Title:

Novel usage of micro-focus computed tomography (Micro-CT) for visualisation of human embryonic development – implications for future non-invasive post-mortem investigation

Short Running Title:

Human embryonic development using Micro-CT

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Bulleted Statements:

1) **What is already known about this topic?**

- Accurate pathological assessment of small gestational age fetuses is challenging.
- Dating an embryo/fetus is essential to recognise expected human developmental stages and prevent misdiagnosis.

2) **What does this study add?**

- Micro-CT offers a non-destructive, digital method for dating human embryos
- The micro-CT imaging here is of the earliest gestation human embryo published to date with corresponding pathology. It can provide an alternative to autopsy even at early stages of development.

Research Letter:

Conventional autopsy remains the gold standard for post-mortem examination[1], although early experience with a high-resolution imaging modality known as micro-focus computed tomography (micro-CT) can provide detailed anatomical information comparable to light microscopy. This has the added benefit of allowing non-destructive assessment, with digital storage and manipulation of the information acquired. Recently published work by Hutchinson et al[2] has shown that fetal micro-CT for early gestation pregnancy loss has a sensitivity rate of 93.8% when compared to conventional autopsy, and may provide an alternative option for post-mortem examination[3]. It is potentially helpful when a primary diagnosis or verification of a suspected developmental anomaly is required[4] and can offer information that would be missed if performing autopsy alone e.g. for cardiac anomalies [5],[6],[7].

Nevertheless, interpreting any novel imaging modality requires an accurate knowledge of normal findings, and in this particular setting - the gestational age and thus expected developmental anatomy is of utmost importance to prevent disease misdiagnosis. In this case study we present post-mortem micro-CT imaging of a very early pregnancy loss where accurate dating of embryonal age was possible, and to a similar level as histopathology. The study not only represents the earliest human embryo assessed by micro-CT in the medical literature, but also offers an understanding for the possibilities of this technology in future pregnancy losses.

The specimen was imaged as part of a larger study investigating minimally invasive autopsy techniques and novel methods of post mortem imaging (CE13/LO/1494 and CE2015/81). Fully informed, written parental consent for conventional autopsy, imaging and the use of tissue for research was obtained. The embryo was referred to our institution as fresh tissue following a termination of pregnancy for social reasons, without any history of underlying congenital anomalies. The estimated gestational age was approximately 11-12 weeks (by date of maternal last menstrual period, LMP).

The specimen was first immersed at room temperature in a solution of 10% formalin (to prevent tissue degradation) and potassium triiodide (I_2KI / Lugol's iodine, to impart tissue contrast), with a total iodine content of 63.25 mg / mL (iodine mass of 2.49×10^{-4} mol / mL), in a 1:1 ratio for 48 hours prior to imaging. Immediately prior to imaging, the fetus was removed from the iodine solution, rinsed in water and dried with gauze. The imaging was acquired using an XTH 225 ST microfocus-CT scanner with a multi-metal target (Nikon Metrology, Tring, UK). Scanning parameters included X-ray energies and beam current at 80 kV and 87μA respectively. Exposure time was 354ms, with the number of projections optimized at 3080 with one X-ray frame per projection. The resultant images were reconstructed using modified Feldkamp filtered back-projection algorithms with proprietary software (CTPro3D; Nikon Metrology, UK) and post processed using VG Studio MAX 3.0 (Volume Graphics GmbH, Heidelberg, Germany). The isotropic voxel size was 9.7 μm. Following imaging, the embryo was de-iodinated using sodium thiosulphate pentahydrate dissolved in water (4%w/v) for 12 hours.

The specimen was then embedded within a paraffin block and sectioned. A traditional autopsy was not possible due to the very small fetal size.

In order to date the embryo on imaging, a similar method utilised by pathology was adopted based on the identification of various morphological features as early development occurs. These can be divided into the 'embryonic' (i.e. first 8 weeks post fertilisation) and 'fetal' period, with the former subdivided into 23 'Carnegie stages', as described and developed by O'Rahilly & Muller[8,9] (Table 1). Whilst the presence of different structures do not provide a specific age per se, only a developmental stage, rough approximations of gestational age can be inferred. Interpretation of the imaging findings were made using a computer graphics programme (VG Studio MAX 3.0 (Volume Graphics GmbH)) by consensus reading between a consultant pathologist and a radiologist. Figure 1 demonstrates the external and internal morphological appearances respectively on micro-CT, with the latter compared to the histological examination.

Prior to imaging, the embryo had an estimated gestational age of approximately 11-12 weeks, however detailed analysis showed this to be in fact several weeks younger around 49-52 days post ovulation (Carnegie stage 21) corresponding to approximately 7 weeks. It is well known that estimation of the gestational age from the first day of the LMP of the mother can pose inherent inaccuracies[10] given inconsistencies in dates of ovulation during a menstrual cycle[9] and inaccurate maternal recollection[11]. The difference in this estimated gestation may therefore serves as a reminder for careful re-assessment of age at autopsy.

