions are often referred to as Lugol's solution or in short Lugol and was first described by Jean Lugol in 1829. Lugol's solution is prepared by mixing potassium-iodide solution with metallic-iodine ($KI + I_2$), which easily dissolves and forms potassium and triiodide ions ($K^+ + I_3^-$).

A major strength of diceCT is that it can be used over a wide age range to study developmental anatomy (Fig. 12). We have previously published that imaging of tissues is feasible in embryonic specimens as young as 6 weeks of gestation [69], while others used it to scan

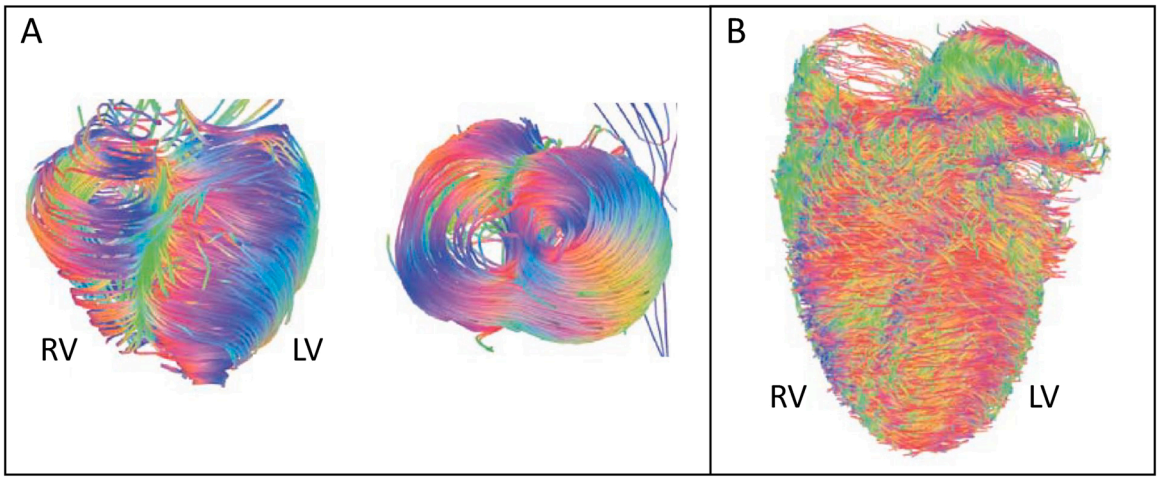

Fig. 9. Diffusion Tensor Imaging (DTI) of fetal heart. Fetal hearts from a fetus around 12 weeks of gestation (A) and around 15 weeks of gestation (B). DTI was acquired using an UHF-MRI to show the muscle fiber tract orientation in the heart. Fiber tracts are visualized from the ventral and apical point-of-view (A) and ventral point-of-view (B). LV indicates left ventricle; and RV, right ventricle. Figure adapted from Nishitani et al. 2020 with permission [62].

