Volatile organic compounds and good laboratory practices in the in vitro fertilization laboratory: the important parameters for successful outcome in extended culture

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Abstract

Purpose This study aims to describe the role of implementing good laboratory practices to improve in vitro fertilization (IVF) outcomes which are of great interest for practitioners dealing with infertility.

Methods Certain modifications were introduced in May 2015 in our IVF laboratory like high-efficiency particulate air CODA system, steel furniture instead of wooden, use of new disinfectants like oosafe, and restriction of personnel entry along with avoidance of cosmetics like perfume to improve pregnancy rates. Volatile organic compound (VOC) meter reading was monitored at two time points and five different places in the laboratory to compare the embryonic development parameters before (group A: July 2014–April 2015) and after (group B: July 2015–April 2016) remodeling.

Results The IVF outcomes from 1036 cycles were associated in this study. Reduction in VOC meter readings, enhanced air quality, improvement in blastocyst formation rate, implantation, and clinical pregnancy rate were observed in the laboratory after implementation of new facilities. Results illustrated that the attention must be focused on potential hazards which expose laboratories to elevated VOC levels. Blastocyst formation rate increased around 18%. Implantation rate, clinical pregnancy rate, and live birth rate increased by around 11, 10, and 8%, respectively.

Conclusion In conclusion, with proper engineering and material selection, we have been able to reduce chemical contamination and adverse effects on culture with optimized IVF results.

Support None.

Keywords VOC · IVF outcomes · Blastocyst · Air quality · Remodeling

Nupur Agarwal and Ratna Chattopadhyay both have equal contribution

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Introduction

Air quality is crucial for the success of in vitro fertilization (IVF), because the presence of volatile organic compounds (VOCs) and particulate materials are harmful for embryo development in vitro [1]. Studies have shown that various factors, such as air quality, temperature, light, and VOCs, in IVF laboratory are known to affect oocytes and embryos and therefore can have detrimental effects on pregnancy rate [1–3]. Embryonic growth and development is affected by direct attachment of VOCs to embryonic DNA and terminating its growth [1]. VOC is also associated with DNA fragmentation in human sperm [4–6]. Many practitioners of assisted reproductive technology (ART) are focusing on VOCs [2, 7–10], many of which can be detected by simple absorption and gas

chromatographic or flame ionization detection and analysis. Construction materials are a major source of VOCs in IVF labs. Wood-based furniture release formaldehyde [11]. PVC flooring materials [12] and carpets [13] also release VOCs. Paints [14, 15] and adhesives (especially vinyl floor tile adhesive) release numerous VOCs, including aldehydes. Many cleaning products are also sources of VOCs, for example vinyl floor liquid wax which can contain lead [16] ammonia-based products such as glass cleaners and aerosol propellants such as butane or isobutane. Presently, many IVF laboratories are using ethanol as a disinfectant as it has a broad effect against bacteria, but it is also a known VOC. Autoclaved materials (e.g., drapes, instrument packs) can release VOCs when packs are opened for use. Cosmetics, especially perfumes, colognes, and aftershaves, are highly toxic to embryos *in vitro*, primarily due to evaporation of their solvent bases [17]. Finally, cigarette smoke contains several hundred volatile compounds including recognized carcinogens and mutagens [18] and high levels can contaminate “fresh” air intakes if improperly located.

It is essential for IVF labs to set up an air filtration system that has the ability to filter hydrocarbon pollutants, chemically active compounds, and airborne pathogens. Air filtration system removes small inorganic gaseous molecules commonly known as aerosolized pesticides, like nitrous oxide (N_2O) and sulfur dioxide (SO_2), as well as heavy metals such as lead, that can adversely affect embryo quality and, hence, clinical outcomes [3]. One of the sources for the transfer of particulate materials is punctuation of walls for plumbing and cables which allows migration of pollution from outside to laboratory area [19]. A carefully controlled sealed environment where the entry of particles and contaminants is significantly minimized may be achieved by highly filtered air being flushed through high-efficiency particle air (HEPA) filters under positive pressure [1]. Recently, oosafe, a quaternary ammonium compound [20], has been introduced as a disinfectant for cleaning CO_2 incubators. William Lee [20] reported in his thesis that oosafe is non-toxic for gametes. Many IVF labs—even entire clinics—are now “perfume-free” zones [3].

