Micro-CT to study and reconstruct fetal and infant coronary arteries: a pilot study on a novel post-mortem technique

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SUMMARY

For a significant part of infant and fetal deaths, specific pathophysiologic processes cannot be recognized. Thus, the scientific community is called to identify novel post-mortem diagnostic tools. This manuscript proposes the results of a pilot study which reports a novel post-mortem technique to study and reconstruct fetal/infant coronary arteries. The study included human fetuses characterized by the absence of macroscopic cardiac abnormalities at post-mortem in situ examination. For the study of fetal hearts, it was used a curing radiopaque silicone rubber compound (which solidified after injection in the coronary arteries) and an X-Ray microtomography (micro-CT). After micro-CT scans, coronary arteries' branches were reconstructed throughout a specific software. At injection, it was possible to macroscopically evaluate coronary arteries' perfusion. The analysis of the three-dimensional reconstructions highlighted that the aforementioned compound reached deep branches too. This approach can be considered a novel post-mortem technique for fetal/infant hearts. Nevertheless, the manuscript also discussed the following limitations: in some spots, coronary arteries' reconstruction appeared interrupted; the compound also perfused parts of internal cardiac chambers. Until now, in the literature there are not methods that allow study with reconstruction of fetal/infant coronary arteries throughout micro-CT. The present paper pointed out the first indications for the application of this technique in human samples.

Key words: Micro-CT – Coronary arteries – Fetus – Infant – Post-mortem technique

INTRODUCTION

According to the Center for Disease Control and Prevention (CDC), each year about 3,400

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sudden unexpected infant deaths (SUID) occur in the United States. Among them, the 37% is attributable to the sudden infant death syndrome (SIDS) and the 34.7% to unknown causes (https://www.cdc.gov/sids/data.htm). It is also important to know that the most common negative outcome of pregnancies is represented by fetal death. 2.6 million stillbirths are globally reported in 2015 (Lavezzi et al., 2019). In 2014, in the United States about 24,000 stillbirths were reported. 30% of them was attributable to unspecified causes (https://www.cdc.gov/ncbddd/stillbirth/data.html).

In the light of the above, it can be stated that, for a significative part of infant and fetal deaths, the specific pathophysiologic processes cannot be recognized, not permitting to communicate the specific cause of death to parents and to identify preventive measures (Lavezzi et al., 2019). In addition, in case of medical malpractice claims and/or inspections requested by prosecutors, the impossibility to identify a certain – or at least probable – cause of infant/fetal death is a significant limitation for forensic operators (Kettner et al., 2014; Lupariello et al., 2019; Lupariello et al., 2021; Rutty et al., 2019).

In order to reduce the number of fetal/infant deaths with unknown causes, the scientific community should be called to explore and identify novel post-mortem diagnostic tools which can allow to give new insights in pathology and forensic routine.

During standard post-mortem evaluations, a meaningful limitation is represented by the extreme difficulty to analyse and reconstruct superficial and deep coronary arteries of fetal/infant hearts. For this reason, this manuscript describes a novel post-mortem technique which can be useful to reconstruct and study these arteries. Even if until now in the scientific literature there are no clear indications about this novel tool, the abovementioned technique (capable to highlight the architecture of fetal/infant coronary arteries) could be commonly used in post-mortem examination.

MATERIALS AND METHODS

Study Samples

The study included 7 human fetuses (15 to 36 weeks of gestational age) characterized by negative prenatal ultrasonography for heart diseases. They had been referred to the Department of Pathology of the University of Turin after spontaneous demise. Parental consent for the study was obtained. All fetuses underwent post-mortem examination (24 to30 hours: interval time between miscarriage and post-mortem examination). In all cases, in situ examination of cardiovascular system confirmed the absence of macroscopic cardiac abnormalities.

Equipment

For the study of fetal hearts the authors used: a) an X-Ray microtomography (SkyScan1172, Bruker; settings: acquisition at 80KV using a 0.5mm aluminium filter at a resolution of 10 µm, 0.4° of rotation step, 180° scan, 4x frame averaging; for each sample, scanning time was fixed to 90 minutes) (Lupariello et al., 2021); b) a curing radiopaque silicone rubber compound (Microfil MV-122, Flow Tech Inc) that – initially fluid – solidifies after about 30 minutes from its injection.

Procedures

Before hearts' removal, pathologists realized a small hole in the superior surface of the aorta – just before the origin of the brachiocephalic artery. Clamping the aorta in order to block the flow towards its distal branches, pathologists injected the curing radiopaque silicone rubber compound (Microfil MV-122, Flow Tech Inc) towards coronary arteries' origins. Then, the 7 hearts were removed, fixed in 4 % aqueous buffered formaldehyde, and stored at room temperature for 20 to 30 days. Hearts were scanned by the X-Ray microtomography (SkyScan1172, Bruker) with the abovementioned settings. Finally, a three-dimensional (3D) reconstruction of hearts' coronary arteries was obtained throughout the CTvox software. Then, all 3D reconstructions were evaluated to identify possible defects and/or anomalies of perfusion. These data are reported in Table 1.