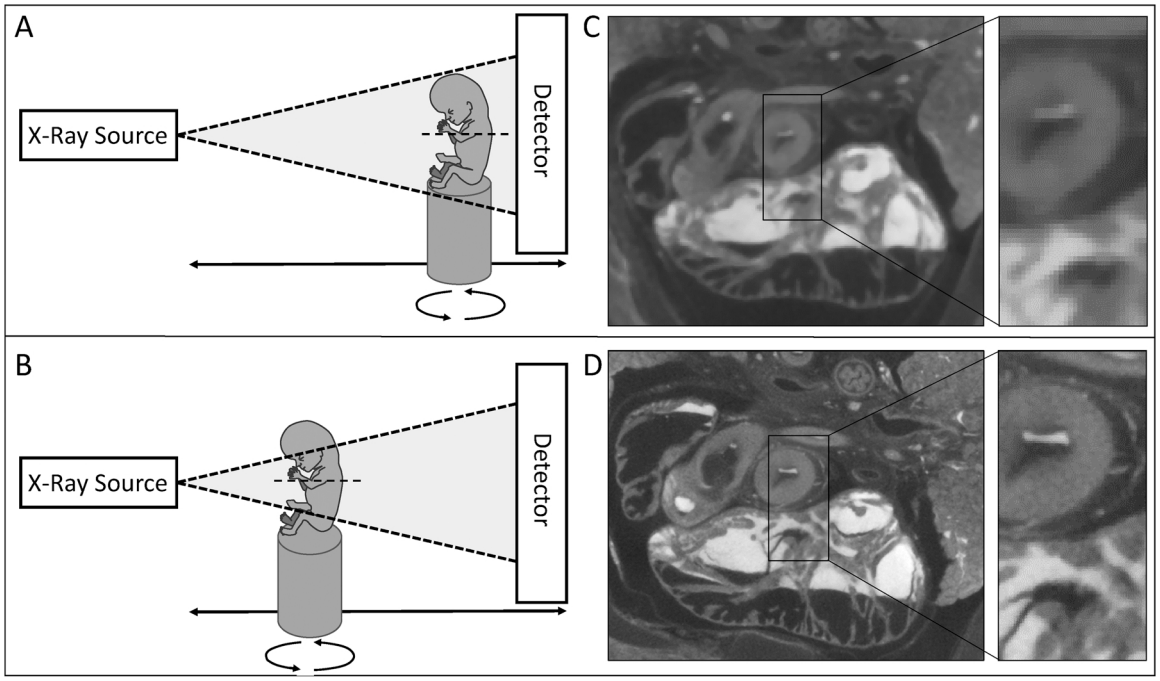

Fig. 10. Schematic representation of a micro-CT cabinet setup. The specimen is mounted on a rotating platform and placed between the X-ray source and the detector. (b) To illustrate the effect of adjusting the source to object distance, we see in the right panel a transverse image around the heart region (height position corresponding with the dashed line). (c) The distance between X-ray source and specimen is adjustable enabling scanning the complete specimen at once (A), or scanning parts of the specimen (B) resulting in a higher resolution. When the specimen is scanned in multiple runs, a high-resolution full body dataset can be reconstructed and stitched afterwards. Scanning the complete specimen resulted in 40 μm resolution (C) and by scanning in parts we could increase the resolution up to 14 μm (D).

specimens up to 24 weeks of gestation [68], with sufficient anatomical detail. Furthermore, because diceCT imaging is minimally invasive it can be used on rare or irreplaceable samples, such as embryos or fetuses with uncommon abnormalities or even unique museum specimens [70]. Moreover, specimens used for diceCT, can subsequently be used in other analysis methods such as UHF-MRI or histology. Also, histology-like resolution can be achieved in scanning time less than an hour, being less time consuming compared to histology and/or UHF-MRI. Although diceCT is very useful to study developmental anatomy, there are some limitations. Firstly, users should bear in mind, that staining is necessary when soft tissues need to be visualized. Until recently, a major drawback of staining with Lugol was that it causes soft-tissue shrinkage, [71]. This shrinkage was shown to be Lugol concentration dependent [71], [72] and varied across tissue types [71]. We have seen extensive shrinkage that varied between 15% and 35% for lung, brain, liver or total body volume after staining of human fetal specimens using Lugol [73]. However, we have recently reported that buffering the pH of the staining solution at 7.2 limits tissue shrinkage to a level that is within biological variation (0–5%) [74]. This adaption of the staining solution marks a major improvement for morphological research, as now also reliable volumetric data can be extracted from diceCT images. Secondly, because triiodide binds to lipids and glycoproteins [67], it is an excellent

