LAB FACTORS THAT INFLUENCE OUTCOMES

MIKE ABEYTA
ASSOCIATE LAB DIRECTOR
CCRM SF

3.20.24
PCRS

DISCLOSURES

NO CONFLICTS TO DISCLOSE

OBJECTIVES

1. CHARACTERIZE THE ROLE OF THE IVF LAB IN A TREATMENT CYCLE
2. IDENTIFY THE MAIN LABORATORY FACTORS THAT INFLUENCE OUTCOMES
3. DISCUSS THE VARIABLES THAT CHALLENGE OUR ABILITY TO STABILIZE/CONTROL THE LAB ENVIRONMENT

THE ROLE OF THE IVF LAB

CLINICAL ~~EMBRYOLOGISTS~~ ALCHEMISTS?

- "MAGIC"
- "SECRET SAUCE"

THE ROLE OF THE IVF LAB

THE REALITY IS THAT THERE IS A MAXIMUM STARTING POTENTIAL OF EACH GAMETE AND THE POTENTIAL CAN ONLY DECREASE THROUGHOUT THE PROCESS, NOT INCREASE

THE ROLE OF THE IVF LAB

GOAL OF THE IVF LAB IS TO LIMIT EXPOSURE TO THE THINGS THAT CAN CAUSE HARM AND TRY NOT TO BE ONE OF THE THINGS THAT CAUSES HARM

WHAT WE IMAGINE THE IVF LAB TO BE

REALITY

CUMULATIVE STRESS

LITTLE

THINGS

ADD

UP

IT IS ESTIMATED THAT THERE ARE OVER 200 VARIABLES THAT CAN IMPACT OUTCOMES IN THE IVF LABORATORY.

THOMAS B. POOL, 2012

STRESSORS

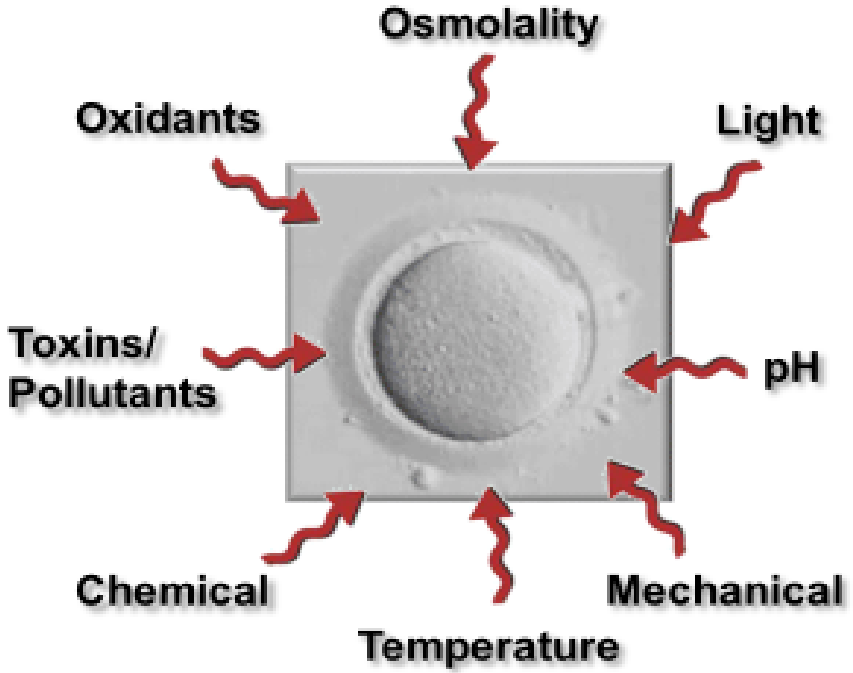
- **THE SCIENCE**: PH, OSMOLARITY, TEMPERATURE, AIR QUALITY, CONTAMINATION
- **THE TECHNICAL COMPONENT**: PROTOCOLS, TOOLS, TECHNICAL SKILL/EXPERIENCE
- **THE LOGISTICS**: TRAINING, TIMING, WORKFLOWS, STAFFING, EQUIPMENT CHOICES, DISTRACTIONS
- **THE HUMAN ELEMENT**: KNOWLEDGE, ASSUMPTIONS, PHYSICAL ERRORS, MENTAL ERRORS
- **THE MACHINE ELEMENT**: EQUIPMENT USAGE, EQUIPMENT MALFUNCTION
- **THE QUALITY COMPONENT**: QUALITY MANAGEMENT PROGRAM – QC, QA

THE SCIENCE

THE MODERN IVF LAB

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
- Modern lab equipment can help with consistency & may reduce stress with higher cycle volumes and evolving techniques
- Proper lab design & QC can be instrumental



