

Addition of Amino Acids (AA) to Early Cleavage Medium (ECM™) Fails to Improve Mouse Embryo Development.

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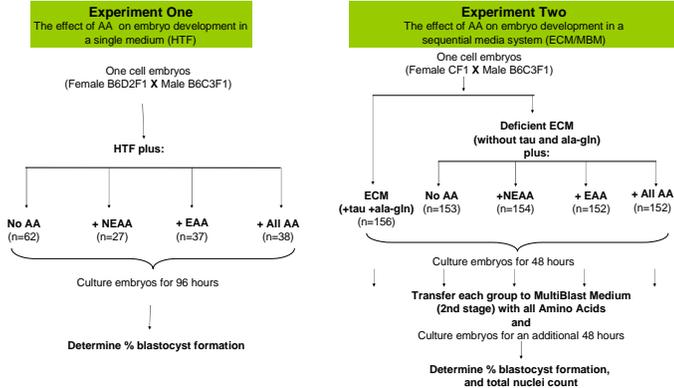
OBJECTIVES

Addition of AA to culture media has been reported to improve development of embryos to the blastocyst stage, though not all AA appear to have the same developmental impact and it is not clear which are required for this improvement. Also, the mechanism of action of AA on embryo development is not well defined, and may involve osmotic regulation. ECM™, a modification of P-1®, contains EDTA (10µM), the amino acid taurine (tau, 0.05 mM) and the di-peptide alanyl-glutamine (ala-gln, 0.50mM). The purpose of this study was to compare the effects of AA addition to Human Tubal Fluid (HTF) and ECM on mouse embryo development.

Design: Experimental Study

MATERIALS AND METHODS

One-cell mouse embryos were collected from super ovulated females and treated with Hyaluronidase, washed and then cultured in groups of 3-4 embryos per 20 µl drop of media under oil. Embryo development was determined for early cleavage (48hr) and blastocyst (96 hr) rates. Embryos were then transferred to MultiBlast Medium (MBM), with all AA for an additional 48 hr. Total nuclei per blastocyst were determined at 96 hr by staining with 0.1 mg/mL Propidium Iodide and 0.025 mg/mL of bisbenzimidazole (Hoechst 33258). Statistics were determined by Chi-Square Analysis (for embryo development) or Student's t-test (for blastocyst nuclei count).



RESULTS

Experiment One

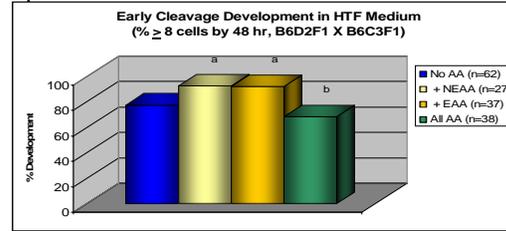


Figure 1: Addition of NEAA or EAA to HTF increased the percentage of embryos reaching or exceeding the 8-cell stage by 48 hr compared to all AA. Bars with different letters are significantly different from each other (p<0.05) by Chi Square test.

Experiment Two

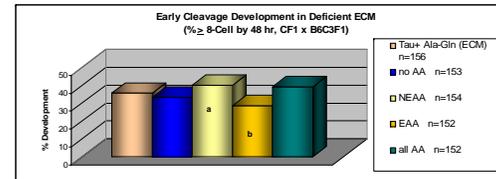


Figure 3: The percent of embryos reaching or exceeding the 8-cell stage in the first 48 hr was significantly lower for those cultured with EAA compared to those cultured with NEAA, but not different from other groups, including ECM containing 2 amino acids. Bars with different letters are significantly different (p<0.05) from each other by Chi Square Analysis.

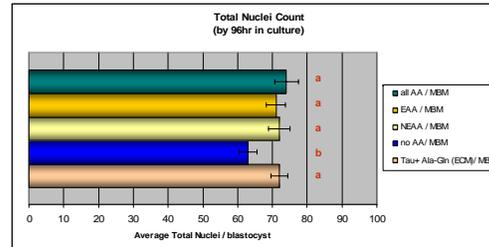


Figure 5: The total nuclei count for blastocysts was significantly lower for the group cultured with no AA initially compared to other groups. The bars with different letters are significantly different from each other (p<0.05) by Student's t-test.

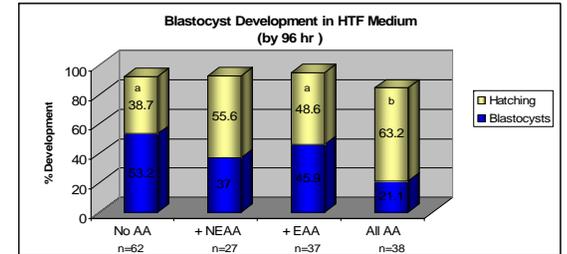


Figure 2: There was no difference in the percent of blastocysts reaching expanded/hatching stages for any of the groups by 96 hr. There were significantly more hatching blastocysts in the group that had all AA compared to no AA or EAA. Bars with different letters are significantly different (p<0.05) by Chi Square Test.

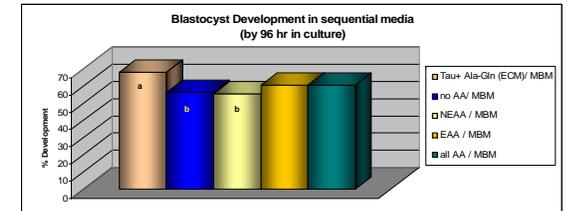


Figure 4: The percent of blastocysts reaching expanded/hatching stage was significantly higher after initial culture in ECM (with tau and ala-gln for the first 48 hr) followed by culture in MultiBlast Medium for an additional 48hr compared to those cultured in no AA or NEAA for the first 48 hr. The bars with different letters are significantly different (p<0.05) from each other by Chi Square Analysis.

CONCLUSIONS

Addition of all essential and/or nonessential AA during early cleavage development does not appear to benefit mouse embryo development if at least taurine and alanyl-glutamine are included in the culture media (ECM™) for the first 48 hours.

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