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Culture Environment: A Delicate Balance

IVF Laboratories and UVC Ionizing Radiation

By Kathryn C. Worrilow, Ph.D.

Human conception involves the coordination of a complex cascade of biochemical and molecular intracellular signaling events between human gametes, resulting in the production of viable embryos capable of implantation. Successful preimplantation embryogenesis is critically dependent upon the culture environment provided by the in vitro fertilization (IVF) laboratory.

Human embryos are largely unprotected because they lack physical barriers typically provided by epithelial surfaces, immunological defense mechanisms, and detoxifying mechanisms provided by a functioning liver. It is highly likely that gametes and embryos grown in vitro are more sensitive to environmental influences than are complex organisms with more developed mechanisms for protection. Despite this high level of sensitivity, the physical laboratory environment for the in vitro culture of gametes and embryos has changed little during the past three decades.[1,2]

Until recently, little information has been available with respect to a significant source of environmental influence, the laboratory ambient air. Most incubators used in the in vitro culture of human embryos consist of 90% to 95% ambient air. The remainder of the incubator environment comes directly from a variable source of gas, which itself can be a source of organic and metallic contaminants. New data indicate that a significant, yet delicate, balance exists between the changing organic chemistry of the laboratory ambient air and the potential effects it may exert on successful embryogenesis, implantation, and conception[3] (*Figure 1*).

Equipment, instrumentation, and personnel activities within the IVF laboratory are significant sources of off-gassing and can emit high levels of volatile organic compounds (VOCs) into the air. Laboratory and clinical personnel constitute the greatest source of bioburden, contributing bacteria, viruses, and mold spores to the culture environment. The media and culture oil, which house and support the human embryo, can serve as a sink for air contaminants, directly compromising embryo differentiation and viability.

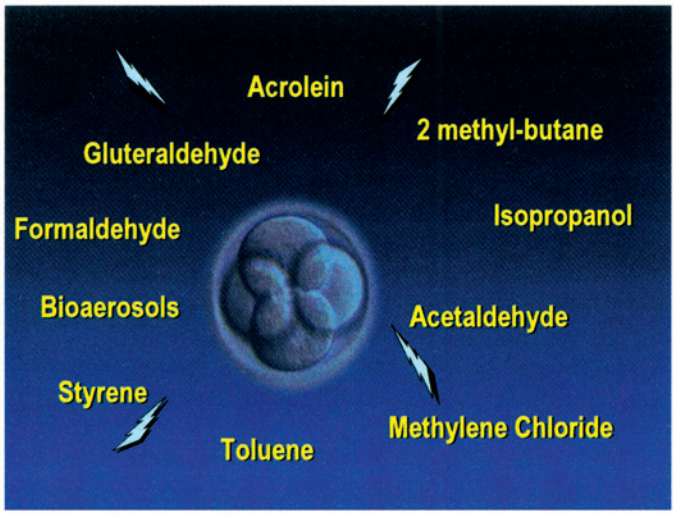
It is often thought that the IVF laboratory is protected and provides relatively clean air for the in vitro culture of human embryos. However, it has been demonstrated that HEPA-filtered laboratory air can carry far greater levels of VOCs and threatening contaminants than unfiltered outside air.[4] This is most likely due to the high level of potential contaminants within the well-sealed IVF laboratory, the low percentage of fresh air, and high percentage of recirculated air serving the laboratory. The evaluation and study of laboratory ambient air and its influence on conception is a new area of investigation within the field of assisted reproductive technologies (ART).

