WHITE PAPER | VITROMED ACADEMY

VOLATILE ORGANIC COMPOUNDS (VOCS) IN *IN VITRO* FERTILISATION (IVF) LABORATORIES

This white paper elucidates the significance of implementing robust laboratory practices for air quality control (AQC) concerning volatile organic compounds (VOCs) in *in vitro* fertilization (IVF) laboratories. By addressing these practices, the paper aims to underscore their contribution to the enhancement of IVF outcomes and the maintenance of high standards in reproductive medicine.

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This white paper elucidates the significance of implementing robust laboratory practices for air quality control (AQC) concerning volatile organic compounds (VOCs) in *in vitro* fertilization (IVF) laboratories. By addressing these practices, the paper aims to underscore their contribution to the enhancement of IVF outcomes and the maintenance of high standards in reproductive medicine.

VOCs

Volatile organic compounds (VOCs) are carbon containing substances with high vapor pressure and low water solubility, ie they are gases that can be released from certain solids or liquids at room temperature. Outdoor VOCs can be emitted by industries, vehicles, pesticides and nature (eg forests, volcanoes). Indoor VOCs can leak from household products (building materials, paints and paint thinners, furnishings, cleaning supplies, aerosol sprays, refrigerants, hydraulic fluids, dry cleaning agents), office equipment (printers, copiers, screens, adhesives), personal care products and combustion (tobacco smoke, cooking).

VOCs are key precursors of ozone and PM2.5* and their outdoor presence plays a vital role in atmospheric chemistry[1] directly affecting the ecosystems, human health, and economic development. EPA (USA's Environmental Protection Agency) studies discovered that levels of approximately a dozen common organic pollutants are 2 to 5 times higher inside homes than outside, regardless of whether the homes are in rural or highly industrial areas. These studies further suggested that using products containing organic chemicals can result in very high pollutant levels, which can linger in the air long after the product use has ended[2]. Interestingly, continuous indoor exposure of users and others to VOCs has been linked to the Sick Building Syndrome (SBS)[3].

*PM is the sum of hazardous solid and liquid particles suspended in the air. They comprise both organic and inorganic particles such as dust, pollen, and liquid droplets. PM can be classified as coarse (diameter 10 µm; PM10), fine (diameter 2.5 µm; PM2.5), and ultrafine (0.1 µm; PM0.1).

Sources of indoor IVF lab VOCs

VOCs' indoor IVF lab presence has been related to toxicity and mutagenicity[4]. Depending on the architectural design and operational infrastructure of an IVF Unit, thousands of indoor VOCs may be present in the IVF lab.

Gamete and/or embryonic VOC exposure in the IVF lab may be from

- the air in the IVF lab (outgassing of building materials, furnishings, lab equipment, fabrics, personnel, consumables, plastic IVF labware, retrofitting and maintenance procedures, disinfection solutions, autoclave packages, medical gases, mold)
- the gases supplying incubators and workstations
- dissolution in the culture medium or mineral oil ('sink effect')[5]

More specifically, VOCs in the IVF lab can be released from

- Human activity: Personnel can introduce VOCs through personal care products (perfumes, nail varnishes), smoking, clothing and use of inappropriate lab and hand disinfectant and cleaning agents
- Building materials: Materials such as polyvinyl chloride flooring, MDF cabinets, paints and adhesives
- Lab equipment: Wooden furniture, plastic components, internal coatings and plastic seals (gaskets) of incubator doors, microscopes, IVF hoods, ICSI workstations, refrigerators, screens, printers and other devices[6,7,8]

- Lab consumables: Plastic IVF labware, semen analysis consumables (fixatives for morphology assessment), autoclaved packages, office equipment (permanent markers used for labelling plasticware and cryovials, glues, correction fluids etc, where applicable) and other disposables[5,6,7]
- Mold: since it produces carbon dioxide, water and VOCs[8]
- Gases supplied to incubators and IVF workstations[9]
- Medical gases in the operating theatre room[10]
- Medical instrument disinfection and autoclave areas[11]

Importance of VOC control in IVF Labs

In vitro culture per se exposes gametes and embryos to external stressors against which they may either not have defensive mechanisms, because they lack epithelial surfaces, immunological defences and robust detoxifying mechanisms or they may adapt resulting in alterations in embryonic gene expression, regulation, or both, including imprinting and transgenerational epigenetic effects[5].