Our study goal was to evaluate IVF outcomes using extended embryo culture after renovation of IVF suite and implementation of good laboratory practices and volatile organic compound filtration.

Material and methods

Study design

In this observational study, we have evaluated the efficacy of blastocyst formation, clinical pregnancy rate, implantation rate, and live birth rate along with VOC assessment before (group A: July 2014–April 2015) and after (group B:

July 2015–April 2016) remodeling of the IVF laboratory of our clinic. One thousand thirty-six IVF and intra-cytoplasmic sperm injection (ICSI) patients were included in our study where our patients had blastocyst transfer. Five hundred fourteen patients were included in group A and 522 in group B. We have also recorded the demographic profile of the patients included in both group A and group B. Long gonadotropin-releasing hormone agonist downregulation was used followed by ovarian stimulation by gonadotropins. Either ICSI or conventional insemination (30,000 to 40,000 motile sperm per oocyte) was done 5 to 6 h of ovum pickup followed by direct swim up technique of sperm preparation. Patients who underwent egg retrieval but had embryo transfer on days 2, 3, and 4 or no embryo transfer at all due to unavoidable reasons were excluded from our study. Frozen cycles were not included as many other factors play a role in embryo quality during freezing. The culture conditions for embryo culture were the same for the groups, that is, temperature, pH, oxygen concentration (5%), humidity, osmolality were maintained. The media used for embryo culture were purchased from Vitrolife Pvt. Ltd. (Göteborg, Sweden). The incubators used were conventional triple gas incubators.

Ethical clearance

Experiments encompassing this study were performed in accordance with the Institutional Human Ethical Committee of Institute of Reproductive Medicine (IRM), HB-36/A/3, Salt Lake City, Sector III, Kolkata 700 106 for conducting the clinical study. The ethics committee operates according to the requirements of Good Clinical practice (GCP), Schedule Y, and Indian Council of Medical Research (ICMR). The protocol number for the clearance is IVFM-11- 02 dated 01 Feb 2012.

Remodeling of IVF laboratory

Reconstruction and addition of new facilities were introduced in the areas where gametes and embryos were handled. This included the embryology laboratory and also the associated areas (oocyte retrieval room, embryo transfer room, sperm process room). The size of the main laboratory is around 120 ft². At the end of construction which was completed in 1 month, we waited for another 1 month to allow off-gassing before starting new IVF cycles. Wood-based furniture was replaced with steel. Anodized aluminum was used in doors. Epoxy paints were used on the floor instead of PVC flooring. Double-shield glass on the windows was used to make the room airtight. Aerosol/ammonia-based cleaning of IVF laboratory was strongly discouraged. IVF lab was established as a “perfume-free” zone. Cosmetics were also avoided inside the laboratory. Earlier, the incubator was brought in use soon after installation (without burning in); however, later on, it was used after a burn-in period of 10 days. One CODA system

was introduced in the main lab away from the incubators. Cleaning of all surfaces and doors, mopping of floors, and emptying of waste were done on a daily basis both in laboratories and adjacent areas. In place of alcohol, a recent disinfecting product named Oosafe® (SparMed, Denmark) was used for cleaning of tables and floors. Sinks were removed and drains were closed in the clean room areas. Separate entry for oocyte retrieval room and laboratory was made with a pass through window between the two. It was used for transfer of gametes and embryos between laboratory and operating room preventing mixture of air between the two. Entry of persons was restricted. Persons were not allowed to smoke in working hours. They had to change their dresses (cotton fabric) before entry and wear cap, mask, and shoe cover. Changing room was just adjacent to the entry door of laboratory. In addition, adhesive-covered mats were used at the entrance to laboratory to remove dirt and dust from the soles of shoes. Care was taken to avoid items which emit VOCs. Powder-free disposable gloves were used to handle gametes and embryos. Our laboratory system design is a stand-alone system that recirculates air.

