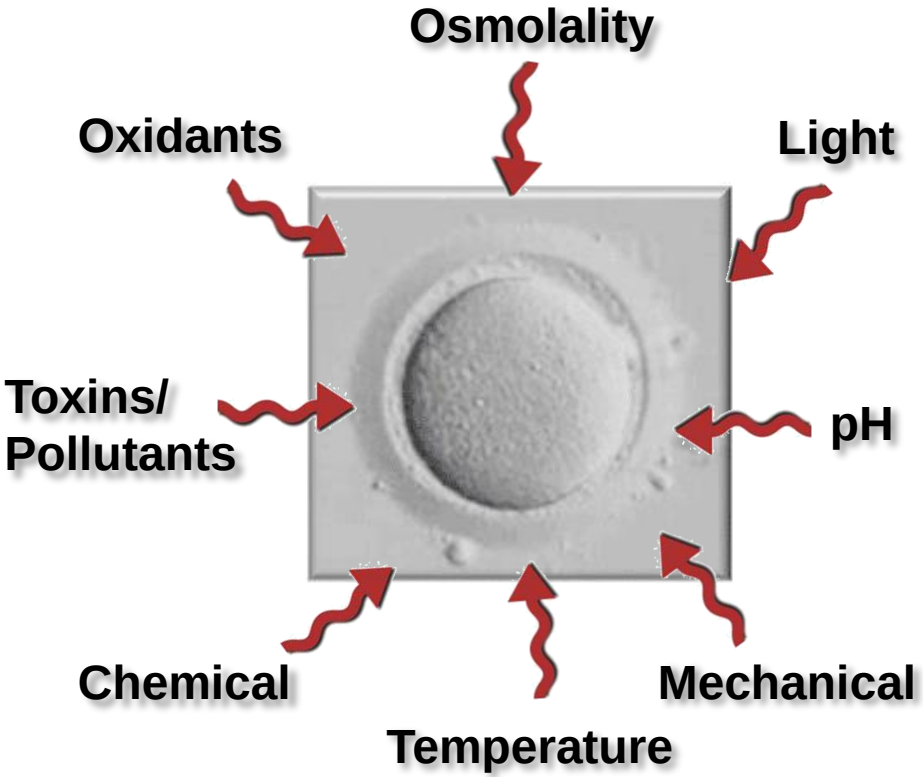
Evidence and/or Common Sense Based Laboratory Design and QC: What Matters and What Doesn't

Jason E. Swain, PhD, HCLD
Fertility Lab Sciences

Minimize *In Vitro* Stress

- Improper lab conditions lead to environmental stress
  - Can compromise cell function and development
  - Especially sensitive cell types
- Consideration of gamete/ embryo physiology can help combat stress
- Proper lab design and QC also instrumental

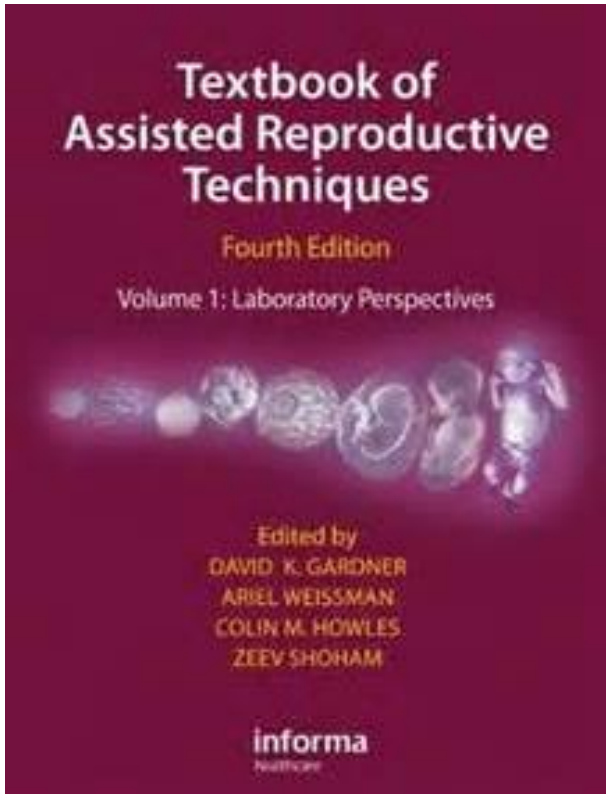


Human Reproduction vol.15 no.10 pp.2241–2246, 2000

ESHRE guidelines for good practice in IVF laboratories

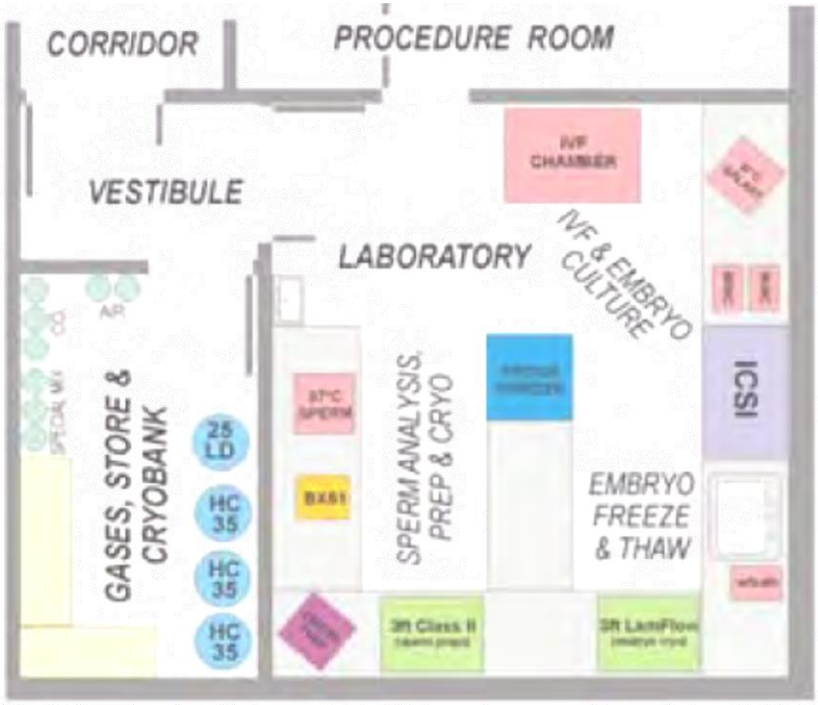
Luca Gianaroli[1], Michelle Plachot, Roelof van Kooij, Safaa Al-Hasani, Karin Dawson, Anick DeVos, M.Cristina Magli, Jacqueline Mandelbaum, Jacqueline Sely and Wouter van Inzen (Commi e of the S Inter t Group on Emb

tute the minimal req or with the aim of i the ryolo furth vel



Laboratory Design

- Consideration of room layout and workflow
  - Proximity of lab to other clinical areas (OR, transfer rooms)
  - Dedicated space for andro/endo
  - Location of equipment in the lab
  - Collection rooms (2+)
  - Cryo storage room
  - Gas storage room
  - Supply storage area
  - Office space



Practical considerations based on budget, space and workflow

Laboratory Design

- Proper building materials
  - Low VOC
    - Insulation
    - Paint
    - Flooring/adhesive
    - Cabinets
- Sealed – positive pressure
  - Limit penetrations
  - Doors, sweeps, etc
- Proper lighting
  - Incandescent, LED, dimmable
- Backup power
- Dedicated HVAC
  - Isolated & cleaned duct work
  - Air exchanges
  - Proper filtration
  - Maintain temp and humidity

Requires careful & constant oversight to verify

Burn-In & Validation

- Clean walls, ceilings, floors, cabinets/counters
- Clean equipment (incubators)
  - Peroxide, etc
- Increase temperature for period of time
  - Room and incubators
- Run HVAC and incubators for ≥ ~2 weeks
- Validate
  - Particle counts, VOCs
  - Monitor/”Dial-in” equipment (proper functioning)
  - Appropriate bioassay (1-cell MEA)

Document the “commissioning” of the lab

Fact vs. Fetish vs. Common Sense

Do we have data to support these suggestions?