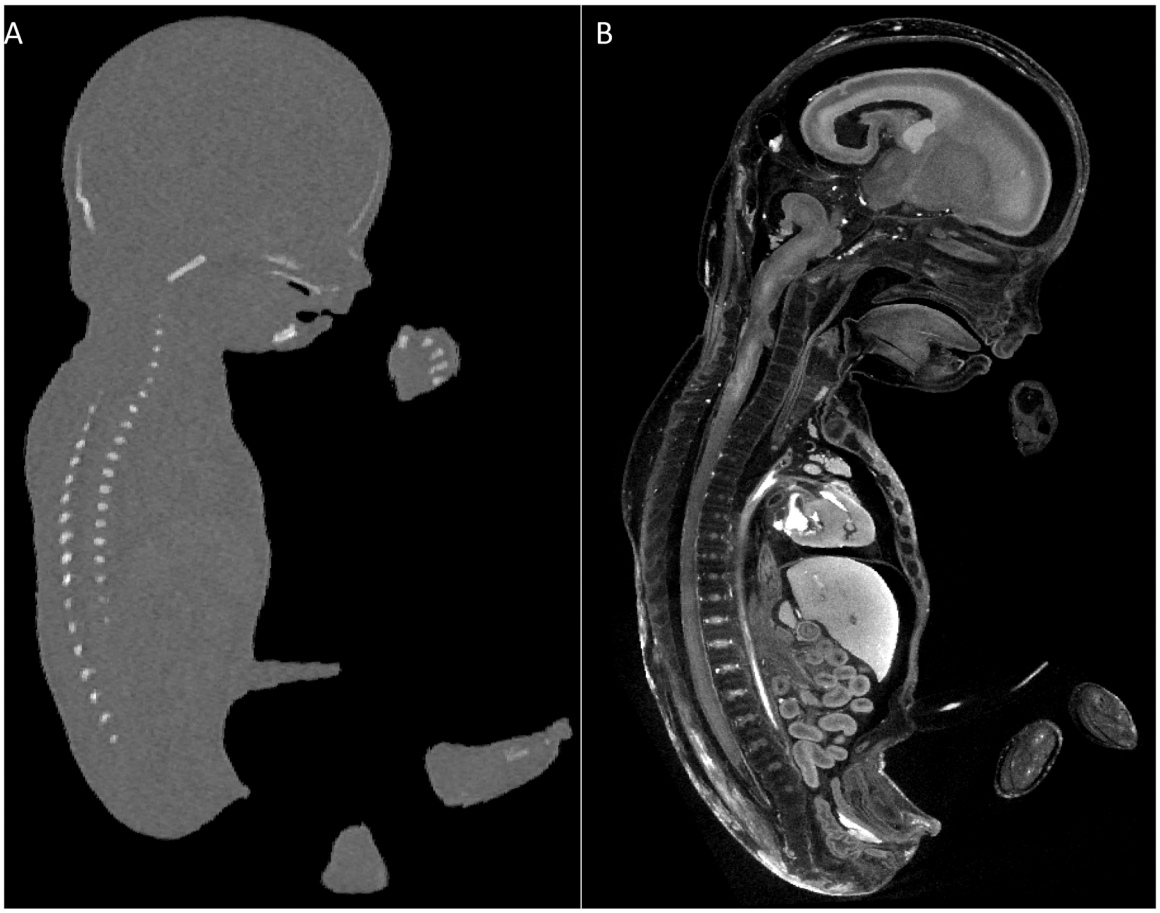

Fig. 11. Increase of soft-tissue visualization due to staining with Lugol's solution. Mid-sagittal view of a 16 week old fetus scanned using a micro-CT before staining (A) and after staining with 3.75% Lugol's solution (B). Due to staining soft-tissue contrast is significantly increased, enabling detailed analysis of the fetal anatomy.

