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Assessing Embryo Development using Swept Source Optical Coherence Tomography

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ABSTRACT

A detailed assessment of embryo development would assist biologists with selecting the most suitable embryos for transfer leading to higher pregnancy rates. Currently, only low resolution microscopy is employed to perform this assessment. Although this method delivers some information on the embryo surface morphology, no specific details are shown related to its inner structure. Using a Master-Slave Swept-Source Optical Coherence Tomography (SS-OCT), images of bovine embryos from day 7 after fertilization were collected from different depths. The dynamic changes inside the embryos were examined, in detail and in real-time from several depths. To prove our ability to characterize the morphology, a single embryo was imaged over 26 hours. The embryo was deprived of its life support environment, leading to its death. Over this period, clear morphological changes were observed.

Keywords: Optical Coherence Tomography, Embryology, Biology, medical and biological imaging

1. INTRODUCTION

Quality evaluation of an embryo based on the assessment of its morphological structure is standard practice in human embryology [1,5]. Refinement of methods to inspect the morphological structure of embryos, applied to assisted reproduction in cows has led to higher pregnancy rates [2]. While visualization is easily applied to human embryos, bovine embryos appear more opaque making the access to morphological data more difficult [3,]. At present, structural embryo screening in the dairy cow is performed by using a stereomicroscope with a magnification up to x100 [4]. This method is simple and non-invasive and has already improved the success rate of assisted fertilization techniques; however, it is not able to measure the vitality of the embryo or even to detect genetically abnormal embryos [3]. To overcome these limitations, time-lapse systems [6,7] have been considered; however, they suffer from poor depth of field, long acquisition time and low portability.

Optical Coherence Tomography (OCT) is an optical imaging method developed in the 1990s that creates a depth resolved image of a sample. It has been mainly used in ophthalmology but has seen applications in a number of other areas [8-9]. OCT can non-invasively produce depth profiles as well as 2D maps and 3D volumes of the internal structures of tissue. Micron-sized axial and lateral resolutions are achievable.

Moreover, OCT has been used to study several aspects of embryo development, such as those connected to the heart or the vasculature system [10,11]. OCT can provide 3D images of the full embryo non-invasively. Furthermore, by using low power light, the risk to damage the sample during the observation is reduced.

Using OCT, summed Voxel Projection (SVP) can be implemented to simulate what a simple camera would do for embryo monitoring. Embryo development over long periods of time using SVP is shown in Fig. 1. General observation can be performed and overall modifications of the structure over long periods of time can be detected. Nonetheless, using a global imaging technique, changes in the inner structure of the embryo might not necessarily be observed and details of the development could be completely missed. Fig. 2 presents three SVP image taken at 1-hour intervals. Barely any morphological modifications are noticeable and it is therefore difficult to ascertain whether the embryo is still alive or dead.

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A better imaging modality is needed to enable us to look inside the embryo at different depths. In this way, more information on the embryo vitality can be gathered. This is what justifies this work in terms of better resolution and more view angles.

In this paper, C-scan images delivered by a Master Slave swept source (SS)-OCT system are produced to monitor the behavior of a single bovine embryo in an unsuitable environment over short and long periods of time.
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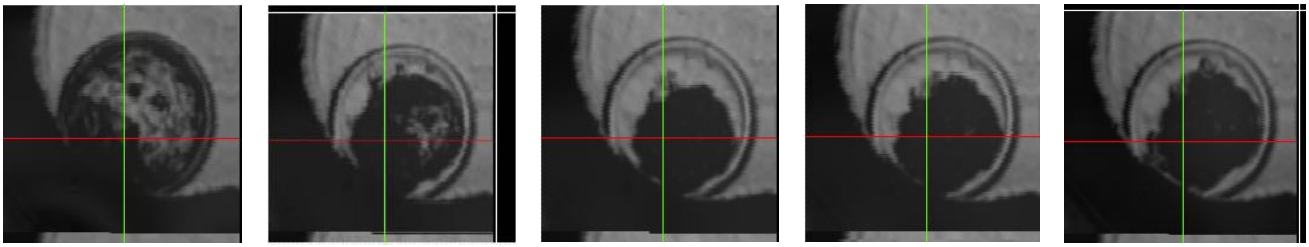


