Micro-computed tomography of isolated fetal hearts following termination of pregnancy: a feasibility study at 8 - 12 week's gestation.

Running head: Post-mortem micro-CT of isolated fetal hearts: a feasibility study

Content Information	
Number of Words	2421
Number of tables:	1
Number of figures:	6

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/pd.5719.

Conflict of Interest: The authors have no conflicts of interest to disclose

Funding information: NA

What's already known about this topic?

- Cardiac evaluation of major anomalies is possible at late first trimester at the time of nuchal translucency scan.
- The postmortem contrast-enhanced micro-computed tomography (micro-CT) was shown to be a promising technique for ex-vivo cardiac evaluation in first- and second-trimester human fetuses and isolated human fetal hearts.
- No studies are available on the feasibility and on the retrieval rate of the heart specimens in late first trimester.
- None of the studies has evaluated the feasibility of virtual cardiac autopsy in very small hearts before 11 weeks' gestation.

What does this study add?

(1) We demonstrated that fetal hearts can be retrieved after first-trimester surgical termination of pregnancy in the vast majority of cases from 8 weeks onwards.
(2) Post-mortem micro-CT can be used to assess cardiac anatomy from as early as 8 weeks.

Data Availability Statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ABSTRACT

Objectives:To assess the feasibility of retrieval of intact human fetal hearts after first trimester surgical termination of pregnancy (TOP) and subsequent anatomical assessment by postmortem micro-computed tomography (micro-CT).

Methods:In a cohort of consenting women undergoing surgical TOP between 8 and 13 weeks' gestation, we attempted the retrieval of the fetal heart from the suction material. Specimens were immersion fixed in 10% formaldehyde, scanned by Iodine enhanced micro-CT and cardiac anatomy assessed by a multidisciplinary team using 3D-multiplanar analysis.

Results:The median gestational age at TOP was 10.7weeks (range 8.3–12.9). In 57 (95.0%) out of 60 suction specimens, the heart could be retrieved. The median cardiac length was 5mm (range 2-8mm), in three (5.3%), the heart was too damaged to assess cardiac anatomy and in five (8.7%) only the four chambers could be examined. In the remaining 49 (86.0%) cases, a detailed assessment of cardiac anatomy was possible, showing a major defect in two (4.1%) and a minor defect in four (8.2%).

Conclusions:Fetal hearts can be retrieved after first trimester TOP being intact in the vast majority of cases. Iodine enhanced, post-mortem micro-CT can be used to assess cardiac anatomy from as early as 8 weeks and to describe heart abnormalities.

Introduction

Over the last decades, advances in ultrasound fetal imaging have significantly improved the ability to diagnose congenital structural anomalies during the prenatal period. Congenital heart abnormalities are the most common birth defects[1-3] and a significant proportion of these can be suspected or diagnosed by obstetric ultrasound towards the late first trimester of pregnancy[4,5]. In cases of early fetal death or termination of pregnancy, autopsy is recommended to verify prenatal findings. Post-mortem assessment of fetal heart through anatomical dissection can be particularly challenging at early gestational weeks as it requires experienced operators due to the small size of the specimens. Alternative, non-destructive approaches to postmortem assessment of first trimester human hearts have been investigated, such as high-field magnetic resonance imaging (MRI)[6-11] and micro-computed tomography (micro-CT)[12-14]. Recent studies have shown that postmortem micro-CT provides similar diagnostic accuracy to conventional autopsy across multiple body systems and in isolated fetal hearts. Votino et al. examined six fetal hearts at 11-14 weeks and showed that cardiac structures could be assessed with high-field 9.4 Tesla MRI but not with lower field strength magnets (1.5 and 3 Tesla)[11]. The feasibility of postmortem contrast-enhanced micro-computed tomography (micro-CT) has been demonstrated in ex-vivo first- and second-trimester human