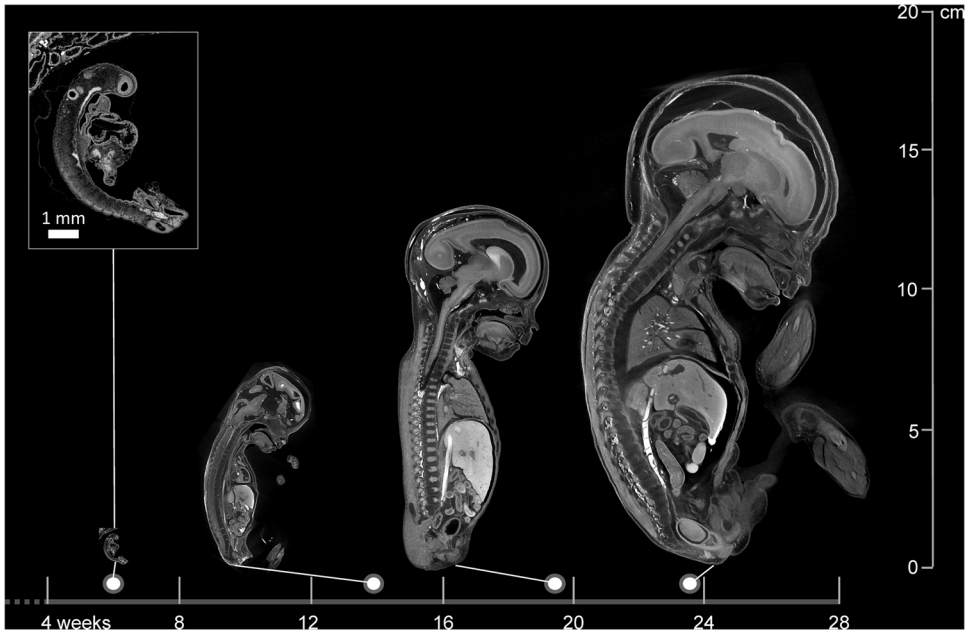

Fig. 12. Age range applicability of micro-CT to study developmental anatomy. One of the major strengths of micro-CT scanning is the wide age range applicability to study developmental anatomy as illustrated in this figure. From left to right: an embryo of 6 weeks of gestation, a fetus of 13 weeks, 20 weeks and 24 weeks of gestation. All specimens were stained using Lugol's solution and subsequently scanned using a micro-CT scanner. Resolution varied between 3 μm and 40 μm depending on specimen size. Panel scale bar represents 1 mm.

all-round staining, though less suitable to target specific tissue types. Interestingly, several research groups are currently developing more specific contrast-enhancing staining agents (CESAs) for contrast enhanced micro-CT, [75]. Thirdly, due to the high resolution, the size of micro-CT files is large, being in the order of tens of gigabytes (GB) per specimen. Consequently, this requires more costly data storage compared to UHF-MRI (<1 GB per specimen). Handling these datasets also requires high-end computers with adequate computing power, large

data storage, a high-end graphics card and enough working memory. Lastly, micro-CT imaging can be challenging for larger specimens, especially fetuses beyond 24 weeks of gestation, for a variety of reasons. For instance, homogeneous and sufficient penetration of Lugol is more difficult in large fetuses. Also, the immobilization of the fetus during the scan, preventing movement artifacts, is more difficult with larger specimens.

4. Future in imaging fetal anatomy

4.1. The use of artificial intelligence

The field of Artificial Intelligence (AI) has made remarkable progress during the last decade, especially due to Deep Learning (DL) algorithms [76]. DL algorithms excel in pattern recognition and, therefore, it is presumed that medical professions, which rely on imaging, will be the first to see the benefits of this tool [3]. One of the largest driving forces behind AI in medical imaging is the enormous amount of digital data generated around the world that may be useful in training algorithms. Therefore, AI methods have already shown their potential to qualitatively and quantitatively analyse images (classifying and measuring structures, organs, lesions, etc) in e.g., radiographs [77], brain MRIs [78] and chest CTs [79].

The potential use of AI applied to fetal imaging has recently been reviewed (Table 1) [3], [80], and several groups have evaluated its use for various reasons, such as fetal diagnosis [81] and gestational age estimation [82], [83]. Most researchers working on implementing DL techniques in fetal imaging focus on 2D-US. For example by facilitating automatic 2D-plane detection [82], [84] and probe-motion tracking, or by improving 2D-biometry measurements such as head-circumference delineation [85], [86], [87], [88]. We expect that AI could also aid in embryonic and fetal imaging in various other ways, especially in applications for high complexity classification and diagnosis of congenital defects. We speculate that in the near future 3D embryonic and fetal models, for instance from the 3D embryology atlas [1], can be used to train DL algorithms to aid sonographers to assess anatomical structures (Fig. 13). In such a setting, sonographers collect 2D and 3D images of the fetus which will be analysed by the algorithm and compared to the existing training models. Red flags or warnings would appear if structures are deviating from normal fetal anatomy, pointing to a potential birth defect. A recent publication already showed the possibility to use DL for prenatal detection of complex congenital heart disease [89], stating that it could increase the prenatal detection of congenital heart disease in comparison to normal screening. This clearly demonstrates that AI has the potential to dramatically increase the efficacy of prenatal imaging and thereby decrease the number of prenatally undetected abnormalities and allow earlier detection of congenital abnormalities and, as a consequence, improve postnatal care.

