SRBT WEBINAR

Practical pH for the IVF Lab

Jason Swain, Ph.D., H.C.L.D.,

February 28, 2013

Hosts

SRBT | Tom Turner, President, SRBT

CRYOPORT | Shannon Curiel, Business Development Manager, Cryoport

Jason Swain, Ph.D, HCLD

Scientific Director of ART Laboratories

University of Michigan

Center for Reproductive Medicine

- Jason is a leader in the field of optimizing culture media and pH regulation.
- The discussion will feature how pH dynamics can affect gamete/embryo development and function.

Learning Objectives

After attending this webinar, the participant will be able to:

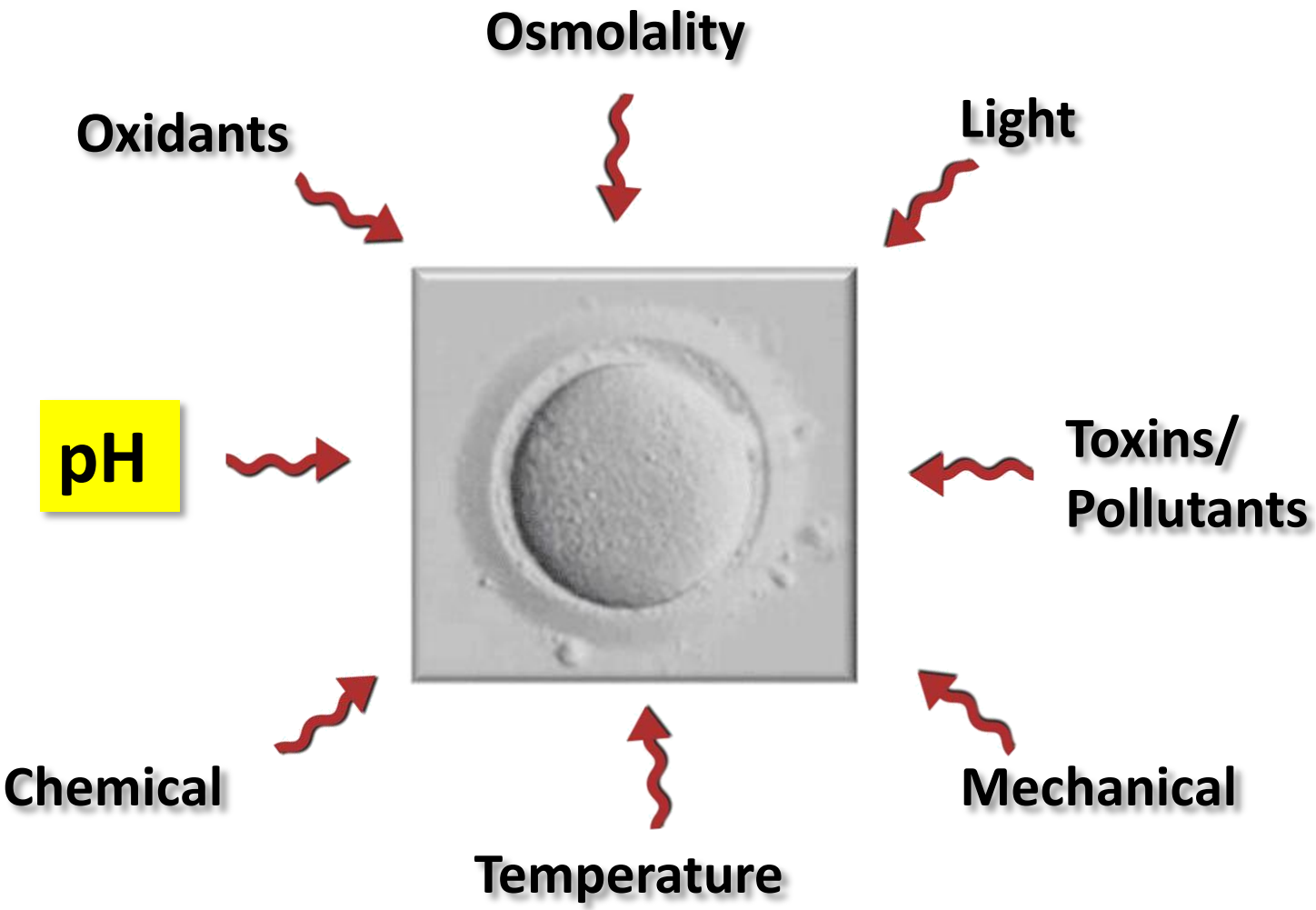
1) Explain gamete /embryo physiology and pH regulation
2) Understand the impact of pH on gamete / embryo development and function.
3) Solve problems relating to pH dynamics in the IVF laboratory

Webinar Instructions

pH and the IVF Laboratory

Jason E. Swain, PhD, HCLD
University of Michigan

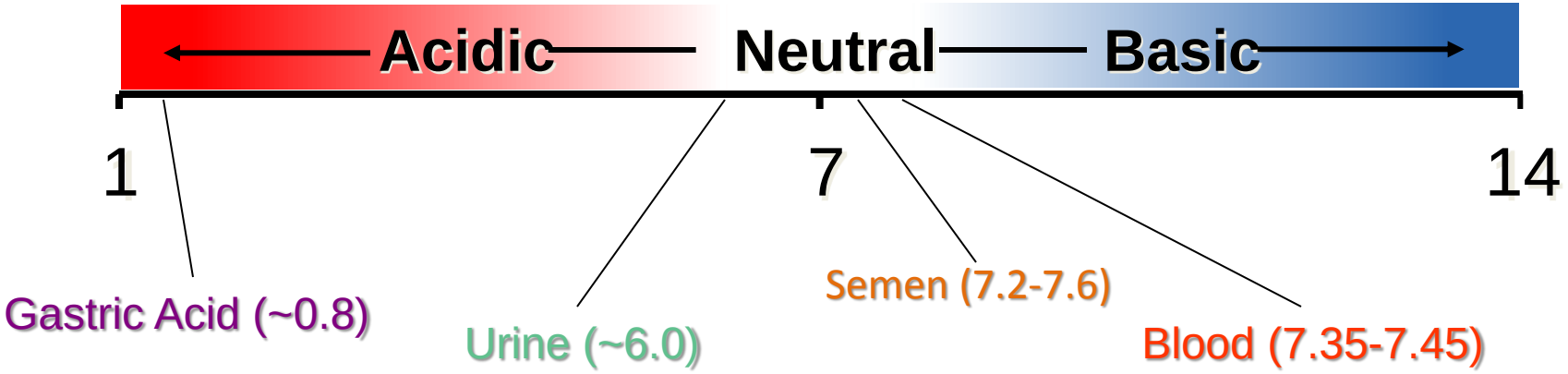
In Vitro Stressors



Objectives

- Define pH
- Explain gamete/embryo pH regulation
  - pHi vs. pHo
- Demonstrate importance of pH on gamete/embryo development and function
- Address practical pH issues in the IVF Lab
  - equilibration, set point, stabilization (buffers)

What is pH?

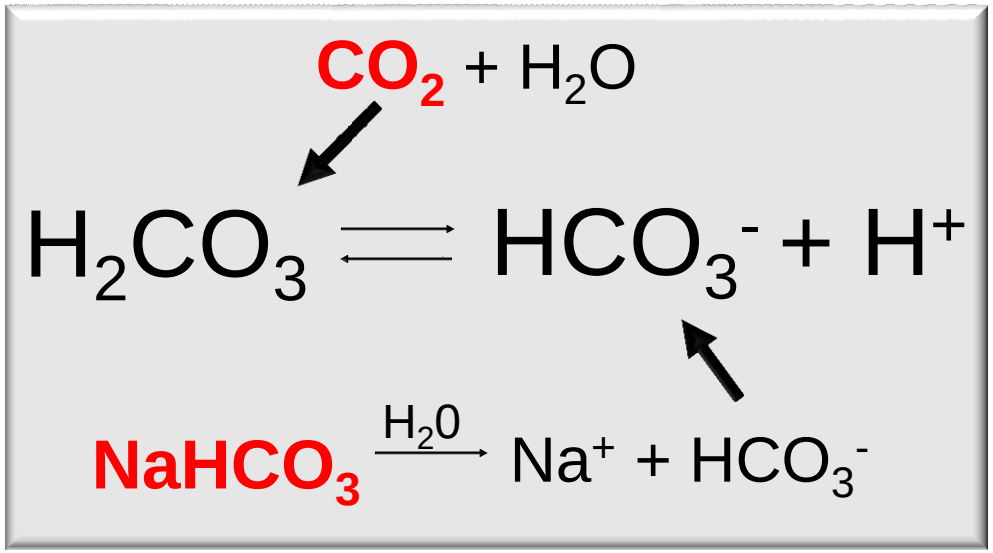


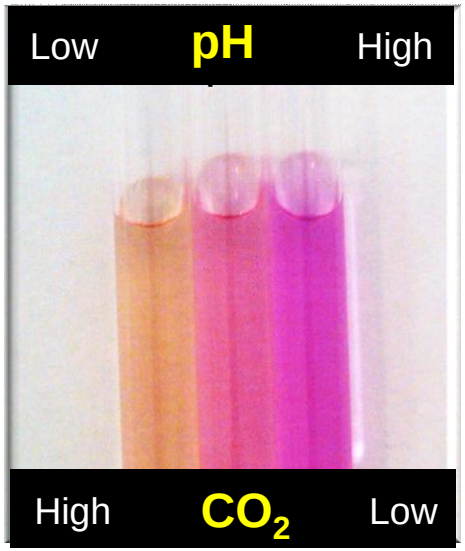
- Acids increase the concentration of hydrogen ions
- Bases decrease the concentration of hydrogen ions

pH is the measure of hydrogen ions

Outside/Media pH (pHo)

Incubator vs. Media

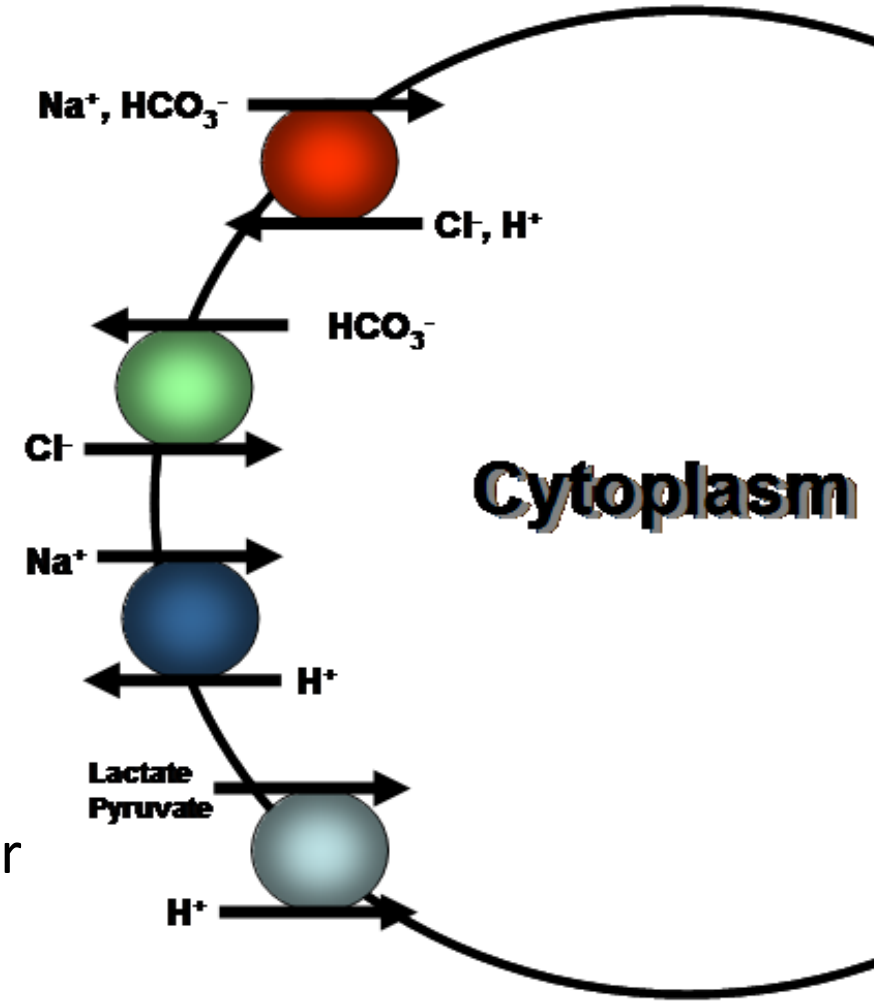




As CO_2 increases, pH decreases

Internal pH (pHi)

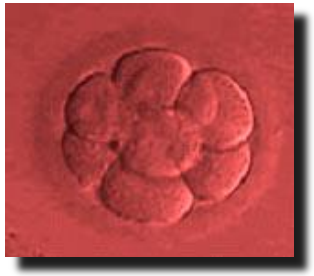
- Limited pH buffering of cytoplasm
- Cells contain pHi regulatory mechanisms
  - HCO_3^-/Cl^- exchanger >7.2-7.3
  - Na^+/H^+ antiporter <6.8
  - Na+ dependent HCO_3^-/Cl^- exchanger <7.0
- pHi follows pHo initially
- Influenced by amino acids and other media components



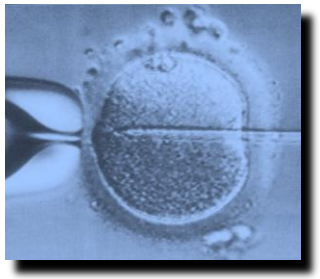
Cells can develop/function over a range of pHo

pH and ART

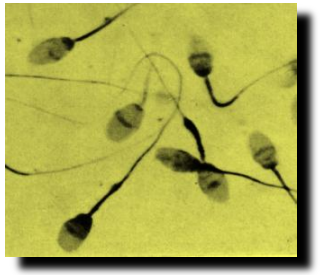
- Embryo development can be influenced by medium pHo



- Cryopreserved/thawed embryos have reduced ability to regulate pHi
  - ~3h recovery (Lane et al. 2000)



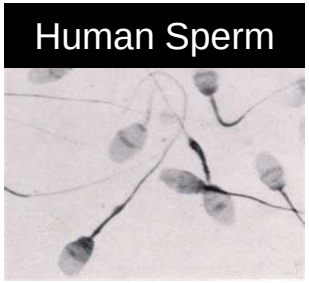
- Denuded mature oocytes lack robust internal pH_i regulatory mechanisms
  - Conveyed by cumulus cells
  - Activated ~6h after fertilization (Phillips et al. 1998, 2000, 2002)



- Sperm motility and binding to the zona pellucida is influenced by medium pHo (Hamamah et al 1996, Dale et al. 1998)

