Impact of *in vitro* culture and embryo transfer on cardiac metabolism in early postnatal life

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Background: The developing oocyte and embryo are vulnerable to any perturbations in the nutritional environment during the periconceptional period. It has been shown that assisted reproductive technologies, one example of manipulations during the periconceptional period, have been associated with altered cardiometabolic health in postnatal life. In this study, we aimed to determine if *in vitro* culture and transfer of the embryo, which are part of assisted reproductive technologies as well as manipulations to the nutritional environment during the periconceptional period, alter cardiac metabolism in postnatal life.

Methods: All experiments were approved by the University of South Australia/IMVS Animal Ethics Committees and performed according to the guidelines of the Australian code of practice for the care and use of animals for scientific purposes. Embryos were either transferred to an intermediate ewe (ET) or cultured *in vitro* in the absence (IVC) or presence of human serum (IVCHS) and a methyl donor (IVCHS+M) for 6d. Naturally mated (NM) ewes acted as controls. At 24 weeks, sheep were humanely killed with an overdose of Lethobarb and the hearts were collected. mRNA expression of receptors and signalling molecules involved in cardiac metabolism were measured using qRT-PCR. Plasma non-esterified fatty acids (NEFA) and glucose concentrations were also measured at 24 weeks.

Results: The mRNA expression of Peroxisome proliferator-activated receptor-α (PPARα), a master transcriptional regulator of genes involved in fatty acid metabolism was decreased in females of all treatment groups compared to controls. An increased plasma NEFA concentration was found in IVCHS+M females only. However, there was no change in other signaling molecules involved in fatty acid metabolism such as CD36, acetyl CoA carboxylase, fatty acid transporter protein 1 and carnitine palmitoyltransferase-Ib. There plasma glucose concentrations remained unaltered in any of the treatment groups. An increase gene expression of pyruvate dehydrogenase kinase-4 (PDK4) was observed in IVCHS+M females only but no difference in other signaling molecules involved in cardiac glucose metabolism such as glucose transporter-4 and Pyruvate carboxylase.

Conclusions: This study demonstrates that culture and transfer of the embryo results in a decrease in cardiac PPARα gene expression in females, suggesting a decrease in fatty acid metabolism. The increase in plasma NEFA concentrations in females of IVCHS+M with a decrease in PPARα gene expression suggests an abnormal balance between fatty acid availability and fatty acid oxidation, which may result in a vulnerability to lipid accumulation by increasing the flux of NEFA in the heart in later life. The increase in PDK 4 in the females of the IVCHS+M group suggests a potential decrease in glucose oxidation with no change in glycolysis and this may lead to acidosis and impaired contractility.These results suggest that early nutritional manipulation of the embryo can alter cardiac metabolism in females, but not males, in early postnatal life.