Clearing the Air:
Does Air Quality Matter?

Sangita Jindal, PhD, HCLD
Professor & IVF Lab Director

Albert Einstein College of Medicine, Bronx NY
Montefiore's Institute for Reproductive Medicine and Health
ASRM Board of Directors, Publications Committee
Immediate Past President SART

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MARCH 20-23, 2024
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Disclosures

- Nothing to Disclose

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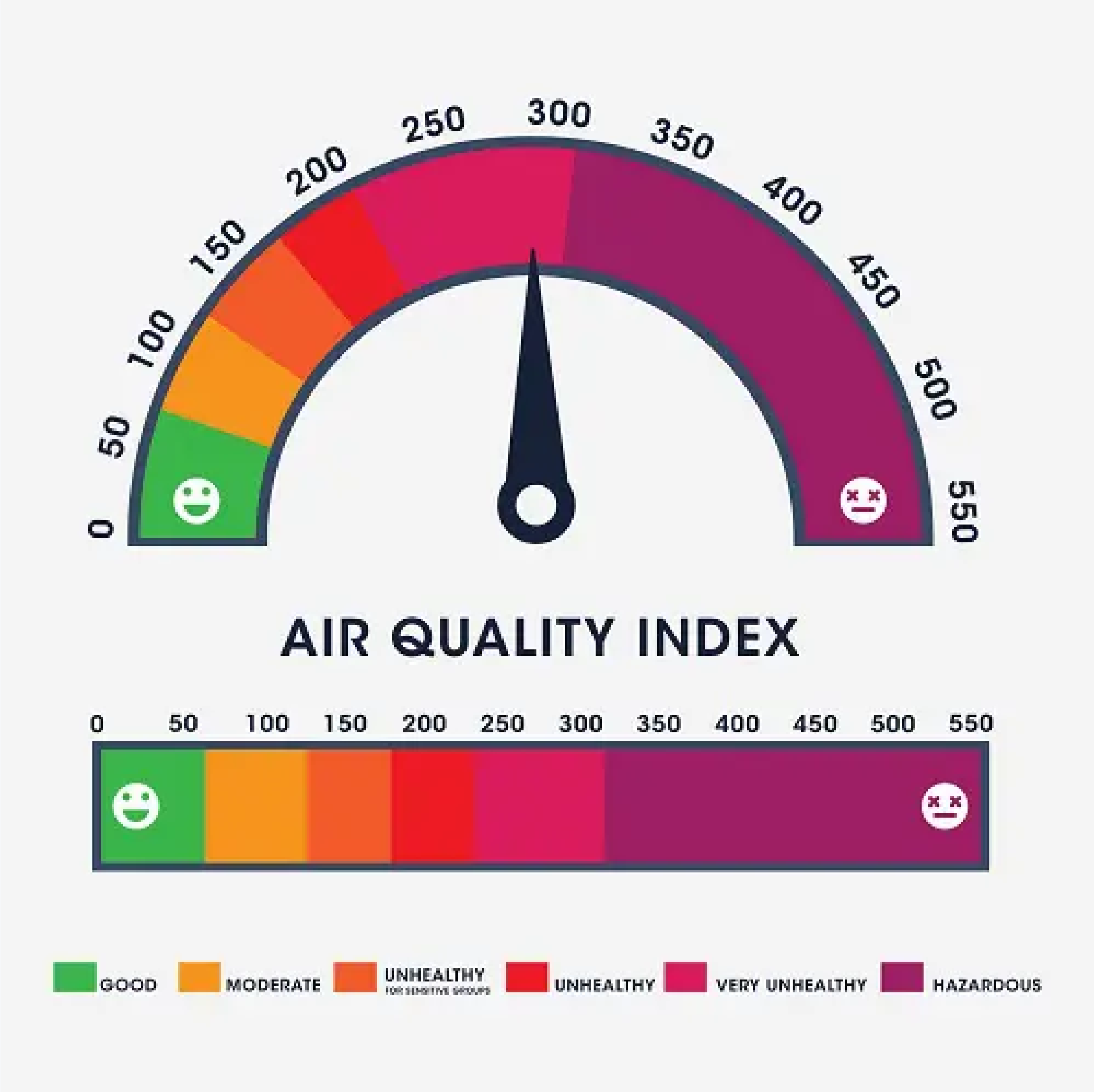
Learning Objectives

- List Sources of air pollution in the lab
- Define what engineering controls contribute to clean lab air
- Apply good laboratory practice ensures clean lab air
- Practice Measuring lab air & associations with lab procedures


ASBESTOS
VOCs
TOBACCO SMOKE
MOLD
NITROGEN OXIDE
CARBON MONOXIDE
INDOOR AIR QUALITY
STALE AIR
PARTICULATE MATTER
FORMALDEHYDE
RADON
CONDENSATION
BACTERIA


0
50
100
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200
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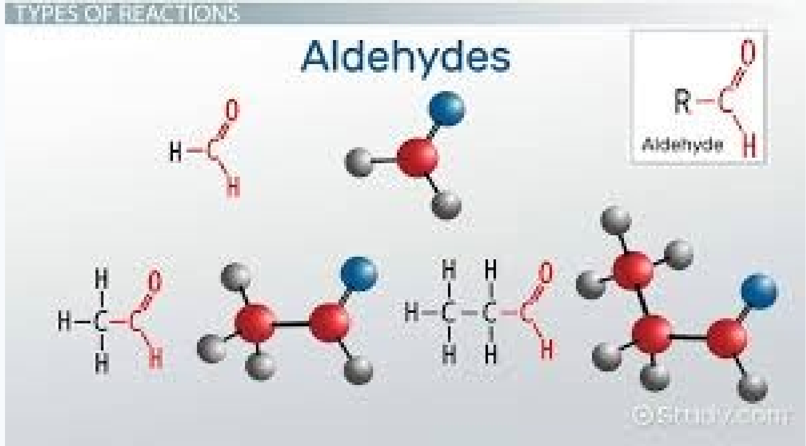
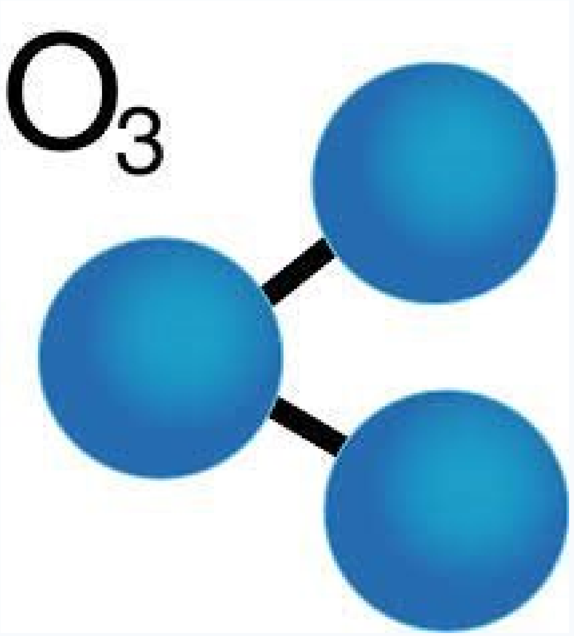
550
AIR QUALITY INDEX
0 50 100 150 200 250 300 350 400 450 500 550
GOOD
MODERATE
UNHEALTHY
UNHEALTHY
VERY UNHEALTHY
HAZARDOUS

Chemical Sources of Lab Contamination

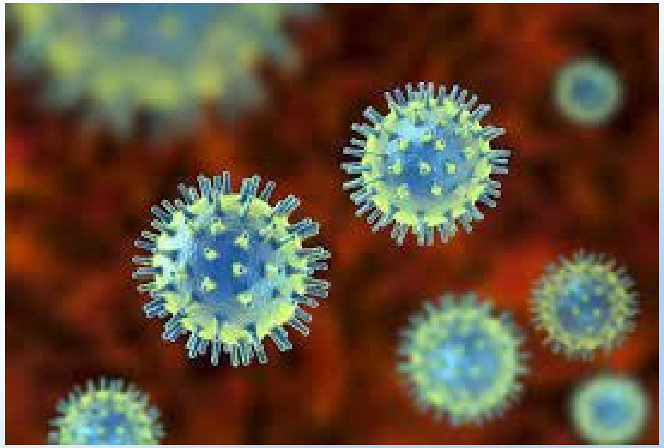




Sources of Lab Bioburden





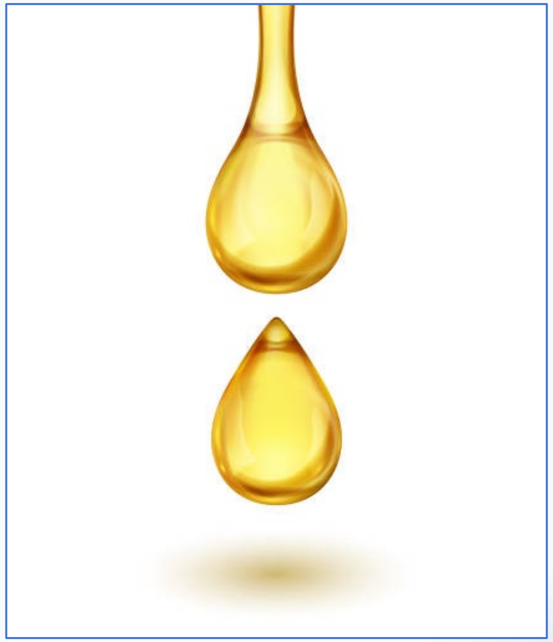



Lab Contaminant Source #1: Foot Traffic

- **Lab staff & visitors**
- **1 B Human skin cells shed daily**
- **>10 M bacteria/cm^2 human hand**
- **Dust is skin cells & bacteria** 🤢
- **Aerosolized viruses such as COVID-19**