VOC measurement

VOC levels were measured using a SASWELL VOC meter AQC910.1V-DO. It has an internal mix gas sensor which is VOC sensitive. VOC was measured in various locations within the embryology laboratory and other adjacent areas before and after remodeling. Monitoring was performed in three locations as shown in Table 3 (a.—beside the work station, b. and c.—in areas 1 and 2 between the incubators) inside the embryology laboratory and two locations in the ICSI room adjacent to the laboratory (d.—ICSI room near microscope, e.—ICSI room near incubator) throughout the study period (July 2014 to April 2016) but as an example, we have given data for seven consecutive days at the same time, prior to and after the lab remodeling. We prepared Table 5 considering two time points, one in the morning during ovum pick up and another in the evening. In the morning, 3–4 persons were present in the laboratory whereas only 1–2 persons were present during evening time. We have also measured that the humidity level in the VOC meter was around 40 to 45% throughout the day.

Assessment of fertilization and embryo quality

Following the changes to the laboratory, various embryogenesis parameters were assessed (i.e., blastocyst formation, clinical pregnancy, embryo implantation rates, and live birth rates) in our laboratory and compared with the same parameters before remodeling. Blastocyst was characterized by development of blastocoel in compacted embryo. One to two embryos were transferred on day 5 and other good quality supernumerary embryos were cryopreserved.

Pregnancy testing

Positive urinary β -human chorionic gonadotropin (β -HCG) after 14 days of embryo transfer was defined as biochemical pregnancy. Clinical pregnancies were confirmed by transvaginal ultrasonography at 6–7 weeks of gestation.

Statistical analysis

Data were presented as the mean $\pm$ SEM. Student's *t* test was done wherever applicable, and significance was expressed as a *p* value and 95% confidence interval of the difference. Statistical analyses were done using the software GraphPad InStat Version 3.06 (La Jolla, CA, USA).

Results

Out of 1036 patients that were included in our study, 514 underwent IVF/ICSI cycle before remodeling of the laboratory (group A) whereas 522 had IVF/ICSI cycle after renovation (group B). Baseline demographic parameters like age of the female partners, duration of infertility, basal hormonal profile, and number of embryo transferred are mentioned in Table 1. No significant variation was found in the different baseline parameters between group A and group B.

Table 2 includes cycle-specific details like number of oocytes retrieved, fertilization rate, number of embryos at cleave stage, blastocyst formation rate, and number of frozen embryos whereas Table 3 depicts IVF outcome in terms of clinical pregnancy rate, implantation rate, and live birth rate. Evaluating results over this period, there was demonstrable benefit of operating under these optimum laboratory environmental conditions, which resulted in improvement in all the parameters. Blastocyst formation rate increased around 18% after making changes in the laboratory. Previous to remodeling, the blastocyst formation rate was around 44.94% and it increased to around 62.83% afterwards with $p \leq 0.0001$. Consequently, the implantation rate also improved from 31 to 42% with $p \leq 0.0015$. Clinically successful pregnancies before and after VOC reduction (27.27 vs. 37.80%) varied significantly ($p \leq 0.0001$). Simultaneously, the live birth rate also increased from 23 to 31% with $p \leq 0.0090$.

Table 4 represents the variation in VOC meter readings in parts per million (“ppm”) at different locations in the IVF laboratory before and after renovation. Renovation reduced the VOC levels in the IVF as well as the ICSI laboratory significantly. According to the VOC meter readings, workstation was found to be relatively free of organic compounds after remodeling. Figure 1 represents the locations where VOC was measured.

The variations in VOC meter readings (“ppm”) during morning and evening time before and after renovation are shown in Table 5. Reconstruction depicts reduction in VOC