Fig.1 Confocal imaging over time

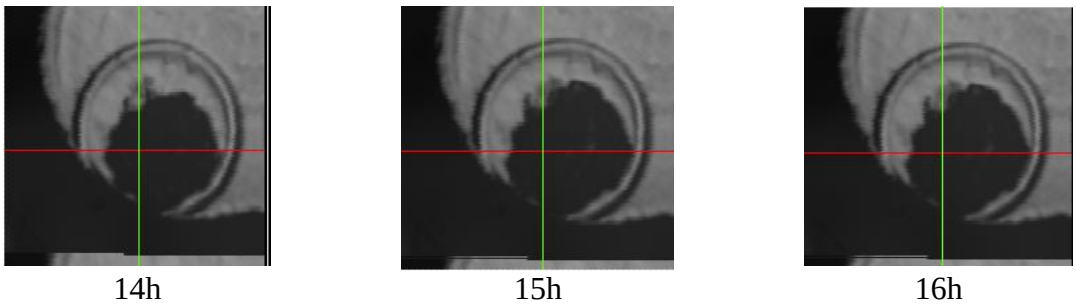


Fig. 2 Limitation of used SVP images (14,15 and 16 hrs)

2. METHOD

Bovine oocytes from an abattoir were matured *in vitro* and then fertilized with frozen/thawed sperm. The resulting embryos were cultured in Synthetic Ovarian Fluid (SOF) for 7 days after the IVF [12].

The OCT system (schematically represented in Fig. 3) employs a swept source centred at 1310 nm with a tuning range of 106 nm. The interferometer is composed by a 2 x2 fiber coupler (C1). After it, the light is split 80% towards the reference arm and 20% towards the sample arm. Light arriving at the sample arm, after collimation, is directed to a pair of galvanometer scanners which scan the sample in the lateral plan (x,y). Beams from both arms are combined in a coupler C2 50:50 and guided to a balanced photo-detector (Thorlabs Model PDB460C, DC 200 MHz). The digitized signal (digitizer AlazarTech ATS9350 - 500 MS/s) is processed with the complex Master-Slave interferometry (cMSI) protocol [13-15]. In air, the axial resolution is approximately 15 µm and the lateral resolution 4.2 µm.

The Master-slave interferometry (MSI) method consists of two steps: the master and the slave ones. Firstly, the system needs to be calibrated - this corresponds to the master step. After placing a mirror at the sample position, several (at least two) channelled spectra (CS) (called “masks”) are acquired at different optical path differences (OPDs). These masks are then used to generate a complex function which takes into account the sampling non-linearities and the unbalanced dispersion from the interferometer. Once this function is known, it is possible to generate an arbitrary number of CS for any depth. Due to being complex-valued and in order to distinguish them from the CS employed to generate the complex

function, these generated CS are labelled complex masks. After this step is completed, the mirror can be removed and replaced by the sample, thus starting the slave step. In order to correctly reconstruct the depth profile from the sample, each CS acquired is compared to the set of complex masks, where the degree of similarity between them represents the reflectivity of a scattering centre in the sample at an OPD equal to that of the complex mask. In this specific application, the axial range is defined by the depth value of the first and of the last complex masks in the generated set.

This method allows to display simultaneously three types of images in real time (Fig. 4): B-scan images, C-scan views and summed voxel projection (SVP). B-scans are the depth embryo profiles (z direction) whereas C-scan and SVP are top view *(en-face)*. The volumes produced are of 200 x 200 lateral pixels over 300 depths (number of OPD values used to calculate the same number of complex masks). The axial range is adjusted to cover the full blastocyst.

