IN-VITRO CULTURE OF SPINY MOUSE EMBRYOS

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BACKGROUND

The reproductive biology of the spiny mouse (*Acomys cahinirus*) is relatively unique amongst mammals as current *in vitro* embryo culture methods are unable to support embryo growth from 1-cell through to the implantation stage[1]. Embryos grow normally until the 4-cell stage where they fail to progress further. This phenomenon has been termed the '4-cell block'[1].

Developmental 'blocks' have been identified, and then subsequently overcome, in a number of species, including mouse (2-cell), humans (4-cell) and bovine (8-16 cell)[2]. In each instance an improvement over the existing culture conditions was required to facilitate successful embryo growth. Overcoming the 4-cell block in spiny mouse embryos by modifying culture conditions may provide an improved rodent model for human embryology compared to the mouse.

HYPOTHESIS

We hypothesize that altering human embryo culture media by manipulating levels of energy substrates, amino acids, antioxidants, chelators, macromolecules, osmolarity, pressure, pH and temperature, will allow us to successfully culture Spiny mouse embryos from 1-cell through to the implantation stage.

METHODS

Female Spiny mice (n=30) between 40 and 75 days old were used. Superovulation was induced as previously reported using 20IU PMSG (administered as 2 X 10IU doses 9h apart) and 20IU hCG (administered 60h later)[1]. Embryos were flushed from the oviduct and assigned to predefined culture conditions (shown in Table 1). F1 (C57BL/6 X CBA) mouse embryos were cultured alongside spiny mouse embryos at >95% blastocyst rate throughout the experiment.

RESULTS:

At 33 h post-hCG an average of 10.2 ± 0.73 1-cell (a) and 2-cell (b) embryos were recovered per successfully mated animal (mean±SEM).

Table 1 Experimental Treatments

Treatment Conditions	Embryos	Treatments	Description of treatments	Rationale	End Result
Energy substrates: glucose, pyruvate and lactate	**63**	**21**	**Glucose** 0.22mM, 4mM, 6mM; **Pyruvate** 0.1mM, 0.32mM; **Lactate** 0.1mM, 10.5mM	Metabolism in the embryo changes over time: achieving the correct balance of each substrate can greatly improve the potential for normal growth[2,4]	No difference
Amino Acids: addition of MEM Essential Amino Acids (EAA)	**24**	**3**	**50X MEM EAA** 0mM, 2.4ul, 4.8ul per 30ul media	Addition of extra EAA's ensures endogenous amino acids within the media aren't exhausted[2,4]	No difference
Antioxidants: reduced-L-glutathione / taurine	**38**	**11**	**Glutathione / Taurine** 0mM, 0.6mM, 1mM, 2mM, 3mM, 10mM	Antioxidants are now used commercially: Vitrolife® G5 Series™ embryo culture media contains an antioxidant	No difference
Chelators: EDTA	**6**	**3**	**EDTA**: 0.01mM, 0.05mM, 0.1mM	Pivotal in overcoming the 2-cell block in mice; chelators reduce oxidative stress caused by transition metal ions[2]	No difference
Macromolecules: sucrose and PVP	**10**	**5**	**Sucrose** 0M, 0.1M, 1M; **PVP** 0M, 0.1M	Osmotic tension is altered by the presence of molecules that are not able to cross the cell membrane (more accurately mimics the *in vivo* environment)[3]	No difference
Osmolarity: salts/water balance	**36**	**23**	**Measured (**using an osmometer) from 201mosm to 340mosm	A critical factor in regulating molecular pathways necessary for development[4]	No difference
Pressure: 3d gel matrix encapsulation	**39**	**1**	**Encapsulated** and cultured 24 embryos successfully	Alginate matrices are shown to mimic the *in vivo* extracellular matrix improving oocyte growth[3]	No difference
pH: 7.16 – 7.33	**248***	**72***	**pH** 7.16 – 7.33	Not manipulated directly in this study the pH of each medium was altered by various treatments	No difference
Temperature: 37.0ºC – 39.0ºC	**248***	**5**	**Temperature**: 37.0ºC / 37.5ºC / 38.0ºC / 38.5ºC / 39.0ºC	Higher temperatures in other species,(eg porcine 38.5ºC), are necessary for normal development[2]	No difference

* Denotes the total number of Embryos or Treatments in this experiment: in these instances all embryos or treatments provided an individual measurement for this variable

DISCUSSION

We were unsuccessful in culturing embryos past the 4-cell block in each of the 72 experimental treatments. This is a remarkable outcome, as every mammal in which *in vitro* embryo culture has been attempted (mongolian gerbils[4], tigers[5] and primates[6] are a few examples) has been successful in overcoming a developmental block using one of the modifications outlined in Table 1.

In other species there is a noted correlation between a developmental block and the first stages of the embryonic genome activation[3]; this is likely the underlying cause of our inability to culture through to 8 cells and further examination of this process by analysing maternal and embryonic gene expression in individual embryos at different stages of development will be a future direction of this project.

CONCLUSIONS

We have been unable to successfully culture spiny mouse embryos *in vitro* using these modifications of human embryo culture media (Table 1). This finding establishes the spiny mouse as being relatively unique, as these modifications have proven successful in all other mammals investigated. Further investigation into the underlying molecular biology and metabolism of the spiny mouse embryo is warranted as discovering the necessary conditions will likely provide an improved model for human embryology compared to the mouse.

References

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