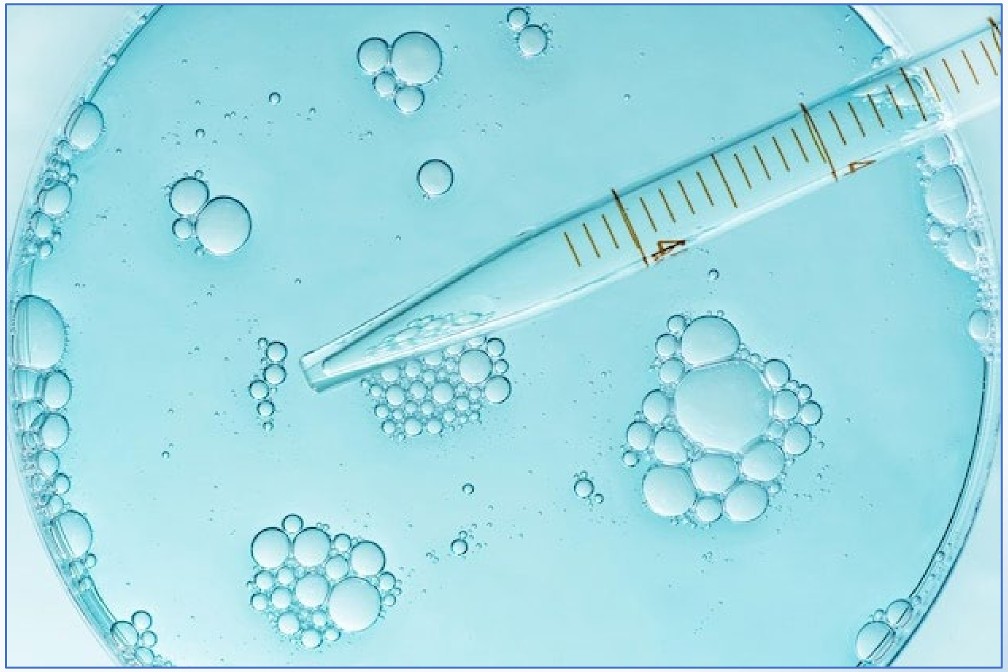
Lab Contaminant Source #2: Culture Conditions

- Oil
- Media, reagents, supplements
- Single-use plastic ware

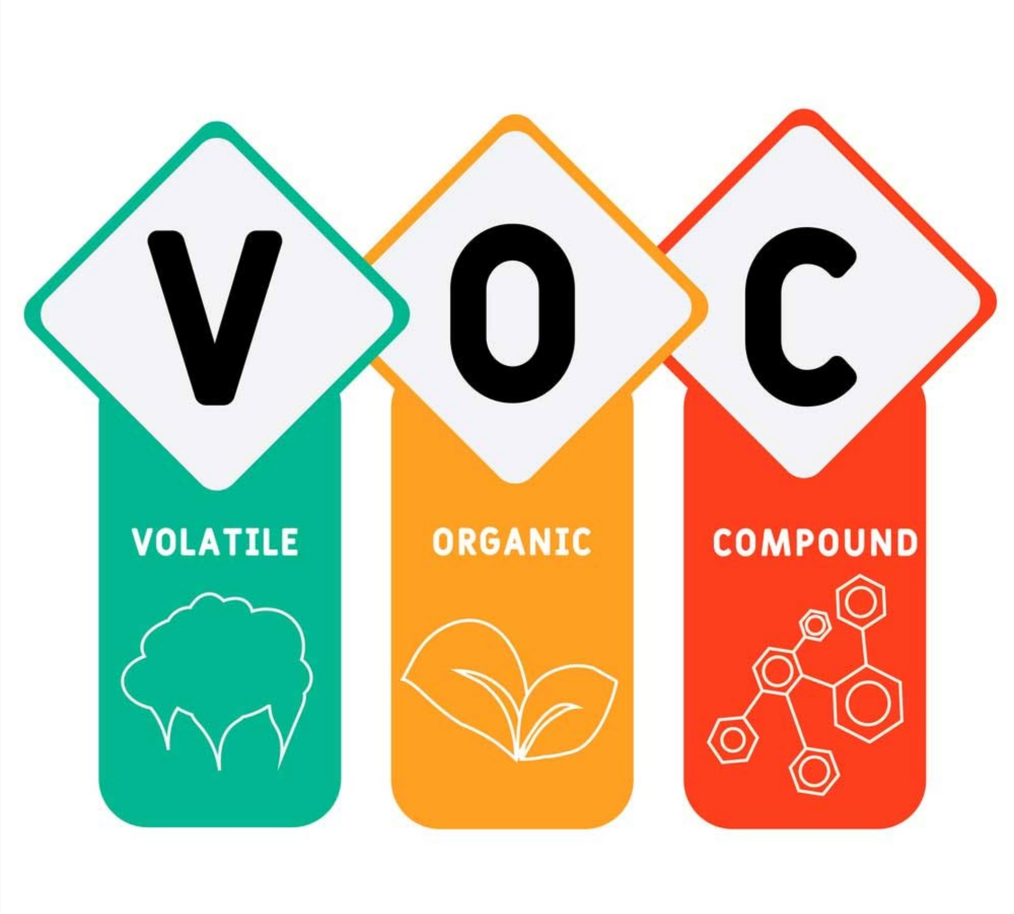


Lab Contaminant Source #3: Patients

- **Biological fluids from female & male**
- **Bacteria, fungus, viruses**
- **Bacteria is a killer, fungi can be washed out**



Lab Contaminant Source #4: VOCs



Cairo Consensus 2018

RBM ONLINE

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Article

Cairo consensus on the IVF laboratory environment and air quality: report of an expert meeting

D Mortimer [a,*], J Cohen [b], ST Mortimer [a], M Fawzy [c], DH McCulloh [d], DE Morbeck [e], X Pollet-Villard [f], RT Mansour [g], DR Brison [h], A Doshi [i], JC Harper [j], JE Swain [k], AV Gilligan [l]

Typical Organic Contaminants in ART Labs

Table 3 – Odour thresholds of organic contaminants typically found in assisted reproduction technology laboratories (American Industrial Hygiene Association, 1989).

Organic compound	Geometric mean AIHA	Comment
Ethanol (ethyl alcohol)	18–100 ppm	Most common VOC in ART laboratories.
Isopropyl alcohol (2-propanol)	19–43 ppm	Second most commonly found VOC.
Acetone (2-propanone)	62–130 ppm	
Propene (propylene)	23–68 ppm	Plastic.
Hexamethylcyclotrisiloxane	No data	Silicone from gaskets.
Acetonitrile (methyl cyanide)	1160 ppm	Plastics.
Formaldehyde	0.03–9970 ppm	
Acetaldehyde	0.067 ppm	
d-Limonene	0.5 ppm	Scent of lemon.
α-Pinene	0.005 ppm	Scent of pine.

AIHA, American Industrial Hygiene Association, ART, assisted reproduction technology; VOC, volatile organic compound.

Mortimer et al., 2018

VOCs Mechanism of Action

- **Numerous studies showing association of high VOC levels with impaired embryo development**
- **VOCs are both aqueous and oil soluble**
- **VOCs can attach directly to DNA during embryo development**
- **Early studies showed lower clinical pregnancy rates were associated with high VOC levels**

Studies of Lab Air and ART

Table 2 – Studies on the effect of air quality (adapted from Esteves and Bento, 2016).