AIR QUALITY

TOXINS/POLLUTANTS

VOCs

INSIDE

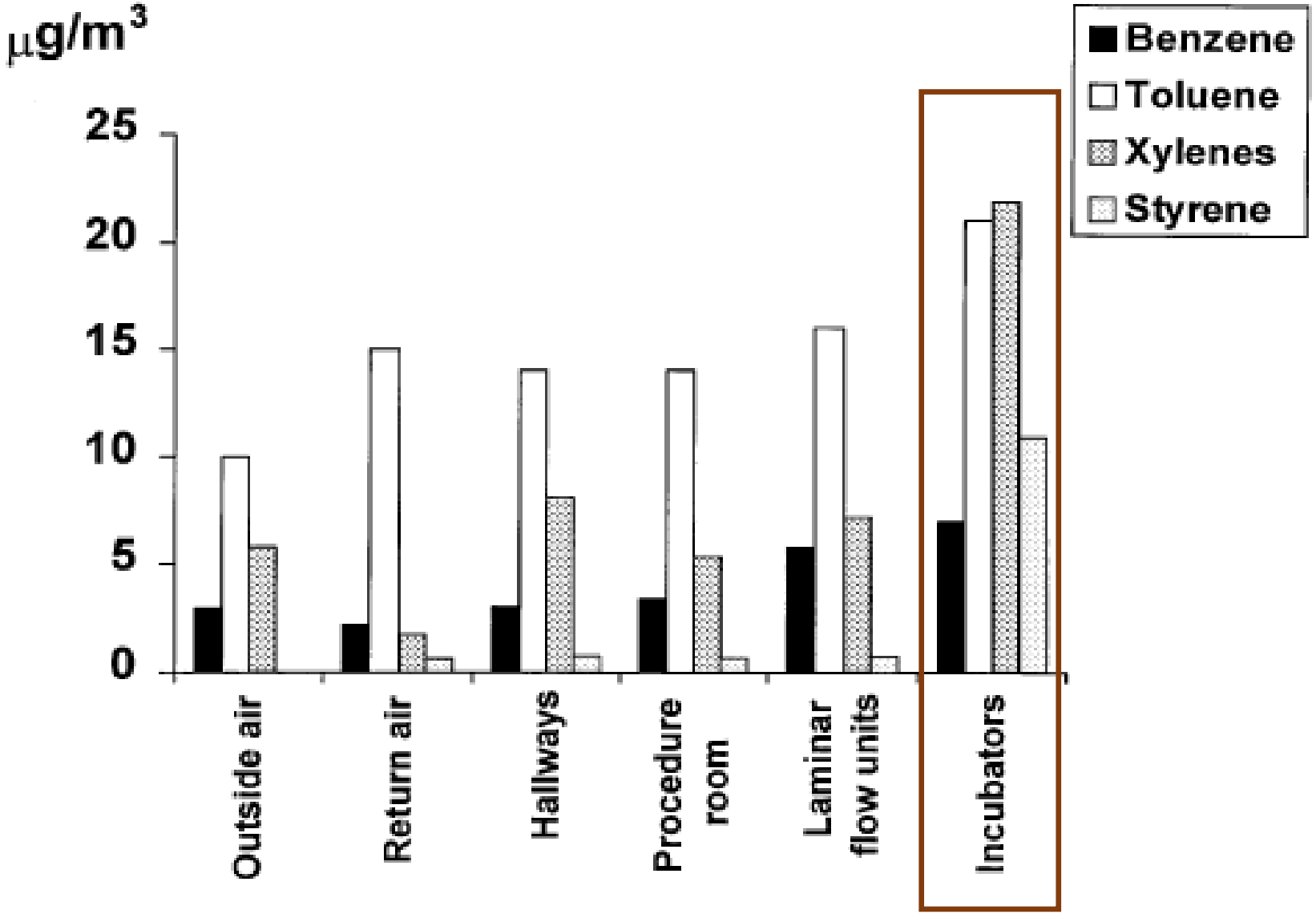
OUTSIDE

POSITIVE PRESSURE

AIR CHANGE OVER

AIR QUALITY

VOCs by Location



Cohen et al. 1997

VOC SOURCES

Contributors to Incubator VOCs

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

Volatile organic compound	µg/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

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

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 ($p < 0.018$)

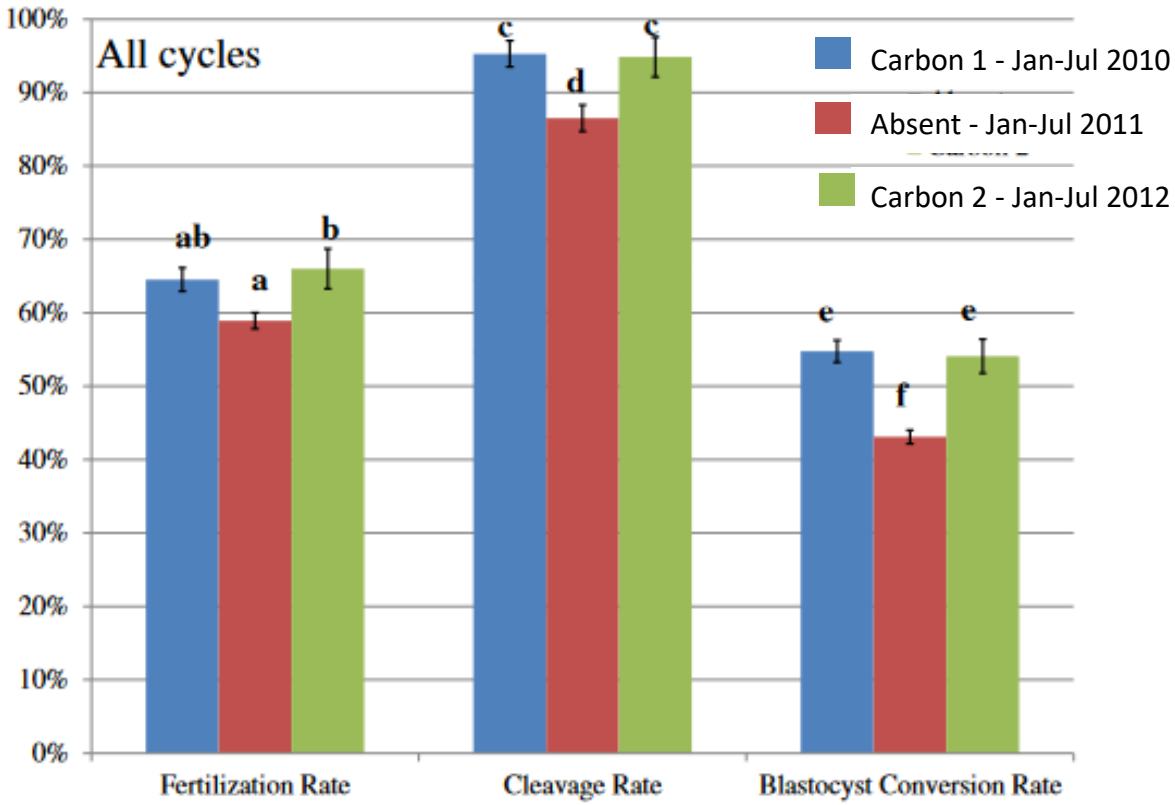
* ± = SEM, S = significant; NS = not significant

J Assist Reprod Genet (2015) 32:1009–1017
DOI 10.1007/s10815-015-0495-1

EMBRYO BIOLOGY

Lack of carbon air filtration impacts early embryo development

Erika M. Munch[1] · Amy E. Sparks[1] · Hakan E. Duran[1] · Bradley J. Van Voorhis[1]



PRACTICAL AIR QUALITY MANAGEMENT

- CARBON FILTERS SATURATE
  - How many?
  - How often to change?
- INCUBATOR VOCS
  - Change water pans
- GAS TANK CHANGES
  - Bottom of tanks “dirtier”?

TEMPERATURE

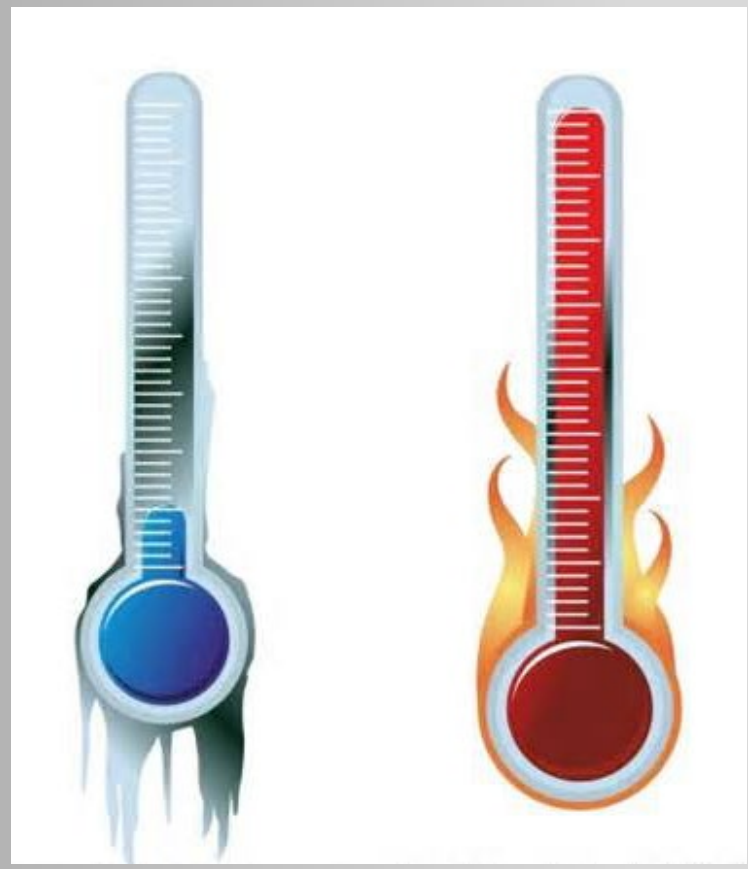
SURFACES

INCUBATORS

ROOM AIR

IMPORTANCE OF TEMPERATURE

- Impacts enzyme kinetics and cell metabolism
- Influences spindle dynamics
- Possible effect on cell division and morphokinetics



