The heterogeneous nature of cow embryos: sorting out the mixed bag of blastomeres

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In vitro embryo production (IVP) is increasingly utilised in cattle breeding for rapid transmission of superior genetics. Time-lapse equipment used in human clinics to select the best embryos for transfers is not applicable to the cost/time-sensitive constraints in cattle IVP, therefore selection based on morphology remains the only option. A recent concept suggests metabolic heterogeneity between blastomeres exists within pre-compaction embryos, and is associated with quality [1]. Therefore, we investigated if (non-invasive) fluorescence microscopy can determine embryo quality when cultured under optimum vs. stressed (7% vs. 20% O_2) conditions.

Cumulus oocyte complexes (COCs) were matured and fertilised as per standard methodologies [2]. Between D1-8, presumptive zygotes were cultured in 7% or 20% O_2. On-time blastocyst development (D8) was significantly reduced in embryos cultured in high O_2 levels (7% O_2=41.3±1.8% vs. 20% O_2=27.3±2.3%, $P<0.05$).

D5 embryos were stained with fluorophores sensitive to reduced glutathione (GSH; MCB), reactive oxygen species (ROS; PF1) and active mitochondria (Mitotracker Red). Within compact morulae, blastomere polarisation was associated with cortical localisation of GSH, and increased overall staining was related to further development. ROS and mitochondrial activity was highly co-localised. Poor quality/arrested embryos were characterised by a range of blastomere metabolic phenotypes, underlying their reduced capacity to develop further. The degree of stain heterogeneity was associated with embryo quality.

The autofluorescence (AF; FAD and NAD(P)H) profiles of embryos on D5 were determined as a non-invasive predictor of D8 development. Individual culture and brief exposure to imaging did not affect developmental competence (7% O_2 control=50%, AF=71.4% and 20% O_2 control=43%, AF=50%; n=56) and preliminary results suggests NAD(P)H levels in D5 morula predict D8 blastocyst development.

In summary, metabolic heterogeneity exists within pre-implantation embryos, with increased heterogeneity associated with compromised development. Furthermore, non-invasive AF measurement of blastomere metabolism offers a new tool for determining embryo health.

[1] Brison DR, Sturmey RG & Leese HJ (2014) Metabolic heterogeneity during preimplantation development: the missing link? Human Reproduction Update, In press DOI: 10.1093/humupd/dmu018

[2] Sutton-McDowall ML, Mottershead DG, Gardner DK, Gilchrist RB & Thompson JG (2014) Metabolic differences in bovine cumulus-oocyte complexes matured in vitro in the presence or absence of follicle-stimulating hormone and bone morphogenetic protein 15, Biology of Reproduction, 87 (4): 1-8.