Proper and stable pHo is crucial

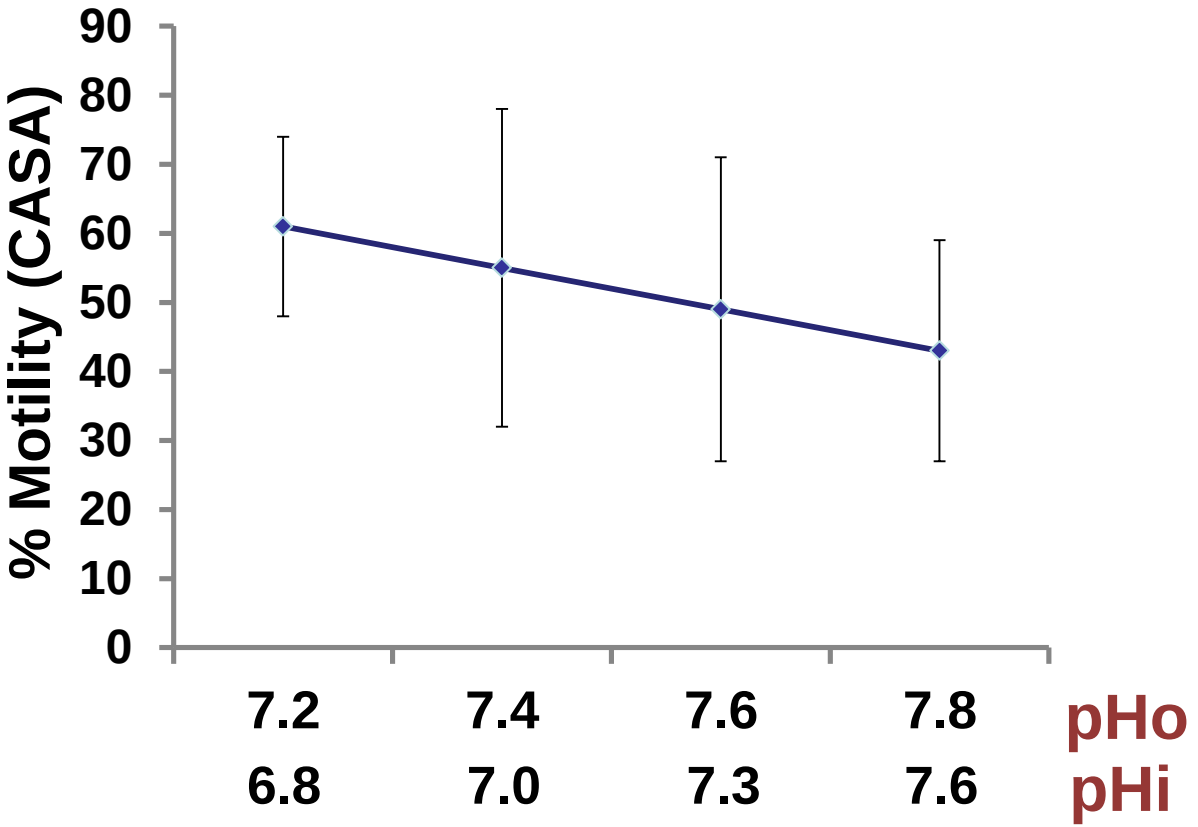
pHo and Sperm Motility



Media
(pHo 7.2-7.8)
2.5hr

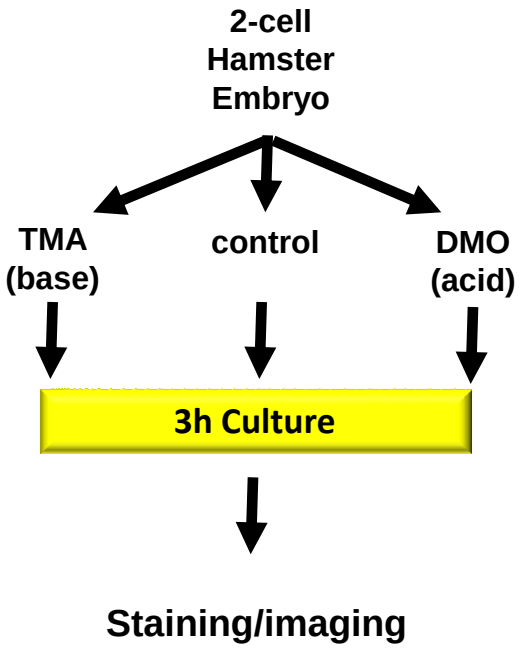
CASA
Sperm Motility

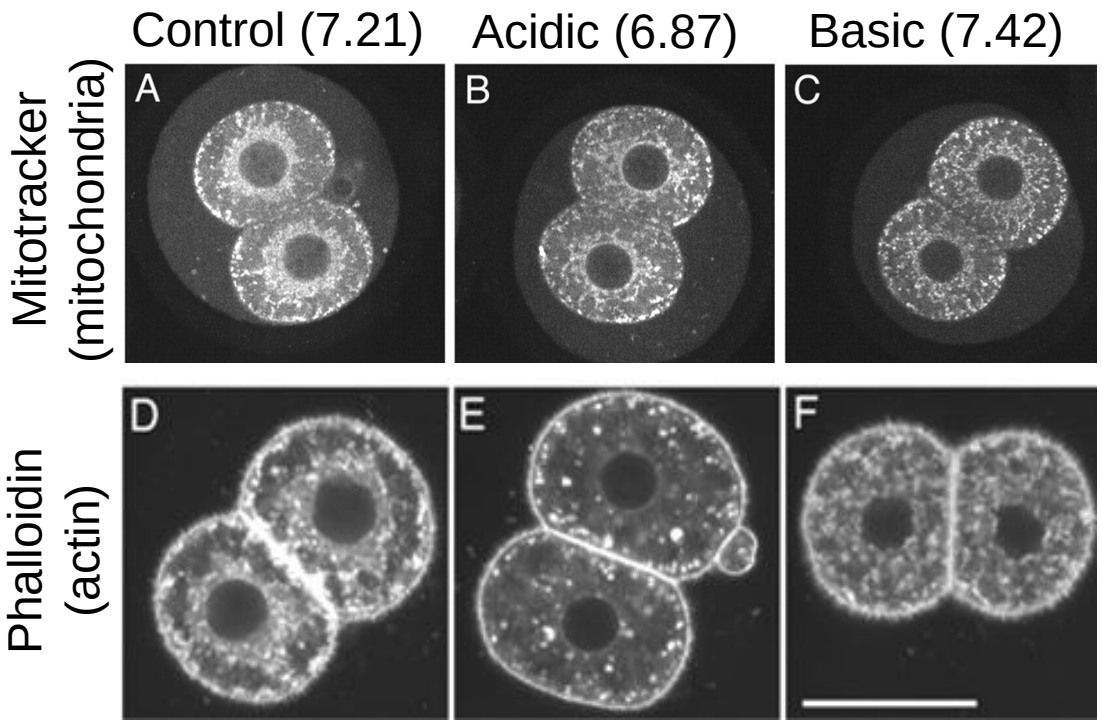
Measure
pHi



Hamamah & Gatti 1998

pH_i and Cellular Organization

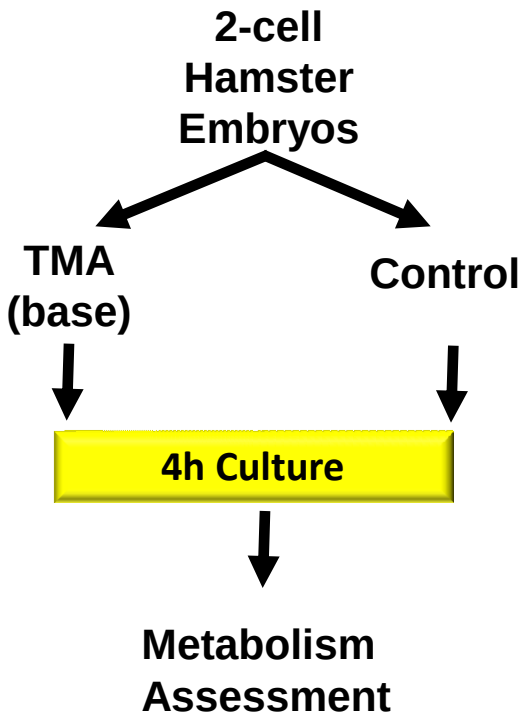


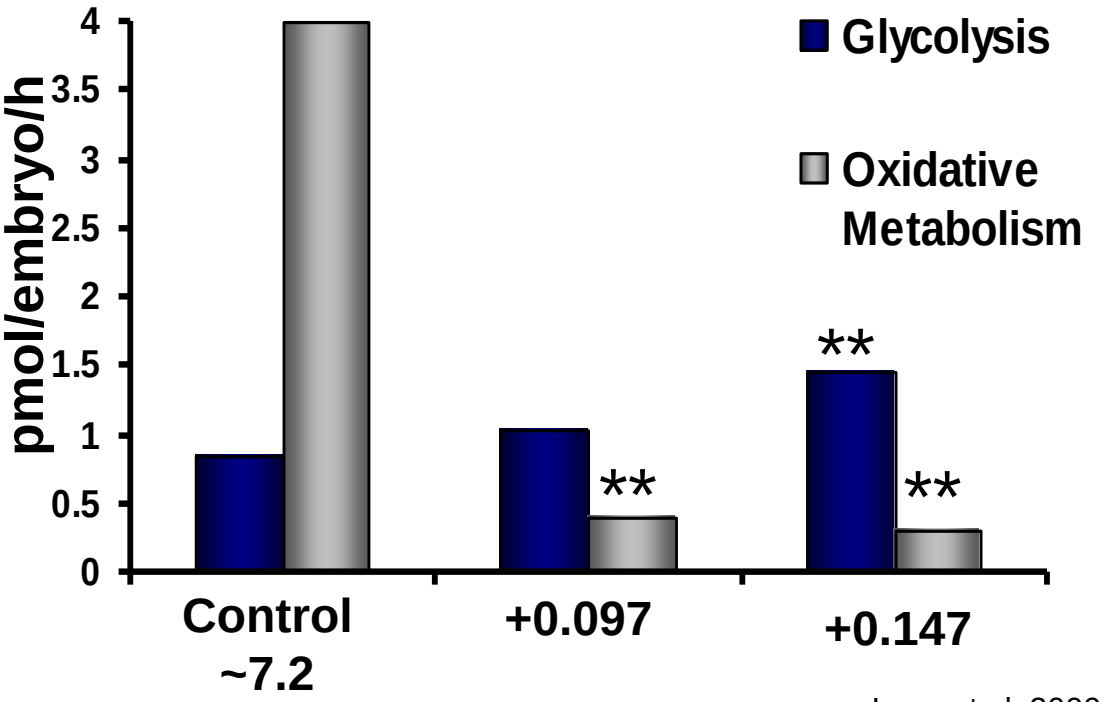


Squirrell et al. 2001

- Cytoskeletal components regulate meiotic/mitotic spindle and chromosome positioning (Zhu et al. 2003, Lenart et al. 2005)
- Mitochondria distribution is correlated to oocyte and embryo developmental competence (Bavister & Squirrell 2000, Nagai et al. 2006)

pH_i and Embryo Metabolism

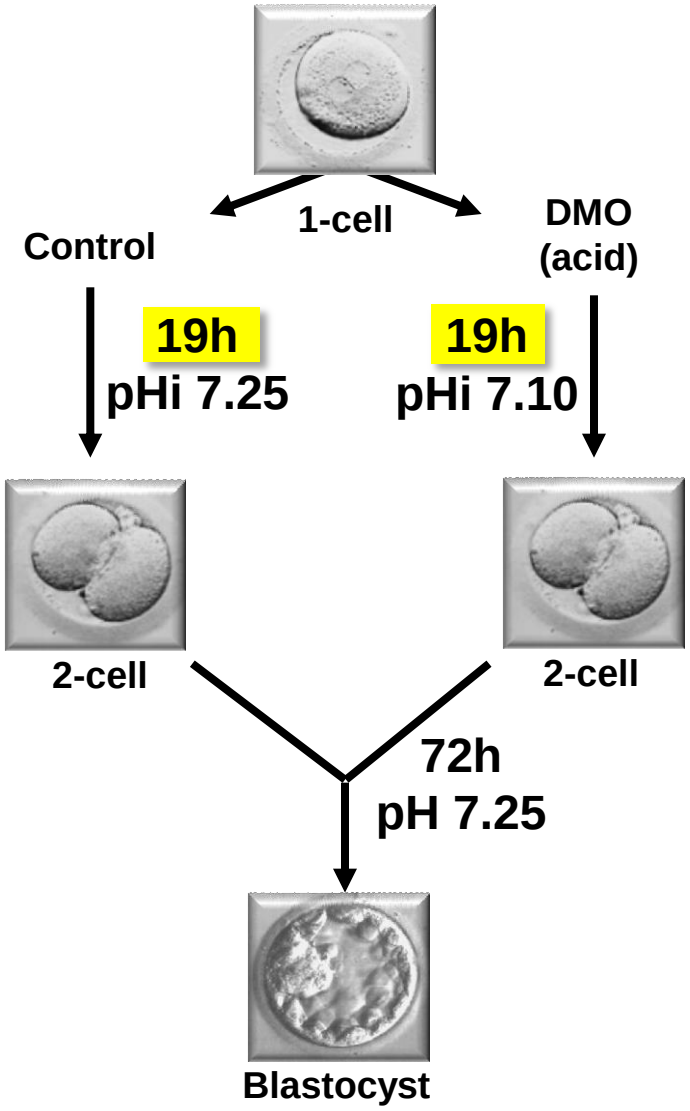




Lane et al. 2000

- Lowering embryo pHi lowers glycolytic activity (Edwards et al. 1998)
- Embryo metabolism is correlated with developmental competence (Lane & Gardner 1996)