fetuses and isolated human fetal hearts[12]. Another report on the diagnostic accuracy of post-mortem micro-CT examined 20 human fetuses between 11 and 21 weeks' gestation and showed a high level of agreement with conventional autopsy across multiple organ systems[13], suggesting that micro-CT might be a promising technique for cardiac evaluation, with near histological levels of detail. In a recent report on the assessment of isolated fetal hearts between 12 and 22 weeks' gestation the authors conclude that micro-CT is at least equal to conventional autopsy for post-mortem confirmation of congenital heart disease and that it is superior to conventional autopsy in small specimens[14]. Moreover, micro-CT imaging has the advantage of generating 3D data sets of the whole fetus or isolated organs that can be reviewed at any stage for clinical, research and teaching purposes. Episcopic microscopy was shown to provide high-quality three-dimensional original volume datasets without the artifact produced by the staining procedure used in µCT, however the latter technology is destructive and time consuming[15-16]. X-ray Phase Contrast Imaging, an imaging modality for biological tissues that does not destroy or alter the properties of the specimen, has been investigated in early human hearts[17], but requires a special source of energy that is produced by synchrotron beamlines of which there are a limited number worldwide.

Micro-CT technology appears to be the most acceptable compromise between costs, turnaround time and diagnostic accuracy. Regardless of the technology used, among the non-destructive methodologies, none of the studies has evaluated the feasibility of virtual cardiac autopsy in very small hearts before 11 weeks' gestation. In addition, no studies have examined the ability to retrieve first trimester human hearts in women undergoing surgical evacuation of the uterine cavity.

The aim of this study was to assess the ex-vivo retrieval rate of first trimester embryonic and fetal hearts in women undergoing surgical termination of pregnancy and to evaluate cardiac anatomy by postmortem micro-CT imaging.

Materials and Methods

The study population comprised single center (Ospedale Maggiore Policlinico, Milan, Italy) patients undergoing first trimester surgical termination of pregnancy. Inclusion criteria were all consecutive cases for which informed patient consent for specimen retrieval and conservation was available, including consent for postmortem micro-CT imaging. According to the Italian Law (no. 194/1978), women have the option of termination of pregnancy for personal or social reasons within 90 days' gestation (12.9 weeks). This study was approved by the Ethics Committee of Maggiore Policlinico Hospital of Milan, Italy (Protocol n. 397, approval date: 24/02/2015).

Specimen retrieval and isolation

Pregnancy termination was carried out by cervical dilatation, uterine suction evacuation followed by curettage (D&C). Immediately after the procedure, the suction material was inspected and searched with the objective to find structures that were likely to be the embryonic or fetal heart. The retrieved specimens were first immersed in an isotonic sodium chloride solution with the purpose to wash out the blood. If needed, this procedure was repeated until no blood clots were visible. The remaining material was placed on a medical gauze to facilitate inspection and identification of the heart. In a few cases in which either the whole fetus or heart and lung en-bloc were recovered, the heart was removed from the chest and isolated from the lungs. Specimens were measured from the cardiac base to the apex (Fig. 1), immersed in 10% aqueous buffered formaldehyde and stored at room temperature until micro-CT scanning. The entire procedure was carried out by a single operator (I.S.) in all cases.

Micro-CT scanning procedure

First the specimens were stained by immersion in lugol solution 15% (10g potassium iodide and 5g iodine in 100mL water) for 24 h. Our choice to use a iodine concentration of 15% (while previous studies refer to as 5%) is related to a lower sensitivity of the micro-CT equipment we used in this study. Subsequently, the heart was washed with alcohol to remove free iodine, blotted dry and scanned according to previously described protocols[12]. All scans were performed with a micro-CT scanner SkyScan 1176 (Bruker, Kontich, Belgium) at a resolution of 9 µm. Acquired images were rendered and exported as a three-dimensional (3D) volume. Post-processing analysis was performed using CTVox volume rendering 64bit version, DATAVIEWER 64bit version (Bruker, Kontich, Belgium) and Horos (v 2.4) software (free and open source code software at Horosproject.org). All scans were performed by a radiologist (C.L.) with extensive experience in micro-CT imaging[12,14].