Reference	Year of publication	Study design	Location/population	Method	Main outcome
Cohen *et al.*	1997	Descriptive VOC quantitative levels.	IVF laboratory.	Air sampling VOCs/aldehyde.	High VOC levels found in laboratory air and inside incubators.
Schimmel *et al.*	1997	Descriptive VOC quantitative levels.	IVF laboratory and gas cylinders.	Gas cylinders VOCs/aldehyde.	Varying levels of VOCs/reduction with activated carbon/ KMnO4.
Hall *et al.*	1998	Observational analytic cohort.	In-vitro cultured mouse embryos	Air sampling Acrolein bioassay.	Embryo development affected .
Mayer *et al.*	1999	Prospective randomized crossover.	Human treatment cycles (*n* = 110).	Incubators with and without filters.	Increased pregnancy rate with filters.
Boone *et al.*	1999	Observational analytic cohort.	Human couples (*n* = 275).	Centralized particle filtration.	Reduced particulates, improved embryo development.
Worrilow *et al.*	2001	Descriptive qualitative.	New IVF laboratory.	Central HVAC/ VOC filtration.	Significant reduction in particulates with the new HVAC system to achieve a US Fed Standard class 100 cleanroom (equivalent to ISO 14644-1 Class 5).
Worrilow *et al.*	2002	Observational analytic cross-sectional.	IVF cycles 2 year.	Outside/inside sampling.	Seasonal VOC variation affecting pregnancy rates.
Esteves *et al.*	2004	Observational analytic cohort.	Human ICSI cycles (*n* = 468).	Two laboratories: conventional versus HVAC/filter.	Improved embryo development, increased pregnancy/ decreased miscarriage rates.
von Wyl *et al.*	2004	Descriptive Qualitative.	IVF laboratory air sampling.	Old/new laboratory particle filter.	Reduced particulates and VOC.
Esteves *et al.*	2006	Observational analytic cohort.	Human male factor ICSI cycles (*n* = 399).	Two laboratories: conventional versus HVAC/filter.	Improved embryo development, increased pregnancy/ decreased miscarriage rates.
Knaggs *et al.*	2007	Observational analytic cohort.	IVF cohort.	Key performance indicators study/EU Tissues and Cells Directive.	Increased pregnancy and implantation rates.
Merton *et al.*	2007	Randomized controlled trial.	Bovine.	Incubator filter.	No effect on embryo development, slight increase in pregnancy rate.
Souza *et al.*	2009	Observational analytic cohort study.	Human ICSI cycles (*n* = 123).	Comparing class 8 and class 5 incubators.	No differences.
Khoudja *et al.*	2013	Descriptive qualitative observational analytic cohort.	Human IVF–ICSI cycles (*n* = 1403).	Standalone filtration versus novel Landson system.	Significant improvements in laboratory performance.
Esteves *et al.*	2013	Observational analytic cohort.	Human ICSI cycles in ISO 5 clean room laboratory (*n* = 2060), *cf* 255 ICSI cycles in older-style laboratory.	New ISO 5 clean room laboratory compared with older-style laboratory.	Increased proportion of high quality embryos on day 3.
Munch *et al.*	2015	Observational analytic cohort.	Human fresh IVF cycles (*n* = 524) and frozen embryo transfer cycles (*n* = 156).	Laboratory with and without carbon filter.	Decline in laboratory performance when filter removed.
Heitmann *et al.*	2015	Descriptive qualitative observational analytic cohort.	Human IVF–ICSI cycles (*n* = 820).	Old laboratory with standalone filter/new laboratory with HVAC and central filter .	Decreased VOC; Improvements in laboratory performance.

HVAC, heating, ventilation and air conditioning; ICSI, intracytoplasmic sperm injection; VOC, volatile organic compounds.

Mortimer et al., 2018

Air Quality Associated with Lab Metrics

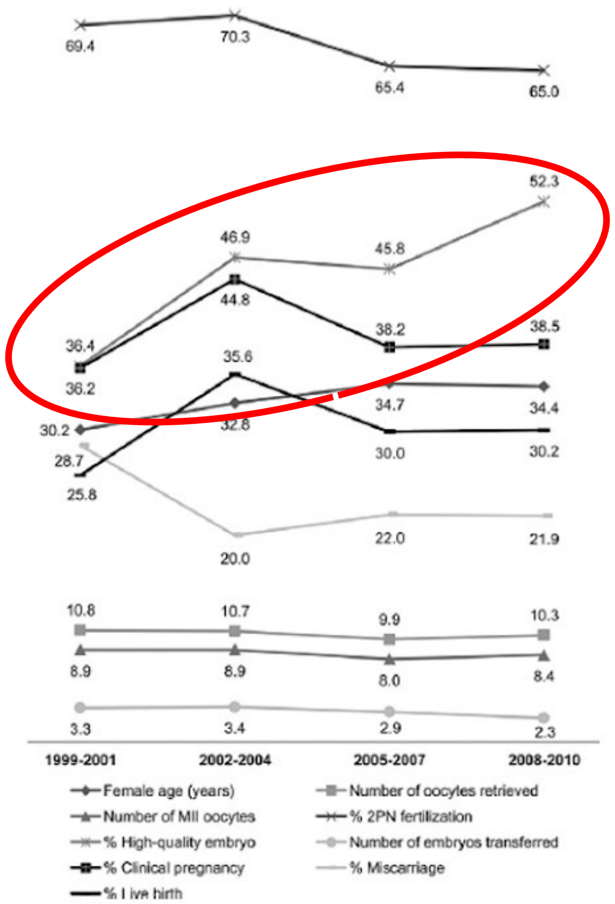

Figure 3 Stratified data analysis by triennium periods of intracytoplasmic sperm injection cycles performed at standard IVF facilities (1999–2001) and cleanroom facilities (2002–2010). Values are expressed as means. MII = metaphase II; 2PN = two pronuclei.

Esteves and Bento, 2013

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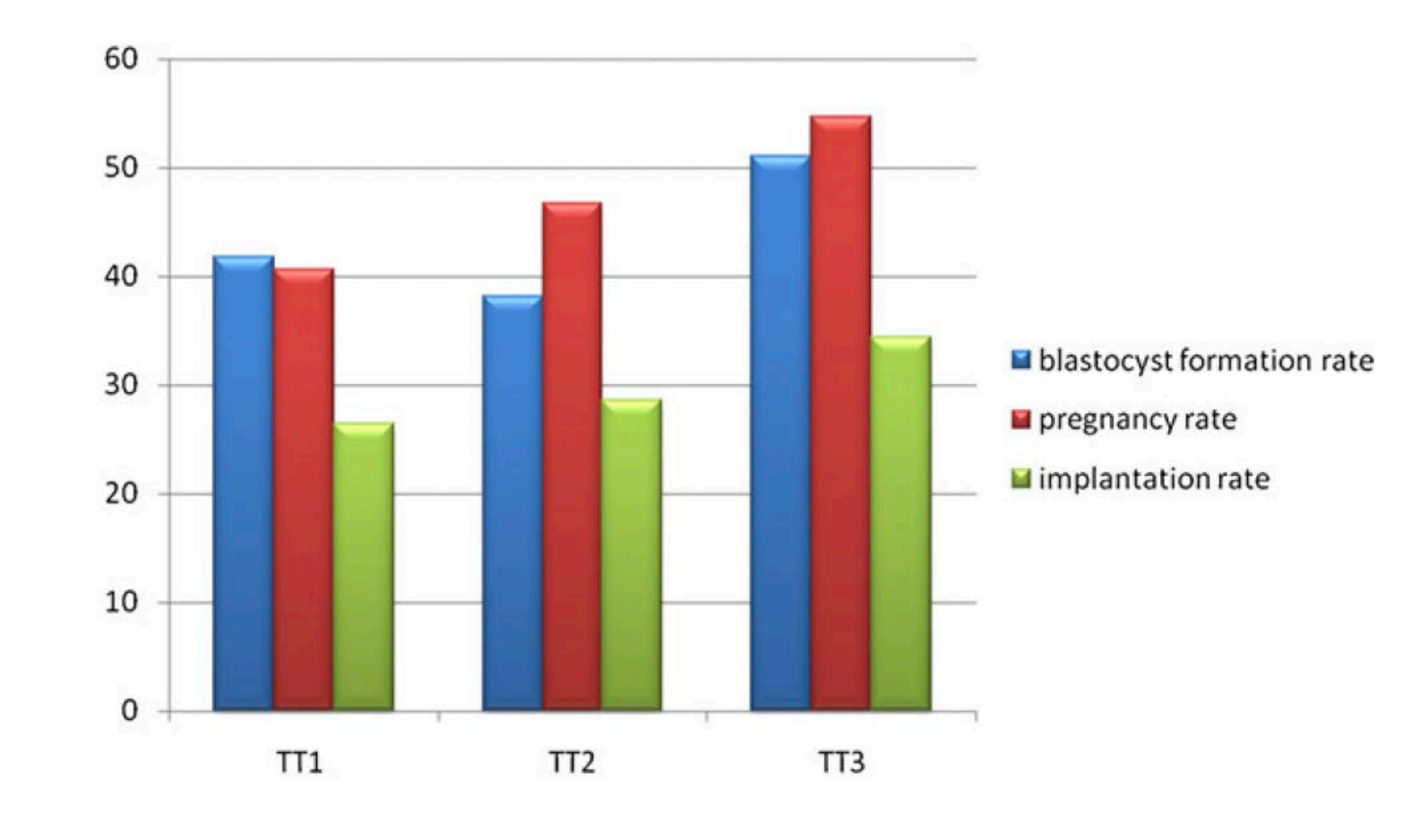
Air Quality Associated with Lab Metrics



