

Webinar

How to monitor pH dynamics

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Hosts:
Matt Holmes
Blood Cell Storage Inc.



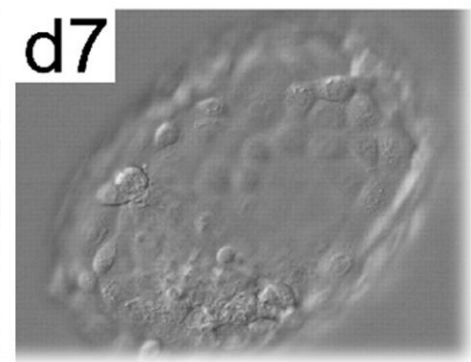
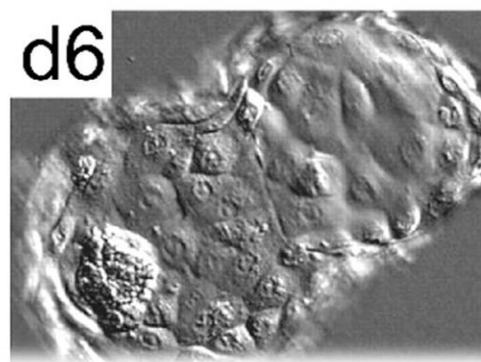
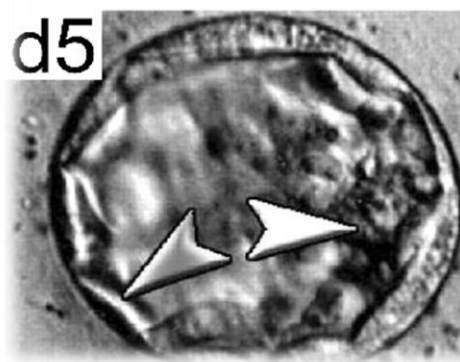
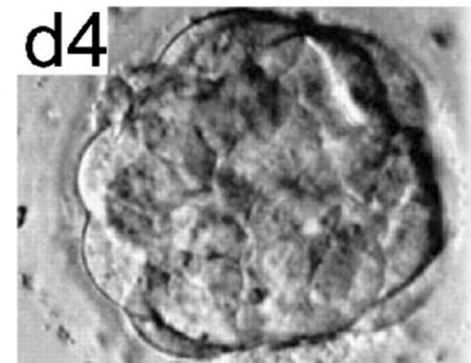
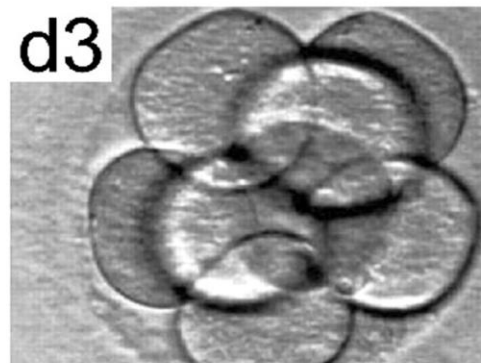
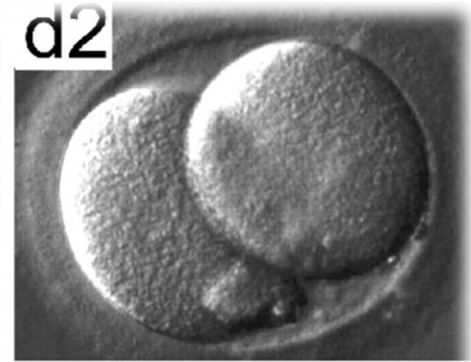
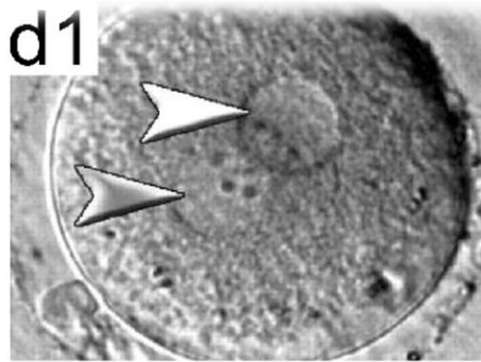
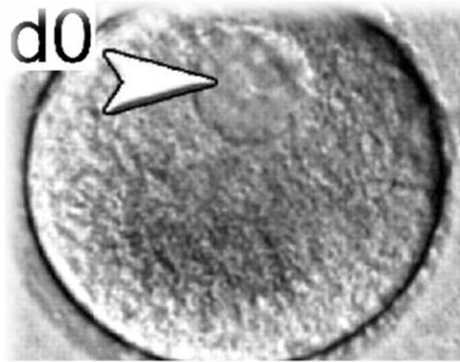
The presentation will focus on how to monitor pH dynamics to avoid negative effects on gamete/embryo development and function.

