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Control of air quality in an assisted reproductive technology laboratory

[Communications-In-Brief]

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Abstract

Objective: To investigate the effect of improved air quality on IVF and subsequent embryo development.

Design: Retrospective cohort study.

Setting: Hospital-based IVF facility composed of an anteroom, a cleanroom, and an adjacent operating room.

Patient(s): Two-hundred seventy-five couples requesting IVF between 1993 and 1997.

Intervention(s): None.

Main Outcome Measure(s): Particle counts (sizes 0.3, 0.5, 1.0, and 5.0 [micro sign]m); IVF rates; and embryo quality (stage and grade).

Result(s): Clinical pregnancy rates decreased from 35% in 1993 to 16% in 1994 (numerous construction odors were detected during 1994) and increased steadily after the cleanroom was built (rates for 1995-1997 were 20%, 32%, and 59%, respectively). Fertilization rates decreased between 1993 (74%) and 1994 (60%) and then steadily increased after cleanroom installation (62% in 1995, 71% in 1996, and 69% in 1997). The proportion of embryos past the four-cell stage decreased from 66% in 1993 to 61% in 1994 but then increased steadily in the years after the cleanroom was built (78%, 77%, and 83% in 1995, 1996, and 1997, respectively). During the same 5-year period, there were no differences in embryo quality or number of embryos transferred.

Conclusion(s): Construction of a Class 100 cleanroom improved air quality and IVF rate and increased the number of embryos past the four-cell stage available for transfer. (Fertil Steril[registered sign] 1999;71:150-4.)

Key Words: Air quality, cleanroom, particle counts, in vitro fertilization, embryo development

For many years, scientists performing embryo culture procedures shared accounts of decreased cell development after construction of laboratories or remodeling of facilities. Researchers also told anecdotes of improved cell development after removal of shelved chemicals from the laboratory. However, with a few exceptions [1], investigators have not reported a correlation between levels of toxicants (e.g., bacteria, dust, particulate matter, and volatile compounds [odors]) and decreased embryo development.

In 1994, construction of additional operating rooms adjacent to our assisted reproductive technology (ART) laboratory and renovation of the emergency room area near our facilities produced detectable odors in the laboratory. We noted increased levels of dust and other particulate matter associated with drywall installation, as well as odors from paint and floor tile glue. Simultaneously, the clinical pregnancy rate (PR) for IVF procedures decreased from 35% (25 of 71) in 1993 to 16% (11 of 68) in 1994. Having proved to ourselves that poor air quality leads to poor embryo development [2], we constructed a cleanroom that provides a unique oocyte/embryo

culture environment, with a sophisticated air-handling system separate from the standard hospital air-handling system.

In this report we describe our cleanroom and associated facilities, prospective results from monitoring air quality within the cleanroom and associated facilities, and retrospective results from culturing human embryos in our facility.

MATERIALS AND METHODS

Cleanroom and Associated Facilities

Anteroom

The anteroom (4.4 m width x 5.1 m length x 2.4 m height [53.8 m^3]) includes three ultra low penetration air (ULPA) filters and three return vents in the ceiling (Figure 1). Walls are painted with low-odor epoxy paint. Ultraviolet-free fluorescent lights illuminate the room. The anteroom undergoes 47.5 air exchanges per hour.

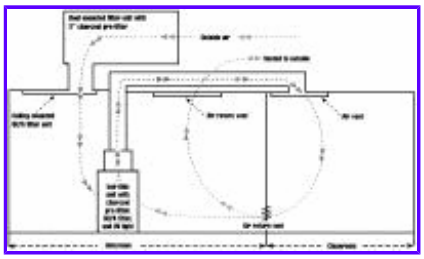

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Figure 1. Air-flow pattern for the anteroom and cleanroom.

Cleanroom

The cleanroom (2.6 m width x 5.1 m length x 2.4 m height [31.8 m^3]) has three UV-free fluorescent light fixtures and two ULPA-filter air diffusers in the ceiling. The air diffusers are above the two most active areas of the cleanroom: the work station for incoming oocytes and outgoing embryos and the station for micromanipulation. The walls, ceiling, and floor of the cleanroom consist of nonshedding materials. The shared wall between the anteroom and the cleanroom contains two floor-level return vents. In the cleanroom, there are 20.5 air exchanges per hour.

Air Flow

Roof-top air-handling units (model YCM-O3; Borg Warner, York, PA) draw outside air through a charcoal prefilter before it enters operating room 24 (OR-24) or the three ULPA filters (Clean Rooms International, Inc., Grand Rapids, MI) in the ceiling of the anteroom. An Isol-Aide unit (model AETP2093-13; Component Systems, Inc., Cleveland, OH) pulls anteroom air through a second charcoal prefilter, past an ultraviolet light for irradiation of bacteria and fungus, and through a 0.12-[micro sign]m ULPA filter. Then the filtered air enters the cleanroom through two ceiling diffusers located over prime working areas. Floor-level vents in the wall of the cleanroom return air to the anteroom, to be remixed with existing air.