Khoudja et al, 2013

Air Quality Associated with Lab Metrics

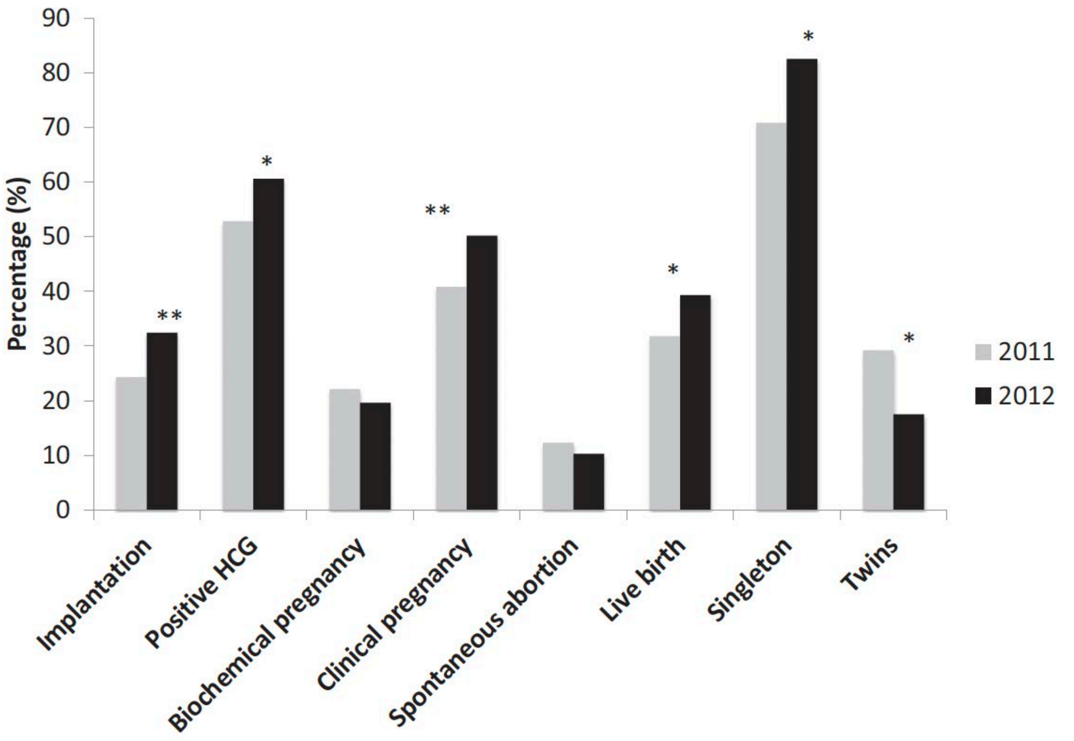
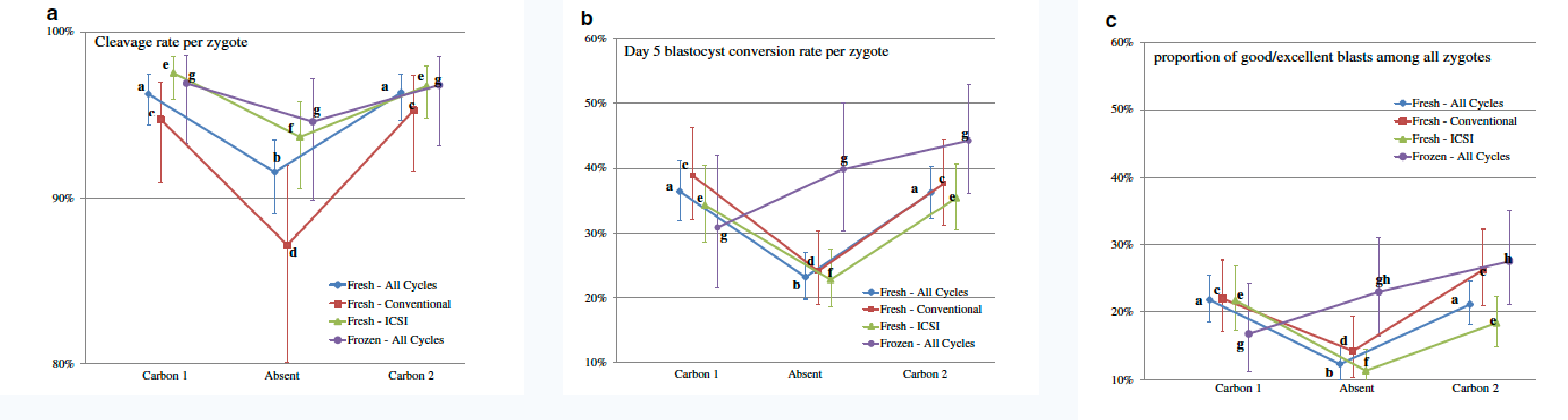


Figure 1 Comparison of IVF cycle outcomes per embryo transfer by cycle year (2011 versus 2012). Implantation reported as average implantation per patient. Chi-squared test was used for analysis. $*P < 0.05$; $**P < 0.01$.

Heitmann et al, 2015

Air Quality Associated with Lab Metrics



Munch et al, 2015

Modeling VOCs in the IVF Lab

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ARTICLE

CrossMark

Modelling the equilibrium partitioning of low concentrations of airborne volatile organic compounds in human IVF laboratories

BIOGRAPHY

John Fox, PhD, PE, is an associate professor in the Department of Civil and Environmental Engineering at Lehigh University, Bethlehem, PA. John received his BS in civil engineering from the Virginia Military Institute and earned his MS and PhD in environmental engineering from the Pennsylvania State University.

John T. Fox[1,*], Pan Ni[1], Alicia R. Urrutia[2], Huey T. Huynh[2], Kathryn C. Worrilow[2]

Fox et al, 2023

VOCs in Air-Oil-Water-Embryo

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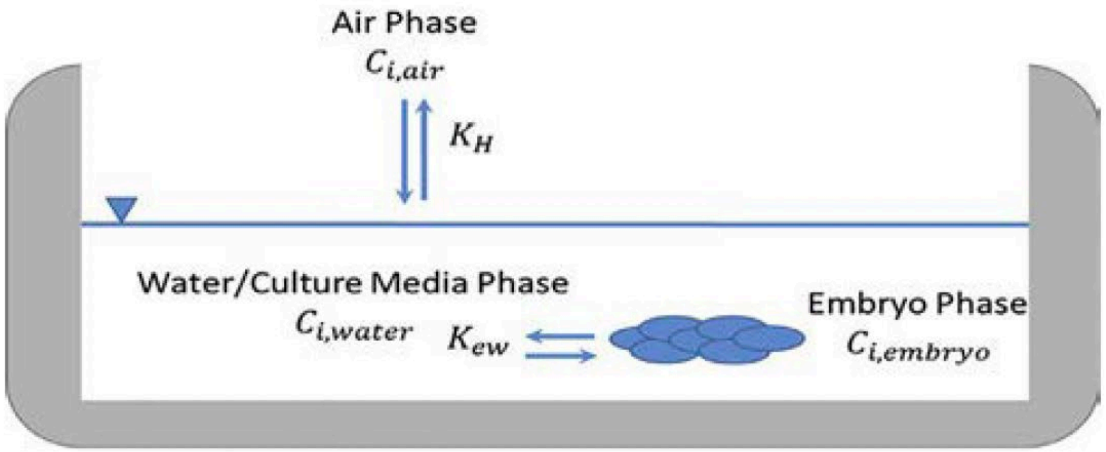


FIGURE 1 Air–water/culture model: when culture media is mixed or exposed to air, ppb concentration VOC in the air phase will partition into the water/culture media phase, governed by Henry's law (K_H) and organic compounds now in the water/culture media phase are defined as the concentration, C_i, where 'i' is chemical in the culture media. K_{ew} = embryo–water partitioning coefficient.