Table 1 Profile of demographic variations in two groups

Parameters (mean $\pm$ SEM)	Group A	Group B	<i>p</i> value	Mean difference (95% confidence interval of the difference)
Total no. of cycles performed in the study period	1370	1420	—	—
Total no. of cycles included in the study	514	522	—	—
Age of female partner (mean)	33 $\pm$ 5	35 $\pm$ 5	0.7774	0.2000 (−11.870 to 15.870)
Infertility time (years)	5.2 $\pm$ 3.2	5 $\pm$ 2.8	0.9624	0.2000 (−8.120 to 8.520)
Basal FSH (IU/ml)	6.8 $\pm$ 2.3	6.5 $\pm$ 1.9	0.9197	0.3000 (−5.532 to 6.132)
Basal LH (IU/ml)	3.4 $\pm$ 2.6	3.6 $\pm$ 2.2	0.9531	0.2000 (−6.461 to 6.861)
Basal E ₂ (pg/ml)	32.2 $\pm$ 15	34.6 $\pm$ 16	0.9129	2.400 (−40.668 to 45.468)
Days of stimulation	10.2 $\pm$ 1.8	10.5 $\pm$ 3.2	0.9352	0.3000 (−6.968 to 7.568)
Total dose of gonadotropins used (IU)	2252 $\pm$ 720	2154 $\pm$ 550	0.9137	98.000 (−1670.8 to 1866.8)
E ₂ on hCG day (pg/ml)	3100 $\pm$ 1250	2900 $\pm$ 1500	0.9186	200.00 (−3642.3 to 4042.3)
P ₄ on hCG day (ng/ml)	0.5 $\pm$ 0.2	0.6 $\pm$ 0.4	0.8250	0.1000 (−0.7866 to 0.9866)
Mature follicle (>18 mm) on HCG day	7.5 $\pm$ 3.6	8.0 $\pm$ 4.2	0.9282	0.5000 (−10.380 to 11.380)
Endometrial thickness on the day of hCG (mm)	10.6 $\pm$ 2.4	11.2 $\pm$ 2.3	0.8567	0.6000 (−5.915 to 7.115)
Number of day 5 embryos transferred	2	2	Not applicable	—

The *p* values indicate that the differences between the two groups are insignificant

meter reading at both the time points for all 7 days. However, in comparison to the evening time, readings are higher in the morning. The panels a, b, c, d, e mentioned in the figure corresponds to the locations mentioned in Table 4.

There was increase in the number of good quality blastocysts; that is, blastocoels' cavity size was good and it appeared on time, increased cell number in inner cell mass, and increased compactness of trophoctoderm cell layer. We have used Gardner classification system for grading the blastocysts [21].

Discussion

This article describes the importance of VOCs on the IVF outcomes in the laboratory. Embryos are sensitive to the environment and VOCs are one of the vital factors affecting

embryonic development. VOCs are hydrocarbon-based organic compounds that have a high vapor pressure at room temperature and so are detrimental for IVF outcomes. They are emitted by various laboratory instruments, furniture, perfumes, aftershave, cosmetics, smoking, etc. used by the persons working in the laboratory. According to the present day findings, VOCs (over 1 ppm) are directly toxic to embryos [22]. Ideally, VOC levels should be below 0.5 ppm for acceptable blastocyst development and reasonable pregnancy rates, but preferably zero. So, IVF laboratory should be constructed in such a manner that minimizes the production of VOCs and also where other conditions like temperature and humidity are controlled. Similarly, it is important to have valid monitoring system from time to time.

This IVF laboratory was established in 1999 and renovation was done in May 2015. A detailed study of various

Table 2 Variations found in the different baseline parameters in two groups

Parameters (mean $\pm$ SEM)	Group A	Group B	<i>p</i> value	Mean difference (95% confidence interval of the difference)
No. of cycles	514	522	—	—
Total no. of oocytes	11.038 $\pm$ 3.2	11.17 $\pm$ 2.8	0.9752 (NS)	0.1320 (−8.204 to 8.468)
Dysmorphic oocytes	1.038 $\pm$ 0.03	1.17 $\pm$ 0.42	0.0109 (S)	0.1320 (0.0304 to 0.233)
No. of oocytes included	10	10	Not applicable	—
Pronuclear stage	6.799 $\pm$ 1.1	7.6 $\pm$ 0.98	0.5865 (NS)	0.8010 (−2.088 to 3.690)
Cleavage stage	5.573 $\pm$ 0.4	7.31 $\pm$ 0.36	0.0013 (S)	1.735 (0.6818 to 2.792)
Blastocyst formed	4.494 $\pm$ 0.37	6.28 $\pm$ 0.82	0.0485 (S)	1.786 (0.0115 to 3.560)
Grade A transferred	2	2	Not applicable	—
Frozen embryos	1.363 $\pm$ 0.05	3.588 $\pm$ 0.87	0.0114 (S)	2.225 (0.501 to 3.948)

