CONCLUSION: Our results suggest that blastomere symmetry is an important predictor of blastocyst development and is sufficient for morphological selection of cleavage stage embryos.

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RENEWING OF MEDIUM IN A SINGLE STEP MEDIA CULTURE PROTOCOL PROVIDES NO ADVANTAGE TO MOUSE EMBRYO DEVELOPMENT WHEN OBSERVED CONTINUOUSLY THROUGH TIME LAPSE MICROIMAGERY. M. D. VerMilyea, J. R. Graham, M. J. Tucker. Shady Grove Fertility Reproductive Science Center, Rockville, MD.

OBJECTIVE: Extended embryo culture and single blastocyst transfer has resulted in the reformulation and development of new in-vitro culture media. Sequential media protocols require a disruption of embryo culture whereas medium in a non-interrupted single step medium (SSM) protocol is used continuously. There has been debate as to whether refreshing of SSM may be beneficial for embryo development; however, some argue against the disturbance to embryo(s) in culture. We applied time-lapse microimagery technology to the comparison of mouse embryos cultured in sequential, static and renewed culture media protocols.

DESIGN: Prospective Animal Study.

MATERIALS ÅND METHODS: Thawed 1-cell mouse embryos (Embryotech) were cultured individually in 25µl of either a prototype SSM (Irvine Scientific) or Quinn's Advantage Cleavage and Blastocyst media (QA, Sage) at 37°C in reduced oxygen. Development was assessed using the EmbryoScope Monitoring System (Unisense, Fertilitech). Images were acquired at timed intervals for key morphologic stages as Mean Development Time (MDT). Traditional scoring of the degree of hatching (>50%) at 120hrs was also documented.

RESULTS: The MDT of 1-cell embryos, cultured to the hatched blastocyst stage in assigned media were all similar (P>0.15, Student's t-Test). At 120 hrs, by traditional scoring methods 69% (25/36) Sage, 61% (22/36) SSM and 55% (20/36) SSM Renewed were graded as >50% hatched (not significant, Sage vs. SSM (P=0.62); Sage vs. SSM Renewed (P=0.33); SSM vs. SSM Renewed (P=0.81, Fisher's exact).

CONCLUSION: The detailed data on cleavage events and subsequent stages of development, provided by time-lapse imagery, show no statistical difference in MDT of 1-cell embryos cultured in Sage sequential, SSM or SSM renewed media. No differences in hatching rates, by traditional scoring, were also noted. Our results support the use of a SSM static protocol in IVF laboratories which may offer cost advantages and simplified culture management when compared to sequential culture media protocols.

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EFFECTS OF ALPHA-LIPOIC ACID ON THE MOUSE EMBRY-ONIC DEVELOPMENT *IN VITRO*. J. Yoon, K. Juhn, S. Yoon, Y. Ko, J.-H. Lim. Maria Research Center, Seoul, Republic of Korea; Korea University, Seoul, Republic of Korea; Maria Fertility Hospital, Seoul, Republic of Korea.

OBJECTIVE: Alpha-lipoic acid (LA) was reported to reduce the level of extracellular reactive oxygen species, which resulted in increase in the cell number of blastocyst stage embryos (Linck et al., 2007). The objective of the present study was to determine optimal concentration of LA when supplemented to culture medium under either low or high oxygen tension.

DESIGN: Experimental animal study

MATERIALS AND METHODS: Zygotes derived from BD F1 mice were developed in MRC#D01 medium (Biosupply Co., Korea) in the absence or presence of LA  $(0, 1, 10, 100~\mu\text{M})$  under either 5%  $O_2$ , 6%  $CO_2$  and 89%  $O_2$  or 6%  $O_2$  in air. Embryo development was monitored on day 4 and 5. Efficacy of LA supplementation for blastocyst development was compared using a t-test and data were analyzed by SPSS.

RESULTS: Regardless of  $O_2$  tension,  $100~\mu M$  of LA significantly reduced the development of expanding blastocysts on day 4 and hatching or more developed blastocysts on day 5. Under  $20\%~O_2$  tension, 1- $10~\mu M$  of LA were significantly effective for the development of hatching or more developed blastocysts on day 5 (P<0.05), but not for the expanding or more developed ones on day 4. Under  $5\%~O_2$  tension, however,  $10~\mu M$  of LA significantly decreased the blastocyst development on both day 4 and 5 (P<0.05).