Table 1
Artificial intelligence (AI) in obstetrics: reported and expected future applications.

AI application	Clinical utility
Structure identification[83], [84]	Automatic identification of fetal limbs, facial structures, thoracic and abdominal organs to facilitate sonographer trainer and aid non-experts in performing basic scanning.
Automatic measurement [85],[86],[87],[88]	Automatic measurements in standard anatomical planes of NT, CRL, AC and other biometric parameters. Evaluation of fetal weight and gestational age. This will reduce operator bias and reduce repetitive caliber adjustment.
Anomaly highlighting and diagnosis[81],[89]	Unusual anatomical findings are identified and highlighted to aid the sonographer with the diagnosis of congenital disease such as craniocerebral malformations, congenital heart disease, polycystic kidneys.

NT = Nuchal translucency, CRL = crown-rump-length, AC = abdominal circumference.

4.2. Experimental imaging techniques

For ex utero embryonic and fetal imaging, the synchrotron radiation-based X-ray phase-contrast tomography (sCT) technique might become a powerful modality. The phase-contrast arises because both the amplitude and phase are modified as an X-ray beam propagates through an object. Since the probability for X-ray phase shift can be 1000 times larger than for X-ray attenuation, phase-contrast imaging is almost 1000 times more sensitive with respect to density resolution and permits visualization of soft tissues that have near to identical attenuation characteristics and as a consequence cannot be detected using conventional radiographs or (micro-)CT (Fig. 14) [90], [91], [92]. As a result, it enables clear soft-tissue visualization at micrometer resolution without the use of contrast agents or staining, making this imaging technique useful for biomedical research.

Proof-of-principle studies showed the ability to image disorganized myocardial architecture in a fetus with a complex heart disease [93] and ductal tissue distribution in specimens with coarctation of the aorta [90]. The latter study, showed that the contrast resolution of sCT images is comparable to that of histological assessment [90]. Furthermore, sCT allowed to discriminate the local predominant direction of myocyte organization using a 3D structure tensor approach, similar to MR diffusion tensor imaging, however with much higher resolution [93]. Although sCT is very promising for high-resolution soft-tissue imaging, it has its limitations. For instance, it is currently limited to synchrotron-facilities and therefore not easily accessible. Moreover, most sCT scanners have a limited field-of-view. Depending on the facility, the field of view is in the order of 10–15 mm for one shot imaging. Using stitching approaches, larger samples or small samples at higher resolution can be visualized, although at the expense of an increased scanning time. Recently, the first high-energy, fourth-generation, synchrotron source, named the Extremely Brilliant Source (EBS), was installed at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. The EBS has resolved some of the limitations of earlier versions, which now enabled 3D imaging of large biological samples. Using the EBS in combination with Hierarchical Phase-Contrast Tomography (HiP-CT), even enables 3D imaging of multiple intact adult human organs. This improvement allows high imaging quality from whole human organs down to individual organotypic functional units and specialized cells at any location within the organ (Fig. 14) [4]. We expect that such new modalities, in the near future, will enable embryonic and fetal imaging to map the complete human development. Further details on the various principles and techniques behind phase-contrast tomography are outside the scope of this review and are discussed in excellent reviews elsewhere [91,94].