To follow the behavior of the embryo, a bovine embryo day 7 after fertilization was monitored over 18 hours.

The sample was placed on a translation stage (TS). Using the TS, the position of the blastocyst selected was adjusted laterally and vertically while monitoring the SVP image. Afterwards, the length of the reference arm is modified to bring the C-scan image visualized into focus. Then the TS is kept at the same position and a similar protocol presented in our previous work [16] is followed during all the experiment. Ten sets of data were acquired every minute followed by 20-minute breaks (when no data was acquired). In Figure 4, the whole depth of the embryo (225 µm) is represented by 9 C-scan images (230 µm x 230 µm) separated in depth by 25 µm (measured in air) while on the B-scan image, the depth range is 1 mm (measured in air). In the B-scan views, orange arrows delineate the embryo shape and blue arrows the glass plate.

In the rest of this communication, only *en-face* images will be presented to show the morphologic embryo evolution.

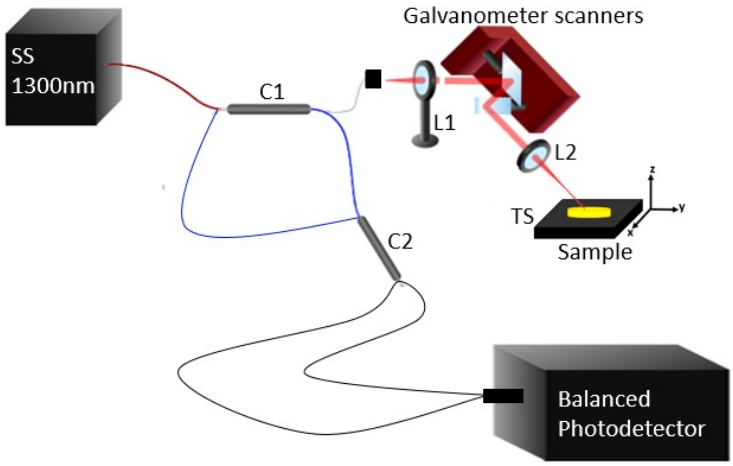


Fig. 3 Experimental set-up of the 1300 nm SS-OCT system. SS: Swept source, C1, C2: optical couplerx, TS: translation stage, L1, L2: lenses