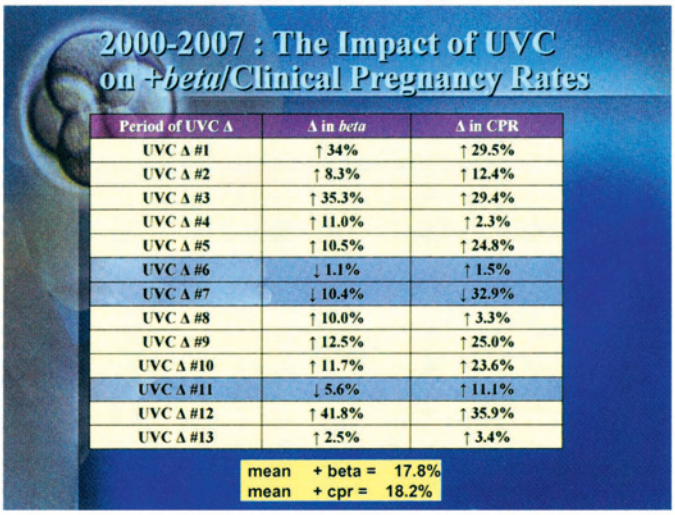
IVF Laboratory—HVAC Design

The design of the IVF laboratory and subsequent culture environment is critical in determining the future success of human embryogenesis and implantation. The necessity of the exceptional air quality and therefore an independent air-handling system also is influenced by the progressive development of ART technologies. One such technological advancement involves extended in vitro culture of the human embryo. Human embryos can be successfully cultured outside of the in vivo environment for up to seven days. Those embryos that reach the blastocyst stage, the most advanced developmental stage of the embryo, are thought to have a higher implantation potential.

Extended culture can allow the return of fewer embryos to the patient, increasing implantation rates, decreasing a potentially high order multiple rate, and improving the obstetrical and neonatal outcome. [5] Extended periods of embryo culture dictate enhanced culture environments including laboratory and incubator ambient air and the supporting media. A suboptimal laboratory or culture environment will compromise preimplantation embryogenesis, the production of a viable blastocyst, and successful implantation.

The goal was to design an IVF laboratory and HVAC system that would provide:





Period of UVC Δ	Δ in *beta*	Δ in CPR
UVC Δ #1	↑ 34%	↑ 29.5%
UVC Δ #2	↑ 8.3%	↑ 12.4%
UVC Δ #3	↑ 35.3%	↑ 29.4%
UVC Δ #4	↑ 11.0%	↑ 2.3%
UVC Δ #5	↑ 10.5%	↑ 24.8%
UVC Δ #6	↓ 1.1%	↑ 1.5%
UVC Δ #7	↓ 10.4%	↓ 32.9%
UVC Δ #8	↑ 10.0%	↑ 3.3%
UVC Δ #9	↑ 12.5%	↑ 25.0%
UVC Δ #10	↑ 11.7%	↑ 23.6%
UVC Δ #11	↓ 5.6%	↑ 11.1%
UVC Δ #12	↑ 41.8%	↑ 35.9%
UVC Δ #13	↑ 2.5%	↑ 3.4%

Figure 1 (left): Human embryos cultured in vitro are sensitive to common VOCs contained in their environment. Figure 2 (right): The impact of UVC replacement on IVF outcomes.

- A cleanroom environment and optimal ambient air to all areas housing human gametes and embryos;
- A dedicated fresh air source, independent from the hospital HVAC system; and
- Isolation from external environmental influences exerted within a large hospital setting.

Optimal ambient air was defined as air low in airborne particulates, VOC levels, viruses, bacteria, and bioaersols. We sought to design an ISO Class 5 cleanroom (Class 100 under Federal Standard 209E).

The design and new construction of the dedicated HVAC system and prototype IVF laboratory has been successful in providing optimal ambient laboratory air, which has met all National Environmental Balancing Bureau (NEBB) specifications as an ISO Class 5 cleanroom.[6] The ISO Class 5 IVF laboratory also has provided a laboratory and incubator culture environment low in VOCs, viruses, bacteria, and bioaersols.[7,8]

The custom HVAC system was designed to process a dedicated outside air source by exposing it to a series of gross particulate and gas phase molecular filters, potassium permanganate, ultraviolet germicidal light, and ultra low penetration air (ULPA) filtration with increased velocities at critical points of process. Each of the five rooms of the laboratory suite is monitored for positive pressure gradients, temperature, humidity, and supply air. Software-driven variable drives maintain all established air parameters.

For optimum effectiveness, UVC germicidal lights were added at critical points of the process within the HVAC system. The selected UVC device delivers 6 μWatts/cm² to 8 μWatts/cm² per inch of glass measured at 1 m (3 ft), when tested in a 500 fpm (2.54 m/s) airstream of 55°F (13°C). This is approximately 4 to 5 times the output of conventional UVC lamps to ensure the delivery of sufficient germicidal energy to produce desired results.