pH_i and Fetal Development

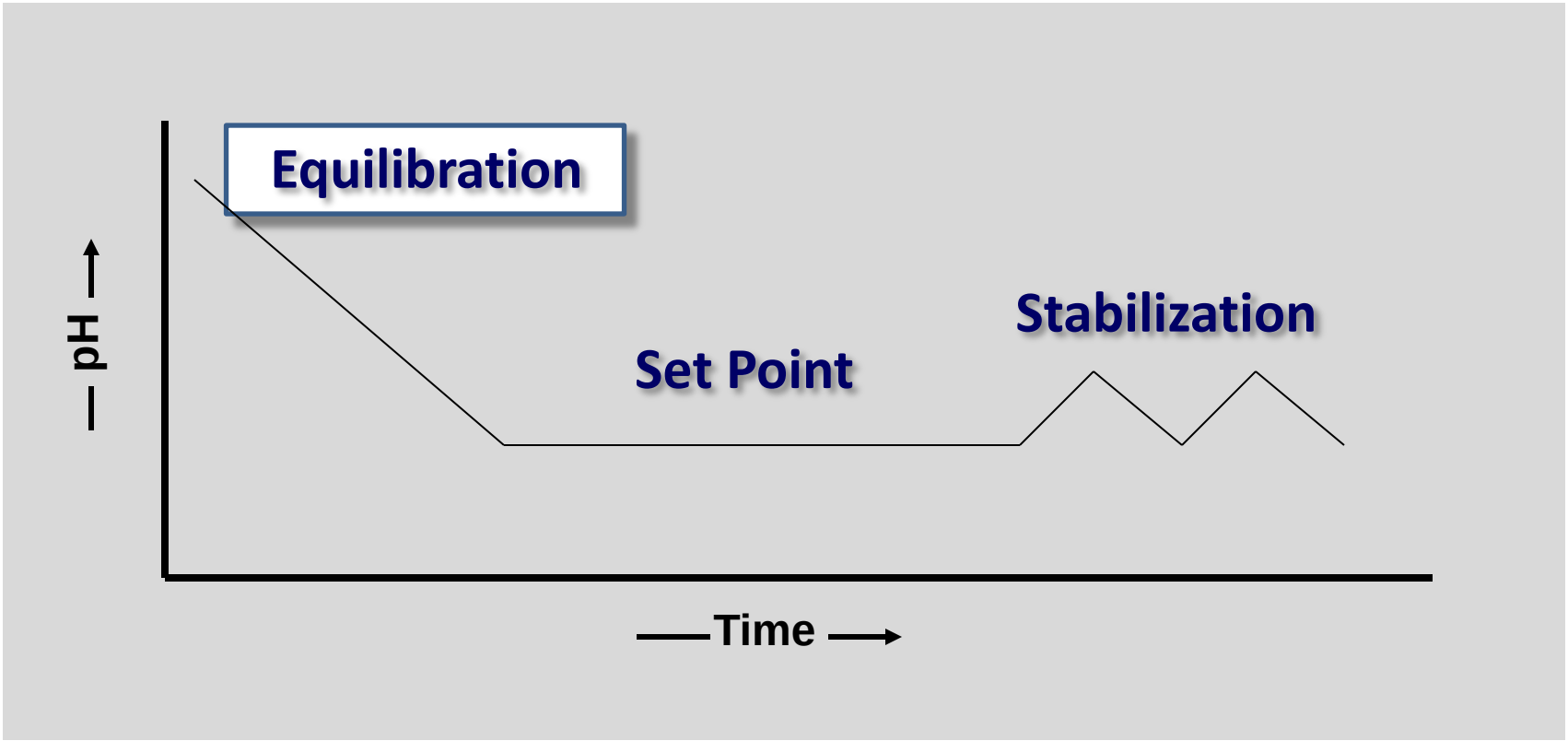


	Control	DMO
Total Blast Cell #	83.0	63.6
ICM #	30.6	20.2
Apoptotic index	1.9	3.2
% Blast	ND	ND
Implantation	ND	ND
Fetal Weight	1058.9	949.1
Fetal Length	21.9	20.6

Zander-Fox et al. 2009, 2010

Practical pH for the ARTisan

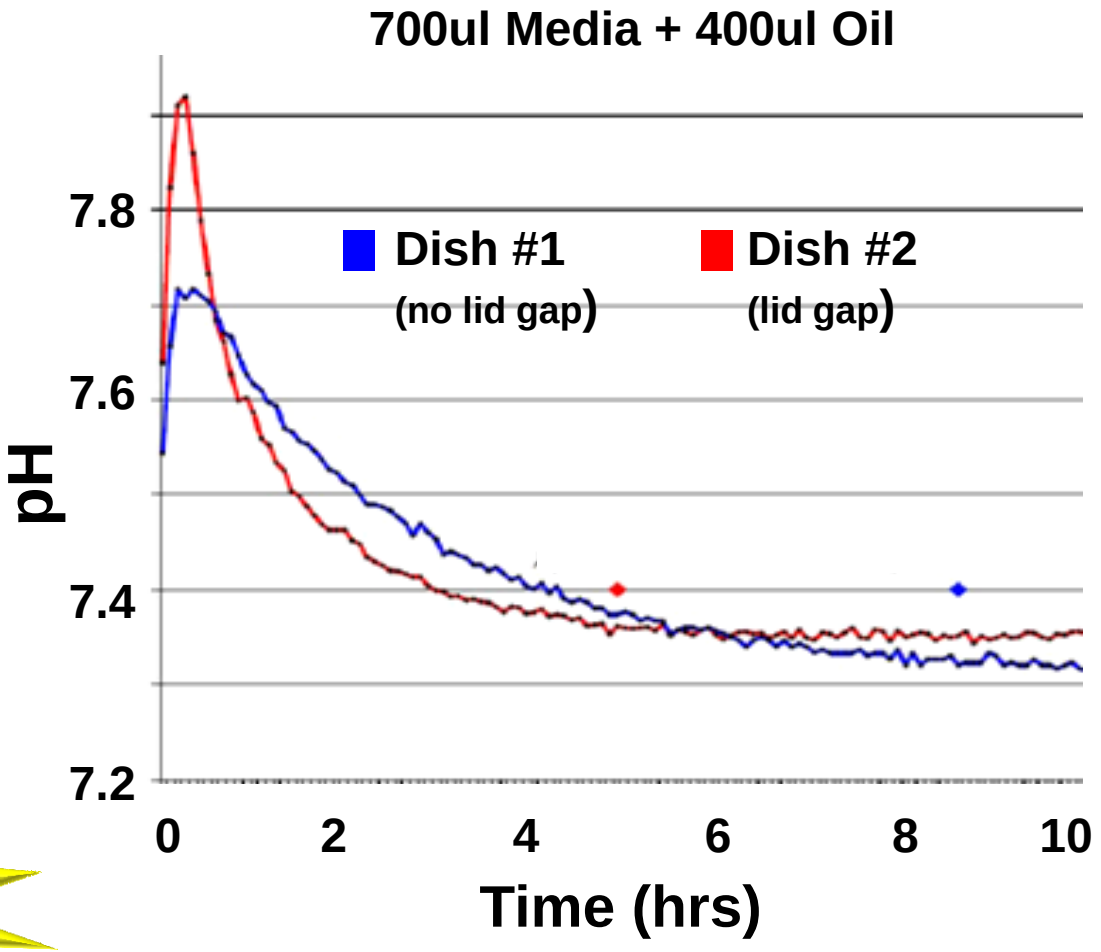
3 Phases of Media pH

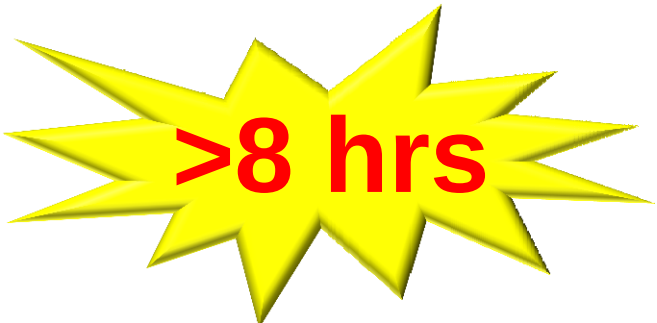


pH Equilibration

Important Factors

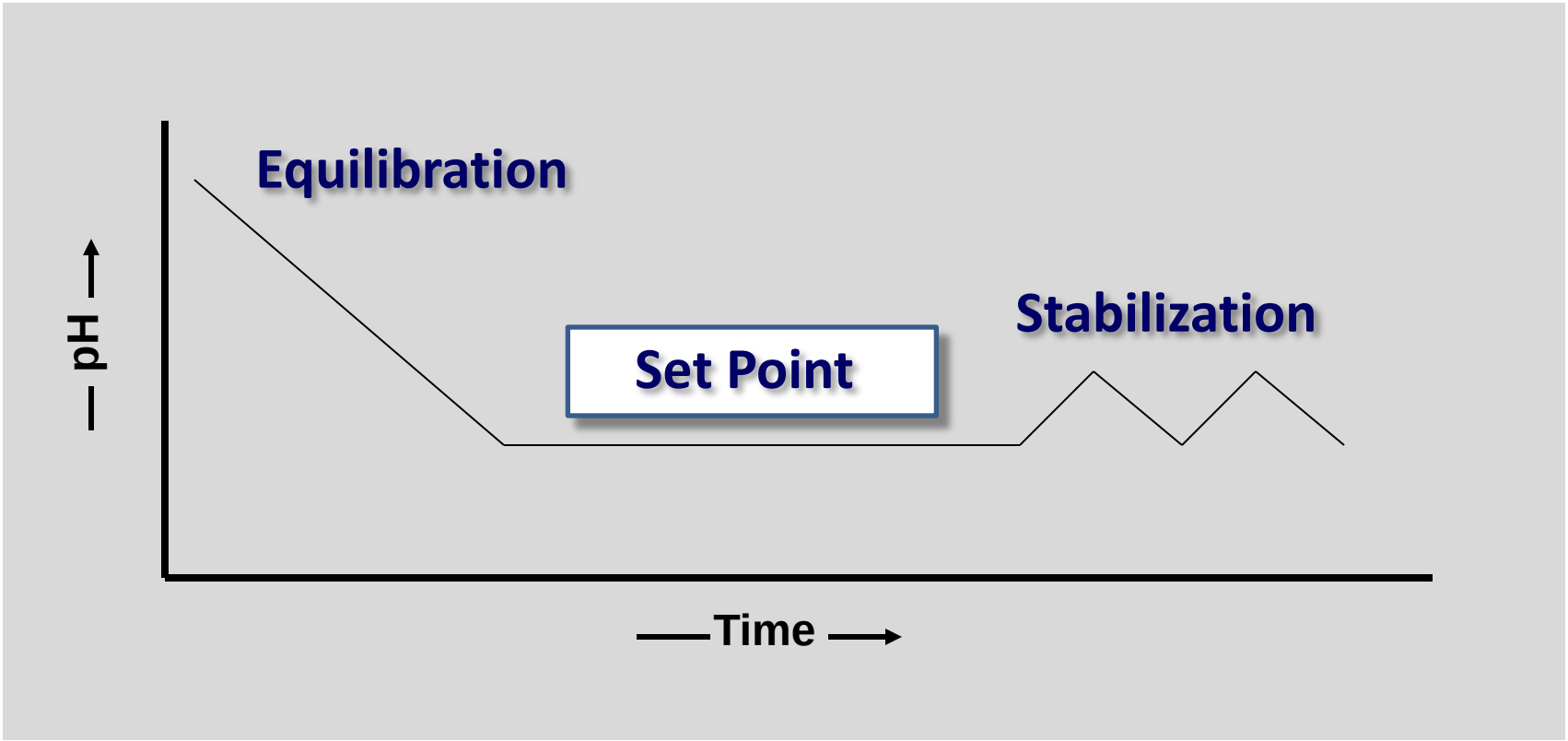
- Dish/lid
- Oil volume
- Media volume
- Start/End pH





Practical pH for the ARTisan

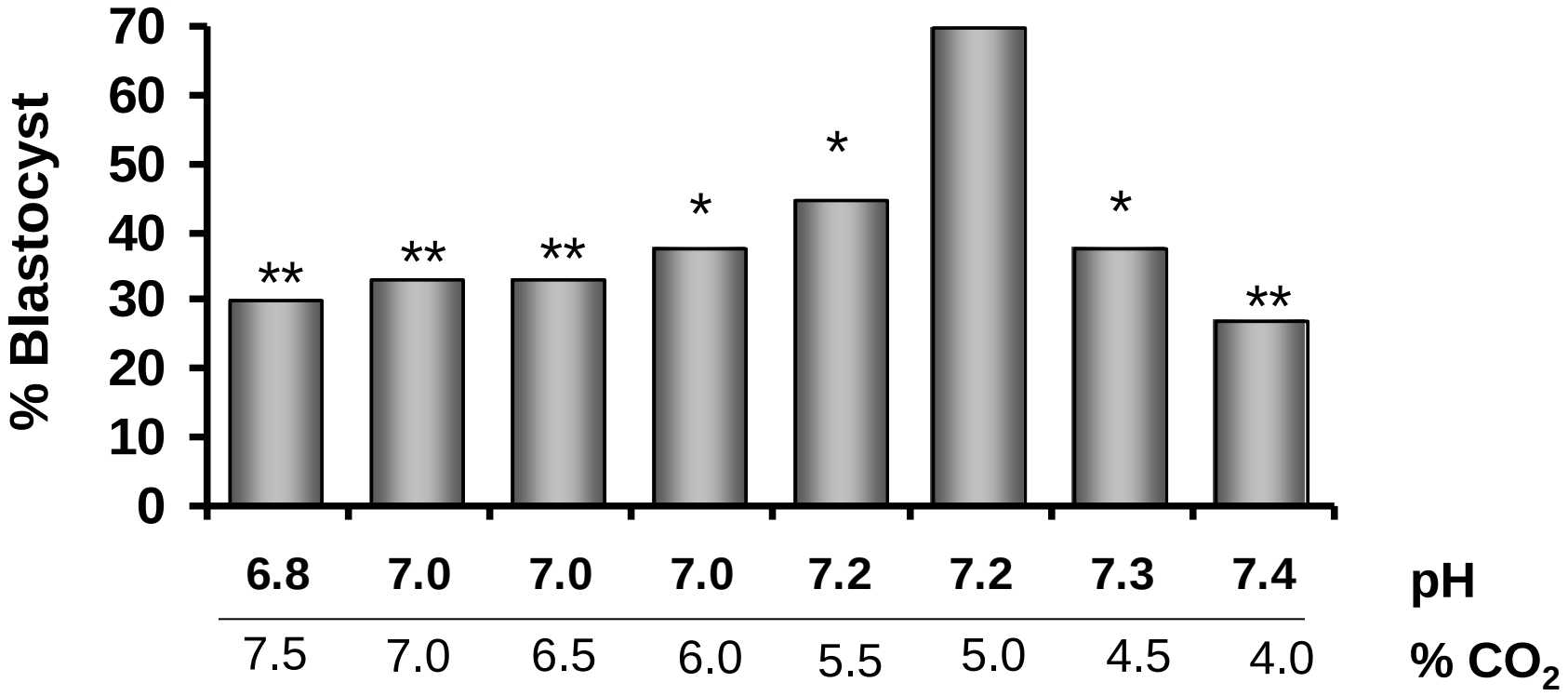
3 Phases of Media pH



The Effect of CO_2 Concentration and pH on the In-Vitro Development of Mouse Embryos

by Avner Hershlag, MD and Huai L. Feng, PhD, HCLD
Center for Human Reproduction, North Shore University Hospital, 300 Community Drive, Manhasset, NY 11030,. U.S.

