Low molecular weight metabolites as possible new non-invasive tool for selecting bovine *in vitro* produced embryos

Nõmm Monika[1], Rando Porosk[2], Pille Pärn[1,2], Ursel Soomets[2], Ülle Jaakma[1,2], Sulev Kõks [1,2,3] Kalle Kilk[2]

[1]Estonian University of Life Sciences, Tartu, Estonia

[2]University of Tartu, Tartu, Estonia

[3]Competence Centre on Health Technologies, Tartu, Estonia

Selecting high quality preimplantation embryo for transfer has been the most difficult task when producing embryos *in vitro*. To date the most used non-invasive method is based on visual observation. Developing a non-invasive method for embryo assessment is essential to have a profitable *in vitro* embryo production (IVP) and embryo transfer system. Molecular characterization of embryo growth media has been proposed as an complementary method to visual assessment of embryo morphology.

In this study we are demonstrating a novel method, allowing sample collection at different embryo development stages, without compromising embryo quality, to determine potential viability markers for bovine IVP.

Single bovine embryos were cultured in 60µl SOF+0.4% BSA droplets under mineral oil. Twenty µl of culture media was removed at day 2, 5 and 8 post-fertilization. A total of 58 samples were analyzed using liquid chromatography-mass spectrometry (Q-Trap 3200), followed by principal component analysis.

Our results indicate that there are significant differences ($p<0,00001$) in concentrations for proline (m/z = 116), inositol (m/z of sodium adduct = 203) and citrate (m/z of sodium adduct = 215) also in the amino acid group of leucine and isoleucine (m/z = 132), phenylalanine (m/z = 165) and arginine (m/z = 211) between the normally developed and retarded in development embryo culture media. Platelet activating factor (m/z = 524) (PAF) was roughly 3 fold increased in day 5 to day 8 embryo culture media. Unfortunately the increase of PAF was not statistically significant between normally developing and retarded embryos.

These results demonstrate that it is possible to remove culture media samples from droplets and not significantly affect embryo development. Applying this method for embryo selection provides a possibility to identify well-developing embryos and provides an opportunity for improving the herds genetic value.

This study was supported by Project 3.2.0701.12-0036 of Archimedes Foundation, Enterprise Estonia grant EU30020, Institutional research funding IUT 8-1 and Horizon 2020 Project SEARMET 692299.