Although UVC ionization is not currently a standard component in cleanroom ventilation designs, it is effective in minimizing another source of ambient air contaminants, the bioaerosol. Bioaerosols are airborne products that include microorganisms, their toxins, and waste products. Such microorganisms include pathogens (viruses, bacteria, and mold products that can cause measles, chicken pox, Legionnaires' disease, aspergillosis, tuberculosis, and other infectious diseases), allergens (bacteria and mold products that can cause allergic rhinitis, asthma, and hypersensitivity pneumonitis), and toxins (mycotoxins and endotoxins that can cause a variety of toxic and allergic reactions, irritations and odors). It has been demonstrated that high levels of VOCs and bioaersols can be a leading cause of sick building syndrome (SBS) and building related illness.[10] Air testing of several IVF laboratories has demonstrated bioaerosol levels similar to those associated with SBS.[3,4]

HVAC systems can serve as the source of and perfect conduit for the spread of microorganisms. By recirculating large volumes of air, the HVAC system can transport system- and space-generated microorganisms room to room and, therefore, person to person, incubator to incubator, and incubator to culture media. The careful placement of an ultraviolet light source within the HVAC system will kill or inactivate harmful microorganisms and prevent their accumulation and proliferation. Placement of each filter layer is critical for optimal efficiency and performance. The UVC devices were installed downstream of the coiling coils and independent humidity system and above the ULPA filters where the air receives final filtration prior to its entry into the IVF laboratory.

Retrospective Study Initiated

A retrospective study was initiated to better understand the potential impact of each stage of filtration and air purification on clinical outcomes. The study examined the time period in which particulate and gas phase filters and UVC emitters were replaced relative to markers of preimplantation embryogenesis and clinical pregnancy rates. Clinical outcome is first detected by the presence of the pregnancy hormone beta human chorionic gonadotropin (beta hCG). The beta hCG is produced by the placenta of the developing fetus. A clinical pregnancy is defined by ultrasound several weeks after the first positive (+) beta hCG. A

clinical pregnancy is defined as the presence of an intrauterine fetal sac demonstrating cardiac activity. Both measures of clinical outcome were analyzed in the retrospective study. The study encompassed seven and a half years of clinical operation and 48 testing quarters. Each testing quarter (TQ) represented 15 to 20 IVF patients.

Particulate and gas phase filter assessment and replacement were dictated by static pressure, particulate reports, and levels of VOCs. Replacement cycles were unique to each HVAC component. Using the time period in which each HVAC component was changed as the dependent variable and fertilization rates, embryo morphology, +beta hCG, and clinical pregnancy rate as the independent variables, the data were subjected to a time series analysis.

Clinical Significance and Outcome

There were no significant differences observed in the fertilization rates, zygote, embryo, and blastocyst morphologies, +beta hCG, and clinical pregnancy rates associated with the replacement of the particulate or gas phase filters in TQ 1–48. In contrast, immediately following 10 of the 13 UVC change outs, the +beta hCG and clinical pregnancy rates increased 17.8% and 18.2%, respectively (*Figure 2*).

UVC energy will destroy 90% to 99% of airborne microbial contaminants. By targeting the DNA and RNA of microorganisms, UVC ionizing radiation functions by degrading and abating the proliferation of airborne and surface embryotoxic organics. Of equal significance to the developing embryo is the suggested impact of UVC ionization on the degradation of VOCs. VOC levels as low as 2.2 ppb can be embryotoxic to the embryo cultured in vitro.[9]

The use of UVC lights represents a departure from the standard HVAC design used in many IVF laboratories. However, the current study suggests that the use of UVC germicidal technology in the HVAC system serving the IVF laboratory may play a critical role in providing optimal ambient air moving towards improved clinical outcomes. The current study demonstrated that a significant relationship existed between the replacement of the UVC device and the associated clinical pregnancy rates.

The study also suggested a relationship between adequate UVC output, change out frequency, and the achievement of optimal results. The study used high-output UVC lamps with a change out schedule of six to nine months. As long as the lamps were functioning properly and were changed on schedule, results were consistently positive. The findings indicate the importance of selecting a device with adequate output and replacing the device consistently at required intervals to maintain that output, or germicidal effectiveness will likely be diminished.

Ongoing studies are exploring the mechanisms of action of UVC ionizing radiation within preimplantation embryogenesis and toxicology.

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