Summary
Thi de



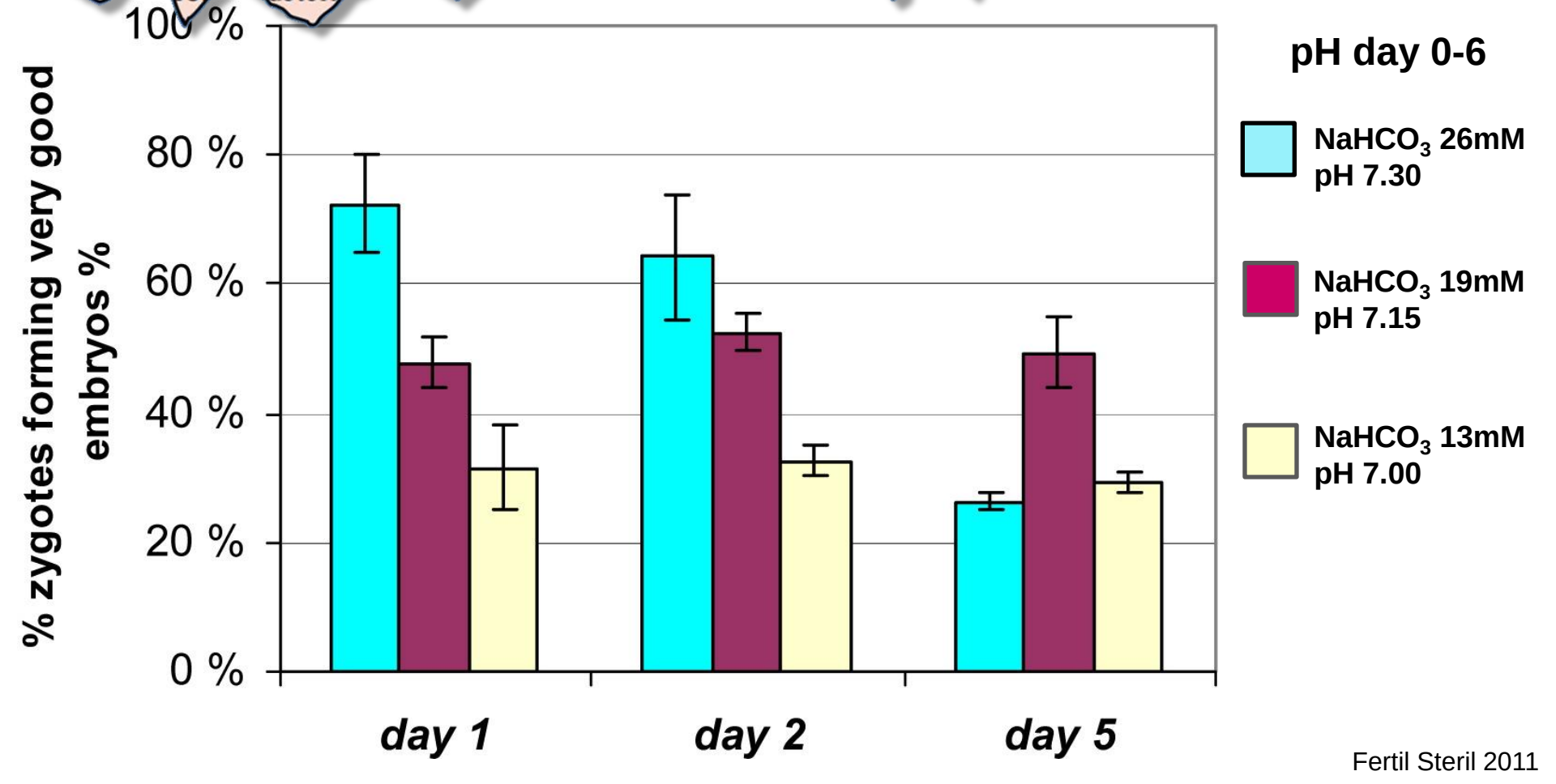
Fertil Mag 2005

Bicarbonate

Differential ~~[illegible]~~in embryo culture

Martha Hentemann, M.D.,[a,b] *Karim Mousavi, Ph.D.,*[b] *and Kjell Bertheussen, Ph.D.*[a,b]

[a] Department of Obstetrics and Gynecology, IVF Unit, University Hospital of Northern Norway, and [b] Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway



Fertil Steril 2011

CO_2/HCO_3^- & the Embryo

- Embryos utilize carbon from CO_2 for biosynthesis of nucleic acids, proteins and metabolic intermediates (Wales et al. 1969; Graves & Biggers, 1969; Quinn & Wales, 1971, 1974)

- Bicarbonate is utilized by various transporters
  - Blastocoel formation (Kane et al. 1975)
  - pHi regulation (Zhao & Baltz 1996, Edwards et al. 1998)

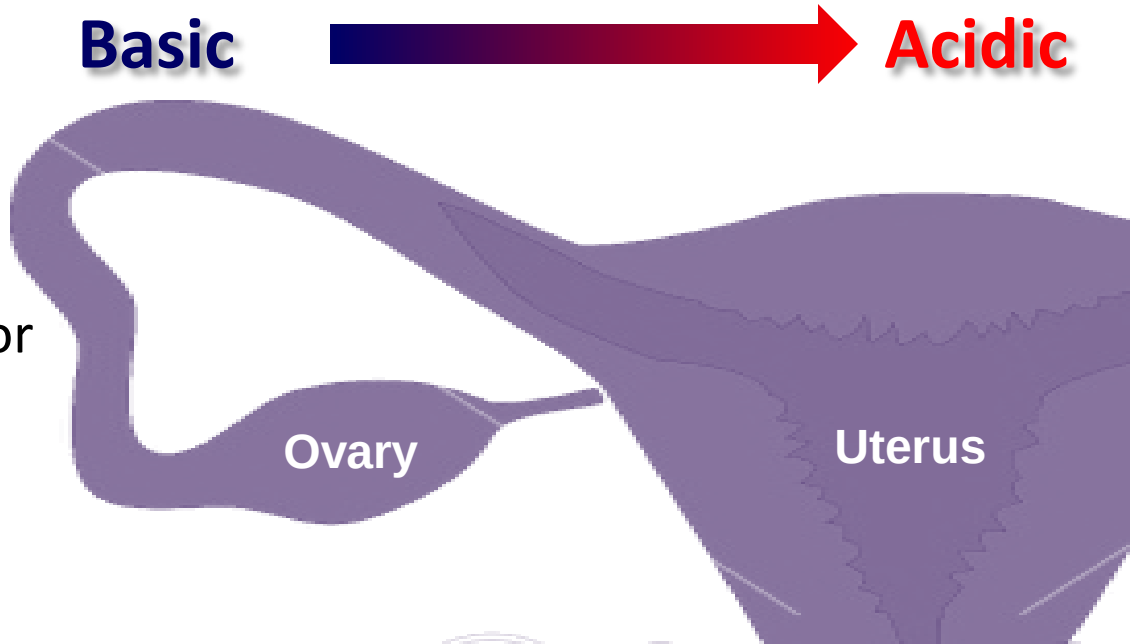
Difficult to isolate pHo as the variable

pHo of the Reproductive Tract

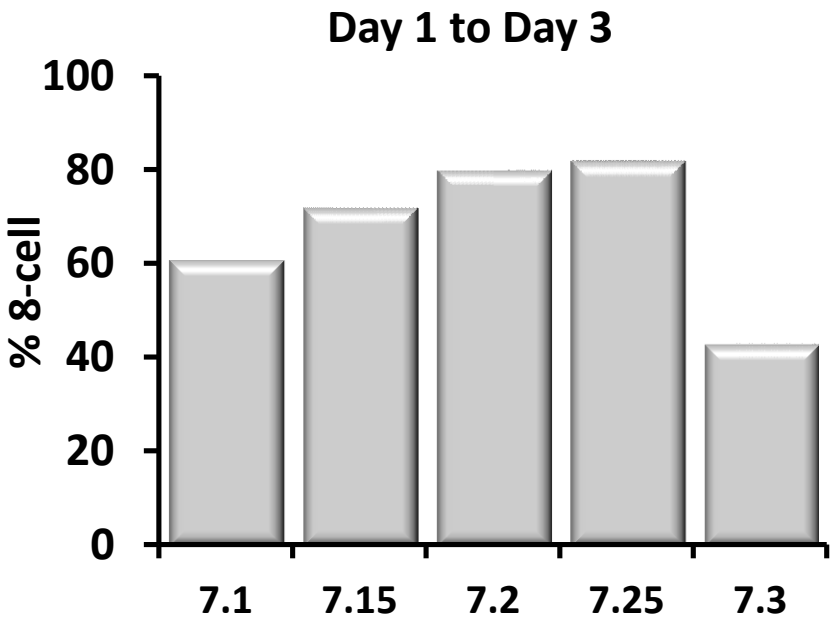
	Follicle	Oviduct	Uterus	Reference
Cow	**7.57-7.64**	**7.6**	**6.96**	Hugentobler et al. 2004
Sheep		**7.4**	**7.0**	Iritani et al. 1969
Human	**7.26-7.24**		**7.12**	Yedwab et al. 1976, Fraser et al. 1973 Shalgi et al. 1972

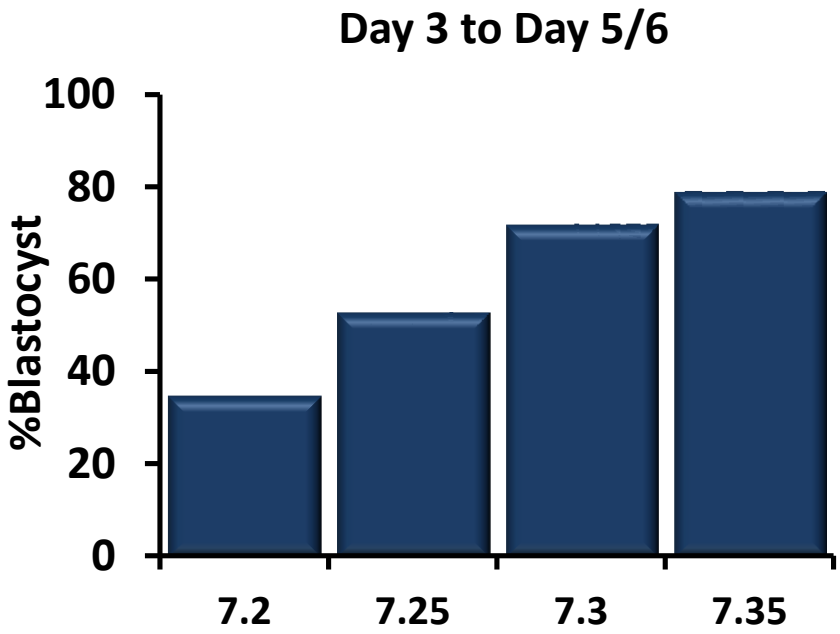
Some companies recommend **High-Low-High** pHe paradigm for fert-cleavage-blast culture

-may not agree with in vivo



Changing pHo





Quinn 2012: Based on 96,431 embryos (K. Miller, unpublished)

Accomplished by adjusting CO_2 in the laboratory

or

Accomplished by altering $NaHCO_3$ by media companies

Changing pHo by altering CO2 ≠ changing pHo by adjusting bicarb

Optimal pHo?

- pHo higher than pHi to combat acidification (~7.2)
  - Human embryo pHi is ~7.1 (Phillips et al. 2000)
- <7.4 to avoid reduced development
- No proven need to change pHo during embryo culture
  - Slightly higher pHo/bicarbonate may benefit sperm/fertilization
  - Later stage embryos may do better with higher bicarb (pHo)
  - Later stages regulate pHi more effectively (tight junctions, etc)
- **Optimum pHo likely varies from medium to medium**
  - **Ingredients, like lacate and amino acids, can impact pHi independently from pHo**

Maintain a narrow and stable pHo

Recommended pHo Values

Irvine

P1	7.27-7.32
ECM	7.2-7.25
CSC	7.28-7.32
Multi-blast	7.3-7.4
HTF	7.2-7.3

SAGE/Origio

Fert Media	7.3±0.1
Cleavage Media	7.2±0.1
Blastocyst Media	7.3±0.1
IVM	7.2±0.1

Vitrolife

G5 Series	7.27±0.07

Life Global

Global	7.27-7.32*
Global Fert	7.27-7.32*
HTF	7.27-7.32*

Origio/SAGE

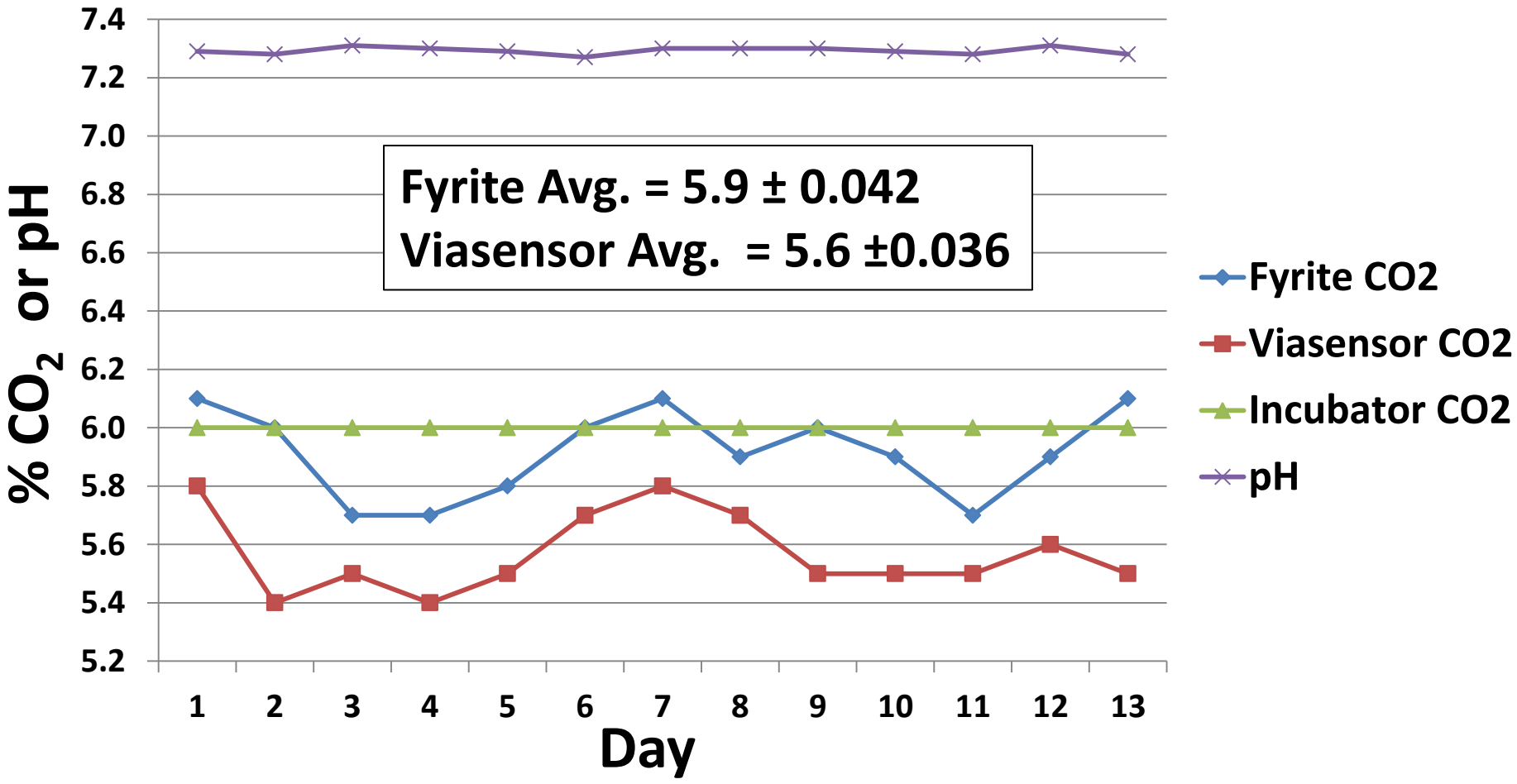
Universal IVF	7.3-7.4
ISM1	7.2-7.3
ISM2	7.35-7.45
EmbryoAssist	7.2-7.3
BlastAssist	7.35-7.45