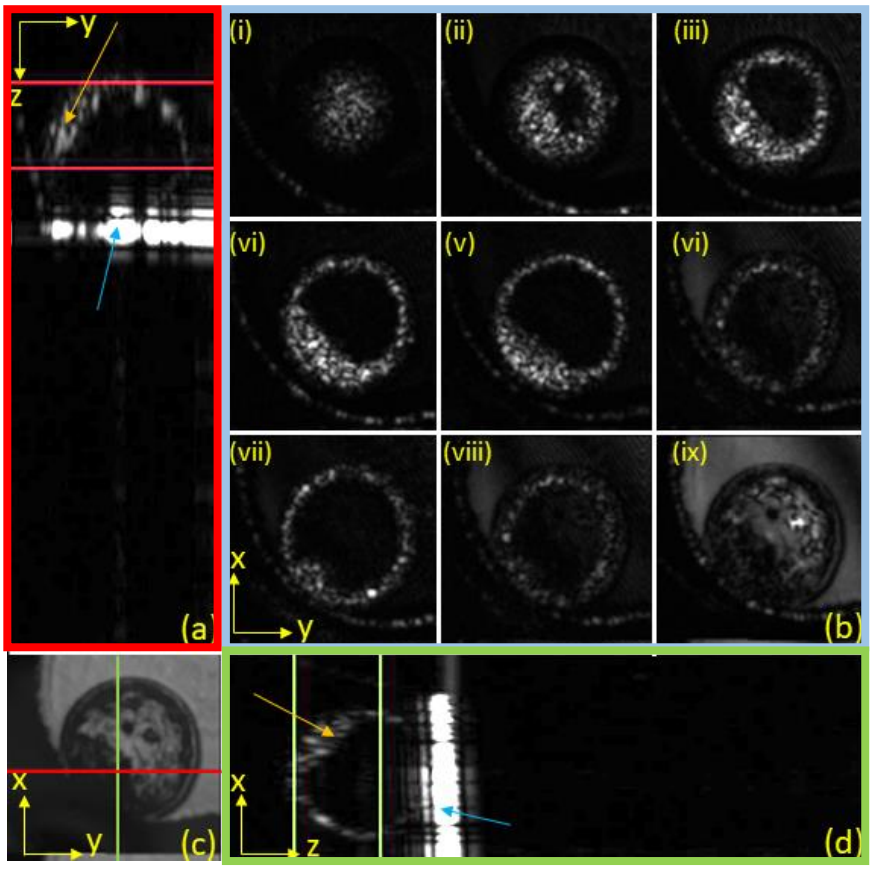


Fig. 4 Screen-shot of real-time display, 3 different image views are simultaneously rendered: B-scan images, C-scan views and SVP image. (a) B-scan image along the red line (y,z), (b) (i-ix) 9 C-scan views, (c) SVP and (d) B-scan image along the green line (x,z).

3. RESULTS

300 C-scan images were used to create a 3D model of the blastocyst (Fig. 5). Two clear structural entities can be distinguished: the trophectoderm which is the ring of outer cells from the embryo and the inner cell mass. From the 3D reconstruction, the two structures can be distinguished and separated giving access to the cell distribution, size and compactness of the embryo [17]. These parameters can be correlated with implementation rates [17-22].

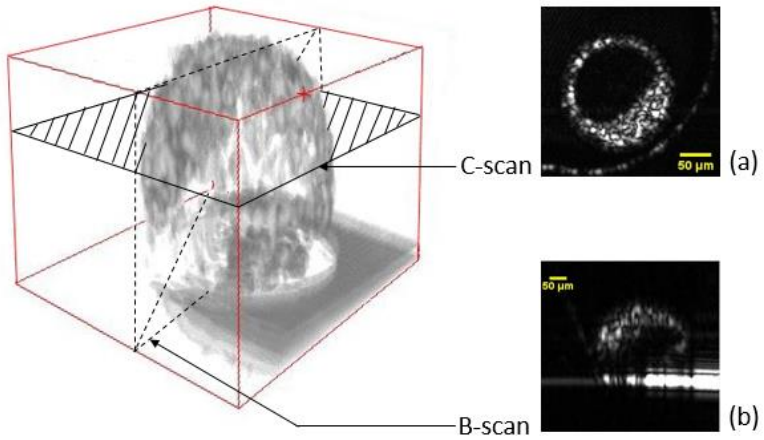
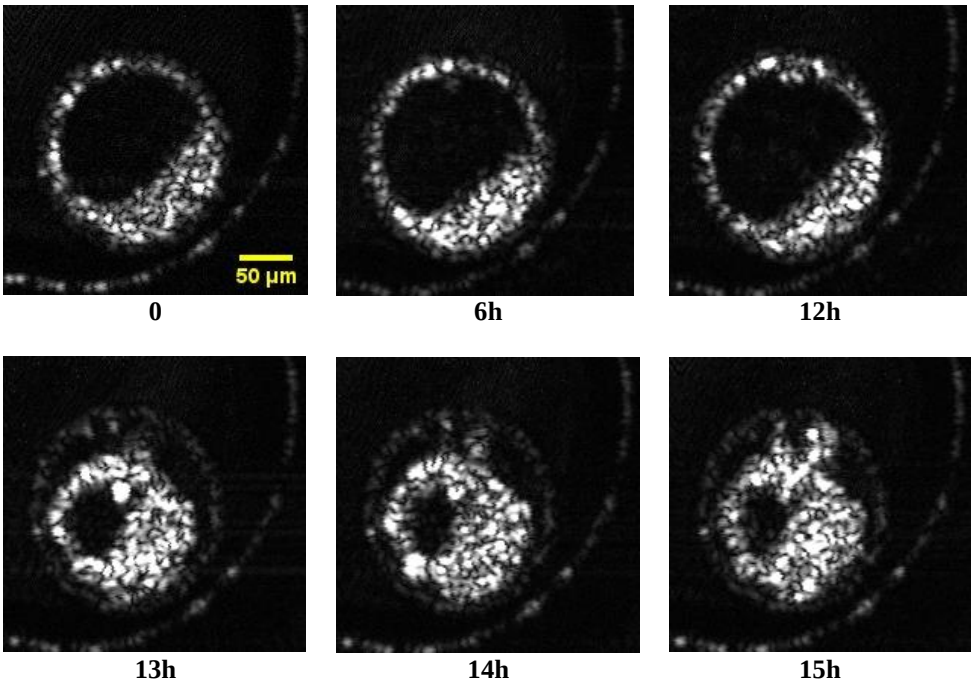
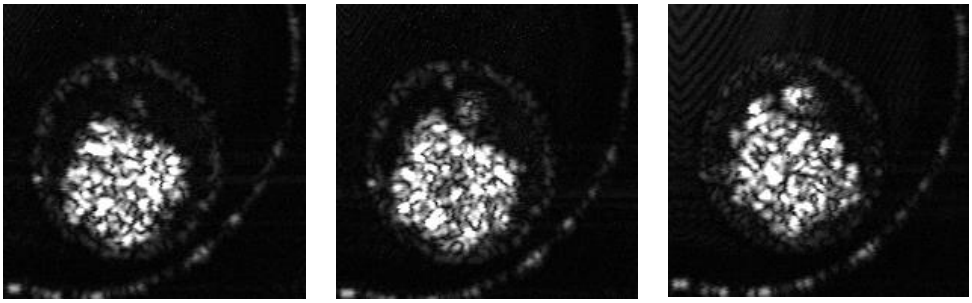