Micro-CT image analysis

The 3D volume was imported into a computer software that allows multiplanar navigation through the three orthogonal planes to examine a region of interest (Fig. 2). A preliminary assessment of the volume was performed to confirm that the specimen was indeed the heart and to ensure that the

image quality was sufficient to allow examination of cardiac morphology. Figure 3 demonstrates micro-CT images of different cardiac views at different gestational age. The analysis of cardiac anatomy was performed using a sequential segmental approach. First, the cardiac chambers and the atrioventricular connections at the level of the four-chamber view were evaluated (Fig.3c). Second, the outflow tracts and ventriculo-arterial connections were assessed in relation to their size, origin and course (Fig. 3d,e). Third, the presence of the atrioventricular and great arterial valves, as well as the atrioventricular and ventricular septums were noted. Image assessment was carried out by a multidisciplinary team that included a cardiac morphologist (A.C.), a radiologist (C.L.) and two obstetricians (S.B., I.S.). All cases were reviewed by the whole team and a consensus agreement was achieved on the diagnosis of a normal or abnormal heart.

Results

A total of 60 patients were enrolled in the study. In this study we included all consecutive cases where women requested termination of pregnancy for personal reasons, therefore no detailed clinical data was available. The median maternal age was 27 (range 18-44) years and the median gestational age at termination was 10.7 weeks (range 8.3–12.9 weeks).

Specimen retrieval and isolation

The time needed to isolate the heart from the suctioned products of conception, ranged from 3 to 10 minutes, with a median of 4 minutes. From a total of 60 attempts, the operator labelled a specimen as the heart in 57 (95.0%) cases. In two cases no heart could be identified in the suction material despite repeated washing-out procedures. This might be probably due to the small size of the heart, both of them being below 9 weeks of gestation (one case at 8.6 weeks; one case at 8.7 weeks). In one case, at 12.4 weeks, the heart was too damaged and it was not suitable for further analysis. In the other specimens (n=57), the median cardiac length was 5mm (range 2-8, Fig. 4). The distribution of retrieval attempts according to gestational age is shown in Figure 5.

Micro-CT image analysis

On micro-CT imaging, all 57 retrieved specimens were confirmed to be the heart. In three (5.3%) (at gestations of 8.3 weeks, 9.3 weeks, 10.6 weeks), the specimen was confirmed to be the heart but was too damaged to assess cardiac anatomy and in five (8.7%) hearts, only the cardiac chambers could be discriminated (at gestations of 9.4 weeks, 10.9 weeks, 11.6 weeks, 11.7 weeks, 12.4 weeks resp.) because the great arteries had become separated, and no evaluation of the outflow tracts was possible. We assume that this damage may have been caused by the high pressure during the vacuum aspiration. In the remaining 49 (86.0%) cases, a full detailed assessment of cardiac anatomy by micro-CT imaging was possible. All the specimens demonstrated good internal contrast on micro-CT examination indicating correct iodination. In 48 (88.9%) of 54 cases suitable for partial (n=5) or complete (n=49) assessment, micro-CT image analysis demonstrated normal cardiac anatomy. A major cardiac defect was diagnosed in two (3.7%) cases. In the first case at 12.3 weeks (a case of thoracopagus conjoined twins), a double outlet right ventricle with pulmonary stenosis, a VSD with overriding aorta and left-sided juxtaposition of atrial appendage was detected

(Fig. 6a,b). In the second at 9.3 weeks, an atrioventricular septal defect (with common AV junction) was demonstrated. In four (7.4%) cases, minor cardiac defects were identified (shown in Table 1).

Discussion

We have demonstrated that embryonic and fetal hearts can be retrieved from suction specimens following TOP in the vast majority of cases. We chose the period between 8 and 12 weeks as routine antenatal ultrasound study of the heart is increasingly shifted to the first trimester. In case of subsequent termination of pregnancy or early miscarriage, retrieval of a specimen is the first necessary step for diagnostic autoptic procedures. Our study is the first to report on the retrieval rates of human heart specimen early in gestation.