Positive Pressure

We maintain a positive pressure differential between the cleanroom and the anteroom with the use of an Iso-Tek Space Pressurization Monitor (model SPM-5100; Tek-Air Systems, Inc., Danbury, CT). The cleanroom is positive to the anteroom, which is positive to OR-24. The operating room is positive to the hallway.

Mechanisms to Reduce Particle Counts

We installed a double-door, pass-through window between the cleanroom and OR-24 that allows for passage of oocytes and embryos between these two locations and minimizes the mixing of air between the cleanroom and the adjacent operating room. Furthermore, we require all laboratory personnel to step on sticky mats (Lab Safety Supply, Janesville, WI) at all anteroom and cleanroom entrances. In the cleanroom, laboratory personnel wear nonshedding Dacron coveralls, hoods, and shoe covers (CleanWear Products, Toronto, Ontario, Canada) as well as masks and gloves.

Operating Room 24

The operating room (OR-24; approximately 7.5 m width x 4.5 m length x 3.0 m height [101.2 m^3]) has eight intake units in the ceiling and two exhaust units in the wall at floor level. Using these passageways, the air in the room undergoes 17.2 exchanges per hour. In addition, OR-24 has a unique portable minihood (Component Systems, Inc.) containing a high-efficiency particulate air (HEPA) filter. During oocyte retrieval, we use the minihood to improve air quality directly over the area where capping and uncapping of aspiration tubes occur. The walls and ceiling of OR-24 are painted with low-odor epoxy paint.

Air-Quality Monitoring

Particle Counts

Particle counts are the standard means for assessing cleanroom classification [3]. The particle counter draws a sample of air across a laser beam and then determines particle size and number. The number of particles of a specified size in a specific volume of air determines cleanroom classification. For example, a Class 100 cleanroom has no more than 100 particles >or=to0.5 [micro sign]m in a cubic foot of air [3].

Particle Count Variation

As part of quality control, we used our Met One analyzer (Grants Pass, OR) to perform particle counts in various locations within the facility. For this experiment, we chose two cleanroom locations (locations 1 and 2), two anteroom locations (locations 3 and 4), one minihood location (location 5, in OR-24), and one operating room location (location 6).

We performed eight particle counts (each count on a separate day) at each of the six locations. For each count, the instrument sampled air for 10 minutes and rested for 50 minutes and then repeated this procedure for 12 cycles. We disregarded the initial cycle from each location, the assumption being that during the first cycle, the instrument was warming up and the room was reaching an at-rest condition. The remaining 11 cycles provided the mean count for four different particle sizes (0.3, 0.5, 1.0, and 5.0 [micro sign]m).

Embryo Culture

Patient Population

We used gametes from 275 couples in this study. Patients signed consent forms indicating awareness that their data might be used to monitor and possibly improve methodologies used for IVF.

Culture and Transfer Conditions

We described the culture conditions earlier [4]. Briefly, most of the culture conditions remained constant from 1993 through 1997. When we did evaluate human tubal fluid (Irvine Scientific, Santa Ana, CA) versus P-1 Medium Medium (Irvine Scientific), no significant differences (P = .812) in IVF potential were observed. Similarly, we found no differences (P = .437) when converting from 1 mL of medium in tubes to 50-[micro sign]L drops of medium under mineral oil (Sigma Chemical Co., St. Louis, MO).

We could not determine PRs for these two studies because the oocytes were assigned to different treatments within patients with the highest-quality embryos being transferred, regardless of the treatment group. In November 1996, we started assisted hatching of all transferable embryos and changed from tomcat catheters (Sherwood Medical, St. Louis, MO) to Wallace catheters (Simcare Manufacturing, Ltd., West Sussex, United Kingdom) for the transfers. In late summer 1997, we added two new physicians to our ART program. These were the only changes recorded between 1993 and 1997.

Embryo Stage and Grade

Embryo stage was defined by the total number of blastomeres present, and grade was defined by blastomere quality. We used a grading system similar to those previously reported [5].

Statistical Analysis

We used the Kruskal-Wallis nonparametric analysis of variance to test for a location effect within each particle size. If significant differences were present, simultaneous Wilcoxon pair-wise comparisons (15 per particle group) were used to assess differences in location. The Kruskal-Wallis nonparametric analysis of variance was used to assess significant differences by year for fertilization rate and for clinical variables that, in our program, affect fertilization (mother's age, prewashed-sperm concentration, and straight line velocity [VSL] of prewashed sperm).

If significant differences were present, Scheffe's procedure for multiple comparisons was used. The chi squared test was used to assess annual differences for categorical variables. For embryo grade and quality, we assumed each embryo was independent. If the overall chi squared test was statistically significant, we then compared 1994 findings with those of other years. P values were not adjusted for the number of comparisons.