Fox et al, 2023

Engineering Controls for Lab Air Quality

- **Air filtration: pre-filters, activated carbon and potassium permanganate, HEPA**

- **Fresh air with majority recirculated, clean, warm air**

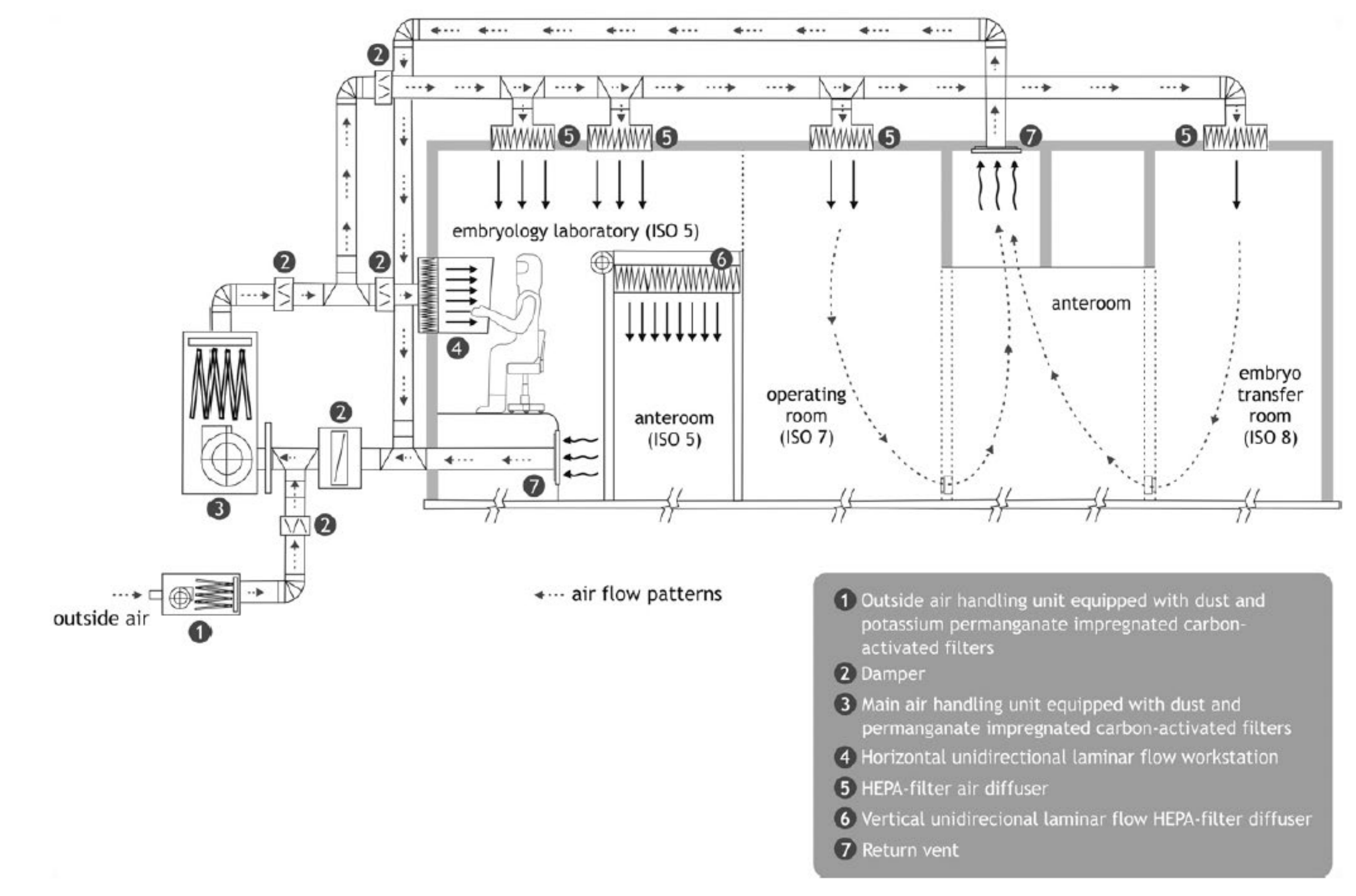
- **Positive pressure**

- **Minimum # of air exchanges per hour**

Filtration for Lab Air Quality

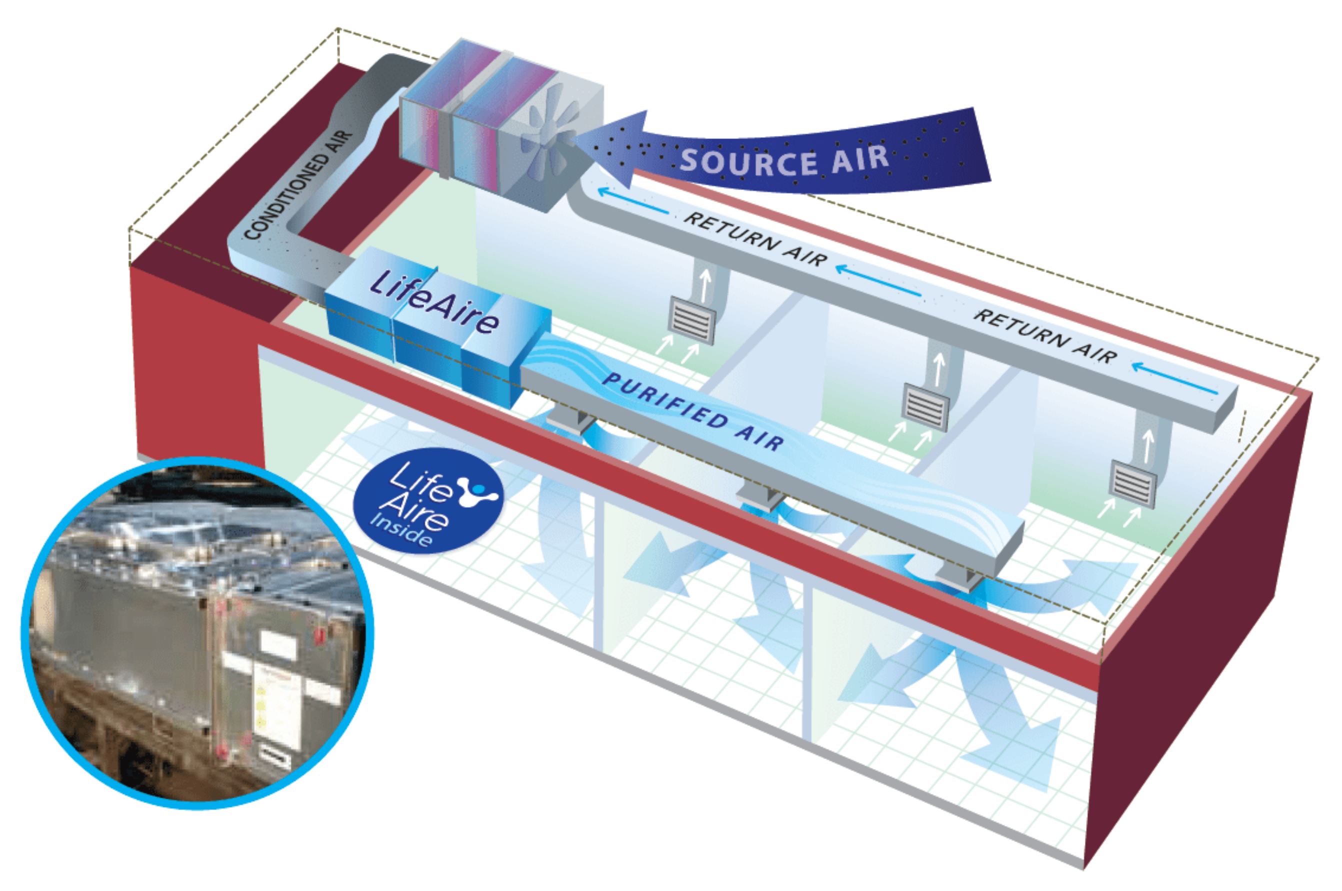
- **pre-filters**
- **activated carbon and potassium permanganate**
- **HEPA filter: high efficiency particulate air filter**

Air Handling System Design



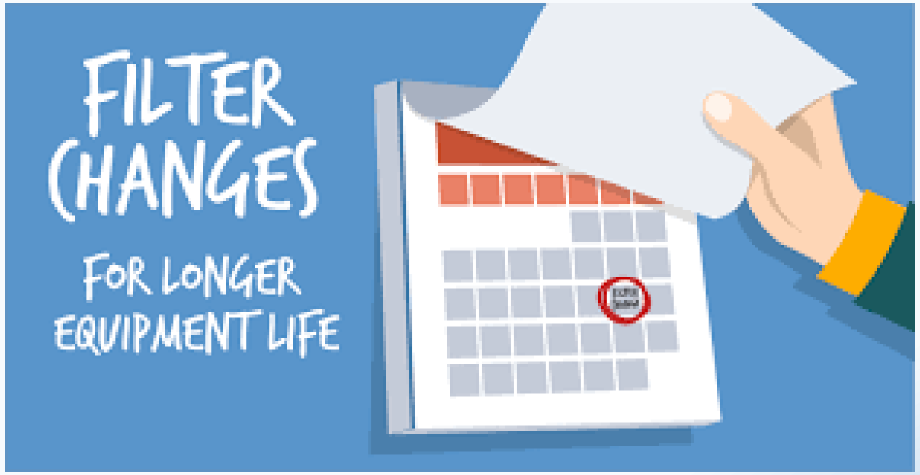
Esteves and Bento, 2013

Life Aire System



Engineering Maintenance for Lab Air Quality

- **Test air handling system for pressure & balance**
- **Change filters as per schedule**
- **Test filter substrate to confirm filter life**