Table 1 - Summary of the results

Case number	Gestational age (weeks)	Methodology	Perfusion's pattern	Reconstruction's anomalies	Perfusion
1	17	Microfil injection towards coronary arteries' origins	Microfil perfused both right and left coronary arteries	Reconstruction's defects of deep branches	Incomplete
2	20	Microfil injection in the coronary arteries	Microfil perfused both right and left coronary arteries	Reconstruction's defects of superficial and deep branches; perfusion of parts of internal cardiac chambers	Incomplete
3	15	Microfil injection in the coronary arteries	Microfil perfused both right and left coronary arteries	Reconstruction's defects of deep branches; perfusion of parts of internal cardiac chambers	Incomplete
4	19	Microfil injection in the coronary arteries	Microfil perfused both right and left coronary arteries	Reconstruction's defects of deep branches; perfusion of parts of internal cardiac chambers	Incomplete
5	25	Microfil injection in the coronary arteries	Microfil perfused both right and left coronary arteries	Reconstruction's defects of deep branches	Incomplete
6	33	Microfil injection in the coronary arteries	Microfil perfused both right and left coronary arteries	Reconstruction's defects of superficial and deep branches; perfusion of parts of internal cardiac chambers	Incomplete
7	36	Microfil injection in the coronary arteries	Microfil perfused both right and left coronary arteries	Reconstruction's defects of deep branches	Incomplete

RESULTS

The most significant results are available in Table 1, in Figs. 1 and 2, and in **Videos 1** and **2**. At injection, thanks to the rubber compound's yellowish color, it was possible to macroscopically evaluate coronary arteries' perfusion. However, the perfusion was incomplete in all cases because the Microfil did not reach all coronary branches (Table 1). Video 1 and Fig. 1 depict the compound filling superficial coronary arteries of a fetal heart (case number one; Table 1). The three-dimensional reconstruction highlighted that Microfil perfusion also reached deep branches. Nevertheless, in some spots coronary superficial and/or deep arteries' reconstruction appeared interrupted. In addition, in some cases the Microfil also perfused parts of internal cardiac chambers (Table 1, Video 2, Fig. 2). Comparing the three-dimensional reconstructions with gross examination' findings, in all cases the X-Ray microtomography (in association with Microfil injection) demonstrated a better capability to highlight the not superficial branches of the coronary arteries.

DISCUSSION

Unlike conventional computed tomography (CT), Micro-Computed Tomography (micro-CT) is not yet implemented in clinical routine. Its resolution ability is under to 1 mm^3 (higher than CT). Nevertheless, it can be used only to analyse small samples. Indeed, in the scientific community it has been commonly used to study (in and ex vivo) animal fetuses and organs (Clark et al., 2014; Degenhardt et al., 2010; Hutchinson et al., 2017; Hutchinson et al., 2018; Lombardi et al., 2014; Liu et al., 2013; Lombardi et al., 2019; Lupariello et al., 2021).

Emerging applications of micro-CT in human samples consist of "the study of fetal/infant organs and whole fetuses, and their two/three-dimension reconstruction, representing an innovative approach because it allows: to facilitate pathologists' role in the identification of causes of fetal stillbirth and of infant death; to create digital two and/or three-dimension representations of fetal/infant organs and whole fetuses which can be easily discussed in civil and/or penal courts" (Lupariello et al., 2021). In particular, the scientific

