Where do Mouse Embryos Thrive Best? Comparison of Mammalian Embryo Development Under Varying Laboratory Environments

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As the field of Assisted Reproductive Technology (ART) expands, so does the need to understand how differing laboratory environments affect development of embryos cultured *in vitro*. More specifically, do different air environments alter embryo development? To answer this question, we obtained mouse embryos from six hyperstimulated mice. The experiment was divided into two arms, with four replicates; half of the allotted embryos went to the ART clean room laboratory and the other half went to the non-clean room laboratory. The results of this experiment demonstrated that the clean room laboratory developed two-cell mice embryos to the blastocyst stage at a higher rate than those in the non-clean room laboratory. In conclusion, the results of this study indicate that improved air quality can promote better embryo development.

Introduction

As the field of Assisted Reproductive Technology (ART) expands, so does the need to understand how differing laboratory environments affect development of embryos cultured *in vitro*. In theory, a sterile environment should provide the best possible chance for embryos to reach their full developmental potential. However, few prospective randomized trials addressing this issue exist in the scientific literature.

Two different laboratory environments, the ART clean room laboratory and the andrology laboratory, are used in the field of Assisted Reproductive Technology (Boone et al., 1999; Proctor et al., 2004). The ART clean room laboratory is a specially designed clean room that eliminates many particles in the air that may be harmful to human embryo development. The room is rated as a Class 100 clean room; therefore, no more than 100 particles of 0.5 microns or larger are in a cubic foot of air (Boone et al., 1999). In contrast, the andrology laboratory is not as efficient as the clean room because it is under HVAC control. Thus, the particle counts can reach in excess of 20,000 particles per cubic foot of air (Boone et al., 1999). These particles may carry unknown contaminates that may enter the culture media and affect embryo development.

The purpose of this study is to determine if air quality affects embryo development.

Materials and Methods

Clean Room Laboratory. The clean room has four ultra low penetration air (ULPA) filters in the ceiling. Air is forced from the clean room through return vents located at the bottom of an exterior wall. The walls, ceiling, and floor of the clean room consist of nonshedding materials. Individuals that enter the clean room wear Tyvex (Clean Wear Products, Toronto, Ontario) clothing to prevent shedding of particles.

Andrology Laboratory. The Andrology laboratory uses a standard airflow environment. The ceiling tiles are made of Celotex (Armstrong, Model Number 861; Armatuff, Greenville, SC). The walls are painted with a standard latex paint while the floor is a vinyl composite tile (Armstrong Imperial Excelon;Bonitzz, Greenville, SC). The ceiling tile, wall paint, and flooring material are not designed for clean room use and thus have a potential for shedding. Individuals enter the Andrology laboratory wearing street clothes.

Incubator Environment. Two incubators (Forma Scientific, Model Number 3158, Marietta, OH) were used for this study - one incubator in the clean room laboratory and one incubator in the andrology laboratory. Both incubators maintain the same, internal environment. The gas concentration for these incubators was 5% CO_2 and air, and the temperature was 36.7^{o} C. Both incubators maintained a relative humidity in excess of 90%.

Embryo Culture. Two-cell embryos were obtained from six hyperstimulated mice (B6C3F1 strain). These two-cell embryos were cultured in 50 μL drops of Human Tubal Fluid (Irvine Scientific, Santa Ana, CA), covered with washed, equilibrated mineral oil (Humaco Mineral Oil; Aaron, Industries, Inc, Clinton, SC) residing in petri dishes (Model Number 3002, Becton Dickinson, Franklin Lakes, NJ). The embryos were grouped ten in each drop. After three days of culture, the embryos were checked for blastocoele development.

Statistics. This experiment was a prospective, randomized study. The experiment was divided into four replicates, performed on four separate days. One half of the allotted embryos were randomized to the clean room laboratory and the other half to the andrology laboratory. The blastocoele developmental rate was determined by the

number of blastocysts divided by the number of embryos allotted to each laboratory times 100%. Statistical tests were performed using Chi-square analysis.

Results

As shown in Table 1, one hundred thirty-seven, two-cell mouse embryos were placed in each arm of the study. The blastocyst rate was significantly different (P=.01) for these two study groups. Overall, two-cell mouse embryo development rates for clean room laboratory and andrology laboratory media were 99% (136/137) versus 93% (127/137), respectively.

Discussion

In this prospective, randomized trial, we demonstrated that the environmental conditions in the clean room laboratory and the Andrology laboratory produced different results when we cultured two-cell mouse embryos to the blastocyst stage. The results of our study indicated that a clean room environment was more appropriate for culturing early mouse embryos than a standard laboratory.

Table 1: Comparison Between Mouse Embryo Development of the Clean Room Laboratory and the Andrology Laboratory.
*P=0.01, Chi-Square Analysis

Replicate	Cleanroom Laboratory	Andrology Laboratory
1	100% (39/39)	95% (37/39)
2	100% (20/20)	90% (18/20)
3	100% (30/30)	80% (24/30)
4	98% (47/48)	100% (48/48)
Total	99% (136/137)	93% (127/137)*

Cohen et al. (1997) performed studies on air quality in ART. As a result, they found common incidences of chemical air contamination caused by air pollutants. These air pollutants include volatile organic compounds (VOC), small inorganic molecules (N_2O, SO_2, and CO), substances derived from building materials, and other polluting compounds possibly released by pesticides or aerosols. It has been determined that ART success rates decreased when embryo culture was performed in low air quality environments (Cohen et al., 1997).

Cohen et al. (1997) have indicated that allowing the emission of gases from new laboratory equipment was crucial. They demonstrated that air extracted from new incubators reveal concentrations of VOC that were 100 fold greater than air extracted from incubators that had been in use. They suggested that outside air may be a cleaner source of air than inside, for outside air is relatively VOC free. They further suggested that fixed and transient laboratory components may produce gaseous emissions. In addition, they cautioned against the use of detergents and alcohol-based scrubs in cleaning laboratory equipment since their efficacy is dubious, especially if they are implemented in order to substitute for poor sterile technique.

In the future, several other studies ought to be conducted. One such study should be a multi-center trial to validate these findings. A second study should investigate development past the blastocyst stage. In order to do this, blastocysts from both arms of the study should be transferred to recipient mice and allowed to develop to term and observations made upon the live offspring to determine if there are significant differences.

In conclusion, our study demonstrated that air quality plays a significant role in the development of mammalian embryos. Thus, we would recommend that individuals that culture mammalian embryos consider monitoring air quality and improving it when necessary.

References

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