Cook

Sydney Cleavage	7.3-7.5
Sydney Blast	7.3-7.5
Sydney Fert	7.3-7.5

Likely can't all be achieved with the same CO_2 setting between labs

CO_2 vs. pH Measurement

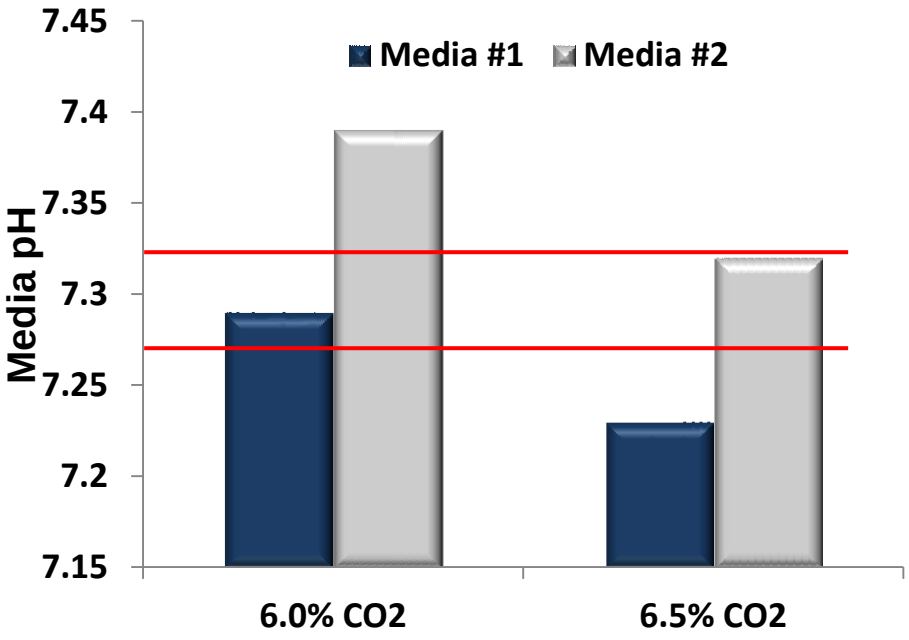


CO_2 readings can differ depending on the method

Measure the pH!

pHo Measurement

Same Medium – Same Company
w/ protein pre-added or adding your own (same concentration)



Same Basal Medium-Different Companies

Commercial Medium (HEPES-HTF)	pH @ 37°C (mean ± SEM)
Medium #1	7.28 ± 0.005
Medium #2	7.27 ± 0.003
Medium #3	7.26 ± 0.003
Medium #4	7.08 ± 0.007
Medium #5	7.08 ± 0.005

Not all media give the same pHo
Lab specific factors can also impact

Measurement of pHo

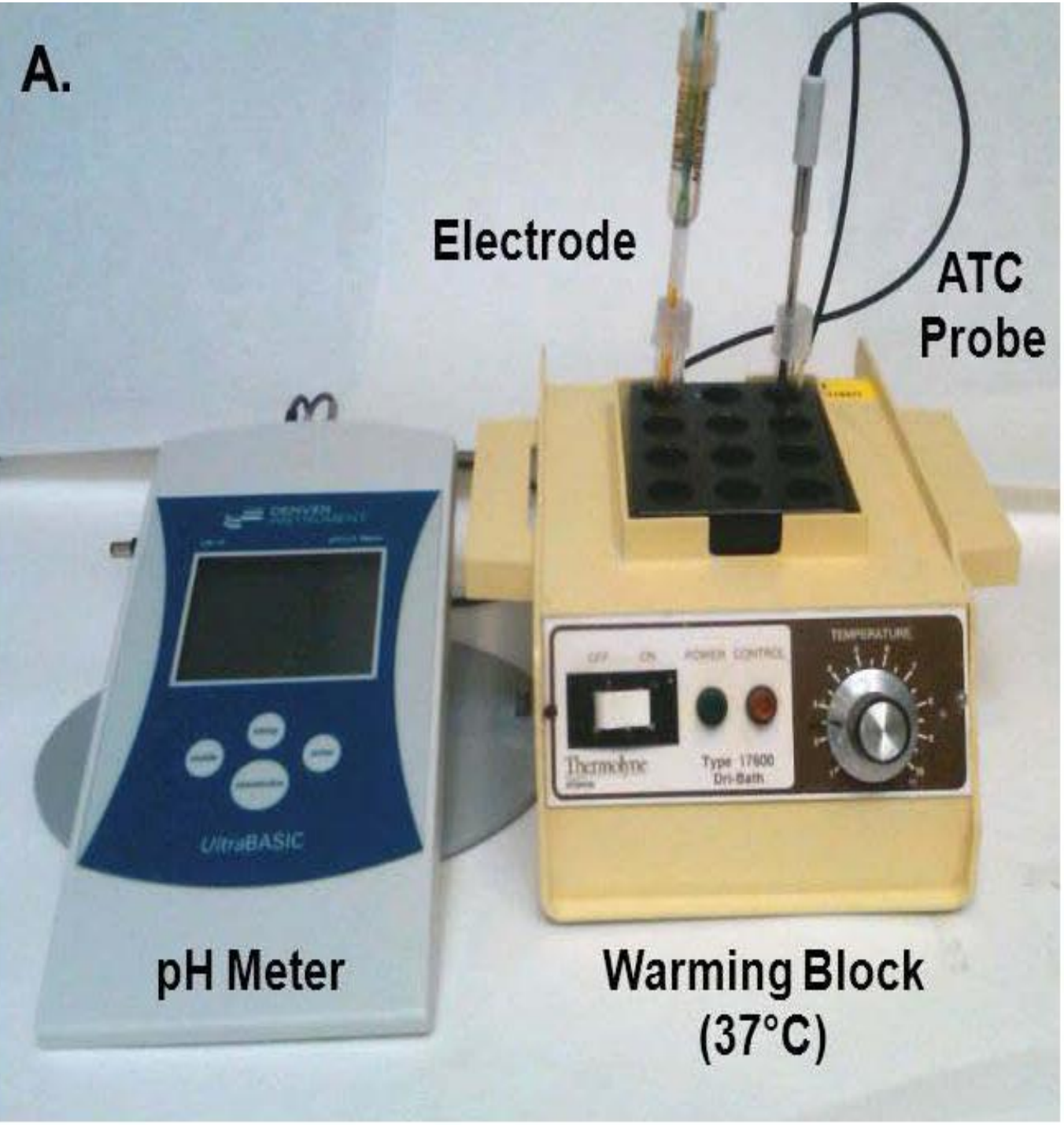
- Proper calibration of device is crucial
  - Fresh standards stored properly
  - Bracket pH range (7 and 10)
- Temperature compensation
- Proper electrode and storage/replacement
- Use appropriate media with protein
  - use your medium, not a “test medium”
- Test each incubator
- Test new lots of media
- Can test at different intervals (daily, weekly)

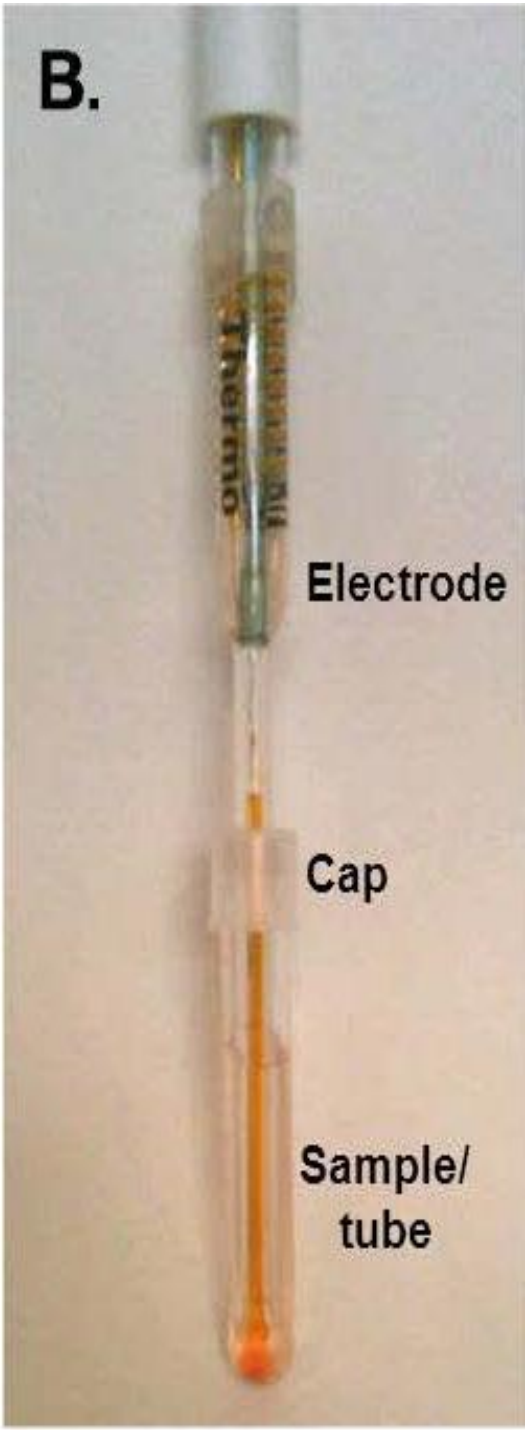
pH Meter/Probe

Inside Incubator

Blood Gas Analyzer

Validate accuracy before clinical use


A.
Electrode
ATC
Probe
UltraBASIC
pH Meter
Thermolyne
Type 17600
Dri-Bath
Warming Block
(37°C)

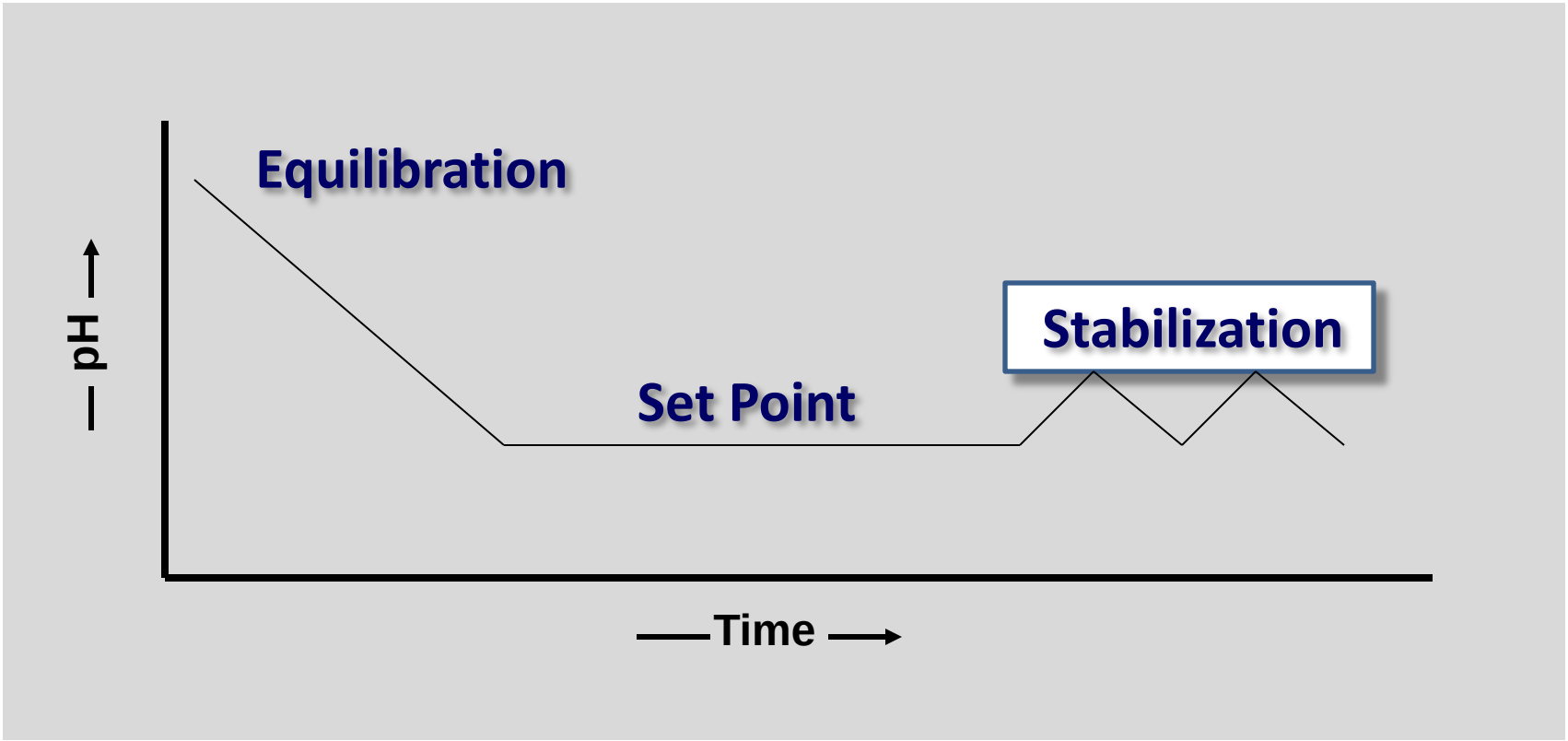

B.
Electrode
Cap
Sample/
tube

Measurement of pHo

- Calibrate using warmed standards 7 & 10
  - Aliquot/warm immediately prior to use
- Rinse probe with DI H2O
- Blot dry - DON'T WIPE
- Remove tubes from incubator and quickly cap – move to adjacent pH meter
- Place the pH probe into the tube with a seal (gasket or through the cap of the tube)
- Wait for the reading to stabilize and record (seconds)
- Can repeat with second tube and average if desired