4.3. Data sharing platform

Scientist globally are producing vast amounts of embryonic and fetal imaging data. To analyse and assess this data (even post-publication), accessibility has been and still is an issue. Some researchers provide access to their datasets online (e.g., https://www.3dembryoatlas.com/, https://transparent-human-embryo.com/, https://hdbratlas.org/). However an overarching data sharing platform where researchers can store imaging data derived from various techniques is thusfar lacking. Regarding this point the biomedical imaging field can learn much from the Human Cell Atlas (https://www.humancellatlas.org) Project. Within this initiative the transcriptional landscape of millions of individual human cells is determined; a process that generates enormous amounts of data that scientists need to store, standardize and interpret. To help coordinate data collection and processing, the HCA established the Data Coordination Platform (DCP), a cloud-based platform where scientists from around the world can share, organize and interrogate single-cell

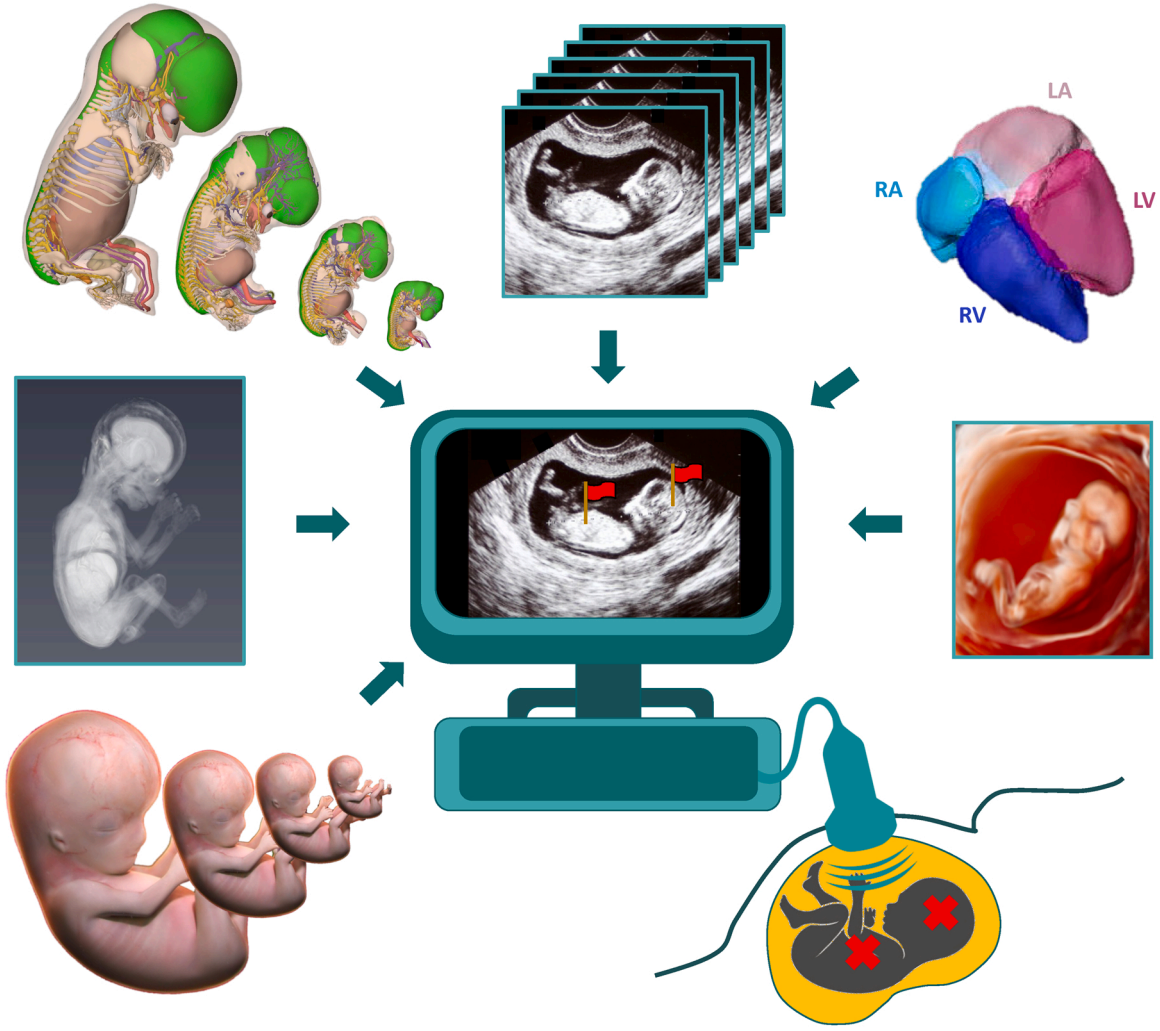

Fig. 13. Use of Artificial Intelligence (AI) in ultrasound. We envision that the ultrasound machine can be trained using input from several clinical and experimental 2D and 3D datasets to learn how normal anatomy of a developing fetus should look like, and warns the sonogapher when a deviation from the default anatomy is detected. In this illustration red flags appear signaling a cranial and an abdominal defect. This way, the number of missed birth defects can decrease dramatically.

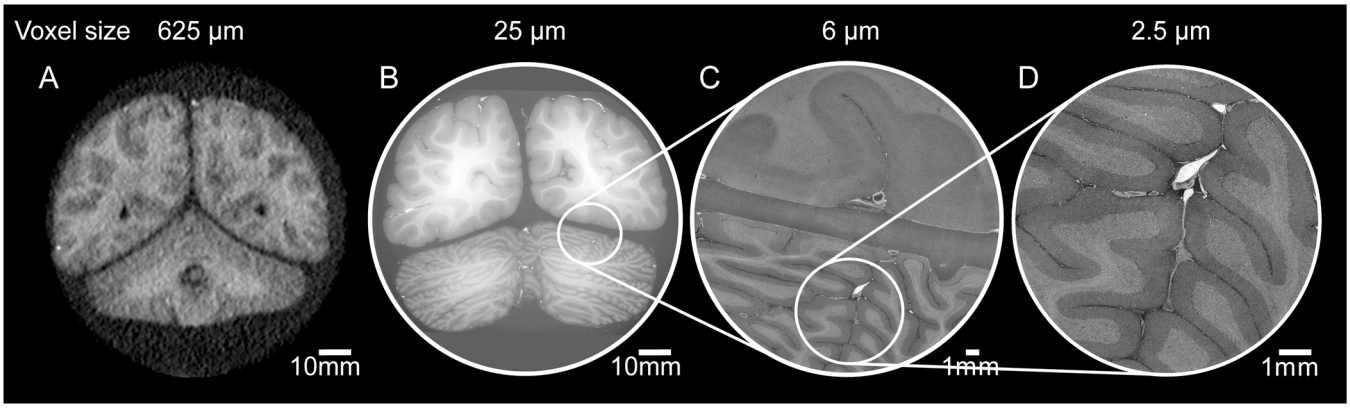

