Microscale Magnetic Resonance Spectroscopy (Micro-MRS) for the Non-Invasive Metabolic Assessment of Individual Mammalian Embryos.

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Non-invasive selection of optimal embryos for transfer is a crucial challenge in assisted reproduction for both humans and animals. Magnetic Resonance Spectroscopy (MRS) emerges as a promising solution due to its chemical sensitivity and non-invasive nature. Despite its effectiveness in analysing large organisms, MRS faces obstacles in small sample handling and sensitivity, particularly with embryos, limiting its clinical and research applications. Our study overcomes these challenges using innovative microchip-based MRS sensors. This approach enabled successful Magnetic Resonance Spectroscopy (MRS) at the embryo scale, revealing metabolic biomarker linked to developmental potential in pre-implantation bovine embryos and opening new possibilities in assisted reproduction technology.

The aim of this study was to demonstrate that rapid, non-invasive analysis of embryos using Micro-Magnetic Resonance Spectroscopy (micro-MRS) can effectively uncover the significance of embryonic metabolites during pre-implantation in-vitro development.

Pre-implantation bovine embryos were obtained through IVF (n=32 samples for cohort one and, n=61 for cohort two). Multiple MRS measuring sessions were conducted on two distinct embryo cohorts. The first cohort involved naturally arrested IVP embryos at early (8-cell) and late (morula/blastocyst) stages, establishing metabolic biomarkers in mammalian pre-implantation embryos. The second cohort involved 8-cell embryos which were further cultured over 10 days post-MRS with imaging at specific intervals (3 hours post-thawing, days 5, 8, and 10). This step was crucial to back-correlate biomarkers with in-vitro development based on bright field microscopy. For both cohorts the embryos were provided vitrified and were subjected to a 50-minute MRS measurement following a 3-hour post-warming culture period. Single bovine embryo analysis was performed using our 1nL micro-MRS probe. Post-MRS, the embryos were cultured in equilibrated IVC medium in a humidified incubator with 5% CO_2 and 6% O_2 at 38°C. Statistical significance was set at $p<0.05$.

Micro-Magnetic Resonance Spectroscopy (Micro-MRS) was utilized to identify metabolic markers in single bovine embryos, providing insights into their pre-implantation developmental stages. The spectroscopy data revealed distinct peak regions in the spectra of both cohorts, indicative of essential life-supporting fatty acids. Notably, up to six lipid markers were identified in the first cohort, five of which displayed statistically significant differences ($p<0.05$). However, the majority of these biomarkers in the first cohort did not show significant expression differences in the groups of embryos assigned to the second cohort. An exception was noted in the case of the Principal Lipid Component (PLC) marker, which was significantly less prevalent in the arrested embryos than in those progressing to the blastocyst stage ($p=0.0016$). Further data analysis based on AI-classification algorithms revealed up to 80% precision in predicting the development to the blastocyst stage solely based on their metabolic profiles at the 8-cell stage.

Magnetic Resonance Spectroscopy (MRS) shows promise for clinical and research use, requiring reliable signal detection from cellular components. While suitable for research, clinical application demands further safety assessments and protocol refinement. Additionally, larger sample sizes and data on post-MRS implantation and pregnancy rates are needed to validate AI-classification algorithms.

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