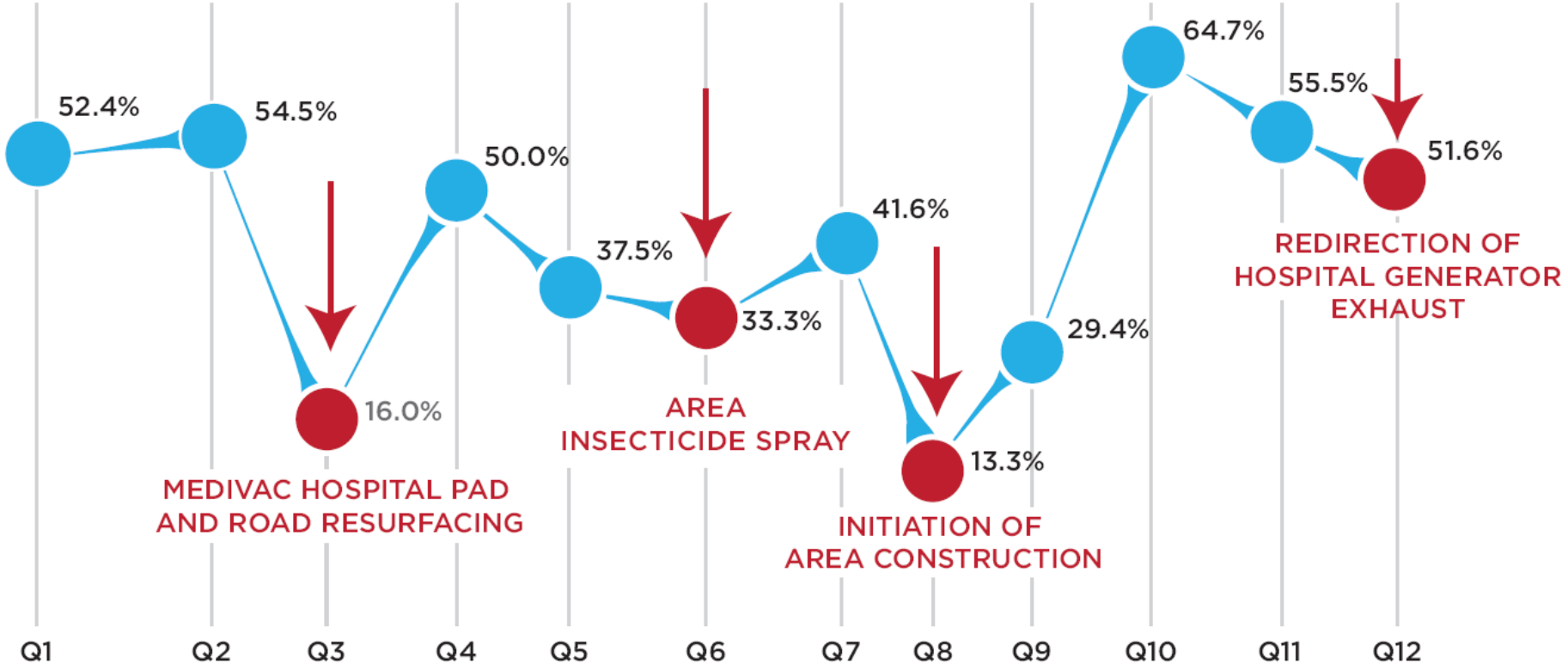
Air Quality & IVF Outcomes

- Several anecdotal accounts of changes in air quality coinciding with decreased outcomes
  - Fertilization, embryo development, pregnancy, implantation, abortion rates

- Has a dramatic impact when it comes to the design and construction of an IVF lab

Are there published data to prove air-quality impacts outcomes?

External Events & Clinical Pregnancy Over 3 Yrs



LifeAire Website

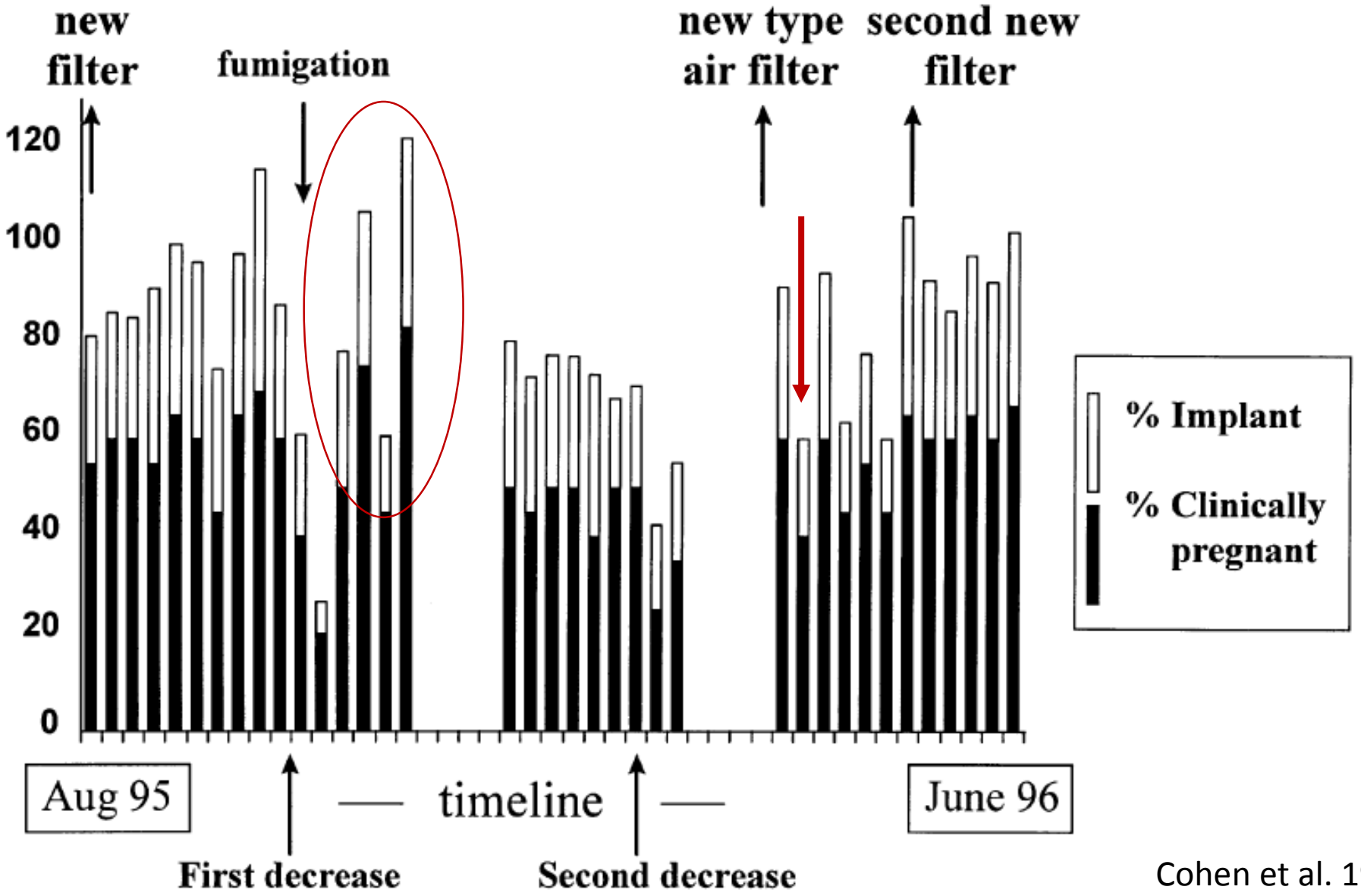
Human Reproduction vol.12 no.8 pp.1742–1749, 1997

Ambient air and its potential effects on conception *in vitro*

Jacques Cohen[1,4], Antonia Gilligan[2], William Esposito[2], Tim Schimmel[1] and Brian Dale[3]

[1]The Institute for Reproductive Medicine and Science of

Air Quality & IVF Outcomes?