Studies examining environmental and airborne pathogens have indicated that laboratory air quality, and more specifically VOCs, may play a significant role on *in vitro* embryogenesis, implantation and conception outcomes.

Interestingly, presence of VOCs in the IVF lab has been related to aggravated reproductive outcomes, as seen by

- low percentages of human embryos reaching the 4-cell and blastocyst stage, as well as low pregnancy rates[5]
- decreased fertilization rates[12]
- decreased live birth rates[13,14,15]
- increased miscarriage rates[16]
- lower sperm quality causing DNA fragmentation and alterations in cell replication[17]

Recommendations of VITROMED Academy to control the effects of VOCs on IVF gametes and/or embryos

"Empowerment through knowledge"

VITROMED Academy embraces evidence based medicine. Evidence-based medicine (**EBM**) is a systematic approach to clinical practice that emphasizes the use of the best available evidence from well designed and conducted research to make decisions about the care of individual patients. It integrates clinical expertise, patient values, and the best research evidence into the decision-making process for patient care. This paradigm shift towards greater patient-centricity strategies is also the core of the EN 15224:2016 Quality Management System for healthcare.

Regulatory compliance to stringent control standards ensuring an environment free of harmful VOCs is vital for maintaining or optimizing IVF success rates. This can be achieved by

1.Education and training of personnel in air quality control (AQC): Educate personnel on the sources and dangers of VOCs, and train them in

- Best *general personal and patient conduct practices* for maintaining a VOC free environment, such as avoidance of perfumes, nail varnishes, dress code, smoking, etc
- *Quality Management tools*, such as SOPs (standard operating procedures) and risk analysis for all lab procedures and VOC control protocols, if not full implementation of EN 15224:2016

2. Appropriate architectural and infrastructural design of the IVF unit:

- *Quality infrastructure:* Use low VOC building materials, such as steel instead of wood where applicable, VOC free paints, appropriate lab furniture, avoid MDF cabinets
- *Certified positive air pressure filtration HVAC* (heat ventilation air conditioning) system for the IVF lab, comprising of HEPA and activated carbon/potassium permanganate filters. Alternatively or concurrently, use of UV for photocatalytic oxidation (UVPCO) of VOCs in 100% recirculating air. Ideally, same conditions should apply for the operating theatre suit
- *Incubator 'inline' and 'indoor' filtering of incoming gases* and air with HEPA and activated carbon/potassium permanganate filters
- *Laminar flow hoods* for embryo handling with HEPA and activated carbon/potassium permanganate filters
- *Stand-alone filtration systems* comprising of HEPA, activated carbon/potassium permanganate filters & UVPCO, where applicable
- *Physical separation* of medical instrument disinfection and autoclave areas, gas supply and cryostorage suites and sperm analysis lab
- *Own high purity gas cylinders*
- *Restricted access to IVF lab*

3. Control of VOC build-up in the IVF lab during routine procedures has proven to be the most effective way of minimising damaging effects on reproductive cells. This can be achieved through

- *VOC monitoring protocols* using 400-800ppb of total VOCs as threshold values, although VOC levels far below 100ppb have been reported to affect preimplantation embryogenesis negatively[5]
- *SOPs for outgassing protocols:* Implement strict protocols for outgassing new equipment and furnishings, retrofitting and maintenance procedures, such as replacing parts or greasing to lubricate hinges, fabrics and consumables, autoclaved packs and plastic IVF labware[16]
- *Mold assessment and gasket replacement for big box incubators*
- *VOC free disinfecting* solutions
- *Gamete and embryo culture design using a) deep column oil overlaid microdrops*, so that the surface area of the exposed oil is small compared to the depth and might be less likely to favour the dissolution of VOCs in the oil[5] b) *shorten length of culture* and c) *minimize incubator door openings*
- *High quality certified plastic IVF labware* with proven low-emitted VOCs

VITROMED addresses the issue of VOC build-up in the immediate surroundings of gametes and embryos, ie culture dishes, offering high quality, soft blister individually packed (single filter pockets), low VOC plastic IVF labware. They are

- Cleanroom manufactured
- Individually soft blister packed
- Particle & scratch free
- Non toxic
- Endotoxin tested
- 96h 1-cell Mouse Embryo Assay (MEA) more than 80% tested
- HSSA more than 70% tested
- Polymers with USP Class VI standard
- Bisphenol A free
- Sterile by radiation
- Shelf life 3 years

Opening single filter pockets of VITROMED IVF labware results in very low VOC levels being released (Figure 1) ensuring sterility conditions (Figure 2), as shown in the following experiment.