NS non-significant, S significant

Table 3 Biochemical rate, clinical pregnancy rate, implantation rate, and live birth rate before and after renovation

Indicators	Group A	Group B	<i>p</i> value	Mean difference (95% confidence interval of the difference)
Beta HCG positive (%)	31.60	43.90	0.0015	12.3 (7.899 to 16.701)
Clinical pregnancy rate (%)	27.27	37.80	0.0001	10.530 (8.522 to 12.538)
Implantation rate (%)	31.60	42.98	0.0015	11.380 (7.314 to 15.446)
Live birth rate (%)	22.94	30.79	0.0090	11.380 (3.264 to 12.436)

The *p* values indicate that the differences between the two groups are significant

parameters was done before and after making changes in the laboratory and found a significant improvement in IVF outcomes after 2015. One of the major challenges was to control air quality inside the laboratory and to ensure that gametes are not in contact with toxic materials. Positive pressure airflow in the laboratory coupled with the use of air purification systems [23] may reduce the concentration of airborne particles and also reduces bacteria and other contaminants, since these microbes attach themselves to the air particles. Removal of these particles requires passing of air using positive pressure through filters having different porosities. High-efficiency particulate air (HEPA) filter is one of such filters which trap most of the microbial contaminants and thus purify air. We have also introduced new CODA filtration unit in our laboratory since the old CODA system filtration was non-functional and the filtration unit was not available. This contributed in improving air quality. According to Munch et al. 2015, poor air quality affects embryo development leading to inferior IVF outcome [24]. Other studies also have demonstrated similar results like ours that is improvements in pregnancy and implantation rates after using the CODA system [25].

In this embryology laboratory, we have installed one electronic VOC meter that can measure VOC level in ppm concentration accurately in the enclosed laboratory environment. VOC monitoring is a part of our quality control and we are recording it daily. We had measured the VOC values over the

entire study period but here we had represented the data for a week as it remained almost similar throughout the study period. There were no drastic fluctuations in levels in either time period. Baseline parameters of the patients included in the study are shown in Table 1. All the parameters were similar in terms of age, hormonal profile, dosage of gonadotropins, etc. The results in Table 3 showed that the number of embryos that developed to the blastocyst stage was significantly higher after changes in IVF laboratory. We had cultured the embryos till blastocyst stage which exposed them for a longer time to the laboratory environment. So, the effect of air quality on the extended culture was reflected more closely. The positive effects were seen not only on blastocyst formation rate but also on clinical pregnancy and live birth rates which increased significantly after renovation. Improvement in all the above parameters was statistically significant cueing towards the deleterious potential of the environmental toxicants. Similar effects were seen by many studies conducted in different parts of the world. Boone et al. [26] observed the increase in high-quality embryos after improvement in air quality. Esteves et al. [27] also demonstrated better outcomes after ICSI cycles with strict air quality control. Clinical pregnancy rate after moving into the new laboratory was significantly higher than the old laboratory—42.6 versus 30.6% [28].

After renovation, visible improvement was observed in the VOC readings at all the places as well as at different

Table 4 Difference in VOC meter readings (“ppm”) at various locations in the IVF laboratory before and after renovation

Locations	VOC reading before remodeling	VOC reading after remodeling	<i>p</i> value	Mean difference (95% confidence interval of the difference)
a. Beside work station	18 ± 0.5	4 ± 0.3	<0.0001	14.00 (12.801 to 15.199)
b. Area 1 in between the incubators	21 ± 2	7 ± 0.5	<0.0001	14.00 (9.763 to 18.237)
c. Area 2 in between the incubators	20 ± 1	5 ± 0.5	<0.0001	15.00 (12.702 to 17.298)
d. ICSI room near microscope	24 ± 2	8 ± 1	<0.0001	16.00 (11.404 to 20.596)
e. ICSI room near incubator	22 ± 1	7 ± 0.7	<0.0001	15.00 (12.491 to 17.509)

The *p* values indicate that the differences between the two groups are highly significant. The number of readings per site is 14 (readings have been taken for seven consecutive days at two time points: morning and evening)