Cohen et al. 1997

Gas Cylinders & VOCs

Table I. Composition of VOC detected in compressed CO_2 used for clinical gamete and embryo culture

Volatile organic compound	$\mu g/m^3$
Benzene	100
Unknown freon	100
Isopropanol	80
n-Pentane	50
Acetaldehyde	50
n-Butane	30
Isohexane + acetic acid	30
Acetone	24
Ethanol	20
Toluene	12
n-Heptane	10
C_9H_{12} alkyl benzene	10
n-Undecane	10
C_7H_{16} alkane	9
$C_{12}H_{26}$ alkane	7
Trichloroethene	4.7
m- & *p*-Xylenes	3.8
Ethylbenzene	1.6

Cohen et al. 1997

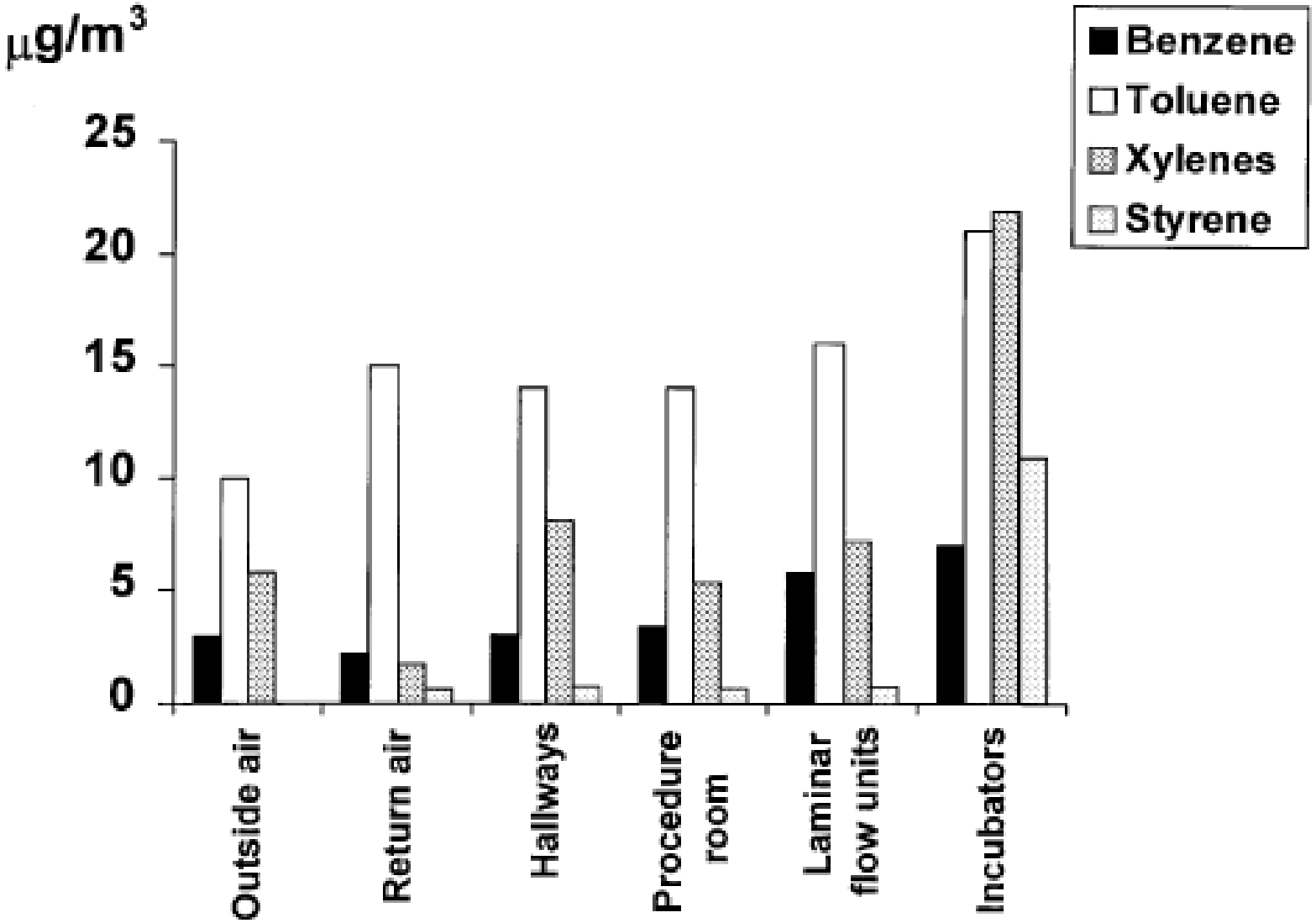
Plasticware & VOCs

Table V. Compounds released from cell tissue culture grade petri dishes

Material	>50 ng/sample	⩽50 ng/sample	
Styrene	920.00	*n*-Pentane	50
Toluene	180.00	3-Methylpentane	50
Acetone	150.00	Nonanal	50
2-Butanone	130.00	Butanal	40
Acetaldehyde	100	3-Pentanone	40
n-Butane	100	n-Hexane	30
Benzaldehyde	100	Butene isomer	30
Hexanal	70	Benzene	23
Ethylbenzene	64.00	*n*-Octane	20
2-Hexanone	58.00	*n*-Nonane	20
		Decanal	20
		Cumene	10
		Propylbenzene	10
		Octanal	10
		m- & *p*-Xylenes	7.5
		o-Xylene	5.80

Cohen et al. 1997

Incubators & VOCs



Cohen et al. 1997

Air Quality & IVF Outcomes?

Table IV. Effects of floor tile adhesive on mouse embryo development *in vitro*

Date	Construction activity in neighbouring space	Zygotes in culture	Expanded blastocysts[a]
26 June 1995	12 days after use of water-based paint	17	17
3 July 1995	During installation of floor tiles	44	3
7 July 1995	One day following bench-top installation.	19	17

[a]Significant differences between second and first/third rows $P < 0.001$ (Fisher's exact test).

Verification of VOC levels during this time?
Were VOCs gone in all spaces by July 7?

Cohen et al. 1997

Aldehydes & Embryos

Table IV. Effect of acrolein on mouse 2-cell embryo development *in vitro*. Comparisons carried out using the χ^2 test

Treatment Group mM (ppm)	No. of embryos	Development state[a] Percentage 8-cell[b]	Percentage blastocyst[c]
Untreated (control)	625	96.1[d]	87.9[k]
0.01 (0.58)	638	94.6[e]	80.1[l]
0.025 (1.40)	521	89.0[f]	41.3[m]
0.0375 (2.10)	502	85.0[g]	3.0[n]
0.05 (2.80)	425	7.7[h]	0.0[o]
0.1 (5.60)	468	0.0[i]	0.0[p]
1.0 (56.00)	192	0.0[j]	0.0[q]

Treatment group mM (ppm)	No. of embryos transferred	No. of pregnant recipients	No. (%) of implants	No. (%) of term fetuses[a]
Untreated (control)	140	14	108 (77.1)[b]	80 (74.7)[d]
0.01 (0.58)	120	12	87 (72.5)[c]	47 (54.6)[e]

Relevant with oil overlay?

Hall et al. 1998

FERTILITY AND STERILITY®
VOL. 71, NO. 1, JANUARY 1999

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Control of air quality in an assisted reproductive technology laboratory

William R. Boone, Ph.D. (HCLD), Jane E. Johnson, M.T. (ASCP),**
*Ann-Jannette Locke, M.S.,† Martin M. Crane IV, Ph.D.,† and Thomas M. Price, M.D.**

Main Outcome Measure(s): Particle counts (sizes 0.3, 0.5, 1.0, and 5.0 μm); 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® 1999;71:150–4. ©1998 by American Society for Reproductive Medicine.)

No other factors changed?