During life in utero, antenatal ultrasound assessment of the greatest length of the embryo (i.e. crown rump length, CRL) is well regarded as the most accurate measure for estimating early gestational age (usually measureable by 6-7 weeks)[12]. Nevertheless, in our study there was no information on CRL from the referring hospital and the measured CRL on micro-CT suggested an embryo of an even younger gestational age (at approximately 6 weeks gestation, 42-45 days post ovulation; Carnegie stage 18). Whilst several pathological reasons may exist for this, it was felt that the discrepancy most likely arose from potential tissue shrinkage after the iodination and fixation process prior to scanning, which has previously been described by Lombardi et al[5] and Vickerton et al[13]. Alternative and less likely explanations (given lack of significant antenatal history and that the tissue was obtained 'fresh') may be from stunted embryonal growth secondary to factors such as poor maternal nutrition, an underlying skeletal dysplasia or maceration. In fact, on initial radiological review a skeletal dysplasia had been an area of some debate, given the long bones of both upper and lower limbs appeared shortened in proportion to the hands and feet. Nevertheless this was later discounted as during normal developmental stages it is usual for long bones to lengthen over several few days subsequent to Carnegie stage 21. Although maceration is known to be detrimental to micro-CT analysis of early gestational embryos[2], in this case our tissue was obtained fresh and there was no maceration on imaging or visual examination.

In conclusion, this case has demonstrated that even at very early gestational pregnancy losses, micro-CT imaging can demonstrate internal and external morphological structures in an embryo similar to histopathology. This may provide a future viable alternative to autopsy where parental consent is refused, however there still remains much work to be established in this field. Research is needed but not limited to the assessment of significant changes in tissue properties induced by our tissue preparation procedures for scanning, production of a micro-CT imaging atlas of 'normal embryonal and fetal development' at different developmental stages and a better understanding for expected radiographic changes in congenital anomalies. Whilst early work in small case series appears promising, application to a larger population and wider range of pathologies is still required. At present in our institution this modality remains a powerful and useful adjunct to pathological assessment.

References

1 Thayyil, S., Sebire NJ, Chitty LS, Wade A, Chong W, Olsen O, Gunny RS et al. Post-mortem MRI versus conventional autopsy in fetuses and children: A prospective validation study. Lancet 2013 Jul 20;382 (9888): 223–233.

2 Hutchinson JC, Kang X, Shelmerdine SC, Segers V, Lombardi CM, Cannie MM, Sebire NJ, Jani JC, Arthurs OJ. Post mortem microfocus computed tomography for early gestation fetuses: a validation study against conventional autopsy. AJOG 2018. Apr;218(4):445.e1-445.e12

3 Kang X, Cos T, Guizani M, Cannie MM, Segers V, Jani JC. Parental acceptance of minimally invasive fetal and neonatal autopsy compared with conventional autopsy. Prenat. Diagn. 2014 Nov; 34(11):1106-10.

4 Cannie, M, Votino C, Moerman P, Vanheste R, Segers V, Van Berkel K, hanssens M, Kang X, cos T, Kir M, Balepa L, Divano L, Foulon W, De Mey J, Jani J. Acceptance, reliability and confidence of diagnosis of fetal and neonatal virtuopsy compared with conventional autopsy: A prospective study. Ultrasound Obstet. Gynecol. 2012 Jun; 39(6): 659–665.

5 Lombardi CM, Zambelli V, Botta G, Moltrasio F, Cattoretti G, Lucchini V, Fesslova V, Cuttin MS. Postmortem microcomputed tomography (micro-CT) of small fetuses and hearts. Ultrasound Obstet. Gynecol. 2014 Nov;44(5):600-9.

6 Vogt C, Blaas H.G.K., Salvesen K.Å., Eik-Nes S.H. Comparison between prenatal ultrasound and postmortem findings in fetuses and infants with developmental anomalies. Ultrasound Obstet. Gynecol. 2012 Jun; 39(6):666-72.

7 Hutchinson JC, Barrett H, Ramsey AT, Haig IG, Guy A, Sebire NJ, Arthurs OJ. Virtual pathology of the human fetal kidney Micro-CT. Ultrasound Obstet. Gynecol. 2016 Nov; 48(5):663-665.

8 O'Rahilly, R, Müller F. Developmental Stages in Human Embryos, Including a Revision of Streeter's 'Horizons' and a Survey of the Carnegie Collection. Washington, Carnegie Institution of Washington 1987

9 O'Rahilly, R, Müller, F. Developmental stages in human embryos: Revised and new measurements. Cells Tissues Organs. 2010; 192(2):73-84.

10 Butt K, Lim K; Diagnostic Imaging Committee. Determination of gestational age by ultrasound. J. Obstet. Gynaecol. Can 2014 Feb; 36(2):171–81.

[11] Newnham J, Evans SF. 'Fetal biometry' in CH Rodeck & MJ Whittle (eds), Fetal Medicine: Basic Science and Clinical Practice. vol. 0, Harcourt Brace and Company Ltd, 24-28 Oval Road, London NW1 7DX, United Kingdom. 1999. Pages 939-953.

[12] Bottomley C, Bourne T. Dating and growth in the first trimester. Best Pract. Res. Clin. Obstet. Gynaecol. 2009. Aug; 23(4):439–452.

[13] Vickerton P, Jarvis J, Jeffery N. Concentration dependent specimen shrinkage in iodine enhanced microCT. J Anat. 2013 Aug; 223(2):185-93

Figure Legends:

Figure 1:

(a) Volume rendered three dimensional image reconstructed from the micro-CT imaging of a 7 week embryo. This image clearly demonstrates characteristic external features expected at Carnegie stage 21, with early eyelid and external ear development, appearance of individual digits at the hand with toe notches at the feet. Of note, the limbs appear relatively shortened compared to expected size for hands and feet which is normal at this stage of development. (b) Sagittal post-mortem micro-CT appearances of a 7 week gestational age embryo imaged at a resolution of 9.7um, with (c) corresponding sagittal histopathology section after hematoxylin & eosin staining. The similarity of internal detail obtainable by micro-CT imaging versus histopathology is clearly visible.