Good Lab Practice Strategies for Lab Air Quality

- Adopt a clean room philosophy
- Testing: MEA, sperm bioassay
- Checklists, checklists, checklists

Checklists, Checklists, Checklists

- Cognitive net that catches mental errors of memory, attention and thoroughness
- Allow for tracking & reliable communication
- Routine but critical items are not overlooked
- Good checklist are precise, efficient, easy to use, practical

Sangita's Clean Lab Maintenance Checklist

- **Hygiene** — ***Did you start by washing your hands?***
- **Cleaning** — ***Did you finish by wiping down surfaces?***
- **Air Flow** — ***Are laminar flow hoods on during the day?***
- **Waste Removal** — ***Are regular & biological waste removed?***
- **Protocols** — ***Are detailed shutdown protocols followed?***

Good Lab Practice for Lab Air Quality

- Frequent hand washing
- Use high quality distilled water
- Use 6% hydrogen peroxide to clean surfaces
- Laminar flow hoods
- Bi-annual cleaning of lab

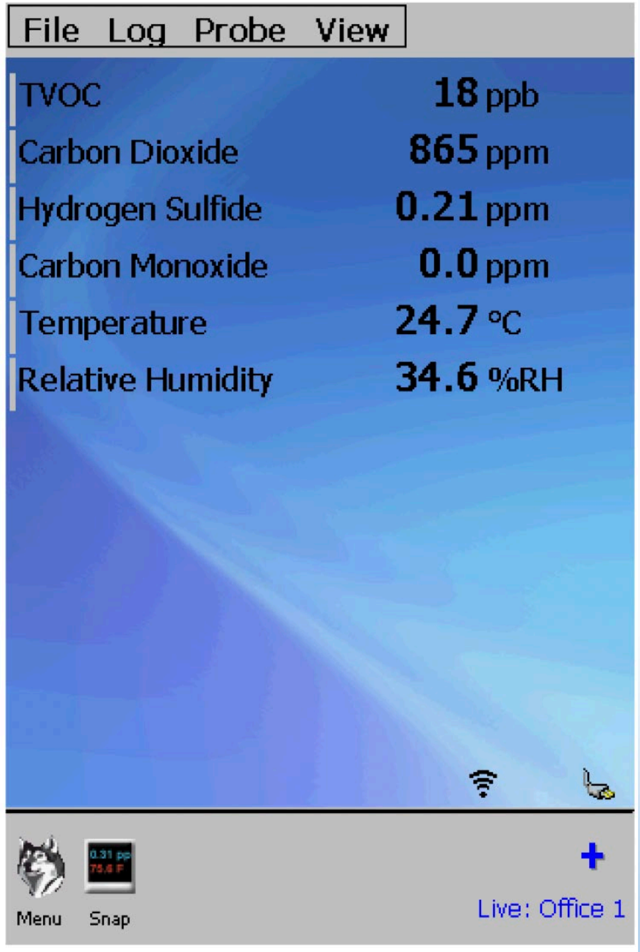
Good Physical Plant Controls for Lab Air Quality

- Lab is in protected area & has secure access
- Maintain scrub discipline
- Sticky or tacky mats at lab entrance
- Be attentive to sterile technique
- Supply unpacking & storage
- No painting, renovation, repair, paving, vibrations

Good QC & QA for Lab Air Quality

- Reduce hazardous materials, remove waste daily
- Schedule & maintain equipment QC & QA
- Change water bottles, H_2O_2 bottles regularly
- Any changes to products? Evaluate before use.
- Cell phone use!

Measuring VOCs in the IVF Lab

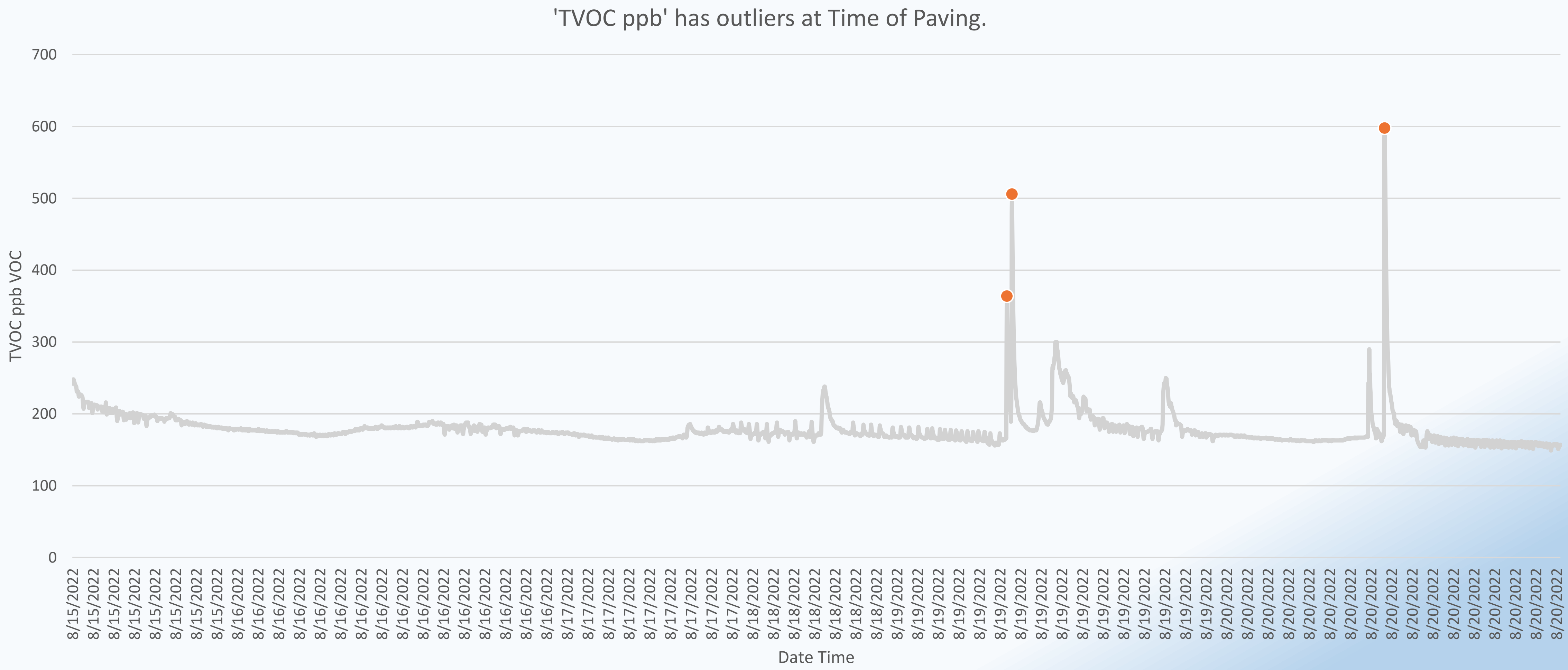


VOCs Limits in the IVF Lab

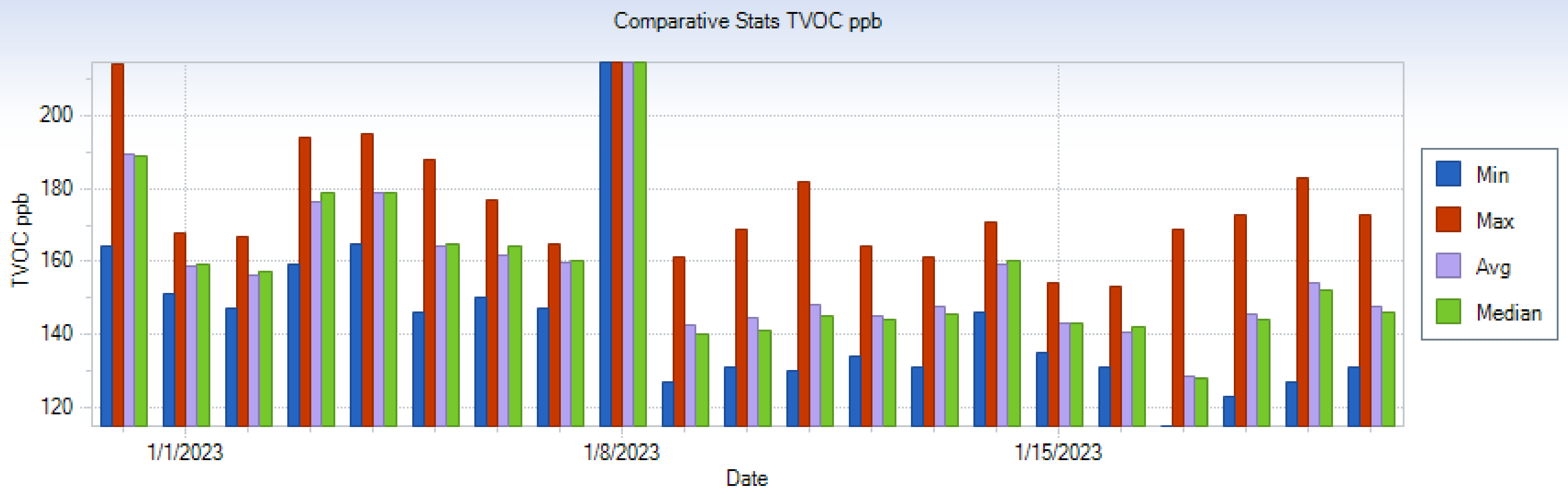
VOCs. Total VOCs less than 500 μg/m^3 (~400–800 ppb total VOC, depending on molecular species); less than 5 μg/m^3 aldehydes.