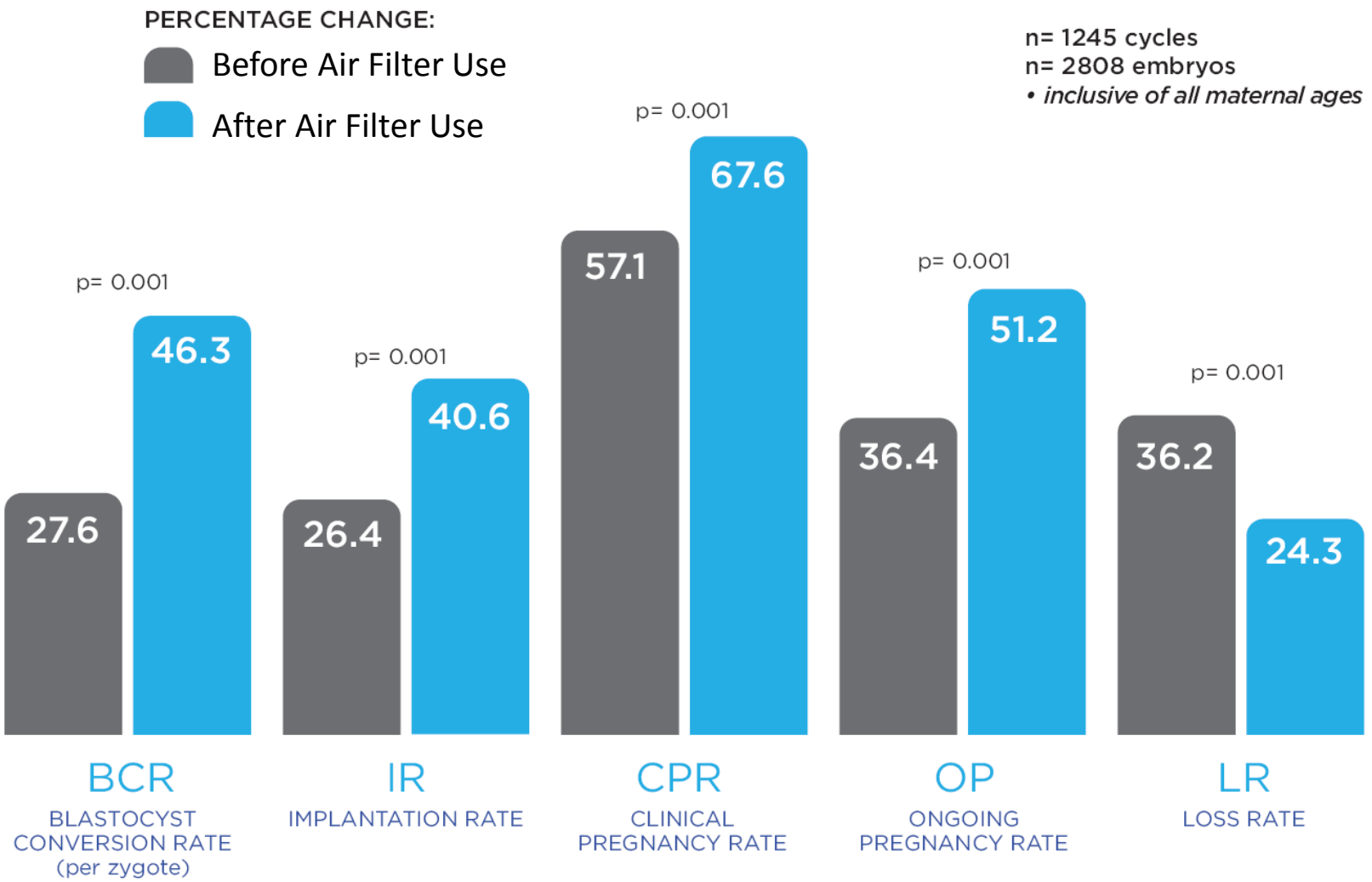
Cleaning of Lab Air

- Methods exist to clean air and reduce VOCs
  - Centralized units
  - Free standing towers
  - Incubator units
  - Gas line filters

- Various approaches
  - HEPA
  - Positive Pressure
  - Activated carbon
  - Photocatalytic oxidation
  - Potassium permanganate

- Various companies with a focus on the IVF lab

Impact of Lab Air Filtration on IVF Outcomes?



Caution in interpretation required

LifeAire Website

J Assist Reprod Genet (2013) 30:69–76
DOI 10.1007/s10815-012-9900-1

ASSISTED REPRODUCTION TECHNOLOGIES

Better IVF outcomes following improvements in laboratory air quality

Rabea Youcef Khoudja · Yanwen Xu · Tao Li · Canquan Zhou

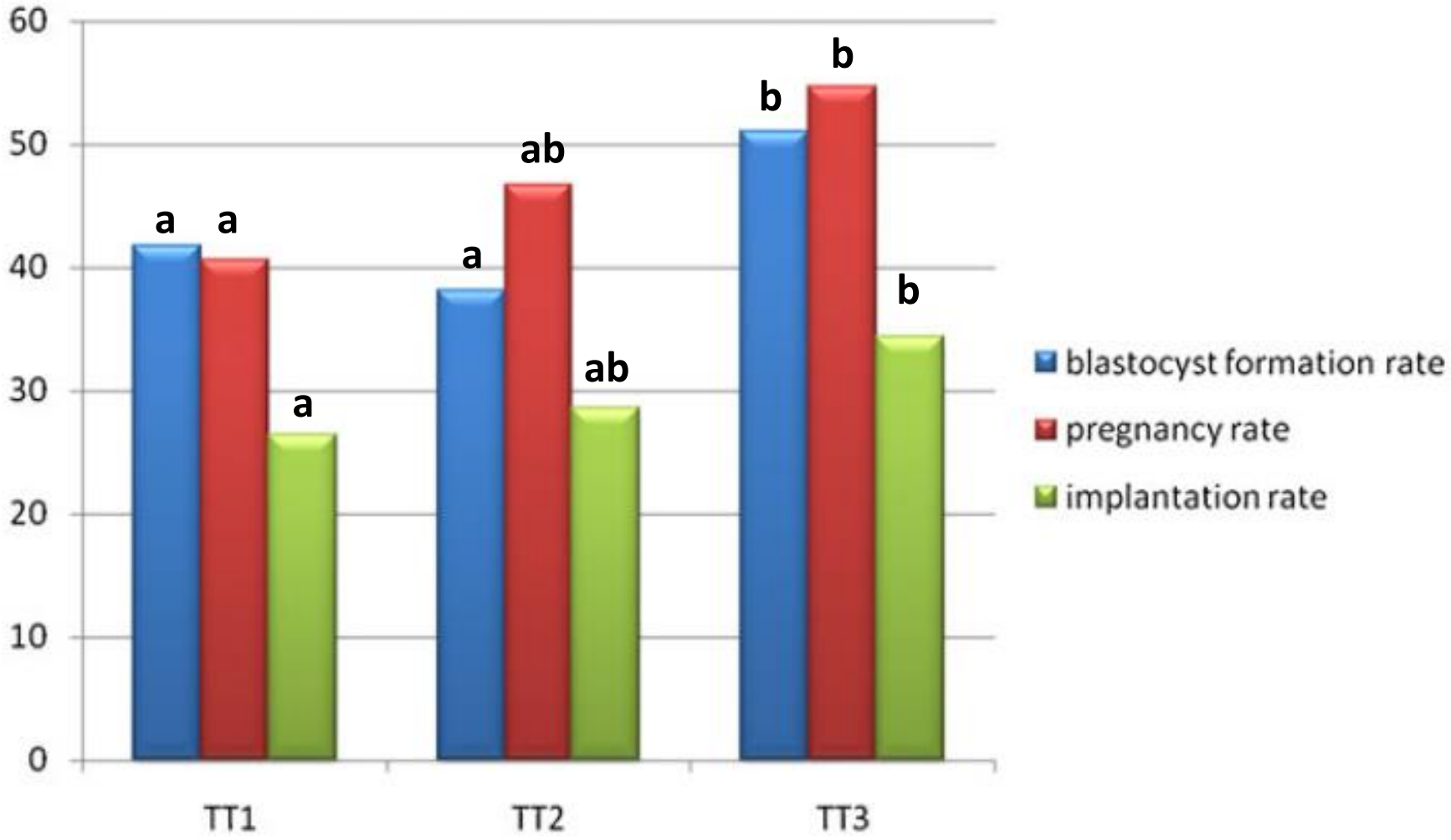
Air Filtration and Outcomes

- Existing HEPA, activated carbon, 4 CODA units, inline gas filters (TT1; April-May 2011)
  - Changed carbon filter (TT2; May-Aug 2011)
- Added a new filtering system (TT3; Aug-Oct 2011)

- VOCs sampled during different times
  - TT1- Unclear how long filters were in place before sampling
  - TT2- 16 days after filter change
  - TT2- 8 days after install

- Found changes in VOC levels between TT1, TT2, TT3
  - Not all VOCs decreased
  - Higher levels of formaldehyde & others in incubator at TT3

Air Filtration and Outcomes



O-110

Prospective Randomized Crossover Analysis of the Impact of an IVF Incubator Air Filtration System (Coda, GenX) on Clinical Pregnancy Rates. J. F. Mayer, F. Nehchiri, V. M. Weedon, E. L. Jones, H. L. Kalin, S. C. Oehninger, J. P. Toner, W. E. Gibbons, S. J. Muasher. The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA.

