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Abstract Title

Non-Invasive Metabolic Imaging Of Early Embryos Is Associated With Altered Metabolic Signatures In Aging

Selecting optimal embryos is pivotal for successful assisted reproduction. Hence, there is a crucial demand for reliable, non-invasive methods to assess embryo quality, enhancing reproductive outcomes. Non-invasive imaging of embryos, evaluating metabolic activity, emerges as a promising avenue. Alterations in metabolism could be the cause of aneuploidy, inducing cell stress, abnormal development, reduced implantation potential and or poor reproductive outcomes. Predicting these issues through non-invasive metabolic measurements is conceivable. However, the influence of aging on embryo metabolic activity, morphology, and reproductive outcomes remains unexplored, presenting an essential area for further investigations. Thus, this study aimed to assess the metabolic activity of blastocyst embryos from both young and old mice, aiming to establish its relationship with embryo morphology and reproductive outcomes. Super-ovulated female mice; young: 5-8 weeks old, n=14; old: 45-52 weeks old, n=35; were subjected to in-vitro fertilisation (IVF). Inseminated oocytes (Young:304; Old:351) were assessed for fertilisation and embryo development to the blastocyst-stage. Embryos from study groups were assessed by non-invasive metabolic imaging to investigate autofluorescence of nicotinamide adenine dinucleotide [NAD(P)H], flavin adenine dinucleotide (FAD) and the optical redox ratio (ORR) levels during embryo development. Subsequently, embryo transfers were performed. F1 (CBA/C57Bl6) mouse strain was used. NAD(P)H and FAD levels were measured during embryo development using confocal microcopy (Olympus FV1200) and a custom-made light-sheet on-a-chip microsystem. A confocal Z-stacking function was used to record 15 focal planes using a 20x/0.95NA air objective of entire embryos, opening the confocal pinhole system completely. Then, images were collected and analysed using FIJI software (v2.0.0-rc-69/1.52n), using arbitrary units (AU). Embryo culture experiments revealed significant variations in blastocyst formation rates between study groups (Young: 73.6%; Old: 22.9%; $p<0.05$). Morphology analysis of blastocysts produced showed no significant difference for good quality embryos between young and old females ($p>0.05$). Embryos that failed to reach the blastocyst stage exhibited distinct NAD(P)H activity profiles during development compared to those that did ($p<0.05$). Additionally, abnormal embryos also presented significantly decreased NAD(P)H activity levels at the 2-cell stage to the morulae stage ($p<0.05$). Furthermore, metabolic images of 2-cell embryos predicted blastocysts formation with an AUC of 0.974. Developed blastocyst showed a significant decrease in NAD(P)H levels in the inner cell mass (ICM) of blastocyst from old females (Young:

424,6±26,0AU; Old: 280,7±43,6AU; $p<0.05$). Furthermore, an increased metabolic activity in the ICM of blastocyst from old females was also observed (ORR Young: 901,5±6,2AU; Old: 937,0±7,3AU; $p<0.05$). On another hand, trophoectoderm cells from old females showed no differences in terms of metabolic activity (ORR; $p>0.05$), however, increased NAD(P) levels (Young: 3431±19.13AU; Old: 4609±346.1AU; $p<0.05$) and decreased FAD levels were observed respectively (Young: 4275±43.56AU; Old: 3615±159.7AU; $p<0.05$).

This study unveils altered metabolic activity levels in embryos that do not form blastocyst and decreased levels of NAD(P)H in the ICM of blastocyst from old females. Increased metabolic activity was similarly observed in the ICM with altered levels of NAD(P)H and FAD in trophoectoderm cells, which it could be indicative of abnormal metabolic activity. Non-invasive measurements of metabolic imaging could be applied to determine the link of embryo metabolic activity with poor reproductive outcomes in aging.

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