Fig. 14. Imaging intact human organs using HiP-CT. 2D slice of a whole brain scan using (A) conventional attenuation based CT at 625 µm resolution and (B) Hierarchical Phase-Contrast Tomography at 25 µm resolution (HiP-CT). As illustrated HiP-CT enables soft tissue visualization at histology-like resolution without the necessity of staining. Moreover, HiP-CT enables the selection of an volume of interest (VOI) from the whole organ scan (B) for scanning at higher resolution (C and D). HiP-CT images are adapted from Walsh et al., 2021 [4] with permission and the data is openly accessible from the ESRF data repository (https://human-organ-atlas.esrf.eu/).

data in a pre-set format with a required minimal set of required information. Anyone can contribute data, find data, or access community tools and application. Such a data sharing platform could be of great value for the biomedical imaging field, and in particular as source to train AI networks.

5. Concluding statement

Since Leonardo da Vinci drew the first fetus within a uterus, a continuously advancing range of imaging techniques has been developed to study the human embryo within the intimacy of the womb. Be that as it may, the early first trimester remains in part a 'black box' due to the limited size of the developing embryo. While ultrasound techniques are being pushed to the limit, other ex utero imaging techniques such as Micro-CT are required to facilitate proper annotation of fetal structures in ultrasound scans of early first trimester embryos. Combining multiple imaging techniques and a large-scale data sharing platform may pave the way for using AI in ultrasound and the first steps towards automatic recognition of aberrant anatomy on a 3D ultrasound sweep, improving the detection rate of congenital abnormalities in the developing embryo.

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Declarations of interest

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