Fig. 1 The variations in VOC meter readings (“ppm”) during morning and evening time before and after renovation are shown in Table 5. Reconstruction depicts reduction in VOC meter reading at both the time points for all 7 days. However, in comparison to the evening time, readings are higher in the morning. The panels **a, b, c, d, e** mentioned in the figure corresponds to the locations mentioned in Table 4

time points compared in the table confirming that the changes in the laboratory were effective in reducing contamination in the vicinity of laboratory as depicted in Tables 4 and 5. Human activities in and around the embryology laboratory are one of the major sources of

Table 5 The variations in VOC meter readings (“ppm”) during morning and evening time before and after renovation on different days

Day	Morning (11 A.M.)		Evening (4 P.M.)	
	VOC in ppm		VOC in ppm	
	Before	After	Before	After
1	19.2	7	14	2.4
2	24	5.4	18.3	4
3	25.4	8	17	3.5
4	22	6	16.5	2.9
5	20	7.3	15	5
6	25.1	6.3	17.4	4
7	24	5.8	18	3

The two sets of data acquired on different days in the morning and evening time were compared separately for before and after renovation. Both the sets were considered extremely significant having $p < 0.0001$. Number of readings per site is 14 (readings have been taken for 7 consecutive days at two time points morning and evening). The mean 95% confidence interval between the two groups during morning is 16.271 and interval of difference between them is 14.106 to 18.437. The mean 95% confidence interval between the two groups during evening is 13.057 and interval of difference between them is 11.571 to 14.543

contamination [15]. Various studies have demonstrated that maintaining clean conditions during oocyte retrieval and embryo transfer is one of the critical steps. This holds true for our study also as VOC readings were higher in the morning during ovum pickups when there were more number of persons around and gradually reduced over the day with decrease in number of individuals. So, after renovation, access was restricted in our laboratory. Separate entry was made for laboratory and operation theater with a pass through window between the two. The present results illustrate that attention must be focused on the construction material, furniture, paints, day to day cleaning, etc. It is recommended to use low odor specialized paints [29, 30]. Not only the main IVF laboratory room but also the adjacent areas like ICSI room, operation theater, and changing room play an important role. So, it is important to design laboratory cautiously [31].

It was observed in a study that “oosafe” had no deleterious effect on gametes and embryos and was better compared to alcohol which itself is a VOC [20, 32]. Oosafe disinfectant was found to be effective in cleaning the surfaces of tables, incubators, and floors and eliminating of microorganisms with no effect seen on embryo morphology and survival rate after the use of oosafe [20]. Further studies are required to establish the effectiveness of oosafe.

The overall effect of renovation of laboratory was prominent on the morphology of the blastocysts formed. According to Gardner and Schoolcraft, there were more grade 1 and grade 11 embryos after changing laboratory

practices in comparison to before renovation; so, the quality of embryos improved [21]. VOCs may significantly affect the embryo quality [1] and proper design of the laboratory is essential [31]. Air quality conditions were better in the new site and were associated with higher blastocyst formation, implantation, and live birth rates in couples undertaking treatment in our new facility.

Effect of air particulate filtration system on embryo development is debatable. While Heitmann and his colleagues [5] and Morbeck et al. 2015 observed higher embryo development after improving air quality on the other hand, according to Esteves et al. [33, 34], little is known about the benefits of particulate filtration system [35–37]. Other important issues like indoor air quality, equipment/furniture, construction and disposable materials, cleaning agents used, and personnel are less explored areas. Therefore, in this study, we have focused on the abovementioned areas rather than the filtration system.

The strength of this study was the large sample size that had helped to draw an accurate conclusion. One of the limitations of this study was its retrospective nature. More of randomized controlled trials are required to assess the impact of air quality on IVF outcomes. Another drawback was the monitoring of VOC in ppm instead of parts per billion (ppb). Use of VOC meter with detection limit of ppb would have made the study more sensitive.