Practical pH for the ARTisan

3 Phases of Media pH

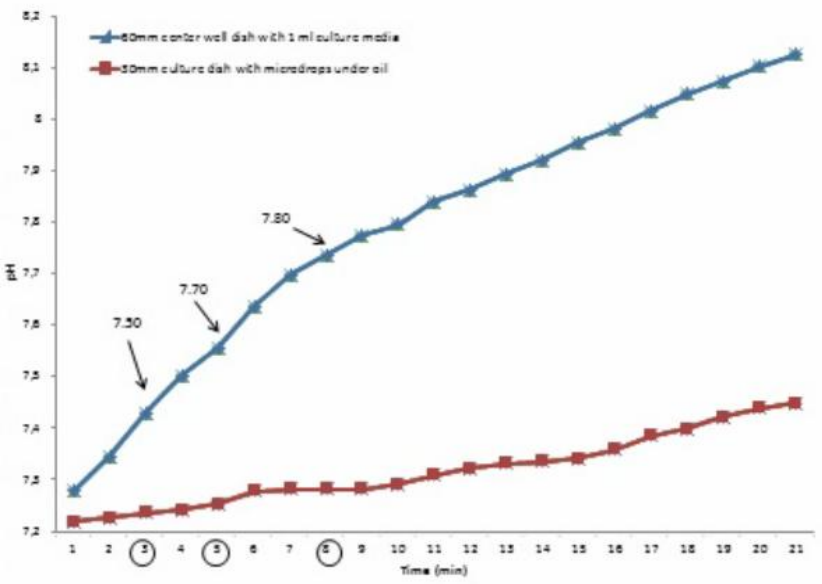


O-195 – Epigenetic consequences of pH stress in mouse embryos

G. Koustas, C. Sjoblom
University of Sydney Westmead Hospital, Westmead Fertility Centre, Department of Obstetrics and Gynaecology

Results

Culture media pH profile

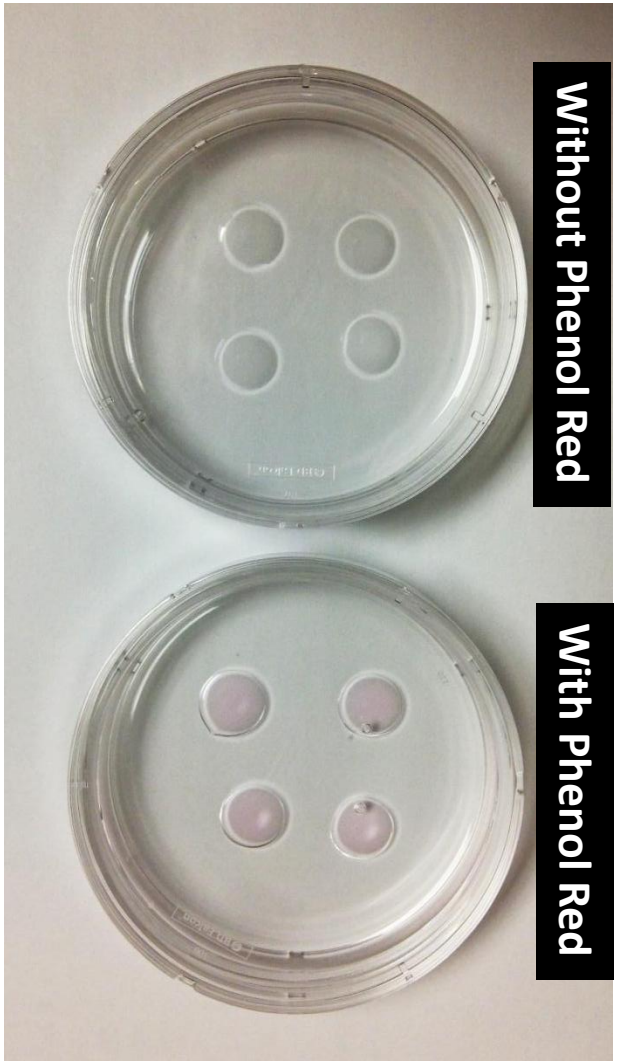


Fluctuations in pH

- Reduced blastocyst formation
- Reduced hatching
- Lowered cell number
- Increased apoptosis
- Altered methylation of *H19* & *Igf2*
- Reduced fetal weight

Stabilizing pHo

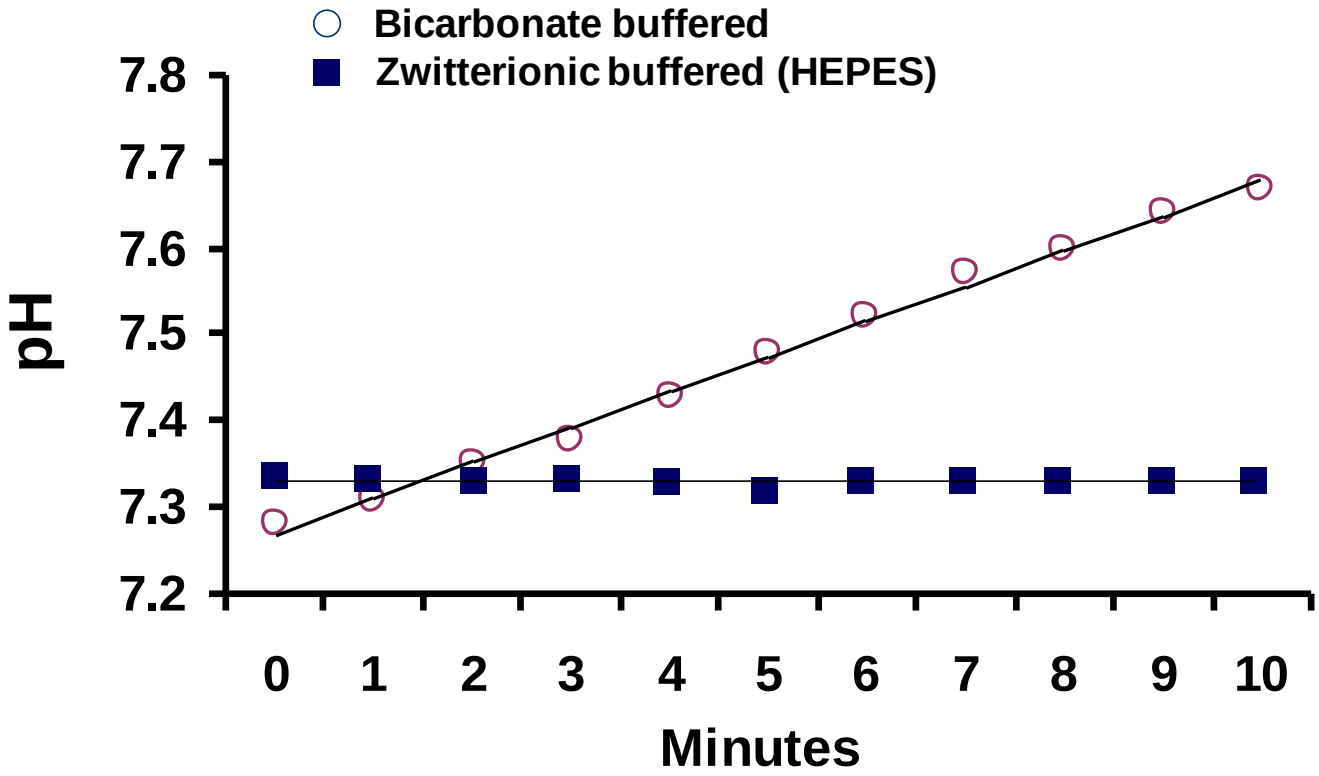
- Minimize incubator openings
- Work quickly outside of the incubator
- Use oil overlay
- Use of isolettes
- Use of proper media
  - zwitterionic buffers



Zwitterionic Buffers

Definition: A buffer is a substance which by its presence in solution increases the amount of acid or base that must be added to cause unit change in pH – act as acid or base

-stabilizes pHo outside confines of the incubator (no CO_2 required)



Concerns with Buffers

- Several **invalid** concerns with buffers:
  - HEPES is toxic:
    - Toxicity of HEPES is due to light exposure and interaction with riboflavin – this is not an issue with embryo culture media (Zigler et al. 1985, Lepe-Zuniga et al. 1987)
  - Injection of buffers may alter pHi:
    - Microinjection of MOPS, TES or HEPES buffered media does not influence embryo pHi (Edwards et al. 1998)
  - Buffers are detrimental to embryo development
    - Low fertilization and embryo development in HEPES and other buffers is likely due to reduced bicarbonate and CO_2 levels, not the buffer (Lee & Storey 1986, Mahadevan et al. 1986, Bhattacharyya & Yanamagachi 1988, Graves & Biggers, 1970, Quinn & Wales 1971, 1974)

0015-0282/06/$32.00
doi:10.1016/j.fertnstert.2005.10.050

Use of a medium buffered with N-hydroxyethylpiperazine-N-ethanesulfonate (HEPES) in intracytoplasmic sperm injection procedures is detrimental to the outcome of in vitro fertlization

Francesco Morgia, B.S.,[a] Monica Torti, B.S.,[a] Monica Montigiani, B.S.,[a] Claudio Piscitelli, M.D.,[a] Annalise Giallonardo, M.D.,[a] Mauro Schimberni, M.D.,[a] Pierluigi Giannini, M.D.,[a] and Marco Sbracia, M.D.[b]

[a] Bioroma, Center of Assisted Reproduction, "P... Hospit... Endocrinolo... and Re... dicine, Rome, Italy

- Increased embryo degeneration (6.5 vs 8.8%)
- Increased triploidy (3.3 vs. 6.1%)
- Reduced pregnancy (37.8 vs. 28.2%)
- Reduced implantation (18.3 vs. 12.3%)

Several problems with the study!!!

Buffers & Handling Media

- Brief exposure to inappropriate handling media can significantly reduce embryo development

-Hamster (Escriba et la. 2001)

-Rabbit (Farrell & Bavister 1984)

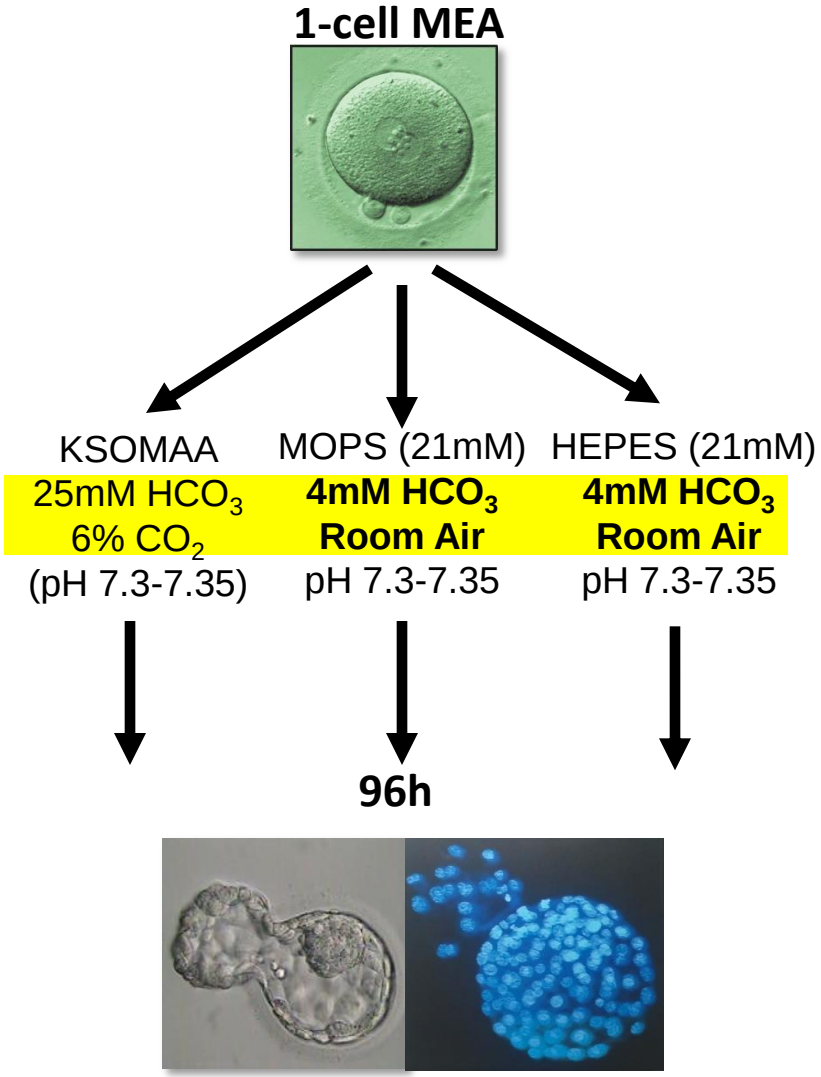
-Cow (Palasz et al. 2008)

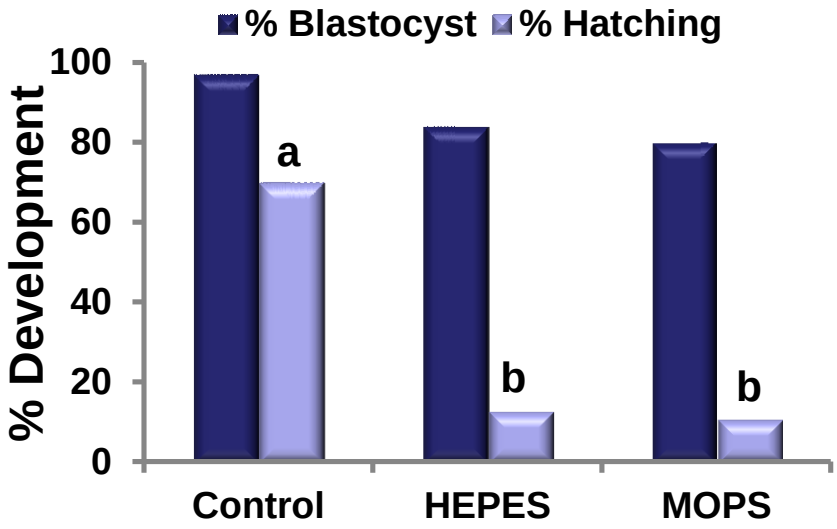
-Mouse (Gardner & Lane 1996)

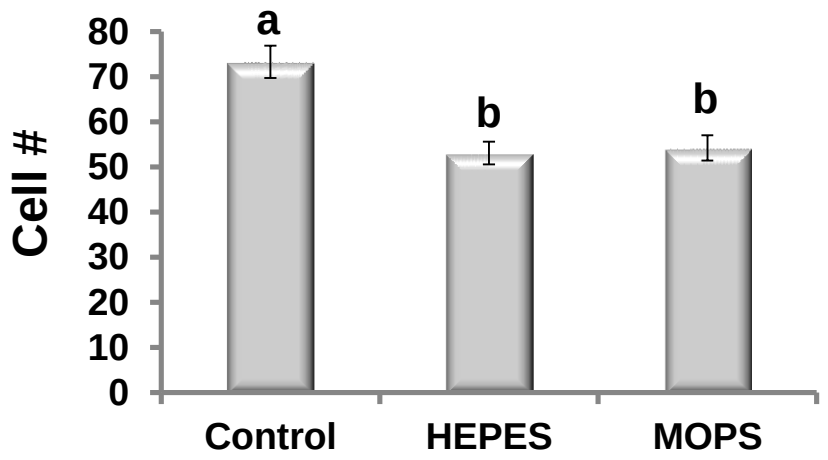
-Human (Morgia et al. 2006)

What is the specific impact of the buffer?