The effect of LA concentrations on the mouse embryo development in vitro

Oxygen tension	LA concentration (µM)	No. of zygotes examined	≥ expanding blastocyst (day 4)	≥ hatching blastocyst (day 5)
20% O2 20% O2 20% O2 20% O2 5% O2 5% O2 5% O2 5% O2	0 1 10 100 0 1 10	228 225 218 220 144 144 136 140	$90.5 \pm 3.9a$ $94.0 \pm 4.2a$ $92.9 \pm 2.3a$ $80.9 \pm 1.3b$ $97.0 \pm 4.7c$ $94.7 \pm 4.1c$ $88.3 \pm 3.9d$ $78.1 \pm 9.6e$	$72.6 \pm 16.2f$ $92.8 \pm 3.7g$ $91.8 \pm 5.7g$ $70.8 \pm 3.0f$ $94.4 \pm 4.4h$ $94.2 \pm 4.5h$ $88.3 \pm 3.9i$ $75.5 \pm 12.7i$

a-b, c-d, c-e, d-e, f-g, h-i indicate significant differences in the column (P<0.05 in t-test)

CONCLUSION: Data from the present study indicate that 1  $\mu$ M of LA supplementation to culture medium is practically useful for mouse embryonic development under both low and high oxygen tensions.

## **OVARIAN STIMULATION**

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SUCCESS OF CLOMIPHENE CITRATE IN A COUNTY POPULA-TION. E. Mellano, K. Brennan, C. Holschneider. Obstetrics and Gynecology, University of California Los Angeles Medical Center, Los Angeles, CA; Obstetrics and Gynecology, Olive View-UCLA Medical Center, Sylmar, CA.

OBJECTIVE: To determine the effectiveness of clomiphene citrate (CC) and timed intercourse (TI) in achieving pregnancy in an underserved county population.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: The medical records of all women who presented to a metropolitan public hospital for infertility between September 2010 and January 2011 and were prescribed CC for ovulation induction or superovulation were analyzed. Data was collected on the 35 women identified including age, body mass index (BMI), infertility diagnosis, day 3 follicle stimulating hormone (FSH) and estradiol (E2) levels, semen analysis, hysterosalpingogram (HSG) results, number of CC cycles, day 21 and 28 progesterone (P4) and BhCG, with pregnancy defined as a positive BhCG.

RESULTS: Of the 35 patients, 31 had normal semen analyses and HSGs, and known ovulatory status and BhCG results of at least one cycle. The total number of CC cycles was 86; of these, 58 were ovulatory as defined by P4 > 3 ng/ml and 2 pregnancies were identified. The ovulation rate per CC cycle was 67.4%. The pregnancy rate per ovulatory cycle was 3.45%. The patients were then subdivided by those who always ovulated (16/31); those who sometimes ovulated (6/31); and those who never ovulated (9/31); no significant differences regarding age, day 3 E2 or FSH were found. However, women with a higher BMI and with polycystic ovarian syndrome or oligomenorrhea were less likely to ovulate with CC (*P*<0.005).

CONCLUSION: The majority of patients ovulated with CC, but few became pregnant. Of the two documented pregnancies, one was an ectopic and the other was lost to follow-up. In this county population, CC and TI alone are not an effective treatment for infertility. Adjuncts, such as dexamethasone or metformin, may increase success in this underserved population.

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DOES EARLIER ADMINISTRATION OF HUMAN CHORIONIC GONADOTROPIN (hCG) IMPROVE THE PROBABILITY OF PREGNANCY IN CYCLES STIMULATED WITH REC-FSH AND GnRH ANTAGONISTS? A PROSPECTIVE RANDOMIZED TRIAL. D. Kyrou, E. M. Kolibianakis, H. M. Fatemi, B. C. Tarlatzis, H. Tournaye, P. Devroey. Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Brussels, Belgium.

OBJECTIVE: The purpose of this trial was to assess the effect of altering the timing of hCG administration on the probability of pregnancy in patients stimulated with rec-FSH/GnRH antagonists for IVF.