AMMONIUM AFFECTS MITOCHONDRIAL DISTRIBUTION AND FUNCTION IN MOUSE 2-CELL EMBRYOS

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Amino acids are key regulators of embryo function and are essential components in embryo culture media. Amino acids spontaneously breakdown and are metabolised by embryos resulting in ammonium build-up in the medium. While ammonium does not affect blastocyst development, the ability of these blastocysts to implant was reduced along with subsequent fetal growth rates. However, the mechanism for the inhibitory effect of ammonium is currently not known. It has been demonstrated in other tissues that mitochondrial bioenergetics can be disrupted by the presence of ammonium in the media which subsequently affects cellular viability. Therefore, the aim of this study was to examine the effects of ammonium on the mitochondria of mouse embryos cultured in the presence of ammonium. Mouse zygotes from superovulated females were cultured in medium G1.2 with or without 300 μM ammonium for 22 h at 37°C in 6%CO_2 : 5%O_2 : 89%N_2. *In vivo*-developed 2-cell embryos were flushed from the reproductive tract and assessed immediately. At the 2-cell stage mitochondrial distribution (Mitotracker) and membrane potential (JC-1) were assessed using confocal microscopy and images were quantitated using IP Lab software package. Differences between treatments were determined using ANOVA and Bonferroni's multiple comparison procedure. Culture of zygotes to the 2-cell stage in medium G1.2 did not affect mitochondrial distribution compared to *in vivo* controls. However, 2-cell embryos cultured with ammonium had a decrease in their mitochondrial nuclear : cortical ratio (97 ± 1 compared to 106 ± 1; $P < 0.05$) indicating that mitochondria were dispersing away from the nuclei. Culture with ammonium also significantly decreased the mitochondrial membrane potential (0.50 ± 0.01 mean pixel intensity ratio) compared to those cultured without ammonium (0.72 ± 0.3 mean pixel intensity ratio, $P < 0.001$). The data presented demonstrates that culture for only 24 h with ammonium disrupts both mitochondrial distribution and membrane potential and supports our hypothesis that mitochondria are an early target for the inhibitory action of ammonium.

10.1071/SRB04Ab233