All could impact embryo development and resulting outcomes

TECHNIQUES AND INSTRUMENTATION

FERTILITY AND STERILITY®
VOL. 77, NO. 6, JUNE 2002

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

Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates

Wei-Hua Wang, Ph.D.,[a] Li Meng, Ph.D.,[b] Richard J. Hackett, Rudolf Oldenbourg, Ph.D.,[d] and David L. Ke M.D.

ion of Reproductive Medicine, Hospital

System 1: 34°C

System 2: 37°C

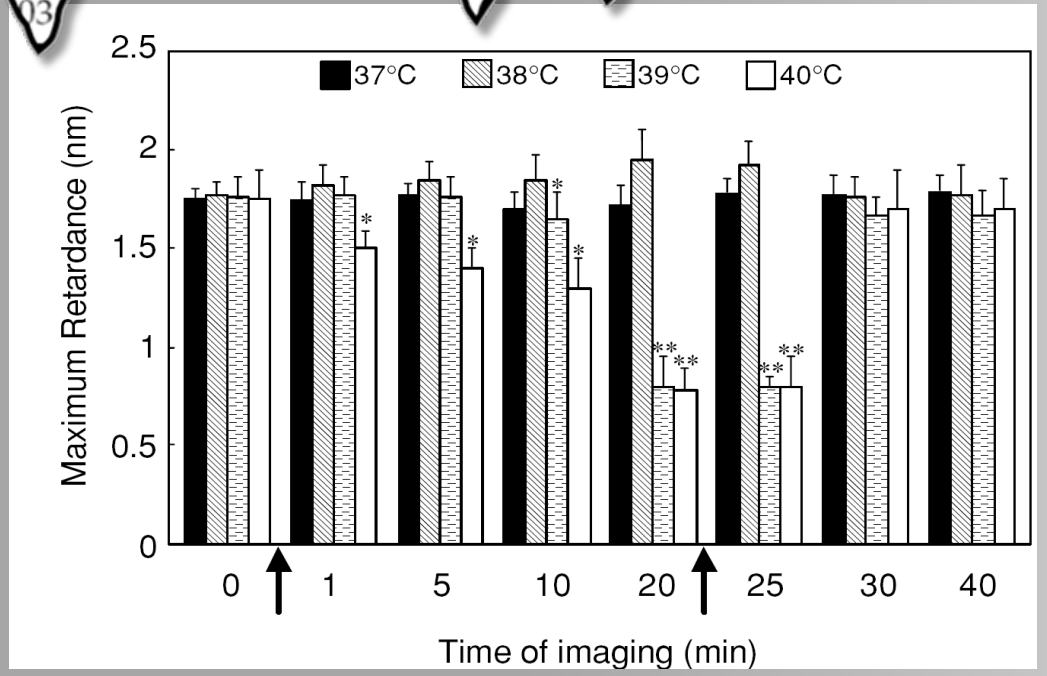
System 3: 33°C

	System 1	System 2	System 3
No. of patients	40	29	52
Average patient's age	33.8 ± 4.4	34.1 ± 4.6	34.1 ± 4.4
Average no. of cycles	2.3 ± 1.4	2.8 ± 1.2	2.6 ± 1.8
Day 3 FSH	6.1 ± 1.8	6.3 ± 2.6	6.2 ± 2.5
E_2 level (pre-hCG)	1346.4 ± 608.3	1344.8 ± 552.4	1417.6 ± 763.5
E_2 level (day for hCG)	1780.3 ± 805.1	1809.0 ± 815.6	1926.8 ± 980.8
No. of eggs examined	402	298	433
No. of eggs/patient	8.3	10.0	10.3
Eggs with spindle (%)	61.4[a]	81.2[a]	NA
Fertilization rate (%)	56.7[a]	78.8[a]	64.0[a]
Pregnant rate (%)	25.0[a]	51.7[a]	23.1[a]

Zygote **12** (February), pp. 65–70. © 2004 Cambridge University Press
DOI:10.1017/S0967199404002631 Printed in the United Kingdom

Overheating is detrimental to meiotic spindles within *in vitro* matured human oocytes

Xiao-Fang Sun[1], *Wei-Hua Wang*[2] *and David L. Keefe*[3]
Guang-Zhou Second Hospital, G[illegible]g-Dong, China; Tomball Regi[illegible]
[illegible]ospital of Rho[illegible]land, [illegible]