Fig. 5 3D volume of a embryo post 7 days after in vitro fertilization. (a) C-scan image at a depth of 225µm (central part of the embryo on the axial direction) and (b) one of the 200 B-scan views (around the center of the embryo along the lateral direction)

Figure 6 is a selection of images from the 18 hours monitored experiment. To describe the morphological changes in the embryo over time, at a specific depth, more exactly at the center of the embryo, a C-scan was monitored during 18 hours. Over the first 12 hours, the structure of the embryos remained the same and this can be qualified as normal. By the 13th hour, the blastocyst shrunk on itself. An outer ring was still visible showing that the blastocyst's zona pellucida kept its shape and position. The process is not instantaneous and lasted for 3 hours (up to and during the 16th hour). This shows that a 7-days post fertilization embryo can still be considered alive and in normal shape after 12 hours outside of the incubator. However, after 16 hours, the embryo has never re-expanded and its shape is similar to that of an embryo at the end of its life.





16h **18h** **26h**

Fig. 6 structural images (*en-face* images) over time at 150 µm depth measured in air from the top embryo surface

4. CONCLUSION

In this study, morphological embryo images have been obtained using a SS-OCT system. At each depth, targeted monitoring can be performed in real time using Master/Slave interferometry. A blastocyst has been monitored over 18 hours for full characterisation of transition to death. Over the first 12 hours, the 7-day post fertilization embryo expanded and afterwards it began to collapse. Observations such as the disappearance of the cavity inside the embryo between the 15th and 16th hour which were not possible with other modalities can be done using the C-scan views. Furthermore, OCT can be used to identify the different structures in the bovine blastocyst and give access non-invasively to the measurement of some parameters such as volume of the blastocyst's inner cell mass or the shape of the inner cavity which can be used to obtain higher pregnancy rates. Further studies should include motion detection software based on phase or amplitude to detect even more minute changes over time.

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