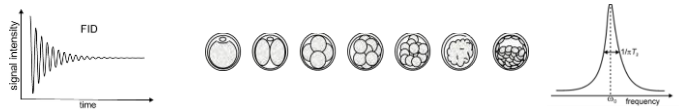
Magnetic resonance spectroscopy of individual mammalian embryos and human microtissues

Giulia Sivelli[a], Gaurasundar M. Conley[a], Carolina Herrera[b], Kathryn Marable[a], Kyle J. Rodriguez[c], Heinrich Bollwein[b], Mateus J. Sudano[d], Marco Grisi[a]

a Annaida Technologies SA, Lausanne, Switzerland; b Clinic of Reproductive Medicine, Department for Farm Animals, University of Zurich, 8057 Zurich, Switzerland;
c Microsystems Laboratory, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland; d Department of Genetics and Evolution, Federal University of São Carlos, São Carlos, SP, Brazil

What is known already: Non-invasive selection of the best embryo to transfer is a great challenge for ART professionals. The chemical sensitivity, resolving power, and, more importantly, the non-invasive nature of Magnetic Resonance Spectroscopy (MRS) makes it an excellent candidate to investigate the building blocks of complex organisms. Although MRS is a well-established technique for the biochemical profiling of large organisms, handling small samples such as embryos and 3D cell cultures alongside sensitivity issues has prevented its adoption for clinical and research applications. Our group has overcome these limitations with a microchip-based sensors to leverage non-invasive MRS technology down to the embryo scale.

Study question: Can magnetic resonance unravel the metabolic fingerprint of samples at the embryo scale?



Summary answer: In this study we successfully utilized MRS on single mammalian embryos and liver microtissues to identify universal metabolic biomarkers. We linked these to a metabolic disease (non-alcoholic fatty liver disease) and earlier or later pre-implantation embryo development in case studies.

Study design:

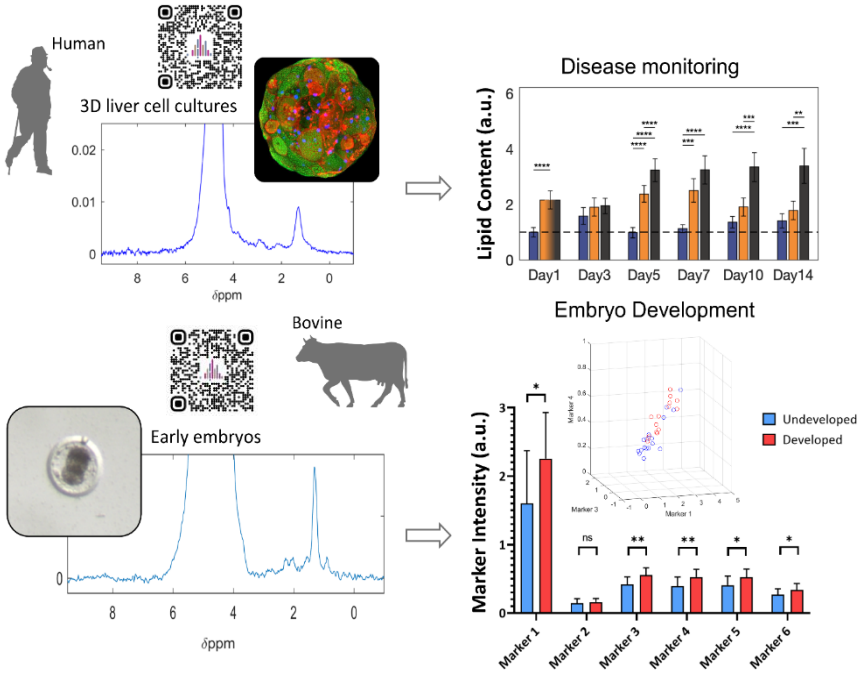
A model of non-alcoholic fatty liver disease was first used to detect MRS-based metabolic biomarkers over multiple time points in 3D human micro-livers. We could discriminate between healthy and diseased livers based on MRS metabolic markers, related in particular to lipids. The microtissues were individually measured fresh in a time frame of 14 days. Results displayed here originate from 117 samples. In a second case study we moved to single arrested bovine embryo analysis. Early or late arrested bovine embryos were obtained through IVF, cryopreserved and measured shortly after thawing. The results here displayed originate from 32 samples.

Methods:

The sample was loaded into a miniaturized proprietary MRS device previously prepared with a culture medium of choice. The MRS device was loaded into a spectroscopy magnet and measurements were performed (analysis time is 50 min/embryo or 10 min/microtissue).

Limitations, reasons for caution: Our method is ready for R&D studies, while its clinical application requires further safety validation and protocol optimization (both upcoming).

Wider implications of the findings: A non-invasive and quick embryo analysis would provide the means to reveal the role of embryonic lipids throughout development. Micro-MRS can further develop into a safe embryo assay for selection and quality control before embryo transfer. This would apply to both human and animal ART, whose success rate is relatively low.



Universal metabolic markers. (Left) Magnetic resonance spectroscopy allows for label-free non-invasive detection of chemical compounds in living matter. In these spectra metabolic markers, more specifically lipid markers, are highly visible. **(Top-Right)** We demonstrated high precision monitoring of fatty liver disease in human 3D liver cell cultures. **(Bottom-Right)** We observed lipid markers in pre-implantation cow embryos correlating with two cohorts: 1) Undeveloped, early arrested at 2 to 8-cell stage, and 2) Developed, late arrested at blastocysts and morulae stage.

Main results and the role of chance:

- Micro-MRS identified biomarkers that were significantly differently expressed ($p<0.05$) according to the biological state of micro-livers simulating non-alcoholic fatty liver disease.
- Three of these markers represent relative concentrations of lipid signatures from fully saturated, mono and poly-unsaturated fatty acids.
- In addition, we analyzed in vitro produced pre-implantation bovine embryos naturally arrested at different developmental stages. This choice arose from the necessity to analyze them at different and fixed developmental stages to investigate our technology discrimination potential.
- The MRS-spectra obtained from the embryos present up to 6 peak regions assignable to fatty acids as observed in microtissues. Furthermore, the amplitude of the peaks varies substantially within the same group, i.e., within the morulae and within the two 2-cell stage arrested embryos.
- Generally, the majority of the analytical assays used in lipid metabolism investigations rely on invasive/destructive methods such as histology and biopsies, which would hinder the continuation of embryonic development.
- A non-invasive in vivo assay like the one presented here would provide the means to reveal the role of embryonic lipids throughout development as well as selection and quality control.

Keywords: Magnetic Resonance Spectroscopy, metabolism, embryo screening, non-invasive