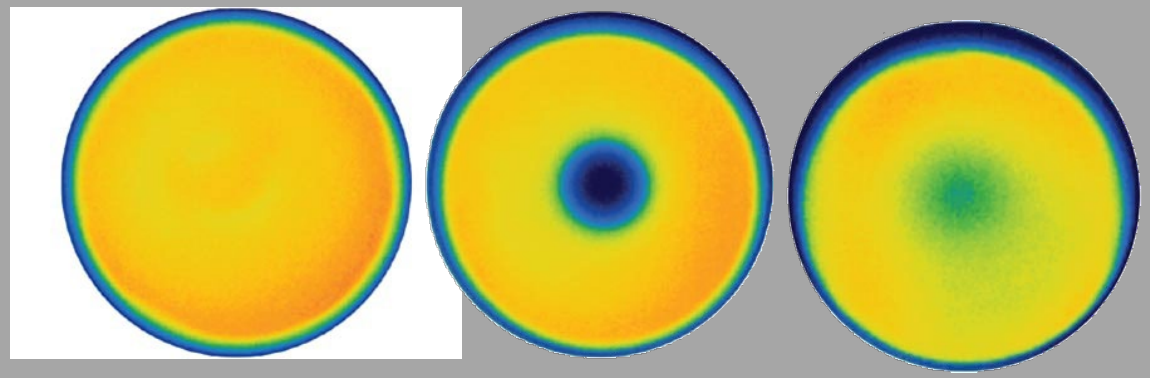


No apparent impact until 39°C

QC IN THE IVF LAB

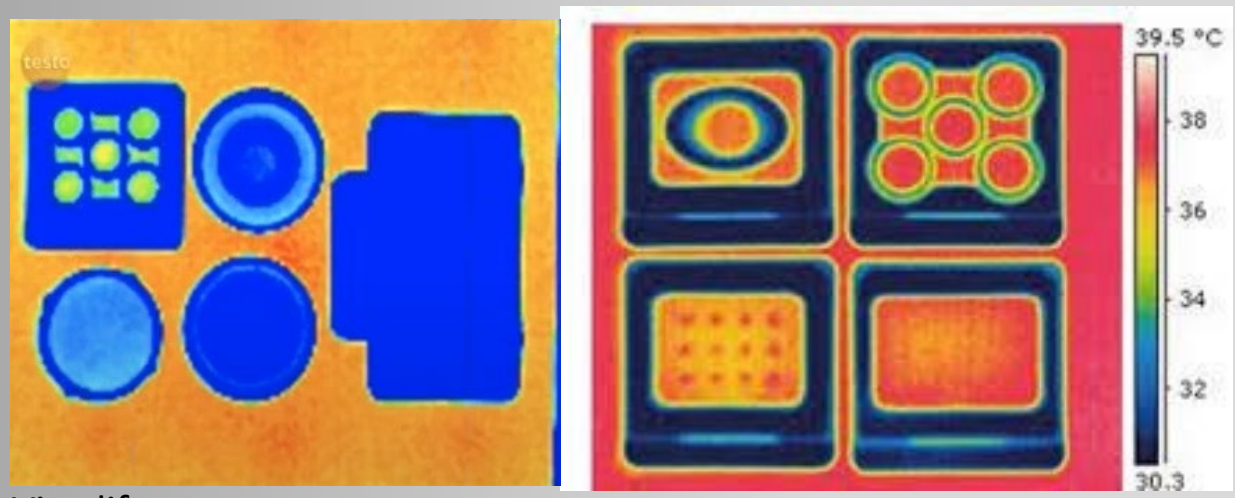
TEMPERATURE

STAGE INSERTS



Stage 1 Stage 2 Stage 3

DISH TYPES



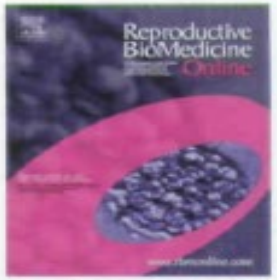
Vitrolife

Stages – Equipment – Dishes – Media Volume - Oil – Lids - Other

SURFACE TEMPERATURE VARIATION

Reproductive BioMedicine Online (2010) 20, 510–515

www.sciencedirect.com
www.rbmonline.com



ARTICLE

Reduction in exposure of human embryos outside the incubator enhances embryo quality and blastulation rate

Jun Qiang Zhang [a], Xiu Ling Li [a], Yuzhu Peng [a], Xirong Guo [b], Boon Chin Heng [c], Guo Qing Tong [a,*]

Open small box incubators to assess embryos
6-times (day 1-6) vs. 4-times day 1, 3,5,6)

Lower total blastocyst formation rate, day-5 blastocyst rate, fewer GQ blastocysts frozen blastocyst per patient from 6x group