Incubators	Filtered	Non-Filtered	Statistics
# Treatment Cycles	57	53	
Age	34.4 ± 0.7*	34.0 ± 0.6	NS
% 2pn Fertilization	73.7%	79.0%	NS
# Embryos Transfer	3.7 ± 0.2	3.5 ± 0.2	NS
# Good quality embryos trans.	2.3 ± 0.23	2.8 ± 0.21	NS
% Clinical Pregnancy	54% (31/57)	29% (16/53)	S ($p < 0.018$)

* ± = SEM, S = significant; NS = not significant

Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 67 (2007) 1233–1238
Theriogenology
www.theriojournal.com

Carbon-activated gas filtration during in vitro culture increased pregnancy rate following transfer of in vitro-produced bovine embryos

J.S. Merton [a,*], Z.L. Vermeulen [a], T. Otter [a], E. Mullaart [a], L. de Ruigh [a], J.F. Hasler [b]

Embryo	Incubator status	No. embryos	Pregnant (%)
Fresh	Control	401	41.0[a]
Fresh	CODA	381	46.3[b]
Frozen/thawed	Control	298	35.6[c]
Frozen/thawed	CODA	337	40.8[a]

Means with different superscripts (a–c) differ ($P < 0.05$).

Were VOCs measured? Other explanations?

Other studies indicate no benefit McLellan et al. 2001; Battaglia et al. 2001; Higdon et al. 2008

Can air quality be isolated as the causative factor?

- Were VOCs measured in the “pre” time period
  - Hard to pinpoint an event as a cause of poor air quality, especially in a large city
- Was air filtration system the only variable changed?
  - No other lab improvements?
  - No differences/trends in staff?
  - No new culture items (dishes, media, incubators)?
  - No new clinical staff or variables (stims, etc)?
- “Post” air quality to confirm variable was changed
  - Could still have poor air quality from items within the lab off-gassing, printers or other sources within the lab, gases/incubator VOCs

What are relevant values?

Common Sense Air Quality

- Air quality/VOCs likely impact embryo development
- Prudent to take preventative measures (Standard of care)
  - HVAC VOC filtration
    - dedicated, proper intake placement , # exchanges, filters, etc
  - Inline gas filters
  - Appropriate design & building materials
  - Burn-in & off-gassing before use

IVF Lab Air Quality Requirements

Table 1 Ambient air quality requirements for IVF laboratories operating under regulatory directives in the European Union and Brazil.

	European Union (EU directive 2004/23/EC; 2006/86/EC)	*Brazil (Anvisa RDC33/2006; RDC23/ 2011)*
Particle filtration	Equivalent to GMP[a] grade A air quality in the critical areas where tissues or cells are exposed to the environment during processing with a background environment at least equivalent to grade D[b]	At least equivalent to ISO class 5 (NBR/ISO 14644-1) in the critical areas where tissues or cells are exposed to the environment during processing
Microbial contamination	Maximum colony forming units (cfu) in grades A and D air quality environments defined as follows: air sample (cfu/m^3: <1 and 200), 90-mm diameter settle plates (cfu/4 h: <1 and 100), 50-mm diameter contact plates (cfu/plate: <1 and 50), 5-finger glove print (cfu/glove: <1 and 'not defined')	Microbiological monitoring required; specifications not defined
Volatile organic compound filtration	Not required	Ventilation systems should be equipped with filters imbedded with activated carbon

Light and the IVF Lab

- Common recommendations include specific lighting or limiting lighting in the IVF lab
  - Use of incandescent bulbs or UV sleeves
- Concern from some about windows in the IVF lab and possible damaging effects of light

Are these concerns based on data?

Cleavage of Mammalian Ova inhibited by Visible Light

JOSEPH C. DANIEL, JUN.

Department of Biology,
University of Colorado,
Boulder.

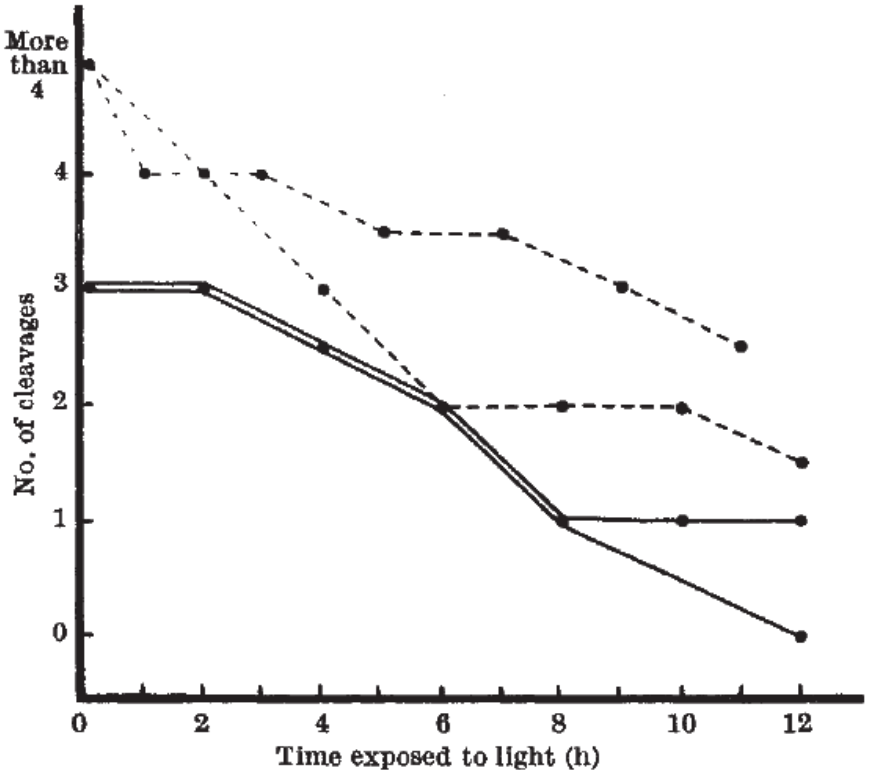


Fig. 1. Effect of visible light on cleavage of rabbit ova *in vitro*. Solid line, experiments terminated after 24 h in culture. Dotted line, experiments terminated after 48 h in culture. The top curve represents an experiment where the ova were shielded against ultra-violet and infra-red wave-lengths

- Fluorescent light 6inches above rabbit embryos
- Red light filters reduced damaging effects

Detrimental Effect of Visible Light on Meiosis of Mammalian Eggs In Vitro

Y. HIRAO AND R. YANAGIMACHI
Department of Anatomy and Reproductive Biology, University of Hawaii School of Medicine, Honolulu, Hawaii 96822 U.S.A.

ABSTRACT Short wavelength visible light (<470-480 nm) emmitted from ordinary light sources is detrimental to unfertilized hamster eggs in that prolonged exposure to the light disturbs the completion of normal meiosis after the eggs are penetrated by spermatozoa. The fluorescent light commonly used in modern laboratories is more harmful than the light from incandescent lamps. In experiments involving the handling of eggs in vitro, minimal exposure to the light or the use of appropriate filters (e.g., red cellophane sheets) is recommended.

Comparison of the detrimental effect of light from four different light sources: % eggs that underwent meiosis and pronuclear development normally

Time of irradiation (min)	Incandescent lamp	Fluorescent lamp	UV lamp	Sun
0.25				43.0
1.0				7.5
1.5				0
5			66.7	
10			51.0	
15	100.0	84.5	10.0	

Light & Embryo Development

TABLE 2

Effects of light intensity[a] during embryo manipulation on preimplantation development of hamster embryos cultured in vitro.

Light strength (lux)	No. of replications (treated hamsters)	Total no. of 2-cell embryos retrieved	No. (%) of embryos developed to		
			4-cell	Morula	Blastocyst
200	6	110	109 (99)	106 (96)[b]	68 (62)[b]
500	6	101	100 (99)	95 (94)[b]	51 (50)[b,c]
900	6	114	111 (97)	96 (84)[c]	44 (39)[c]

Note: Model treatment effect in the development to the 4-cell, morula and blastocyst stages, which was indicated as *P* value, was .5022, .0026, and .0022, respectively.

[a] Light intensity derived from electric bulb of stereo microscope during embryo manipulation in dark room was measured by illuminometer.

[b,c] Different superscripts within the same parameter were significantly different; $P < .05$.

Oh. Optimization of embryo culture system. Fertil Steril 2007.