Buffers & Embryo Development

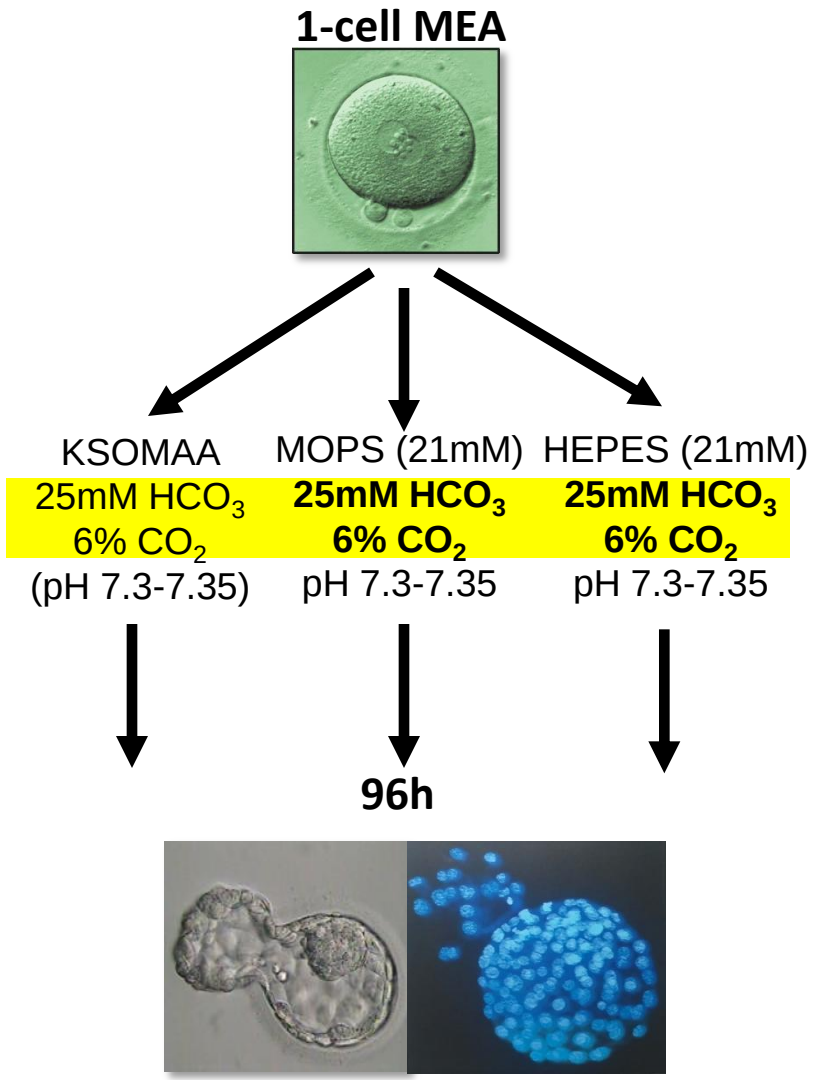


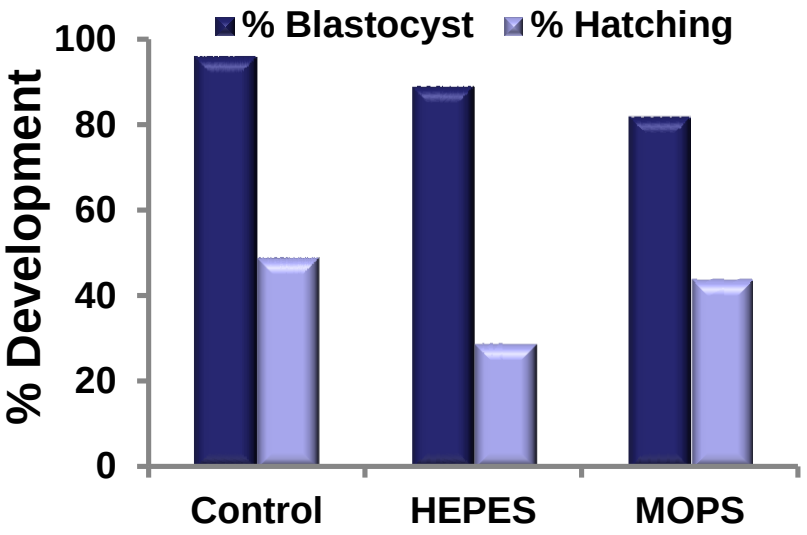


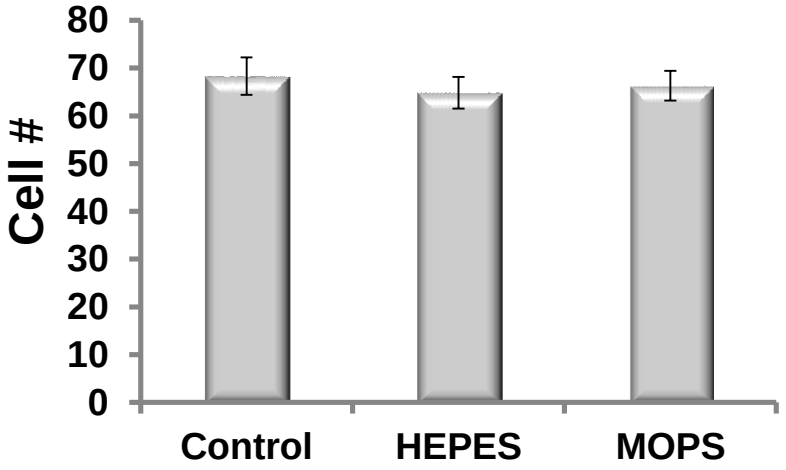


Swain & Pool 2008

Buffers & Embryo Development







Swain & Pool 2008

Concerns with Buffers

- Potential **valid** concerns with buffers:
  - Cell-specific sensitivity to a particular buffer
    - Evaluate various buffers
      - adequate buffering (pKa)
      - examine toxicity

Buffer Selection

Henderson-Hassebalch Eq: $pH = pk_a + \log \frac{[A^-]}{[HA]}$

Maximal Buffering

$pHo = pK_a$

- Less buffer needed to maintain pH when buffer's pKa value is near desired pH (7.2-7.4)

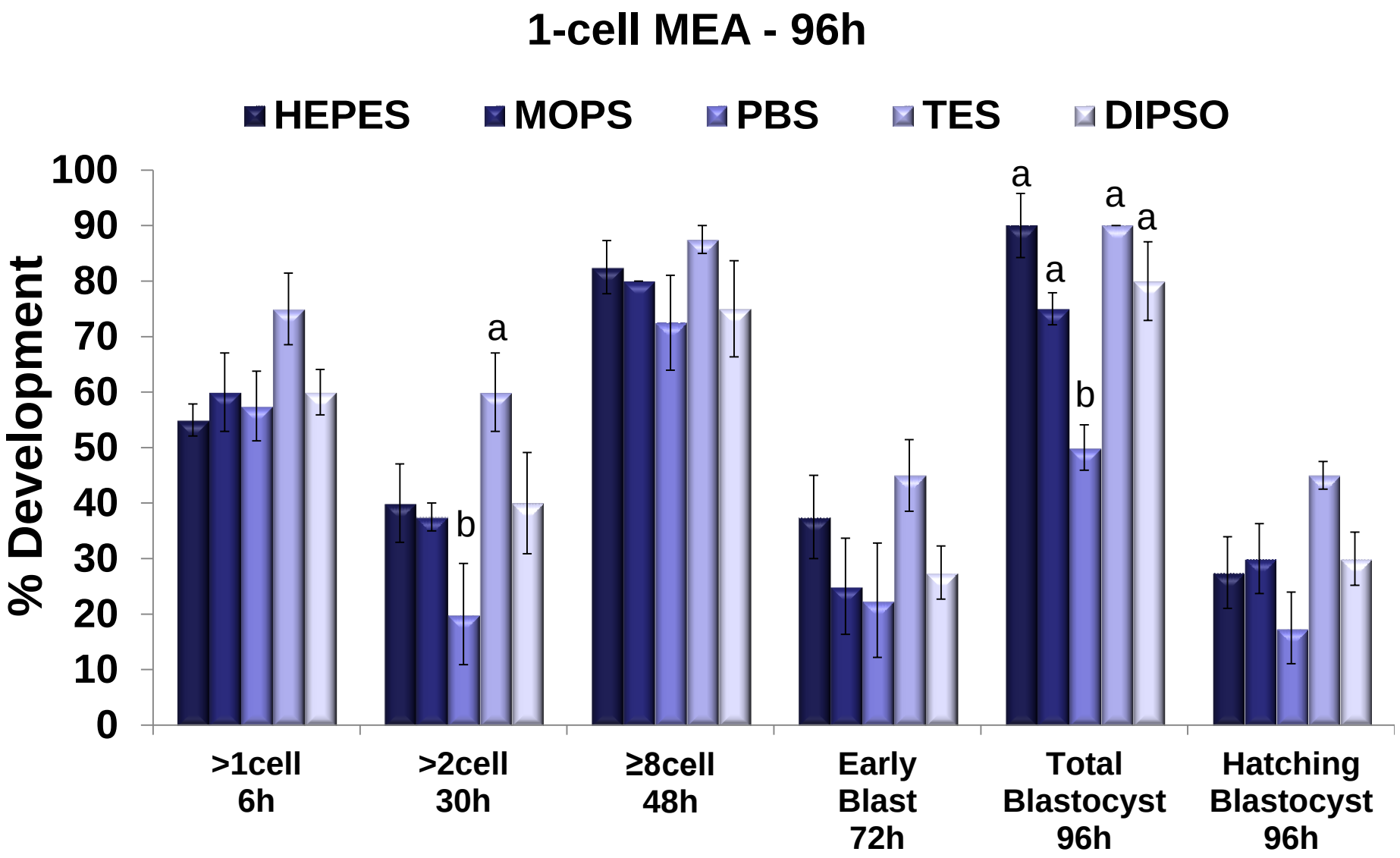
- pKa slightly higher than working pH may be beneficial
  - protonated buffer forms are less harmful (Izawa et al. 1966)
  - added buffering in the sensitive alkaline range

Buffers & pKa

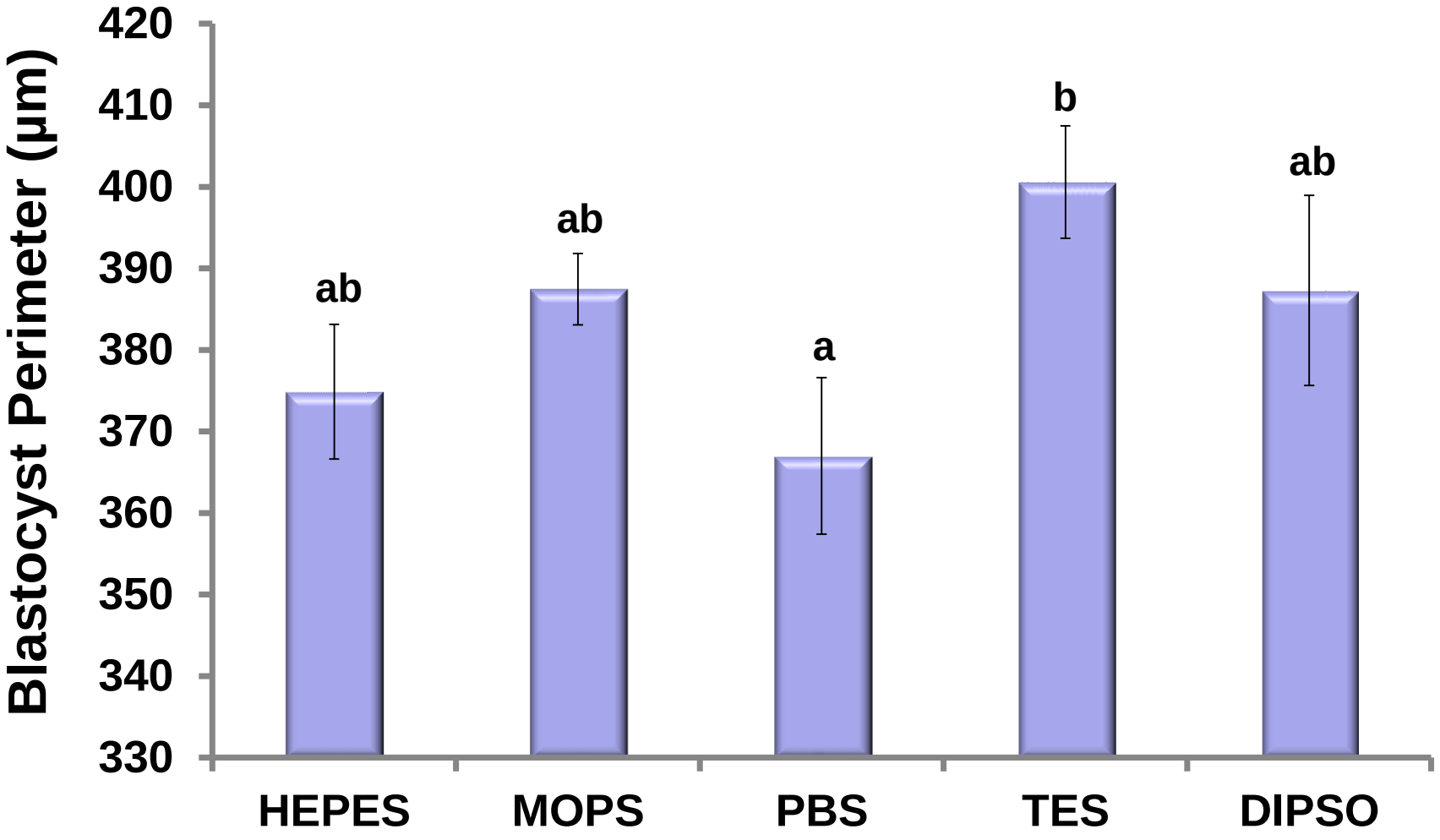
Temperature Impacts Buffering

Common Name	pK_a at 20°C	pK_a at 37°C
TAPSO	7.7	7.39
DIPSO	7.6	7.35
HEPES	7.55	7.31
TES	7.5	7.16
~~Phosphate*~~	~~7.21~~	~~7.19~~
MOPS	7.20	6.95
Carbonate*	6.38	6.30