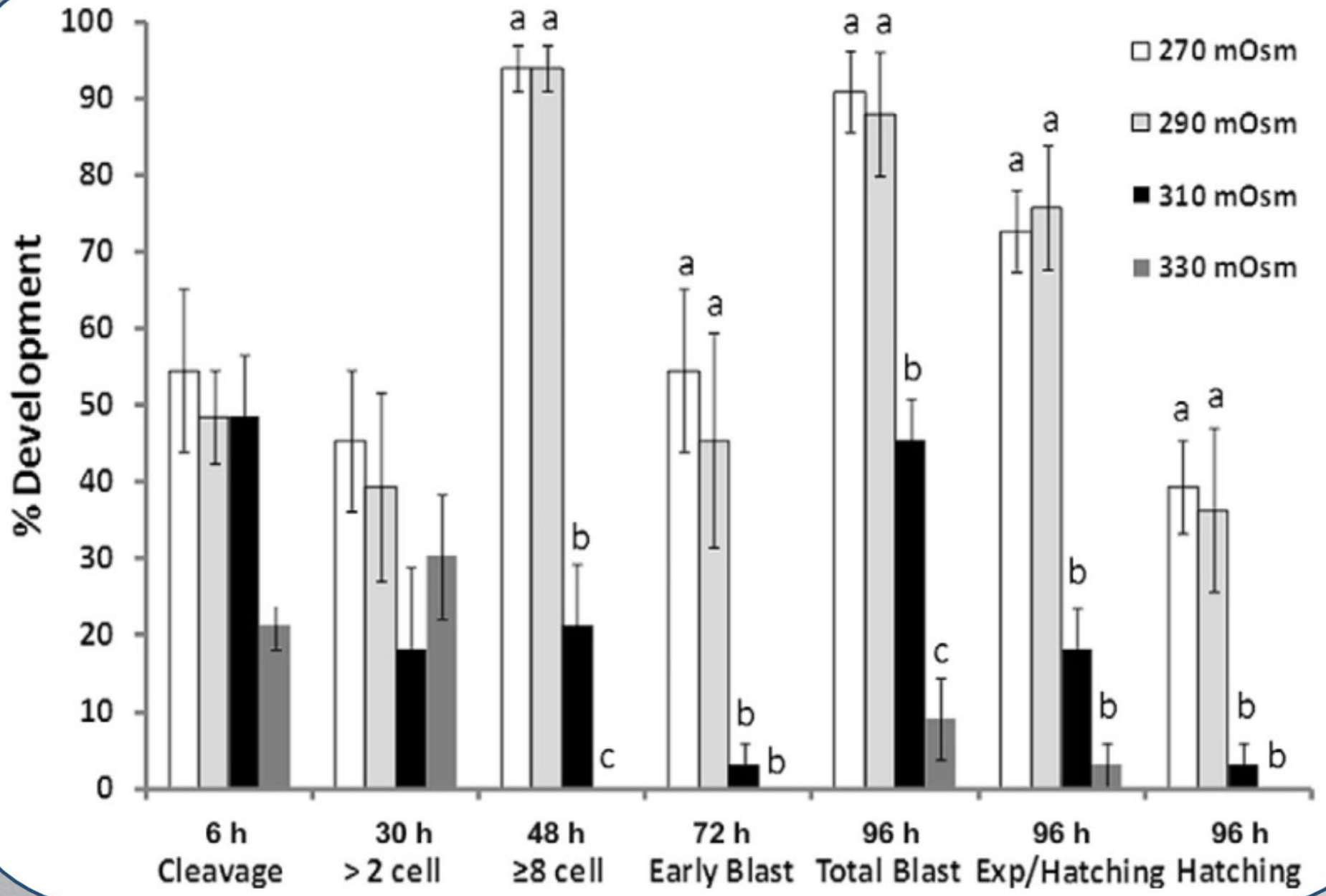
EMBRYO CULTURE

- PH
- OSMOLALITY
- TEMPERATURE

UNINTERRUPTED EMBRYO CULTURE

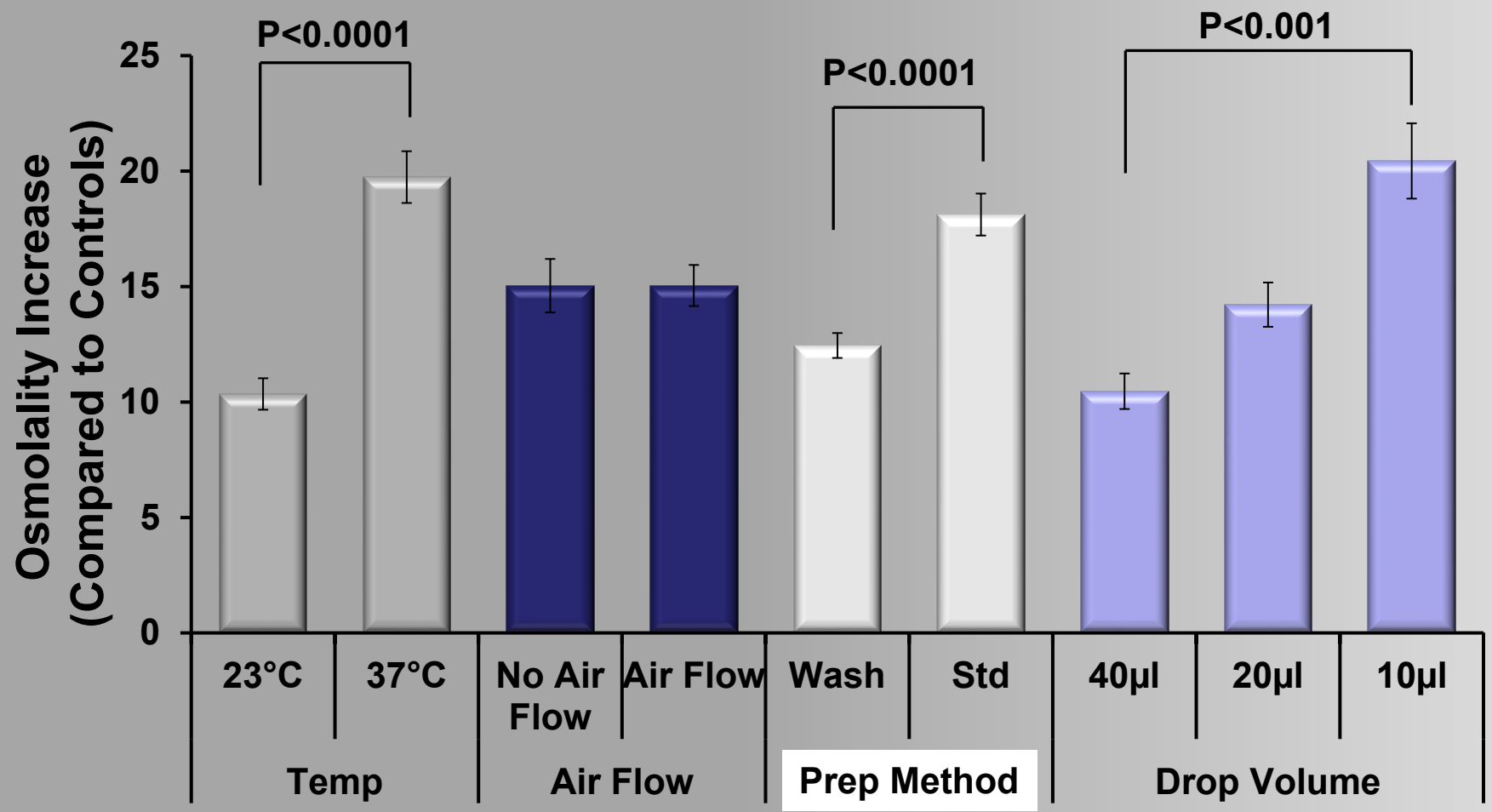
BENEFITS	RISKS
Reduced dish removal from incubator – more stable culture conditions (gas, temperature)	Media degradation (ammonia production, substrate depletion, other component degradation)
Reduced cell handling – reduced risk for cell damage or loss	Missed information or problem detection *(only in non-time lapse systems)*
Accumulation of beneficial autocrine/ paracrine factors	VOC accumulation (oil & media)
Compatible with new time-lapse incubators – new technology, additional selection endpoints	Media evaporation – osmolality increase, pH increase, increase in other solute concentrations (in dry incubators)
Improved workflow – less staff time, possible cost savings	Mineral oil degradation (peroxidation)