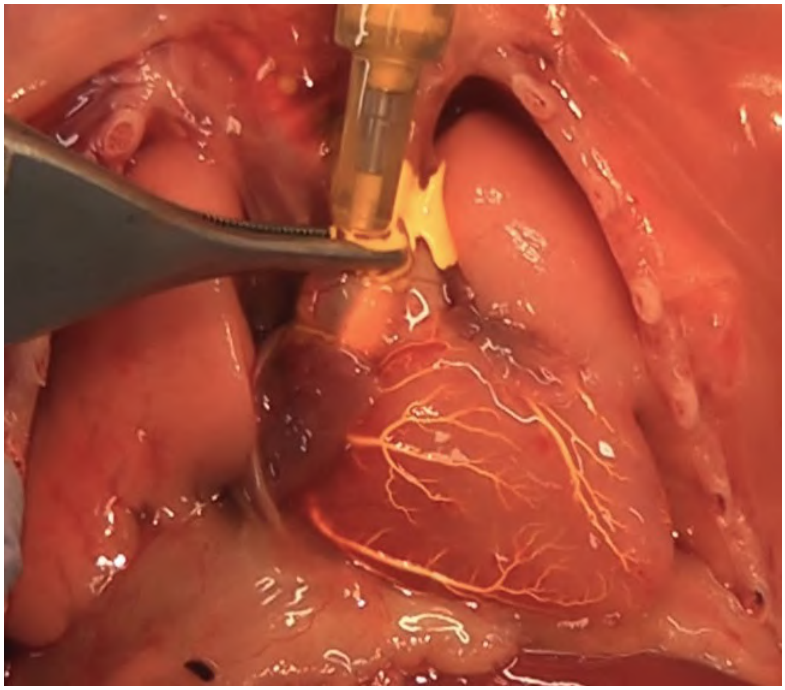

Fig. 1.- A fetal heart (17 weeks of gestational age) during Microfil injection. All superficial coronary arteries appear to be perfused by the compound. ç: aorta; +: right auricle; §: branches of the right coronary artery; *: left anterior descending artery; #: right marginal branch of the right coronary artery.

literature reports the usefulness of micro-CT – in association with Lugol solutions – in post-mortem diagnosis of human cardiac abnormalities (especially the so-called congenital heart defects – CHDs) (Hutchinson et al., 2017; Hutchinson et al., 2018; Lombardi et al., 2014; Lupariello et al., 2021; Sandaite et al., 2020).

On the contrary, until now in the scientific literature there are no indications about techniques which can allow us to reconstruct superficial and deep coronary arteries of human fetal/infant hearts. The results of this pilot study pointed out the possibility to impregnate the aforementioned arteries with the injection of a curing radiopaque

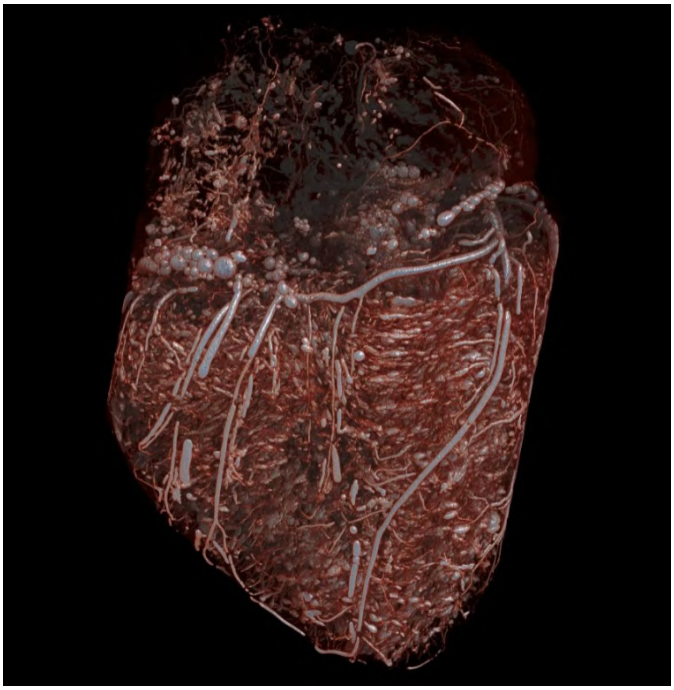

Fig. 2.- Three-dimensional reconstruction of a fetal heart (20 weeks of gestational age). Right ventricle (RV) and left ventricle (LF) show multiple defects in the reconstruction of superficial coronary arteries.

silicone rubber compound (Microfil). In addition, they highlighted the possibility to create – throughout micro-CT scans and specific software – three-dimensional reconstruction of fetal coronary arteries. Therefore, this approach can be considered a novel post-mortem technique for fetal/infant hearts. It could be commonly used in pathologists' routine especially when anomalies of not superficial/deep branches of the coronary arteries could have caused the demise.

The present study demonstrated a better capability of micro-CT to highlight the not superficial branches of the coronary arteries. On the contrary, due to their extremely small diameter, these branches are hardly distinguishable at standard gross examination of fetal/infant hearts, not allowing a complete analysis. The latter is a significant limitation in case of suspected fetal unexplained demise and sudden infant death syndrome, in which it is mandatory to exclude all cardiac anomalies for an accurate diagnosis. Thus, after proper standardization, micro-CT with coronary arteries' perfusion could be implemented as a useful ancillary tool for pathologists.

The results highlighted that the perfusion of parts of internal cardiac chambers and the impossibility to reach all the extent of some coronary arteries represent significant limitations. In particular, the study pointed out defects in the reconstruction of superficial and/or deep arteries, not allowing a complete visualization of the coronary tree. Possible explanations for these effects are, respectively: internal cardiac chambers were perfused because the Microfil was not injected directly throughout left and right coronary arteries' ostia (part of the compound went into heart cambers passing through the aortic valve); in some coronary arteries' branches there were already clots which did not allow us to complete the perfusion (the interval time between miscarriage and post-mortem examination was 24 to 30 hours). However, future studies are planned in order to solve these limitations. Indeed, preventive perfusion of coronary arteries with anticoagulant solutions and direct injection of Microfil throughout right and left coronary arteries' ostia could be suitable remedies to improve results.

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