In conclusion, our study confirms that implementation of air quality control systems and acquisition of good laboratory practices improved IVF outcomes significantly. We therefore suggest that any potential hazards which expose laboratories to elevated concentrations of VOCs should be determined and consequently controlled. So, more sensitive and optimized methods for controlling air contaminations are warranted to improve pregnancy outcomes especially in extended culture. Further research for longer time period, use of more sensitive instruments, and optimized methodology are required to improve our understanding of IVF outcomes due to fluctuations in air quality.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Khoudja R, Xu Y, Li T, et al. Better IVF outcomes following improvements in laboratory air quality. *J Assist Reprod Genet.* 2013;30:69–76.
2. Esteves SC, Bento FC. Implementation of air quality control in reproductive laboratories in full compliance with the Brazilian cells and germinative tissue directive. *Reprod BioMed Online.* 2013;26: 9–21. doi:10.1016/j.rbmo.2012.10.010.
3. Lawrence C, Moritmer S, Havelock J, et al. VOC levels in a new IVF laboratory with both central and in-laboratory photo-catalytic air purification units. *Alpha Newsletter.* 2007;36:1–5.
4. Dadvand P, Rankin J, Rushton S, et al. Association between maternal exposure to ambient air pollution and congenital heart disease: a register-based spatiotemporal analysis. *Am J Epidemiol.* 2011;173: 171–82.
5. Meng Z, Zhang LZ. Chromosomal aberrations and sister chromatid exchanges in lymphocytes of workers exposed to sulfur dioxide. *Mutat Res.* 1990;241:15–20.
6. Rubes J, Selevan SG, Evenson DP, et al. Episodic air pollution is associated with increased DNA fragmentation in human sperm without other changes in semen quality. *Hum Reprod.* 2005;20: 2776–83.
7. Heitmann RJ, Hill MJ, James AN, et al. Live births achieved via IVF are increased by improvements in air quality and laboratory environment. *Reprod BioMed Online.* 2015;31:364–71.
8. Worriow KC, Huynh HT, Gwozdziwicz JB, Schillings W, Peters AJ. A retrospective analysis: the examination of a potential relationship between particulate (P) and volatile organic compound (VOC) levels in a class 100 IVF laboratory cleanroom (CR) and specific parameters of embryogenesis and rates of implantation (IR). *Fertil Steril.* 2001;76:S15–6.
9. Worriow KC, Huynh HT, Bower JB, Schillings W, Peters AJ. A retrospective analysis: seasonal decline in implantation rates (IR) and its correlation with increased levels of volatile organic compounds (VOC). *Fertil Steril.* 2002;78:S39.
10. Von Wyl S, Bersinger NA. Air quality in the IVF laboratory: results and survey. *J Assist Reprod Genet.* 2004;21:283–4.
11. Brown SK. Chamber assessment of formaldehyde and VOC emissions from wood-based panels. *Indoor Air.* 1999;9:209–15.
12. Lundgren B, Jonsson B, Ek-Olausson B. Materials emission of chemicals—PVC flooring materials. *Indoor Air.* 1999;9:202–8.
13. De Bortoli M, Kephelopoulous S, Kirchner S, et al. State-of-the-art in the measurement of volatile organic compounds emitted from building products: results of European inter laboratory comparison. *Indoor Air.* 1999;9:103–16.
14. Sparks LE, Guo Z, Chang JC, et al. Volatile organic compound emissions from latex paint—part 1—chamber experiment and source, model development. *Indoor Air.* 1999;9:10–7.
15. Srivastava PK, Pandit GG, Sharma S, et al. Volatile organic compounds in indoor environments in Mumbai, India. *Sci Total Environ.* 2000;255:161–8.
16. Cohen J, Gilligan A, Esposito W, et al. Ambient air and its potential effects on conception in vitro. *Hum Reprod.* 1997;12:1742–9.
17. Johnson JE, Boone WR, Bernard RS. The effects of volatile compounds (VC) on the outcome of in vitro mouse embryo culture. *Fertil Steril.* 1993;Suppl 1:S98–9.
18. Stillman RJ, Rosenberg MJ, Sachs BP. Smoking and reproduction. *Fertil Steril.* 1986;46:545–66.
19. Hall J, Gilligan A, Schimmel T, Cecchi M, Cohen J. The origin, effects and control of air pollution in laboratories used for human embryo culture. *Hum Reprod.* 1998;13(Suppl 4):146–55. doi:10.1093/humrep/13.suppl_4.146.
20. Effectiveness and biocompatibility of common IVF laboratory disinfectants on embryonic development William Lee (23296712).