OSMOLALITY



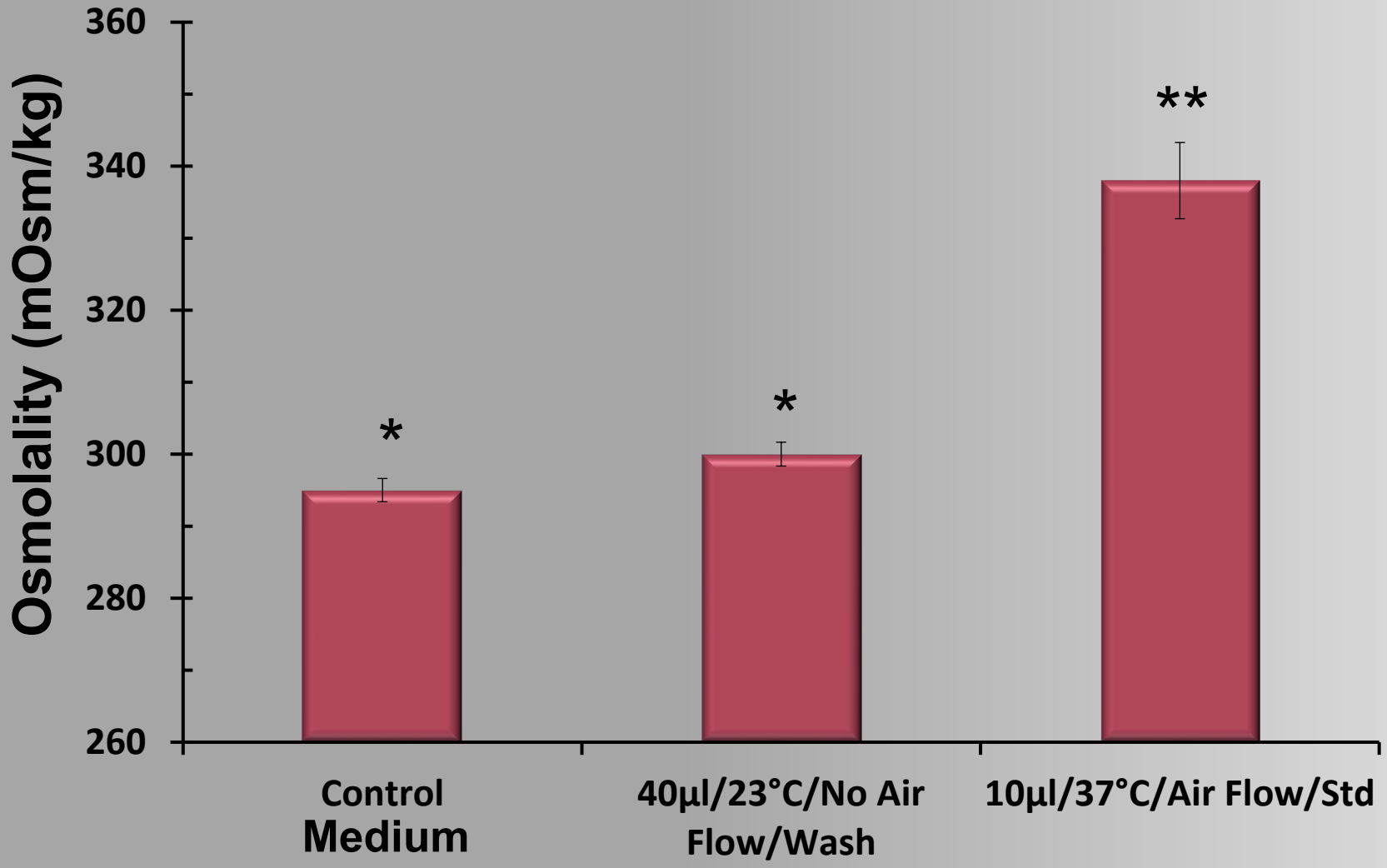
OSMOLALITY IMPACTS CELL VOLUME & EMBRYO DEVELOPMENT

PREPARATION CONDITIONS & OSMOLALITY



Significant interaction of the 4 environmental variables

PREPARATION CONDITIONS & OSMOLALITY



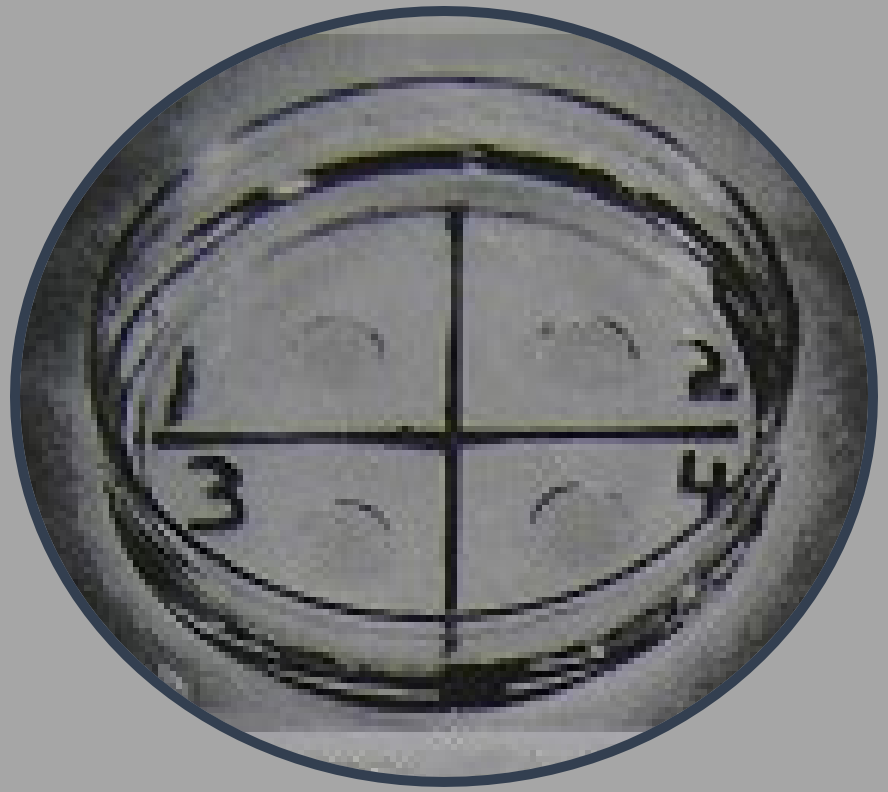
CULTURE MEDIA

Current formulations take osmolality changes into account

Most are formulated near the bottom end of tolerable range

OIL OVERLAY

LONG-TIME STAPLE OF EMBRYO CULTURE



Stabilizer for microdrops

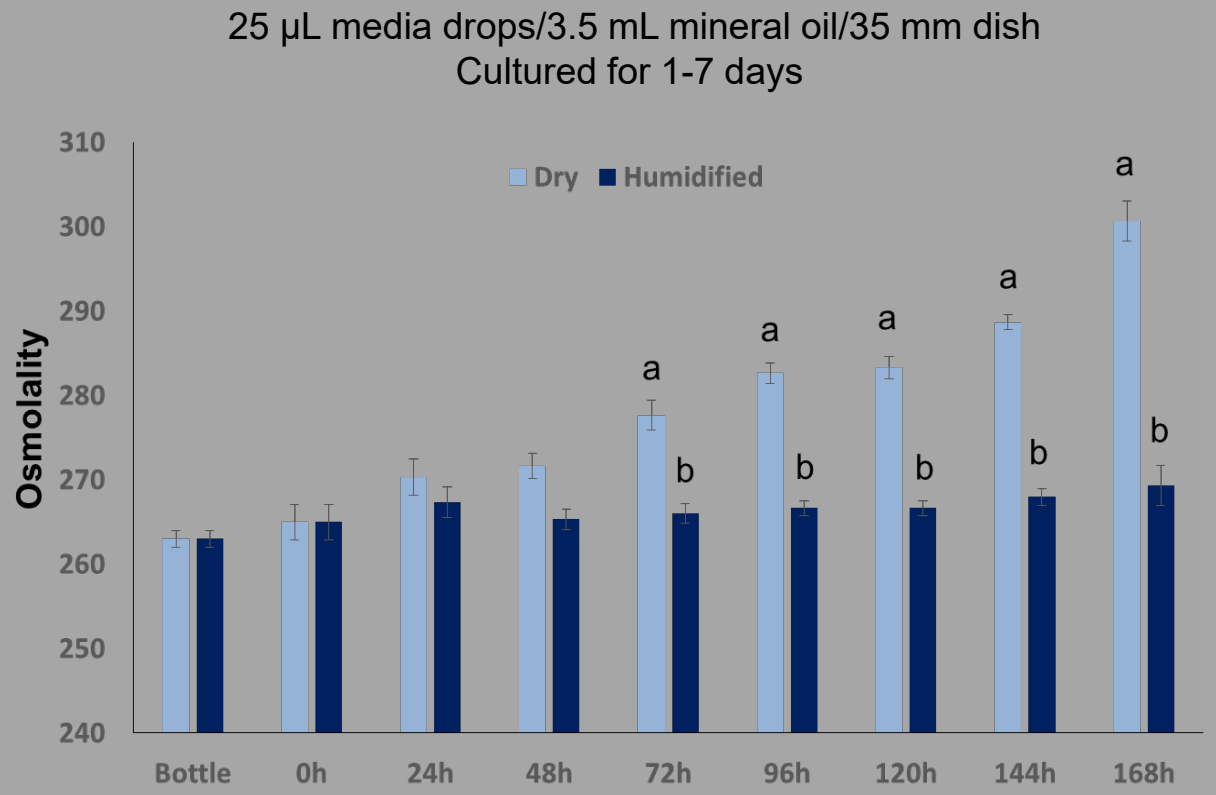
Protectant for cell culture

- -Provides thermal stability
- -Evaporation/osmolality protection
- -Reduce off-gassing/pH stabilization
- -Sink for oil-soluble factors (protective)