Expected Result:

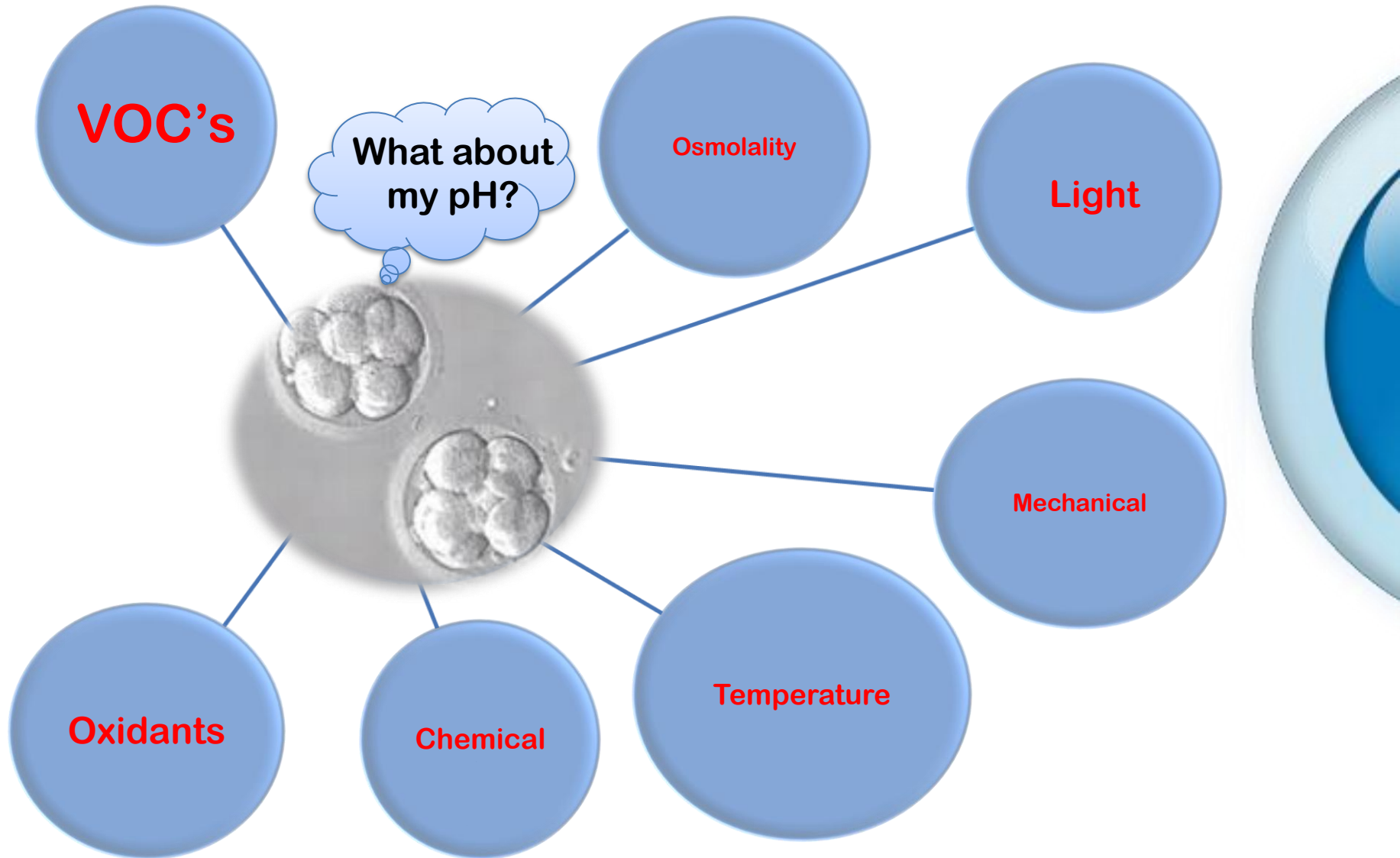
Building a bridge between science and technology.



pH and the cell culture lab - should we care?



pH and the cell culture lab - should we care?