- Supervised by Dr. Jim Catt, Dr. Sally Catt, Dr. Mulyoto Pangestu. Monash Medical Centre Clayton, Graduate Diploma in Reproductive Sciences.
21. Gardner DK, Schoolcraft WB, Wagley L, et al. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. *Towards reproductive certainty: infertility and genetics beyond*. Camforth: Parthenon Press; 1999. 378.
 22. Panizzo R. Air pollution linked to lower IVF success. Progress Educational trust http://www.bionews.org.uk/page_58675.asp (2010).
 23. Mayer JF, Nehchiri F, Weedon VM, et al. Prospective randomized crossover analysis of the impact of an IVF incubator air filtration system (coda, GenX) on clinical pregnancy rates. *Fertil Steril*. 1999;Suppl. 1:S42–3.
 24. Munch EM, Sparks AE, Van Voorhis BJ, Duran EH. Lack of carbon filtration impacts early embryo development. *J Assist Reprod Genet*. 2015;32:1009–17.
 25. Merton JS, Vermeulen ZL, Otter T, et al. Carbon-activated gas filtration during in vitro culture increased pregnancy rate following transfer of in vitro-produced bovine embryos. *Theriogenology*. 2007;67:1233–8.
 26. Boone WR, Johnson JE, Locke A-J, et al. Control of air quality in an assisted reproductive technology laboratory. *Fertil Steril*. 1997;71:150–4.
 27. Esteves SC, Gomes AP, Verza S Jr. Control of air pollution in assisted reproductive technology laboratory and adjacent areas improves embryo formation, cleavage and pregnancy rates and decreases abortion rate: comparison between a class 100 (ISO5) and a class 1.000 (ISO 6) cleanroom for micromanipulation and embryo culture. *Fertil Steril*. 2004;82(Suppl 2):S259–60.
 28. Knaggs P, Birch D, Drury S, et al. Full compliance with the EU directive air quality standards does not compromise IVF outcome. *Hum Reprod*. 2007;22:i164–5.
 29. Cutting RC, Pritchard J, Clarke HS, et al. Establishing quality control in the new IVF laboratory. *Hum Fertil*. 2004;7:119–25.
 30. Cohen J, Alikani M, Gilligan A, et al. Setting up an ART laboratory. In: Gardner D, Weissman A, Howles C, Shoham Z, editors. *Textbook of assisted reproductive techniques*. London: Informa Healthcare; 2012. p. 1–8.
 31. Boone WR, Higdon HL, Johnson JE. Quality management issues in the ART laboratory. *JRSCB*. 2010;1:30–107.
 32. Piggot PJ, Hilbert DW. Sporulation of *Bacillus subtilis*. *Curr Opin Microbiol*. 2004;7:579–86.
 33. Esteves SC, Bento FC. Air quality control in the ART laboratory is a major determinant of IVF success. *Asian J Androl*. 2016;18(4): 596–9.
 34. Morbeck DE. Air quality in the assisted reproduction laboratory: a mini-review. *J Assist Reprod Genet*. 2015;32(7):1019–24.
 35. Dickey RP, Wortham JWE Jr, Potts A, Welch A. Effect of IVF laboratory air quality on pregnancy success. *Fertil Steril*. 2010;94: S151. doi:10.1016/j.fertnstert.2010.07.605.
 36. Jindal SK, Polotsky AJ, Buyuk ER, Lieman HJ, Gilligan A. Improved pregnancy rates following introduction of engineering controls of lab air quality. *Fertil Steril*. 2008;90(Supplement): S403. doi:10.1016/j.fertnstert.2008.07.1412.
 37. Forman M, Sparks AET, Degelos S, Koulianos G, Worrirow KC. Statistically significant improvements in clinical outcomes using engineered molecular media and genomically modeled ultraviolet light for comprehensive control of ambient air (AA) quality. *Fertil Steril*. 2014;102:e91. doi:10.1016/j.fertnstert.2014.07.307.