RESULTS

Particle Count Variation

As shown in Table 1, mean particle counts (sizes 0.3, 0.5, and 1.0 [micro sign]m) for the six locations fell into three groups: low counts, for locations 1-4 (oocyte/embryo handling area, micromanipulation area, two areas of anteroom); intermediate counts, for location 5 (minihood in OR-24); and high counts, for location 6 (OR-24). In the 5-[micro sign]m-particle group, all counts were low. Formal statistical tests confirmed a location effect for each of the four particle sizes ($P < .001$). In the 0.3-, 0.5-, 1.0- and 5.0-[micro sign]m-particle groups, counts for locations 1-4 were not different from one another; location 5 counts differed from location 1-4 counts and from location 6 counts.

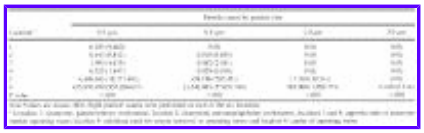

Table 1. Mean particle counts for six locations in an assisted reproductive technology laboratory.

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Clinical Variables

Pregnancy rates decreased from 35% in 1993 to 16% in 1994 when odors and fumes were detected in the ART laboratory (Table 2). Pregnancy rates increased slightly in 1995 (20%) after installation of the cleanroom and then dramatically in 1996 and 1997 (32% and 59%, respectively). Over that period, the age of the mother, prewashed-sperm concentration, and mean prewashed-sperm VSL did not differ by year ($P > .05$), although sperm counts were highest in 1993 and lowest in 1995. The percentage of patients undergoing intracytoplasmic

sperm injection did not increase significantly from 1996 to 1997 (1996, 29% [19 of 66]; 1997, 43% [22 of 51]; P = .121). During these same years, the percentage of females >39 years of age who sought IVF did not increase significantly (1996, 5% [3 of 66]; 1997, 8% [4 of 51]; P = .697).

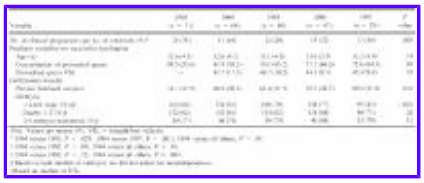

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Table 2. Distribution of variables associated with successful pregnancy, by year.

In addition, during these same 2 years the top four diagnostic reasons patients underwent IVF (tubal disease, endometriosis, male factor, and ovulatory dysfunction) did not change. The rates for tubal disease and endometriosis were almost identical both years and these diseases constituted approximately 60% of the primary diagnoses. A shift downward was noted in the proportion of embryos past the four-cell stage, between 1993 (66%) and 1994 (61%), followed by an increase in 1995, 1996, and 1997 (78%, 77%, and 83%, respectively). Over this same period, there was no appreciable difference in embryo grade or proportion of patients with four or more embryos transferred (P > .05).

DISCUSSION

In this report, construction and monitoring of an ART cleanroom are described for the first time. Furthermore, we demonstrate that improvement in fertilization rates and cell-stage developments takes place when oocytes and embryos are kept in high-quality air.

The initial impetus for investigating laboratory air quality came from a reduced PR in our ART program in 1994. The IVF clinical PR decreased from 35% in 1993 to 16% in 1994. This decrease in PR coincided with the emission of volatile compounds, an emission that resulted from nearby construction.

Before 1995, the air supply for the ART laboratory had been the same as that for OR-24. Since 1995, air has been passing through ULPA filters before entering the anteroom (locations 3 and 4). With ULPA filters, air quality inside the cleanroom (locations 1 and 2) is significantly better than air quality in OR-24 (location 6). Even the minihood, with its HEPA filter, in OR-24 (location 5) improves air quality. The air quality remains high, and the cleanroom has been certified semiannually by an independent agency (Professional Certification Group, Inc., Braselton, GA) and maintains a Class 100 rating.

We observed an increase in fertilization rate and an increase in cell stage and embryo quality (more advanced cell stage) for the transferable embryos, after construction of the cleanroom. Other researchers confirm that cell development increases in a cleanroom environment [1]. In this previous report, volatile compounds hindered cell division in mouse embryos. However, these scientists did not attempt to repeat this procedure to observe IVF results, so whether fertilization in mice decreases when embryos are exposed to off-gassing is still not known. Although these investigators state that "none of the [construction] events inhibited fertilization," no data are present to support this statement. However, these researchers did provide data to support the claim that volatile compounds decreased pregnancy and implantation rates in human IVF programs [1].

Anecdotal evidence indicates that cleanroom facilities undergo a lengthy curing process. This concept is supported by our finding of a continued increase in PRs (1995, 20% [12 of 60]; 1996, 32% [15 of 47]; and 1997, 59% [17 of 29]). Future studies are needed to determine whether a curing time exists and, if it does, what the controlling factors are (e.g., building materials, cleaning regime, facility uses). Also, other factors, such as our

change in ET technique (new transfer catheter, increased use of assisted hatching of transferred embryos, and the addition of new personnel) may have contributed to our continuing increase in PR.

In conclusion, rates of IVF of oocytes and cleavage rates of embryos transferred are improved in an environment with high-quality air. Improved air quality also appears to improve clinical PRs. We acknowledge, however, that there is a need for a prospective study that can control the confounding factors and can test the true direct effect of air quality.

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