Mortimer et al., 2018

They Paved Paradise in NY

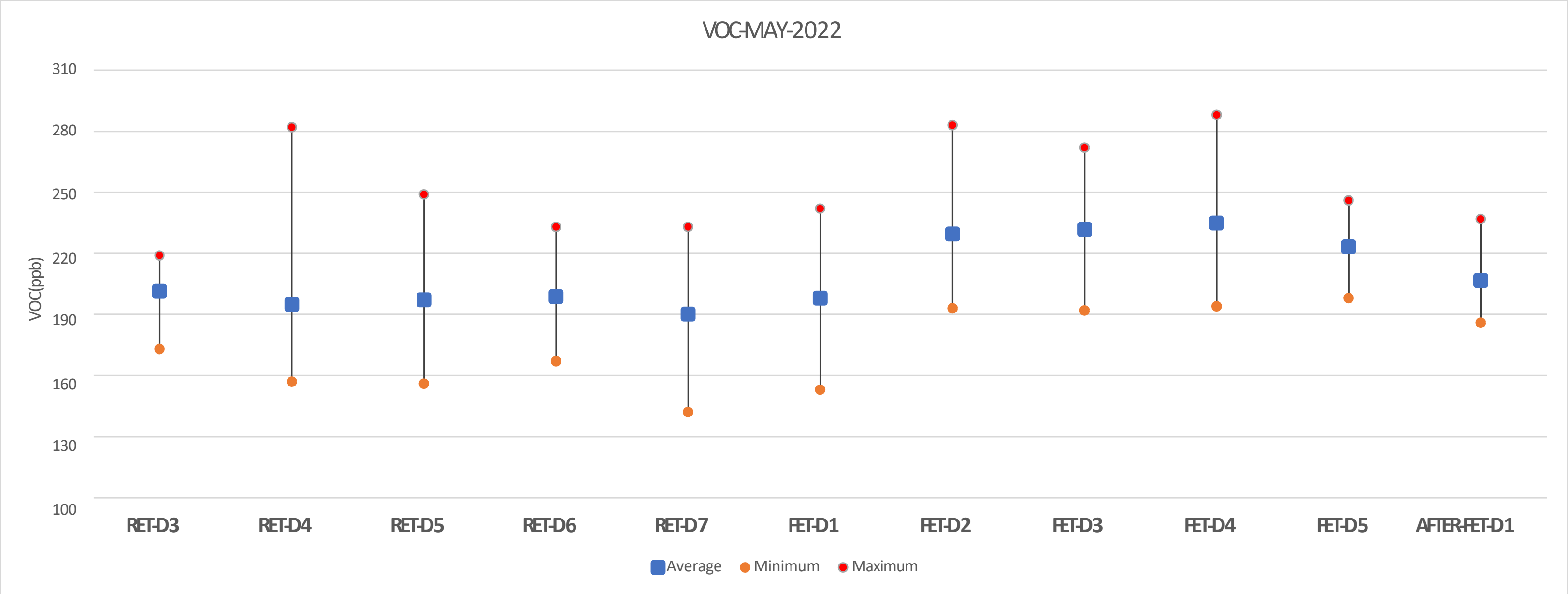


Santa Barbara Patient Series



	12/31/2022	1/1/2023	1/2/2023	1/3/2023	1/4/2023	1/5/2023	1/6/2023	1/7/2023	1/8/2023	1/9/2023	1/10/2023	1/11/2023
Min	164	151	147	159	165	146	150	147	NaN	127	131	130
Max	214	168	167	194	195	188	177	165	NaN	161	169	182
Avg	189	159	156	176	179	164	162	159	NaN	143	145	148