Brinster, Exp Cell Res, 1963

OIL OVERLAY

REDUCES MEDIA EVAPORATION



Oil overly can reduce evaporation & resulting osmolality increase

- -required with modern dry incubators
- -important with uninterrupted culture

Evaporation can occur under oil

Critical variables to consider

Swain et al. 2016

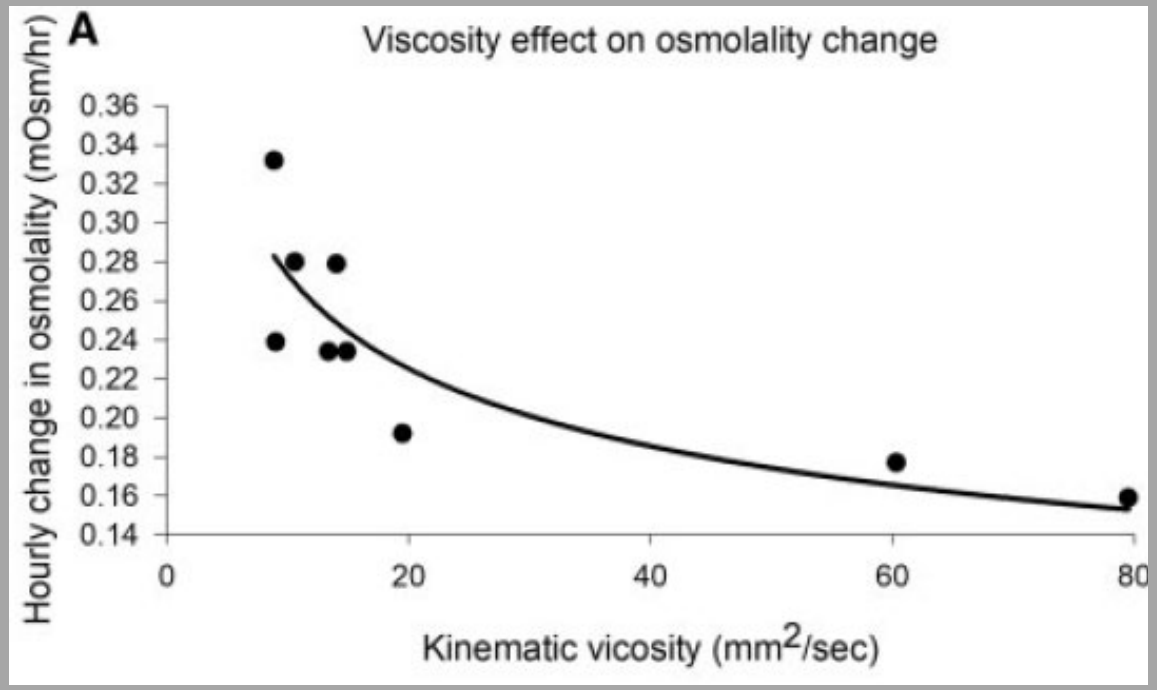
Human Reproduction, Vol.36, No.5, pp. 1230–1241, 2021
Advance Access Publication on February 9, 2021 doi:10.1093/humrep/deab015

human reproduction ORIGINAL ARTICLE *Embryology*

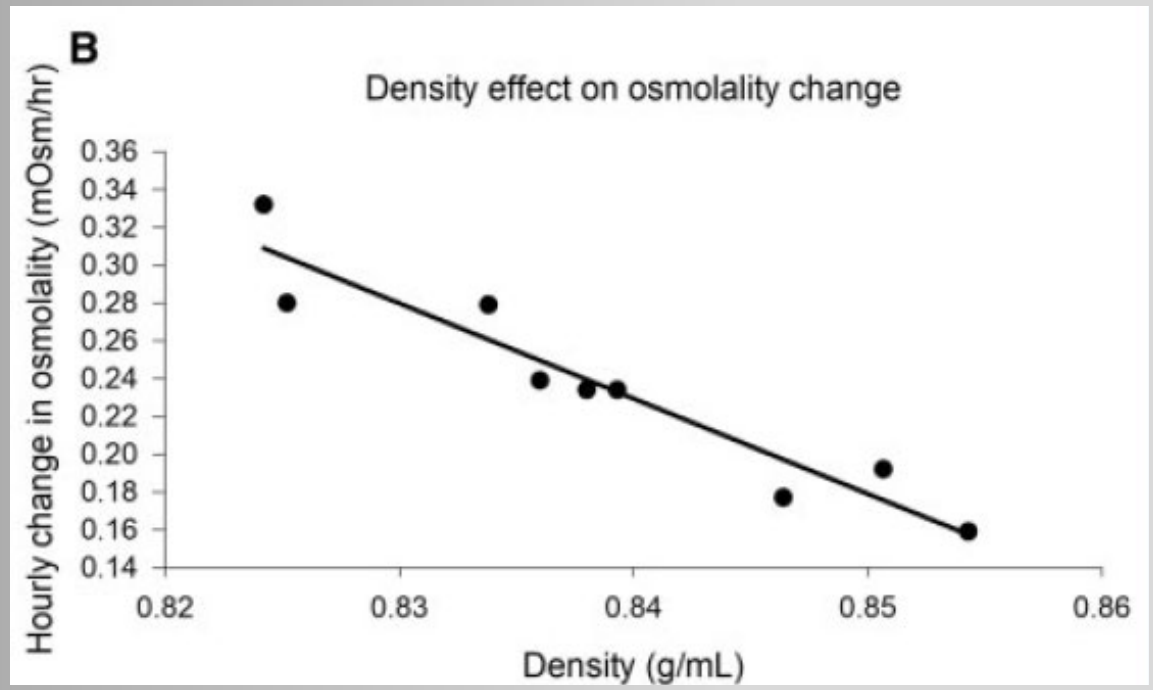
Toward a predictive theoretical model for osmolality rise with non-humidified incubation: a randomized, multivariate response-surface study

