LOW LEVELS OF AMMONIUM DETECTED IN SPENT MEDIA FROM MOUSE OR HUMAN EMBRYOS CULTURED IN CONTINUOUS SINGLE CULTURE™ MEDIUM (CSCM) ARE NOT DETRIMENTAL TO DEVELOPMENT

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OBJECTIVE

There has been debate about the levels of ammonium that may be detrimental to embryo viability and development during extended in vitro culture. Recent publications have shown that human blastocysts from uninterrupted continuous culture are of equal quality to those from sequential media protocols which disturb the embryos in order to refresh the medium. The aim of this study was to determine the level of ammonium present during continuous culture of mouse and human embryos to the blastocyst stage.

MATERIALS AND METHODS

Design: Prospective Study

Fresh 1-cell mouse embryos (n=33, B6D2F1XB6C3F1) were cultured uninterrupted in groups of 3 in 20 µl drops of CSCM medium with 5mg/mL HSA under oil (Irvine Scientific) at 37ºC in 5% CO_2 and air for 96 hours. Development was assessed at the blastocyst stage. Spent and control media (without embryos) incubated under the same conditions were analyzed for ammonium using a commercial kit (Sigma).

Human embryos from 892 oocyte retrievals were cultured in reduced oxygen (6% CO_2, 6% O_2 & 88% N_2) in groups of up to six in 0.5 ml of CSCM medium with 10% SSS (Irvine Scientific) per well. Embryos remained uninterrupted in the same medium until day-7 of culture. The best quality embryo(s) were chosen for transfer on day 3 or 5. Spent media from a random selection of cultured embryos were collected and analyzed for ammonium compared to control media (without embryos) incubated for 1 day (D1) or 7 days (D7) under the same conditions.

RESULTS

Table 1: Ammonium accumulation in mouse embryo culture.

Culture Condition	NH_4^+ (µM)	Expanded Blastocysts	Hatching Blastocysts
No embryos, 0 hr	11 ± 2.1		
No embryos, D1 (24 hr)*	21 ± 2.1		
No embryos, D5 (120 hr)**	48 ± 6.4		
With embryos, D4 (96 hr)	50 ± 3.5	14 (43%)	18 (55%)

• Medium was pre-equilibrated for 1 day (D1=24hr) prior to addition of embryos
** Medium control for total incubation time (D5=120 hr) without embryos.

Table 2: Ammonium accumulation in human embryo culture.

Culture condition	NH_4^+ (µM)	# embryos/ drop of medium	Embryo Developmental Stage
No embryos (D1)	31 ± 3.5	0 (control)	
No embryos (D7)	52 ± 5.0	0 (control)	
Patient A (D7)	66 ± 7.1	3	(2)HBL, (1)BL
Patient B (D7)	61 ± 10.6	1	(1)HBL
Patient C1 (D6)	74 ± 4.2	5	(3)HBL, (2)BL
Patient C2 (D6)	65 ± 3.5	5	(3)XBL, (2)EBL
Patient D1 (D6)	43 ± 0.7	5	(3)BL, (1)EBL, (1)COMP
Patient D2 (D6)	73 ± 1.4	6	(3)HBL, (1)BL (2)DEG
Patient G (D6)	58 ± 3.5	5	(3)XBL, (2)BL
Patient H (D6)	47 ± 7.8	2	(1)XBL, (1)EBL
Patient I (D6)	65 ± 7.8	1	(1)XBL
Patient J (D6)	76 ± 4.2	5	(2)HBL, (2)XBL, (1)BL
Patient K1 (D7)	58 ± 7.8	5	(3)HBL, (2)DEG
Patient K2 (D7)	84 ± 11.3	5	(2)XBL, (3)BL
Patient L (D7)	54 ± 4.2	3	(1)HBL, (1)XBL, (1)DEG

KEY: DEG= Degenerate, EBL=Early Blastocyst, BL=Blastocyst, XBL= Expanded Blastocyst, HBL=Hatching Blastocyst

Ammonium levels in mouse embryo cultures were: control (D1) 21±2 µM, control (D5) 48±6 µM, spent medium (+ embryos, D4) 50±3 µM with development rates of 43% expanded and 55% hatching blastocysts. Ammonium levels in the human embryo study were: control (D1) 31±3 µM, control (D7) 52±5 µM, spent media (+ embryos up to D7) levels ranged from 43-84 µM. Human embryo development rates were 29% hatching, 25% expanded, 25% blastocysts, 8% early blasts, 2% compacting and 10% degenerate.

CONCLUSION

Comparison of ammonium levels for control media (incubated without embryos) and spent embryo culture media showed that ammonium buildup was primarily contributed by the medium components alone (<55 µM without embryos). The ammonium levels detected in the presence of human embryos was not correlated with the number of embryos in group culture and these levels did not compromise embryo development. Ongoing pregnancies were obtained from transfers of human blastocysts grown uninterrupted in CSCM, demonstrating that a single continuous culture protocol supports clinical outcomes that are comparable to sequential media systems (data not shown, VerMilyea et al., 2012). The ammonium levels detected in these studies were well below the proposed cutoff value of 119 µM reported for human embryos by Virant-Klun *et al*. 2006 (Fertil. Steril. 85:526).

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Funded by Irvine Scientific