Intensity is important

Oh et al. 2007

Light & Embryo Development

TABLE 3

Effects of light wavelength[a] during embryo maniplulation on preimplantation development of hamster embryos cultured in vitro.

Light wavelength (nm) selected by color filter	No. of replications (treated hamsters)	No. of 2-cell embryos retrieved	No. (%) of cultured embryos developing to		
			4-cell	Morula	Blastocyst
390–750 (visible ray, no filtering)	4	69	69 (100)	67 (97)[b]	45 (65)[b]
445–500 (blue ray)	4	61	61 (100)	52 (85)[c]	30 (49)[c]
500–575 (green ray)	4	96	94 (98)	94 (98)[b]	69 (72)[b,d]
575–585 (yellow ray)	4	80	79 (99)	77 (96)[b]	57 (71)[b,d]
620–750 (red ray)	4	69	69 (100)	69 (100)[b]	58 (84)[b,e]

Note: Model treatment effect in the development to the 4-cell, morula and blastocyst stages, which was indicated as *P* value, was .4366, .0003, and .0005, respectively.

[a] The same light strength of 200 lux was used.

[b-e] Different superscripts within the same parameter were significantly different; $P < .05$.

Wavelength important

Blue light damaging – red filter helpful

Oh et al. 2007

Embryos & Light

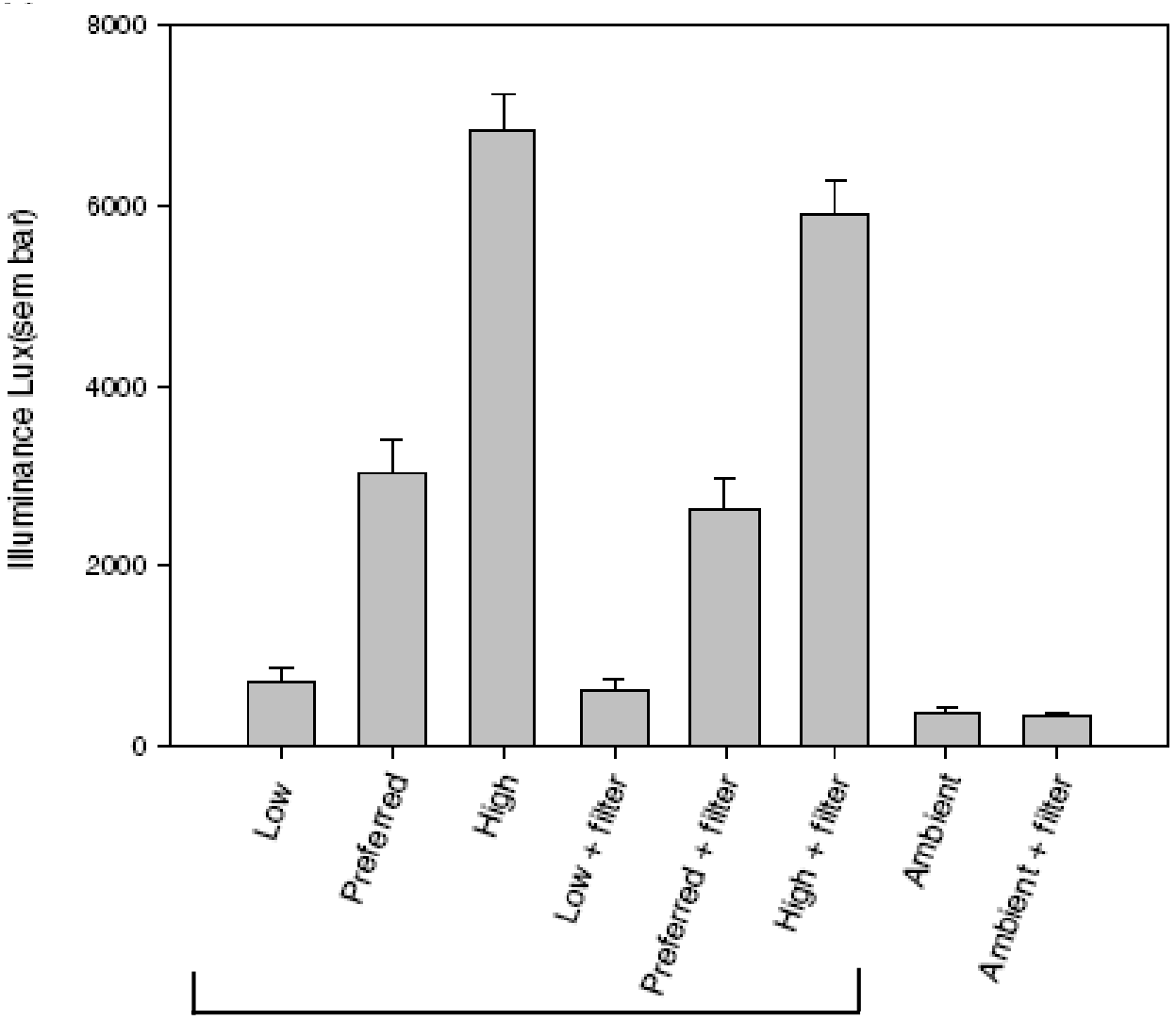
- 1-cell rabbit embryos appear to be more sensitive to light than morula, as evidenced by reduced thymidine incorporation (Schumacher & Fischer 1998, Fischer et al. 1998)
  - 1600 lux fluorescent light – 1hr
  - did not control for incubator effects

- 2-cell mouse embryos showed no impact after exposure to fluorescent light – development to blast (Kruger & Stander 1985)
  - 2900 lux fluorescent light- 30min
  - Did not control for mice/embryo quality

- Mouse oocytes showed no impact after exposure to incandescent light-normal development and live birth (Barlow et al. 1992)
  - 4000 lux – 1, 2, 4 hrs

- GIFT laproscopic procedures performed using a Xenon light with higher UV sprectra yielded fewer pregnancies than using a Halogen light (Evans et al. 1999)
  - -no way to verify the light was the cause

Light Intensity & Source



Ottosen et al. 2007

Light & Embryo Development

- Negative impact of light shown in rodent species
  - Hamster, mouse, rabbit
- Reactions with media?
  - Phenol red (Nakayama et al. 1994)
  - Riboflavin, HEPES (Zigler et al 1985)
- Peroxidation of oil?
  - Sunlight, UV (Otsuki et al. 2007)
- Direct impact on embryos (Daniels 1964)
  - Embryo DNA damage (Takahashi et al. 1999)

Light and the Lab

- Most recommend dimmable overhead lighting
  - Incandescent or LED, UV sleeves on fluorescent?
  - Ensure proper mounting for positive pressure
- Dim microscopes or use filters (red)
  - LED lights on microscopes (heat, longevity)
- Windows likely OK
  - Blackout shades
  - Isolate incubators & working areas
  - Bigger issue is impact on HVAC system and temp
  - Maintenance and lab security

Incubators

IVF specific incubators to improve growth conditions

Reproductive BioMedicine Online (2014) 28, 535–547

ELSEVIER

www.sciencedirect.com
www.rbmonline.com

SYMPOSIUM: QUALITY MANAGEMENT IN ASSISTED REPRODUCTIVE TECHNOLOGY

Decisions for the IVF laboratory: comparative analysis of embryo culture incubators

Jason E Swain

Many considerations for incubator selection and operation

Incubator Comparisons

Reference	Incubators Compared	Environmental Outcomes	Clinical Outcomes
Cooke et al. 2002	MINC vs. Forma (water jacket, TC)	Faster temp recovery in MINC	
Fujiwara et al. 2007	MINC vs. Astec (water jacket, TC)	Faster temp and O2 recovery in MINC.	More "good" blasts 15 vs. 8%
Lee et al. 2010	MINC vs. Forma (water jacket, TC, high O2)	Faster temp, humidity and CO2 recovery	Higher fert in MINC No diff in day 3 or blast formation
Cruz et al. 2011	Embryoscope vs. HeraCell (high O2)		No diff in blast rates or pregnancy
Kirkegaard et al. 2012	Embryoscope vs. Galaxy R (high O2)		No diff in embryo quality or preg/implantation rates