Steven F. Mullen*

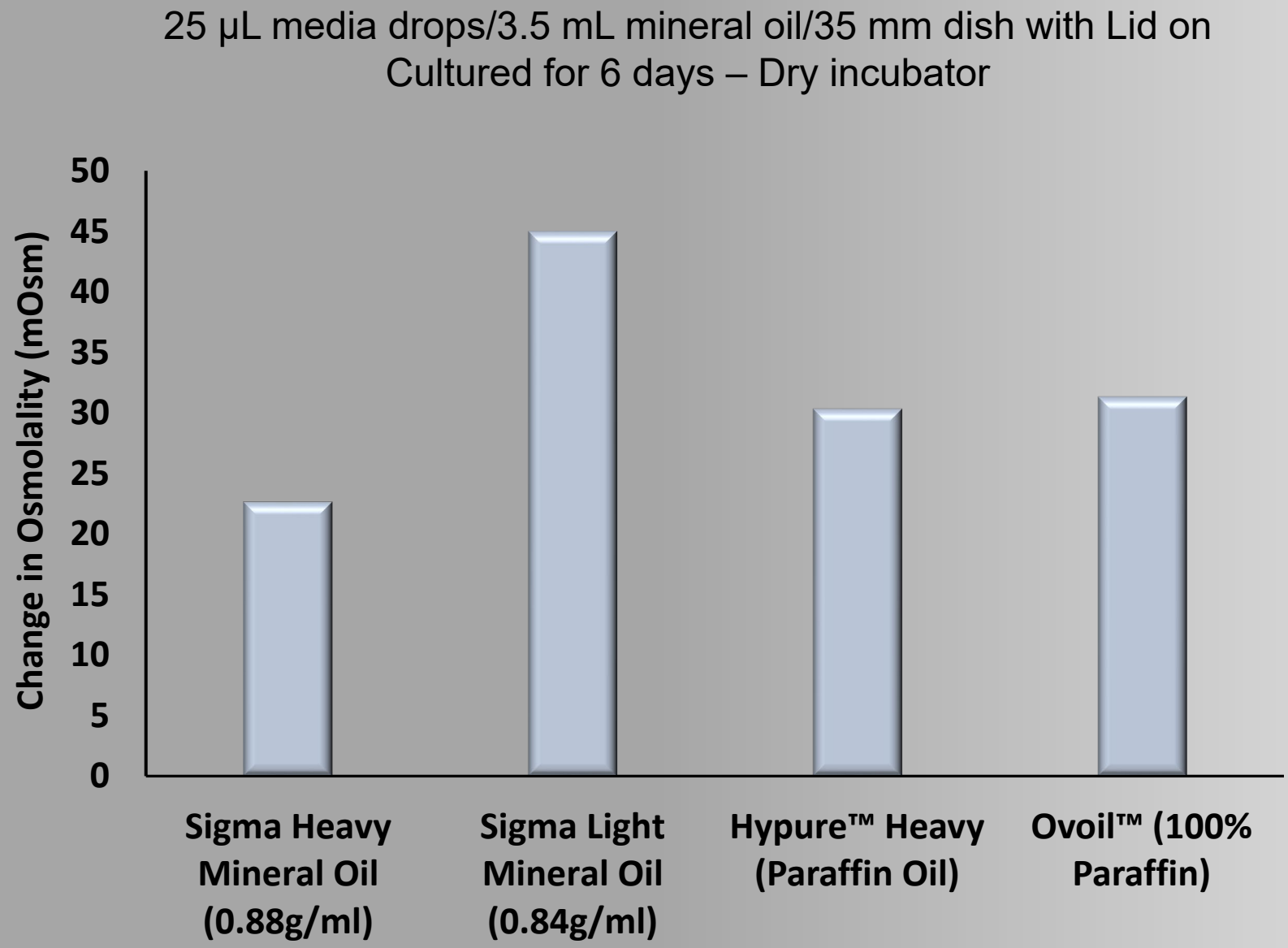
Oil Viscosity



Oil Density



OIL & EVAPORATION



EVAPORATION

VARIABLES TO CONSIDER

Incubator

Humidification

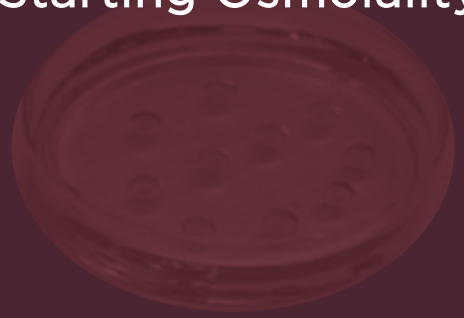
Dish Type Permitted

Media

Surface Area[1]

# of Exchanges
uninterrupted culture

Starting Osmolality



Oil

Volume of Oil
Depth[1]

Type of Oil
Heavy vs. light vs. paraffin
Density/Viscocity[1]

[1] Mullen et al. Hum Reprod 2021
Mestres et al. Hum Reprod 2021
Swain Reprod Biomed Online 2019

TABLE 3 PROPOSED BEST PRACTICES OR CONSIDERATIONS IF IMPLEMENTING OR USING UNINTERRUPTED EMBRYO CULTURE

Use of humidified incubation if possible, especially if culturing to the blastocyst stage, but not required if other variables are optimized.
Monitoring of consistent ambient room humidity (~30–50% recommended)
Use of appropriate medium with a starting osmolality ~255–270 containing the dipeptide form of glutamine (alanyl- or glycyl-)
Use of appropriate medium volume and sufficient oil overlay
Use of appropriate oil type (paraffin or heavy oil)
Use of high-quality oil (low peroxide and volatile organic compounds levels)
Use of volatile organic compounds filtration for laboratory air, medical gas supply and incubator recirculation
Use of high-quality protein supplements with low or no ammonia, and low accumulation
Measurement of medium characteristics (pH, osmolality and electrolytes) before and after uninterrupted culture up to 7 days under the laboratory conditions used to confirm adequacy of the culture system before culturing human embryos. Re-measurement after any changes to the culture system. Adjustments to the culture system can be made if differences in end-point assessments are noted.

REAL WORLD SCENARIOS

MICROMANIPULATION

THE CONSTANTS

- LOW PROFILE DISH
- MICRODROPS (5-25uL)
- UNDER OIL

THE VARIABLES

1. WHERE DO YOU MAKE THE DISH?
2. HOW DO YOU MAKE THE DISH?
3. WHEN DO YOU MAKE THE DISH?
4. WHAT MEDIA IS IN THE DISH?
5. WHERE DO YOU KEEP THE DISH PRIOR TO USE?

REAL WORLD SCENARIOS

LAB X'S USUAL PROCESS

- BIOPSY DISH MADE DAY OF PROCEDURE

THE CHANGE

- SOMEONE IN THE LAB DECIDES TO MAKE DAY BEFORE PROCEDURE BECAUSE NEXT DAY IS REALLY BUSY

OR

- UNUSED DISH KEPT FOR USE NEXT DAY

THE OUTCOME

- DISH PULLED FROM A HUMIDIFIED INCUBATOR AFTER ~18 HOURS OF INCUBATION AT 37C
- EXPANDED BLASTOCYST MOVED FROM CULTURE DISH INTO BIOPSY DISH
- BLASTOCYST IMMEDIATELY COLLAPSES

WHAT JUST HAPPENED?

- **THE SCIENCE**: OSMOLARITY
- **THE TECHNICAL COMPONENT**: PROTOCOLS
- **THE LOGISTICS**: WORKFLOWS
- **THE HUMAN ELEMENT**: KNOWLEDGE, ASSUMPTIONS, MENTAL ERRORS
- **THE MACHINE ELEMENT**:

  HEY, DON'T BLAME ME!
- **THE QUALITY COMPONENT**: QUALITY MANAGEMENT – QC, QA

EACH IVF LAB IS A UNIQUE SYSTEM

WHAT IS APPLICABLE IN ONE LAB MAY NOT BE IN ANOTHER

INFLUENCED BY:

STRUCTURE

EQUIPMENT/TOOLS/CONSUMABLES

LAYOUT

PEOPLE

WORKFLOWS

PROTOCOLS

ASSUMPTIONS MAKE AN…

HOW DO YOU KNOW IT'S DOING WHAT IT'S SUPPOSED TO BE DOING?

HOW DO YOU KNOW THAT THE FOUNDATION OF THAT CONCEPT IS ROOTED IN FACT, NOT ASSUMPTION?

HOW DO YOU KNOW YOU CAN APPLY WHAT WORKS IN ONE PLACE TO ANOTHER?

ASSUMPTIONS MAKE AN…

FAILURE TO UNDERSTAND EQUIPMENT (AND WHAT CAN GO WRONG WITH IT) IS ONE OF THE BIGGEST CAUSES OF SUBOPTIMIZATION

WHY THIS SHOULD MATTER TO YOU

WHAT YOU ASK OF YOUR LAB TEAM LIKELY HAS IMPLICATIONS FOR THE ENTIRE LAB SYSTEM

DISTRACTIONS CAN LEAD TO STRESSORS – CLINIC AND PHYSICIANS HAVE AN IMPACT

WHY THIS SHOULD MATTER TO YOU

TRY TO MAKE THINGS AS CONSISTENT AND REPRODUCIBLE AS POSSIBLE

- RETRIEVALS
- EMBRYO TRANSFERS
- CARE PLANS

“THE ONLY CONSTANT IN LIFE…”

THE SYSTEM IS DYNAMIC!

JUST WHEN YOU THINK YOU HAVE IT ALL DIALED IN, SOMETHING WILL INEVITABLY CHANGE

ACKNOWLEDGEMENTS

JASON SWAIN PHD, HCLD

QUESTIONS?

MIKE ABEYTA

MABEYTA@CCRMIVF.COM

650-814-4566