The availability of the whole fetus or relevant body parts for postmortem examination is strongly dependent on the type of procedure used to remove the products of conception. Following spontaneous or medically induced abortion, there is a high chance that one is able to retrieve the whole fetal body. However, surgical evacuation may be preferred before 14 weeks' gestation[18], either based on patient request, or as a back-up after failure of medical treatment, which occurs in 2-5% of the cases[19]. Nonetheless, following surgical termination of pregnancy, the integrity of the fetal body is not preserved. Therefore, we aimed to assess the feasibility of retrieving first trimester hearts from pregnancy products removed through suction evacuation. When inspecting the suction material, especially early in gestation, it might be challenging for the operator to distinguish the heart. One must rely on subtle signs as an increased consistency of the tissue and identification of anatomical details suggesting the selected specimen to be a heart. Our findings showed that the heart can be easily isolated in 95% of the cases (81.7% completely intact) and that the procedure can be carried out in few minutes. A first check can be performed by inspecting the suction material and typically the heart can be identified. If not, additional washing of the material enables the heart to be found. Based on this experience it seems feasible to perform the heart retrieval in a surgery room immediately after the D&C procedure.

When operator defines the recovered specimen as a possible heart, micro-CT is an excellent non-destructive method to confirm the real nature of the retrieved sample. We demonstrated that post-

mortem micro-CT provides high quality images of cardiac anatomy and can detect congenital heart abnormalities from as early as 8-10 weeks' gestation. In the literature among the studies on cardiac assessment by post-mortem micro-CT so far only one study on the feasibility of micro-CT has included four entire fetuses before 11 weeks, without assessment of isolated organ systems[12]. In the present study, a total of 35 embryonic hearts at 8-10 weeks' gestation were included and micro-CT provided highly accurate imaging of the cardiac structures in most of these cases. The feasibility to retrieve human heart in embryo stage is important to provide specimen to be evaluated by micro-CT, which allows to assess cardiac microstructure at different stages of development. In the future the latter knowledge might be helpful to understand the complexity of heart morphogenesis and the associated functional consequences of congenital heart disease and provide appropriate treatment strategies.

In clinical setting this methodology might be used in the future to validate prenatal diagnosis in case of TOP for cardiac abnormalities. In our series of 49 cases, a major cardiac defect was detected on micro-CT imaging in 4% of our population, which is significantly higher than the known prevalence of 0.7-1.2%[20,21]. This may have been caused by an unintentional selection bias or due to the fact that the prevalence of cardiac defects is higher in early pregnancy and decreases through the first trimester because of a higher loss rate of affected embryos and fetuses[22]. However, diagnostic accuracy of micro-CT for assessment of cardiac defects was beyond the aim of the present study.

Our study has some limitations. First, the feasibility of specimen retrieval is based on a single operator, single center experience. Secondly, the micro-CT procedure was not optimised for each gestational age. The same resolution was used for the entire range gestational ages, as the main purpose of our study was focused on the heart recovery rate at early gestational age and we used micro-CT to confirm the specimen to be a heart. We studied our samples at a fixed resolution of 9 microns as this was the best available in our equipment, considered relatively old. There are advanced microcomputed tomography scanner that provide much higher resolution. We were also able to assess the basic segmental anatomy of the heart, however fine details of the heart such as coronary arteries and subtle valve details, particularly early in gestation, were beyond the limit of resolution of the current micro-CT protocol. In the present study, no other methods were used to verify the diagnosis yielded by micro-CT, and the normality or abnormality of fetal hearts was

decided by consensus alone. However, conventional autopsy is challenging in early gestation fetuses and could be precluded due to very small size of the specimen or when autolysis is present.

In conclusion, fetal heart specimens can be quickly and successfully retrieved from D&C material with a high recovery rate from eight weeks of gestation onwards. Furthermore, postmortem micro-CT allows the rapid demonstration of cardiac anatomy from 8 weeks of gestation and could be a valuable tool for ex-vivo anatomical evaluation of the fetal heart in early gestation.

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Table 1. Pathological findings

Major cardiac defects	
9.3w	AVSD with common AV junction
12.3w	Complex CHD: double outlet right ventricle, VSD, overriding aorta, pulmonary artery stenosis, left-sided juxtaposition of atrial appendage (in thoracopagus conjoined twins)
Minor cardiac defects	
11.9w	muscular VSD
12.1w	perimembranous VSD
12.3w	muscular VSD
12.9w	muscular VSD

AVSD – atrioventricular septal defect; CHD – congenital heart defect; VSD – ventricular septal defect.