Single filter pockets of VITROMED IVF labware and multipacked competitor products were compared. Immediately after opening, the escaping total VOCs and formaldehyde were measured using the photoionization detector BQ16 (Trotec) device, which allows quantification of VOC levels in the ppm

(mg/m^3) to ppb (µg/m^3) range. The measurements took place in the morning for several days, to ensure that lab VOCs, which add up after a while, will not interfere. All items were opened in the same laminar flow hood, which was switched off immediately prior measurements. Attention was paid, that no lab or laminar flow hood cleaning was conducted before the measurements. Before each item measurement, flow hood VOC levels were determined and then subtracted from the final result. The comparisons were made using one-way ANOVA and multiple comparisons were made using the Holm-Sidak method with P<0.05 and results are presented with means and standard deviations.

Two different dishes types were used in this study, 60mm IVF culture dishes and center well IVF dishes. The results for total VOC and formaldehyde levels are shown in Figure 1. Total VOCs in single filter pockets of VITROMED 60mm culture dishes (V-CTD-60) and center well dishes (V-CWD-60) were 0,05±0,02ppm for both types of dishes. Competitor A emitted 0,56±0,09ppm total VOCs from multipacked 60mm culture dishes and 0,46±0,09ppm from multipacked center well dishes. Competitor B emitted 0,14±0,04ppm total VOCs from multipacked 60mm culture dishes and 0,17±0,05ppm from multipacked center well dishes. Formaldehyde, was also emitted at significantly lower levels from single filter pockets of VITROMED V-CTD-60 and V-CWD-60 dishes (0.02±0.01ppm and 0.01±0.01ppm, respectively) compared to multipacked Competitor's A and B 60mm culture dishes (0.28±0.1ppm and 0.08±0.03ppm, respectively) and Competitor's A and B center well dishes (0.25±0.13ppm and 0.13±0.04ppm, respectively).

Single filter pockets of VITROMED V-CTD-60 and V-CWD-60 dishes, emitted significantly lower (P<0.001) total VOCs and formaldehyde compared to both multipacked dish types and Competitors, respectively. Competitor's A products released the highest levels of both total VOCs and formaldehyde.