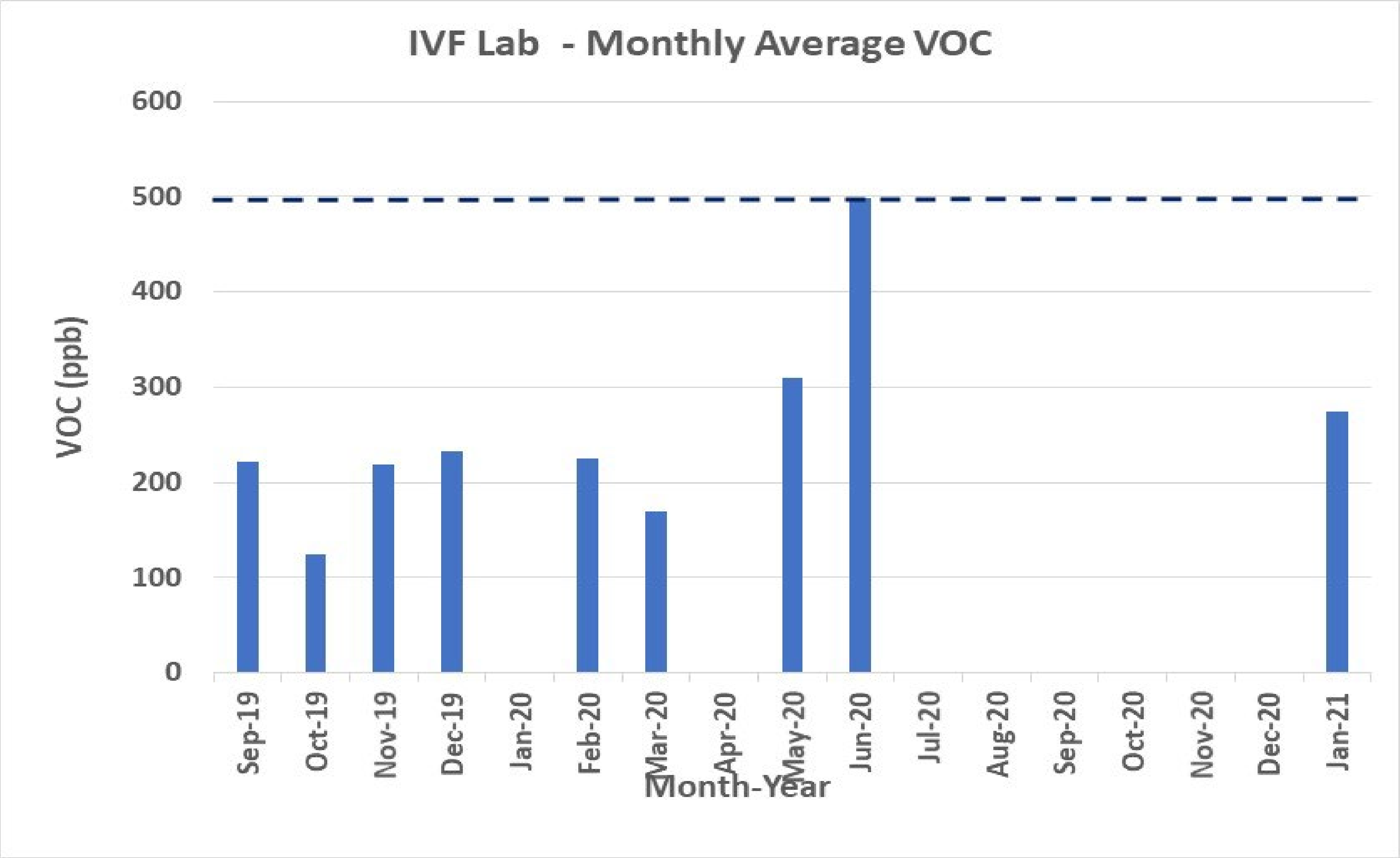
Miami Patient Series



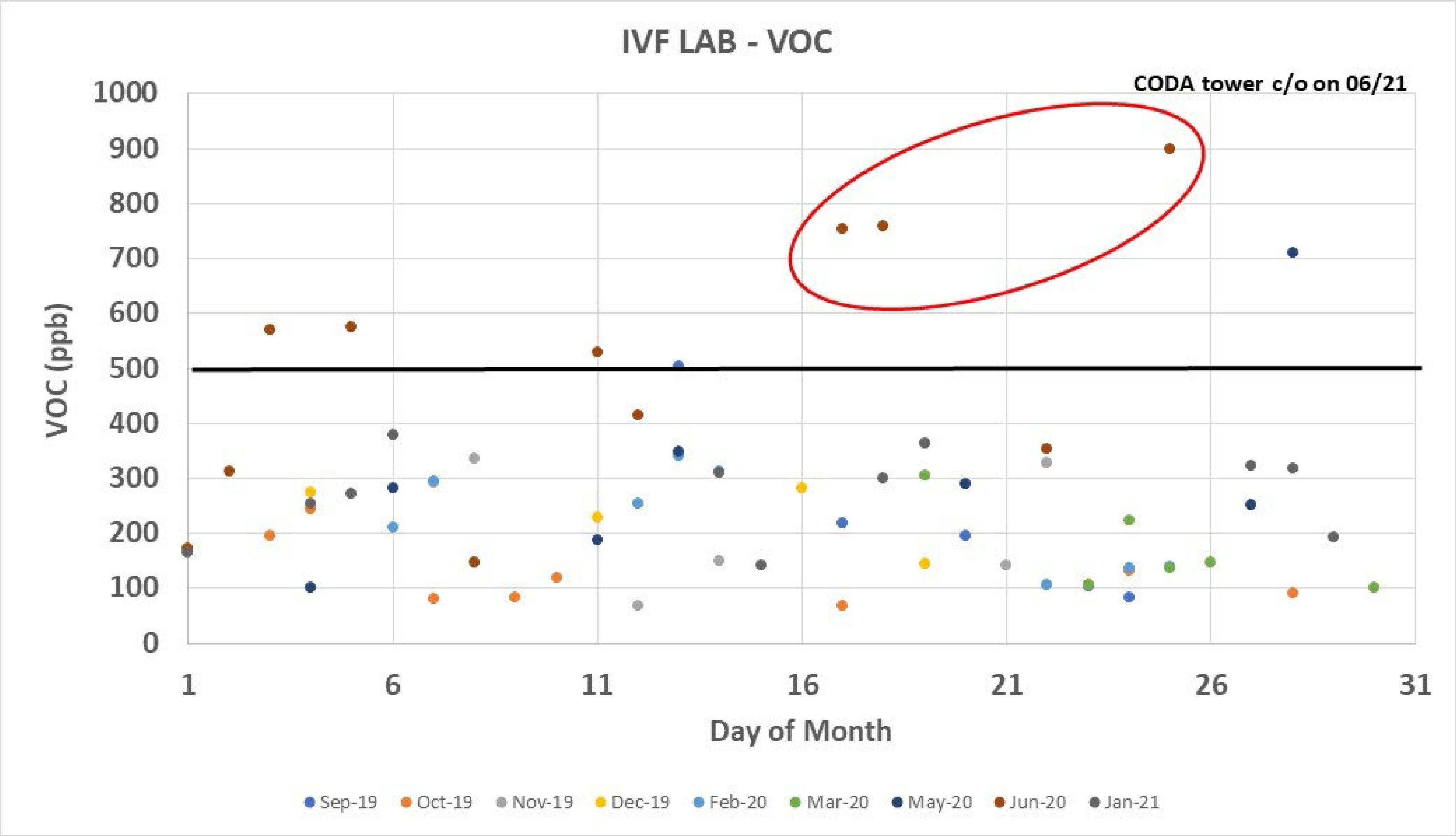
	RET-D4	RET-D5	RET-D6	RET-D7	FET-D1	FET-D2	FET-D3	FET-D4	FET-D5	AFTER-FET-D1
#RET	2	2	0	3	0	4	8	9	0	0
#FET	1	0	0	1	0	0	0	0	0	0

	RET-D3	RET-D4	RET-D5	RET-D6	RET-D7	FET-D1	FET-D2	FET-D3	FET-D4	FET-D5	AFTER-FET-D1
AVERAGE	201	195	197	199	190	198	230	232	235	223	207
MIN	173	157	156	167	142	153	193	192	194	198	186
MAX	219	282	249	233	233	242	283	272	288	246	237
STD	21.74	13.98	18.61	20.00	19.18	13.60	14.83	11.86	16.68	14.57	14.82

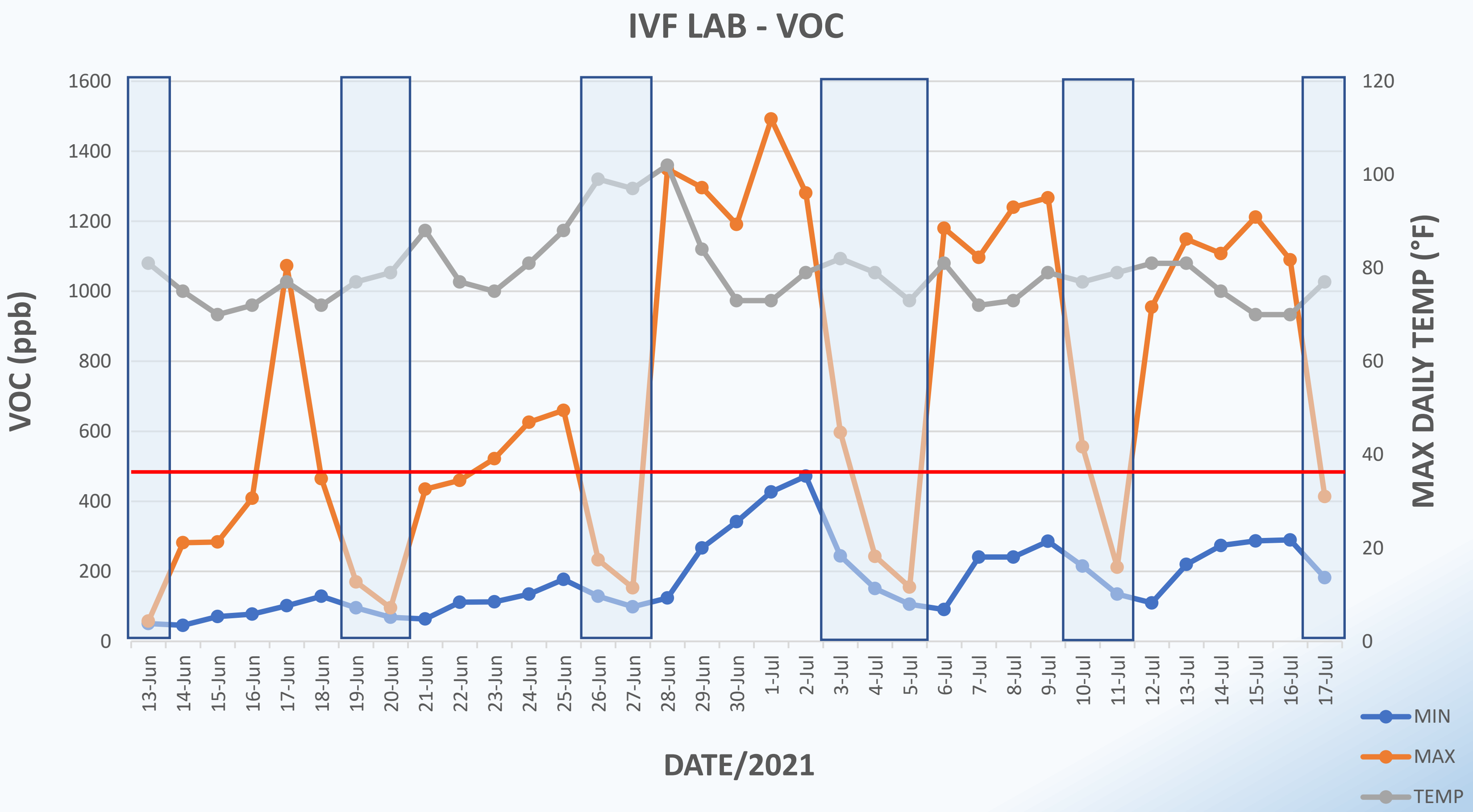
Seattle Patient Series



Seattle Patient Series



Seattle Patient Series



Seattle Patient Series

IVF KPI	MATURITY (%)	FERTILIZATION RATE (%)	BLASTOCYST RATE (%)	EUPLOID RATE (%)
1st QUARTER RESULTS (1/1/21 - 3/31/21)	455/577 (79%)	314/422 (74%)	239/334 (72%)	89/179 (50%)
6/13-7/17/21 OUTCOMES (n=18 cycles)	219/251 (87.3%)	147/219 (67.1%)*	104/147 (70.7%)	18/33 (54.5%)

NB: 6/13-7/17/21 outcomes only for IVF/Fz all patients. Egg cryo/donor egg cycles not included

* Borderline significantly different (p=0.52)

In Summary

- Maintain lab air quality for optimized IVF outcomes
- Most pollutants are aldehydes, VOCs, biologicals from staff, patients
- Use hardware engineering controls to HVAC
- Use software controls of rigorous QC & QA
- Measure VOCs, temperature, humidity
- Track VOC data to lab outcomes & KPIs

Thank You

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www.sangitajindal.com

sangita.k.jindal@gmail.com | sjindal@montefiore.org