Several variables not controlled

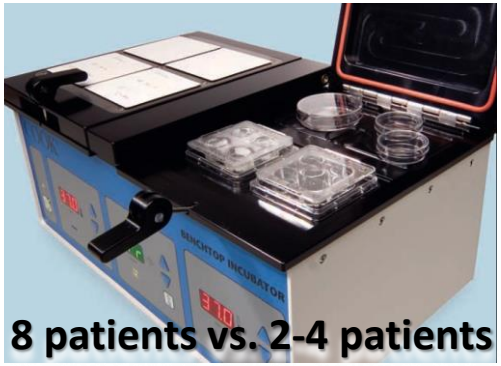
Incubator Features

- Features to consider when selecting a new incubator
  - CO_2 Sensor (IR vs. TC)
  - O_2 sensor (Zirconium vs. Galvanic)
  - Gas mixer or premixed gas supply
  - Water jacket vs. air jacket vs. direct heat
  - Humidity vs. none
  - Other (internal fans, internal HEPA, UV, etc)
    - Recirculation of incubator air through filters
  - Size/capacity
  - Cost

Incubator Management

- Use adjuncts to improve growth conditions in box incubators
  - Inner doors
  - Temp plates
  - Air filters

- Limit on patient #
  - Based on daily/weekly use *(not annual)*



Newer incubators still require proper use

Incubator Management

- A mix of incubators is practical in the modern IVF lab
  - Larger box-type incubators for sperm swim-up and dish equilibration, FBTs, isolation conditions, cameras

Primo Vision™

Eeva™

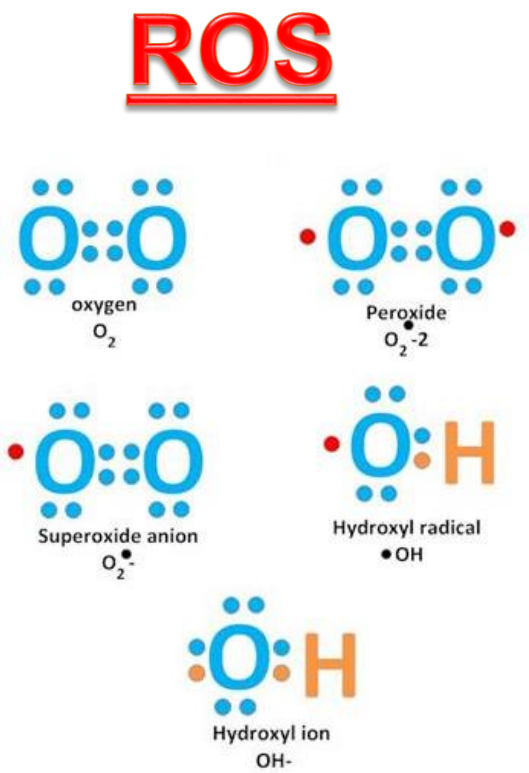
  - Smaller benchtop units for extended embryo culture

Layout/Workflow

- Placement in low traffic area
- Avoid placement under air vents
- Proximity to assessment area

Low Oxygen & Embryo Culture

- O_2 levels in the female reproductive tract are ≤8%
  - Atmospheric O_2 ~21%
- High O_2 may result in increased oxidative stress
  - apoptosis, membrane damage, DNA damage, altered gene expression & epigenetics
- Low O2 improves embryo metabolism
- Low O2 may improve air quality?
  - Filtered N2 instead of air



Are there any publications where low O_2 *decreases* embryonic development or other measured parameters? **NO!**

Low O_2 & Human Embryos

- Dumoulin et al. 1995 Fert Steril 63:115-119
- Dumoulin et al. 1999 Hum Reprod 14:464-469
- Dumoulin et al. 2000 Hum Reprod 15:402-409
- Catt and Henman 2000 Hum Reprod 15(suppl 2):199-206
- Bedaiwy et al. 2004 Fertil Steril 82:593-600
- Bahceci et al. 2005 RBMonline 11:438-443
- Petersen et al. 2005 Acta Obstet Gynecol Scand 84:1181-1184
- Bedaiwy et al. 2006 Fertil Steril 86:304-309
- Kea et al. 2007 Fertil Steril 87:213-216
- Anderson et al. 2007 Fertil Steril 88(suppl 1):S91
- Waldenstrom et al. 2009 Fertil Steril 91:2461-2465
- Kovacic and Vlaisavljevic 2008 RMBonline 17:229-236
- Meintjes et al. 2009 Hum Reprod 24:300-307
- Ciray et al. 2009 Fertil Steril 91(4 Suppl):1459-61
- Higdon et al. 2009 J Clinical Embryology (Fall) 12:6-11
- Nanassy et al. 2010 Fertil Steril 93:579-585
- Guo et al. 2014 Int J Clin Exp Path. 7(9):6191-8
- Kasterstein E. 2013 J Asst Reprod Genet 30(8):1073-9

Human Reproduction, Vol.24, No.2 pp. 300–307, 2009
Advanced Access publication on October 16, 2008 doi:10.1093/humrep/den368

human reproduction ORIGINAL ARTICLE *Embryology*

A controlled randomized trial evaluating the effect of lowered incubator oxygen tension on live births in a predominantly blastocyst transfer program†

Marius Meintjes[1,3], Samuel J. Chantilis[2], James D. Douglas[2], Alfred J. Rodriguez[2], Ali R. G[illegible]rami[2], David M. Bookout[2], Brian D. Barnett[2], J[illegible] Madd[illegible]n[1]

[illegible] Institu[illegible]prod[illegible] [illegible]spit[illegible]gram

Low oxygen Days 1 – 5/6

Parameter	21% Oxygen	5% Oxygen	P-value[a]
Implantation rate	95/267 (35.6%)	122/247 (49.4%)	0.003
Live-birth implantation rate	82/267 (30.7%)	106/247 (42.9%)	0.005
Clinical pregnancy rate	56/115 (48.7%)	74/115 (64.3%)	0.027
Live birth rate	49/115 (42.6%)	66/115 (57.4%)	0.043

Human Reproduction Update, Vol.19, No.3 pp. 209, 2013
Advanced Access publication on February 1, 2013 doi:10.1093/humupd/dms055

human reproduction update

IN A NUTSHELL

Low oxygen concentrations for embryo culture in assisted reproductive technologies

Background : **Results**

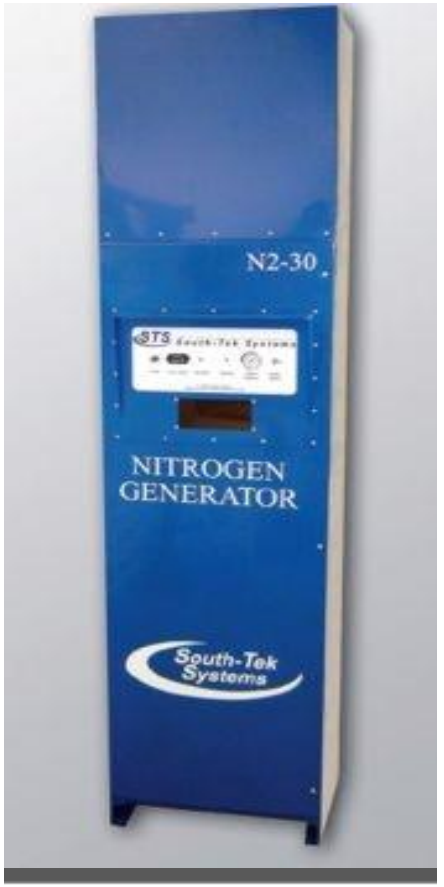
During

Conclusions

The results of this systematic review and meta-analysis suggest that culturing embryos under low oxygen concentrations improves the success rates of IVF/ICSI, resulting in an increase in the live birth rate.