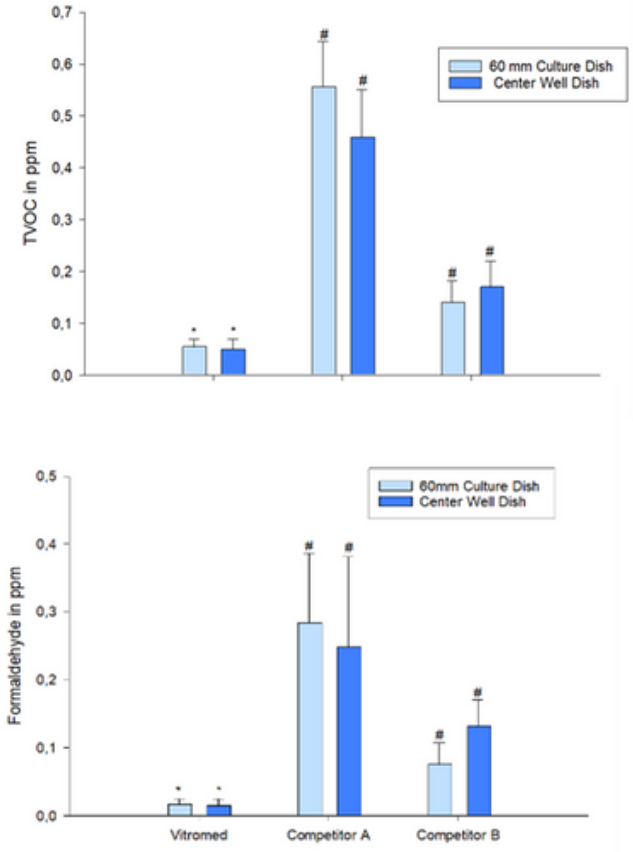

Figure 1: Levels of total VOCs (TVOC) and formaldehyde emitted directly after opening single filter pockets of VITROMED IVF labware and multipacked Competitor's A and Competitor's B 60mm (light blue) and center well (dark blue) culture dishes. The comparisons were made with single filter pockets of VITROMED IVF labware vs Competitor's A and single filter pockets of VITROMED IVF labware vs Competitor's B same type of dishes. Different signs mean statistical difference (P<0.001)

Finally, aerosolized microbial challenge test was performed to compare bacterial contamination after opening Competitor's A and B multipacked culture dishes (45% and 10%, respectively) with single filter pockets of VITROMED IVF labware (0%), as shown in Figure 2.

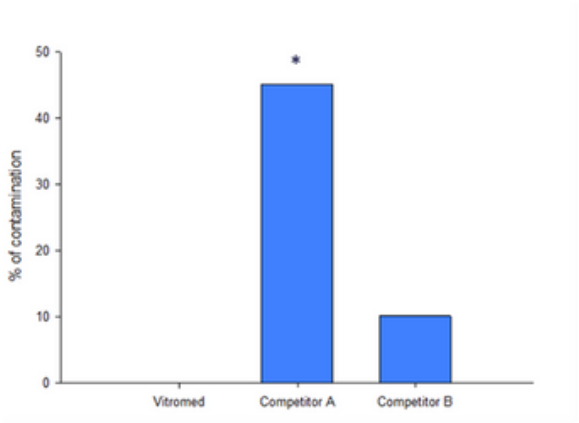

Figure 2: Aerosolized microbial challenge test showing bacterial contamination (%) after opening of multipacked Competitor's A and B 60mm and center well culture dishes compared to the respective single filter pockets of VITROMED IVF labware (P<0.001)

Conclusion

VOCs and plastic IVF labware quality are significant but often overlooked factors affecting IVF success rates. By understanding the sources and implementing mitigation strategies, IVF labs can enhance their success rates and ensure a safer environment for embryo development. Continuous research and adherence to best practices are essential for maintaining the highest standards of care in reproductive medicine.

Plastic IVF labware are one of the main sources of VOC build-up in the IVF lab. Lab personnel have to outgas multipacked plastic IVF labware before use and it is suggested to do so outside of the lab. This way released VOCs will not build-up contributing to the 'sink effect' on culture media and oil. As mentioned earlier, the Cairo consensus recommended that the total VOC levels in an IVF lab should be kept under 400 to 800ppb[5]. Aldehydes commonly found in IVF labs, such as formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, benzaldehyde, n-hexaldehyde, and acrolein are known carcinogens and mutagens and have been implicated in disrupted embryo development. Acetonitrile, released from plastic, has also been suggested as a possible source for the slow release of cyanide[5].

In the experiment conducted became evident that the quality of plastic IVF labware may play a significant role in the 'sink effect', since Competitor's A and B multipacked plastic IVF labware released increased levels of total VOCs and formaldehyde (Figure 1). Competitor's A 60mm culture dishes and center well dishes, released formaldehyde levels of 280ppb (0.28ppm) and 250ppb (0.25ppm), respectively. Data have been provided showing that formaldehyde levels of as low as 25ppb in the IVF lab and 2.8ppb in the incubators resulted in failure to pass the mouse embryo (MEA) quality control assay[5]. Therefore, such high levels of released VOCs impose that Competitors' multipacked plastic IVF labware be outgassed outside the IVF lab for toxicity reasons, albeit undermining their sterility (Figure 2).

Based on the results of the present study, single filter pockets of VITROMED IVF labware can be stored and opened in the IVF laboratory, as their opening releases minimal levels of total VOCs and formaldehyde. Additionally, being individually sealed in filter pockets ensures the maintenance of sterility conditions. Monitoring and control of plastic IVF labware related VOCs, such as formaldehyde, acetonitrile and styrene in IVF labs could contribute to improvement of embryonic development and clinical outcomes.

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