Buffers & Embryo Development



Buffers & Blastocyst Size



Concerns with Buffers

- Potential **valid** concerns with buffers:
  - Cell specific sensitivity to a particular buffer
    - Evaluate various buffers
      - adequate buffering (pKa)
      - examine toxicity
  - Concentration dependent side-effects
    (Downs & Mastropoki 1997, Iwasaki et al. 1999)
    - Minimize concentration when possible
      - maintain adequate pH stability
      - support development

Buffer Conc. & pH Stability

Buffered Media
(MOPS or HEPES)
(10,12,14,16, 21mM)

↓

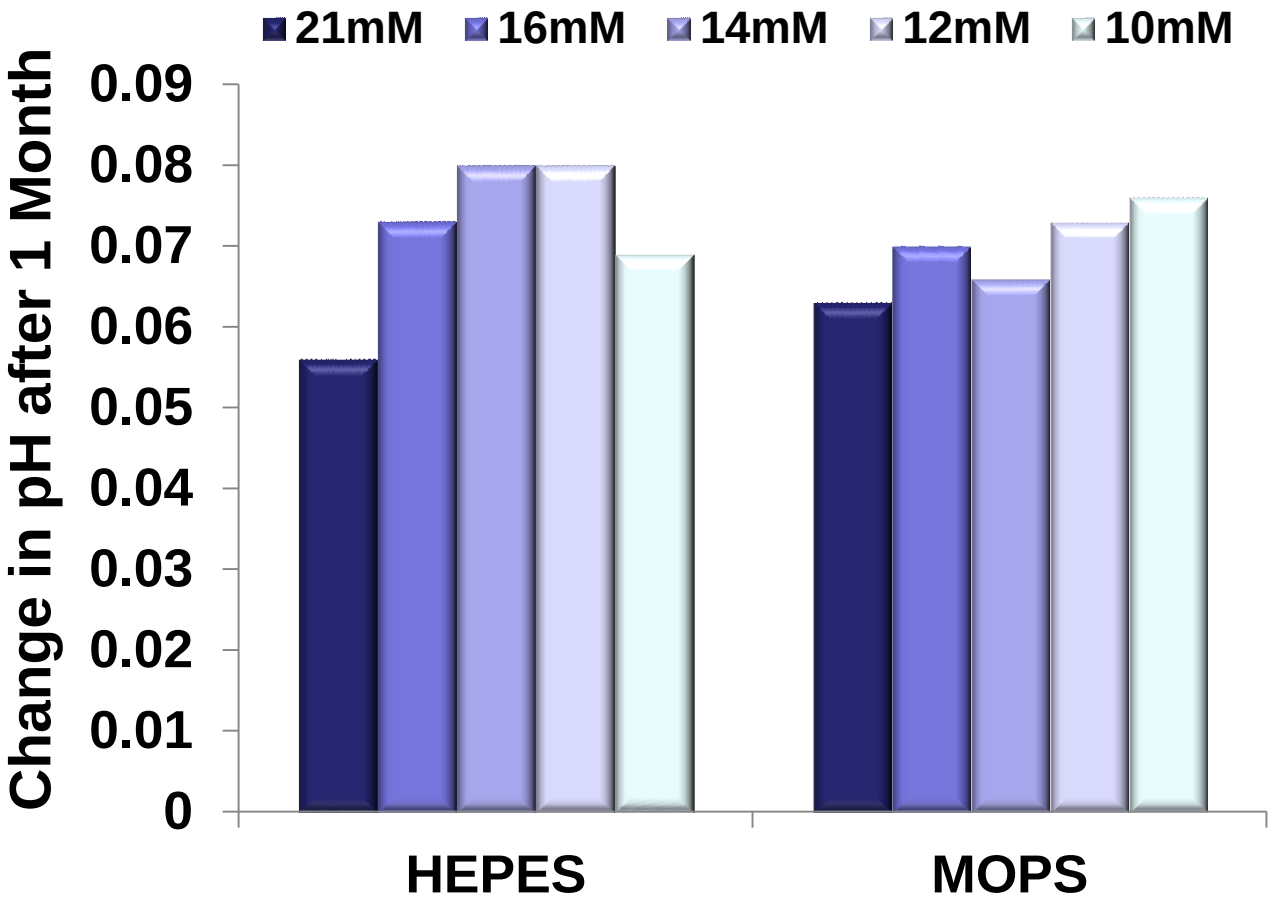
Aliquot 10mL

↓

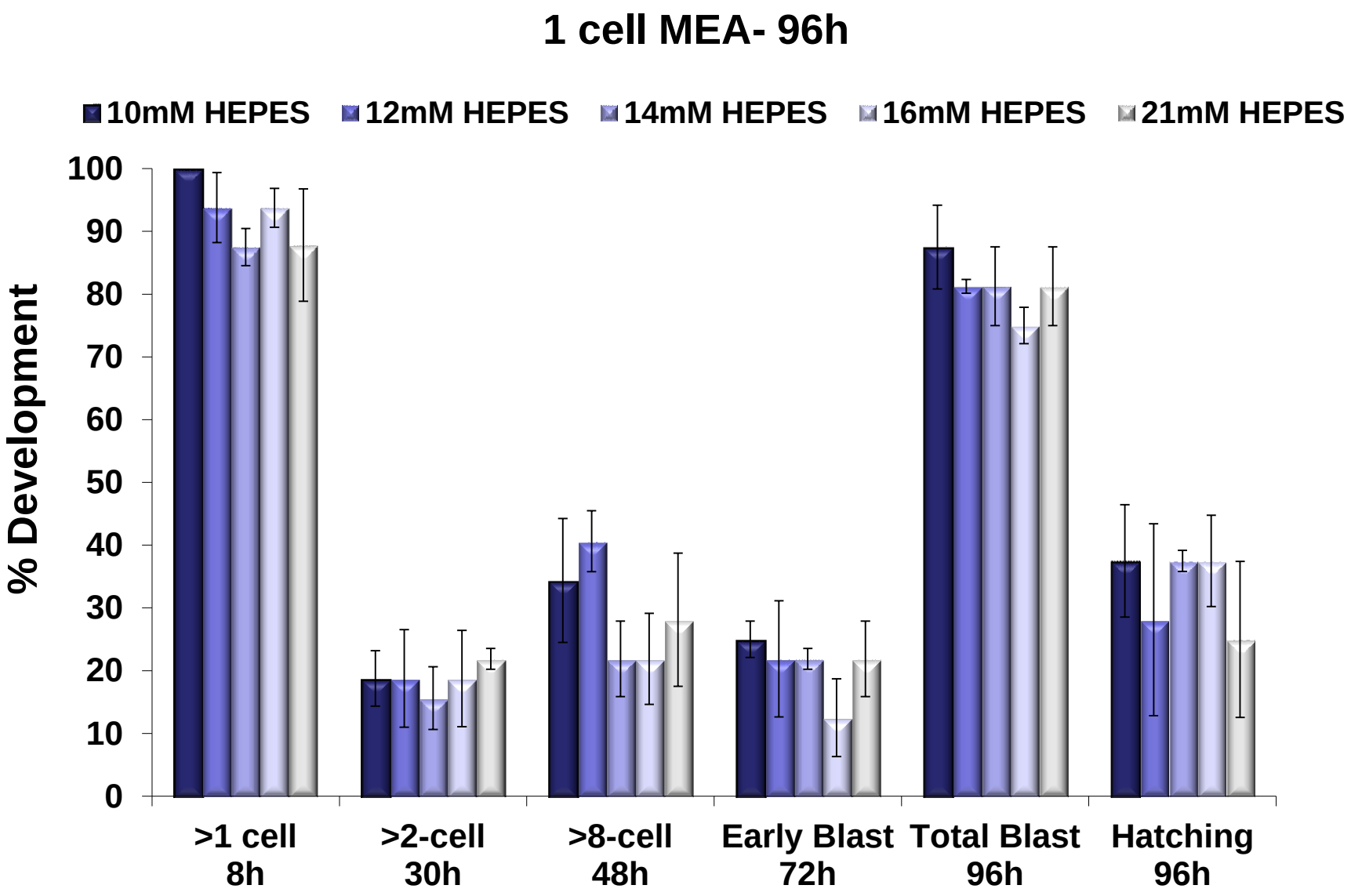
Open/Re-Cap
tube every 4 days
for 1 month

↓

Measure pH @ 37°C



Buffer Conc. & Embryo Development



RBM Online - Vol 18 No 6. 2009 799-810 Reproductive BioMedicine Online; www.rbmonline.com/Article/3860 on web 7 April 2009

Article

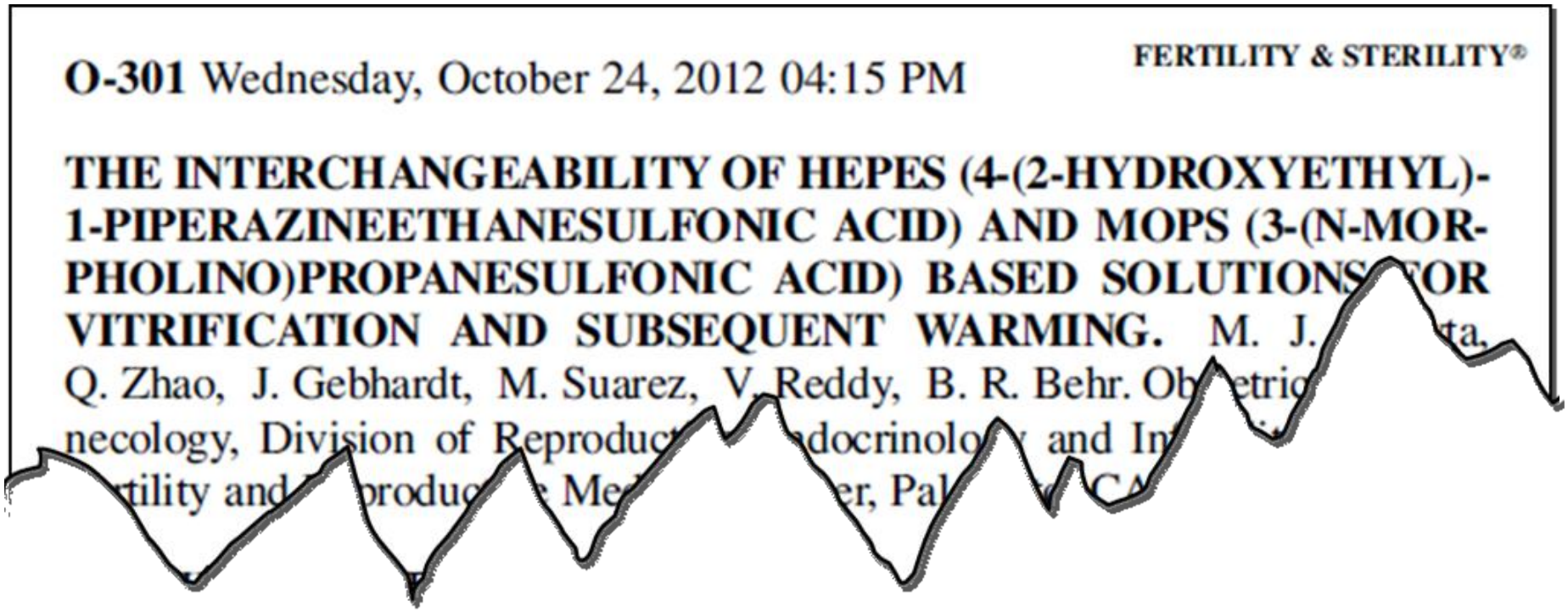
New pH-buffering system for media utilized during gamete and embryo manipulations for assisted reproduction

Jason Swain, PhD, is the ART La[illegible]atory Dire[illegible] the Univ[illegible]ersity of Michiga[illegible]Dr Swa[illegible] completed his post-doctoral tra[illegible] the [illegible]orator[illegible] Th[illegible]mas R[illegible] his Ph[illegible] in Molecular and Inte[illegible] labo[illegible]ry of Dr [illegible]ry [illegible]th, R[illegible] Krisher [illegible] ir[illegible]nclu[illegible]

Combination buffering system

- optimize pH buffering capacity (pKa) and adjust for temperature
- avoid concern with elevated concentration and possible toxicity, or cell-specific sensitivity

Alternating Buffers



FERTILITY & STERILITY®

O-301 Wednesday, October 24, 2012 04:15 PM

THE INTERCHANGEABILITY OF HEPES (4-(2-HYDROXYETHYL)-1-PIPERAZINEETHANESULFONIC ACID) AND MOPS (3-(N-MORPHOLINO)PROPANESULFONIC ACID) BASED SOLUTIONS FOR VITRIFICATION AND SUBSEQUENT WARMING. M. J. ta, Q. Zhao, J. Gebhardt, M. Suarez, V. Reddy, B. R. Behr. Ob etric necology, Division of Reproduct ndocrinolo and In tility and produc Me er, Pal CA

Changing buffers appears safe for embryo vitrification/warming

Minimize pH Stress *In Vitro*

- Educate ourselves
- Good technique
  - Monitor pH, narrow range, work quickly
- Appropriate conditions
  - Oil overlay, buffered media
- Adopt change

Acknowledgements

cryoport

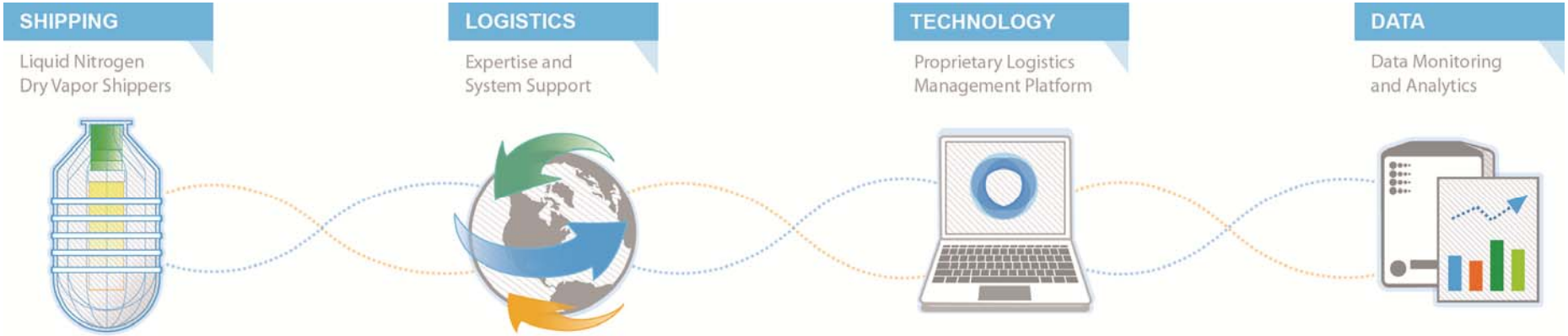
University of Michigan
Center for Reproductive Medicine

- Laura Keller
- Melissa Hiner
- Lisa Gerisch

SOCIETY OF REPRODUCTIVE Biologists & Technologists
ASRM SRBT

swainj@umich.edu

And the winner is ???



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