Low Oxygen Approaches

- Nitrogen Supply Options
  - Compressed gas cylinders
    - Single gas
    - Premixed
  - Liquid nitrogen
  - Nitrogen Generator



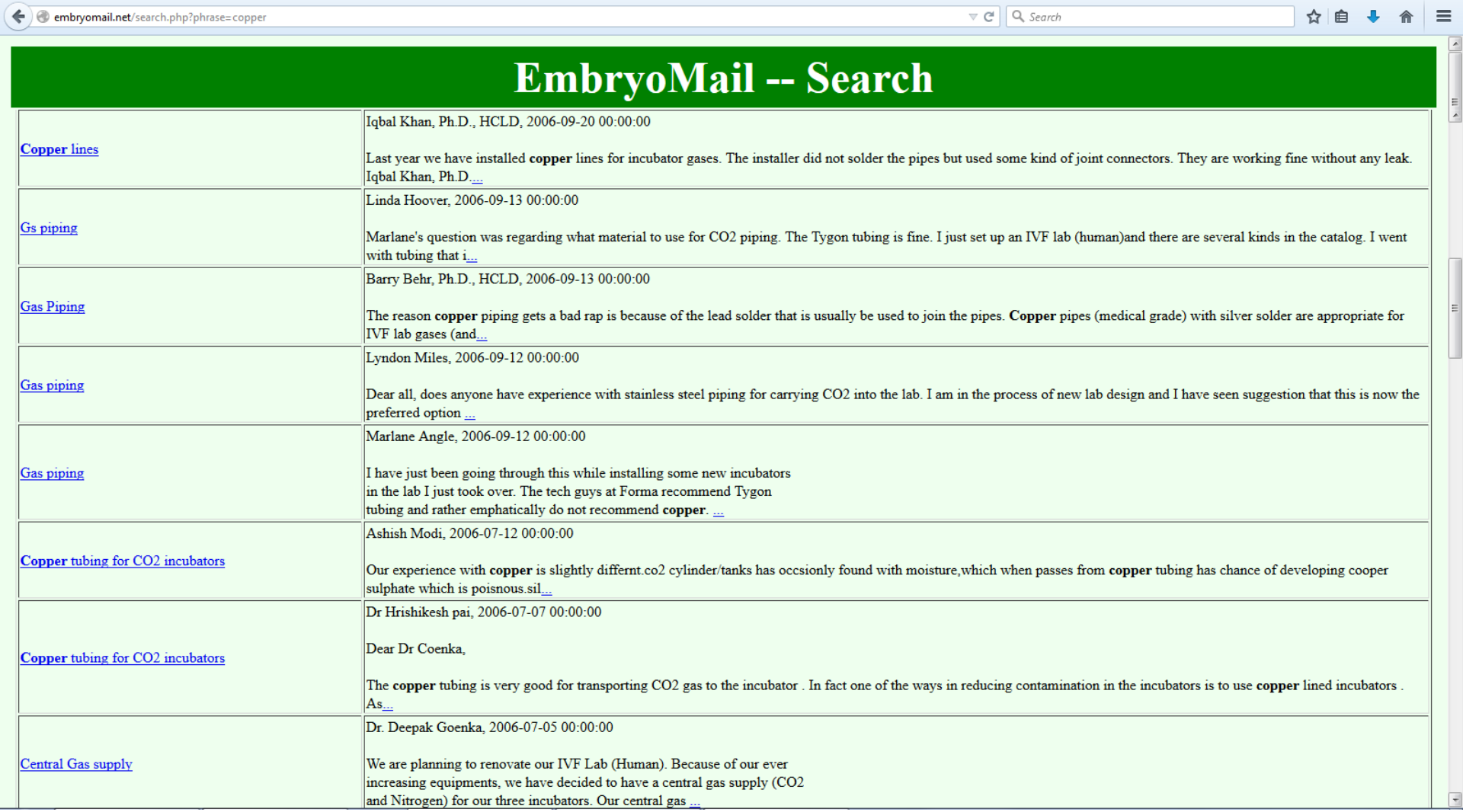
Advantages/Disadvantages to each

Gas Supply

Numerous dedicated outlets

Step-down regulators

Type of gas lines to use?



Numerous discussions on type of piping/tubes to use for incubators
Specific concerns with copper

Copper & Embryo Development

- 2-6ug/ml Copper added to bovine IVM improved blastocyst development and cell # (Picco et al. 2012)
- 0.46-0.68mg/L Copper added to culture media improved bovine morula and blastocyst formation (Gao et al. 2007)
- 100uM Copper inhibited mouse embryo development (Vidal and Hidalgo 1993)
- 0.00005M Copper addition to media lead to rabbit blastocyst degeneration (Verdugo et al. 1981)
- >~0.00001M Copper may impair fluid transport in rabbit blastocysts (Cross 1973)

Are these relevant?

Copper & Toxicity?

- Copper sulfate concerns with moisture in lines?
  - Inline gas filters to address
- Numerous humidified copper lined incubators exist
  - No apparent issues
- Antimicrobial properties (Borkow & Gabbay 2005)
- Avoid soldering?

DEVELOPMENT OF SCREENING SYSTEMS FOR EVALUATION OF MATERIALS USED IN MAMMALIAN EMBRYO TRANSFER

B. E. Lee,[1] W. R. Boone,[2] P. O. Brackelsberg,[1,3] and R. A. Carmichael,[2]
[1]Iowa State University, Ames, IA 50011
[2]Maplehurst Ova Transplants, Keota, IA 52248

Table 2. Effect of co-culture of mouse embryos with various tubing types

Treatment	Embryonic Stage: 4 to 16 cell		Morula		Blastocyst	
	No. Embryos	% Developed[a]	No. Embryos	% Developed[a]	No. Embryos	% Developed[a]
Embryological watch glass	19	89[b]	12	92[b]	9	100[b]
Silastic tubing	16	62[b]	13	92[b]	9	78[b]
Tygon tubing	16	62[b]	12	83[b]	9	89[b]
Latex tubing	16	0[c]	13	15[c]	9	0[c]

Tubing Types

Non-DEHP, Phthalate Free Tubing Products

- Tygon S3™ E-3603 (Replaces Tygon® R-3606)
- Tygon S3™ E-LFL (Replaces Tygon® LFL)
- Tygon S3™ Silver
- Tygon S3™ E-TAAT (Replaces Tygon® TAAT)
- Tygon S3™ M-34-R
- Tygon S3™ Transflow® Vacuum
- Tygon® B-44-3 (No Formulation Change)
- Tygon® B-44-4X (No Formulation Change)
- Tygon® B-44-4X I.B. (No Formulation Change)
- Tygon® ND-100-65 (Replaces Tygon® S-50-HL)
- Tygon® ND-100-80 (Replaces Tygon® S-54-HL)

Applications for Tygon S3™ E-3603

- General Laboratory
- Food and Beverage
- Biopharmaceutical
- Analytical Instruments
- Peristaltic and Vacuum Pumps
- Condensers
- Incubators
- Desiccators
- Gas & Drain Lines

Original Tygon® Tubing Formulations

- Tygon S3™ R-3603 (Replaced by Tygon S3™ E-3603)
- Tygon S3™ LFL (Replaced by Tygon S3™ E-LFL)
- Tygon S3™ TAAT (Replaced by Tygon S3™ E-TAAT)
- Tygon® S-50-HL (Replaced by Tygon® ND-100-65)
- Tygon® S-54-HL (Replaced by Tygon® ND-100-80)

Applications for Tygon® R-3603

- General Laboratory
- Analytical Instruments
- Peristaltic and Vacuum Pumps
- Condensers
- Incubators
- Desiccators
- Gas & Drain Lines

Fertil Steril. 1988 Jul;50(1):110-6.

The gamete and embryo compatibility of various synthetic polymers.

Hunter SK[1], Scott JR, Hull D, Urry RL.

Author information

Abstract

Several popular and well-characterized polymeric materials were evaluated for their biocompatibility toward the cells unique to reproduction. To accomplish these studies, several in vitro tests were developed that evaluated biocompatibility between the polymers and spermatozoa, ova, and embryos. The data indicated significant differences between the materials with respect to their biocompatibility toward sperm motility, the sperm's ability to penetrate zona-free hamster eggs, and the ability of two-cell mouse embryos to divide. Polytetrafluoroethylene (PTFE-Teflon; PTFE, Chemplast Inc., Wayne, NJ), polyethylene glycol (PEG), and polyhydroxyethyl methacrylate (PHEMA) appear to be the most inert of the materials studied. Polyvinyl chloride (PVC; Tygon-Norton, Akron, OH) was found to be the most detrimental material toward gametes and embryos, with gross physiologic and morphologic changes observed in the PVC-exposed cells.

Gas Supply

- Types of gas lines
  - Stainless steel
  - Copper (braized, not soldered)
  - Tygon

- Factors to consider
  - Cost
  - Security
  - Permeability
  - toxicity

Summary

- Conclusive proof for IVF lab design is often lacking
- Many IVF lab design recommendations are common-sense approaches
- Better to err on the side of caution
- Constant oversight required to ensure proper IVF lab construction

Acknowledgements

NATIONAL FOUNDATION FOR
Fertility Research

jswain@fertilitylabsciences.com