Learning Objectives

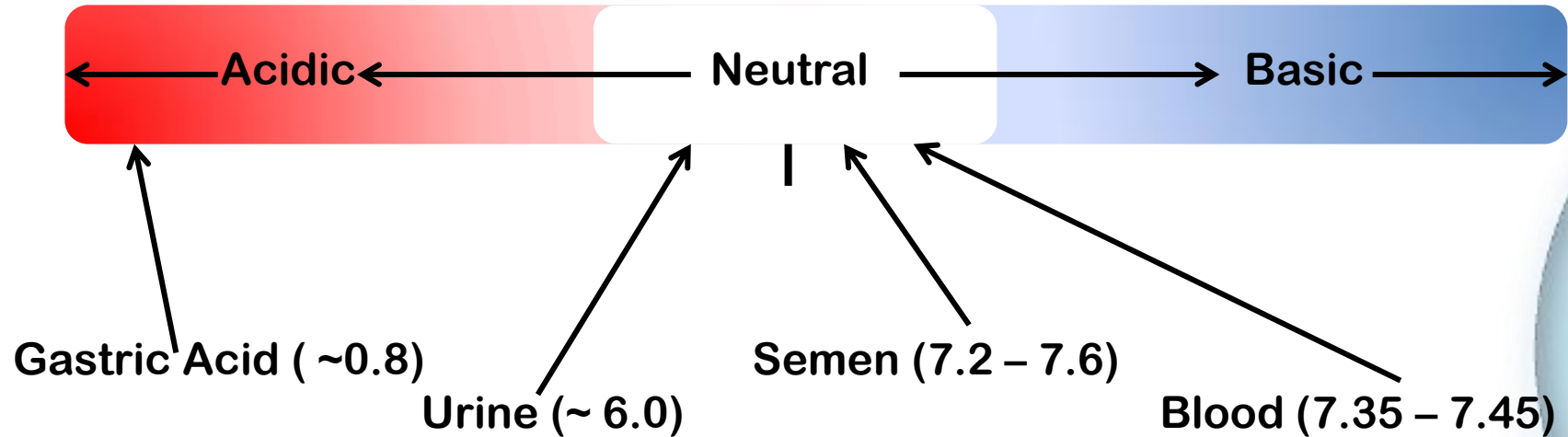
After attending this webinar, the participant should be able to:

1. Explain how important is pH_o in regards to the pH_i
2. Understand the correlation between CO_2 vs. pH Measurements
3. Understand the 3 phases of culture media pH
4. Aiming for the best pH measuring technique

Objectives

1. Optimal pHo vs. pH_i
2. CO₂ vs. pH Measurements
3. The 3 phases of culture media pH
4. Finding the “best” pH measuring technique

A short pH overview



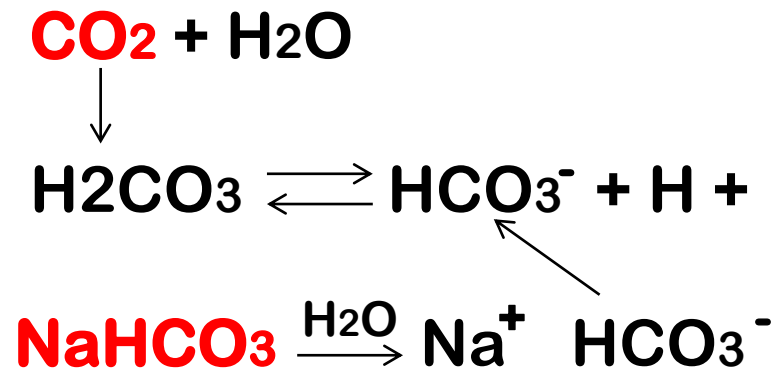
pH is the measurement of hydrogen ions

Therefore:

- An acid impact will increase the concentration of hydrogen ions
- A basic impact will decrease the concentration of hydrogen ions

Objectives – 1. Optimal pHo vs. pH_i

Outside media pH (pH_o)



Therefore we know that:

As CO₂ increases, pH decreases and vice versa

Objectives – 1. Optimal pH_o vs. pH_i

There is a limited pH control of cytoplasm

- Cells do contain pH_i regulatory mechanisms as:
 - $\text{HCO}_3^-/\text{Cl}^-$ exchanger $>7.2-7.3$
 - Na^+/H^+ antiporter <6.8
 - Na^+ dependent $\text{HCO}_3^-/\text{Cl}^-$ exchanger <7.0
- **But**, pH_i will follow pH_o as it will be influenced by amino acids and other media components.

Therefore we can conclude that:

Cells can function & develop over a range of pH_o for a limited period



Objectives – 1. Optimal pHo vs. pHi

- We know that denuded mature oocytes lack robust pHi regulatory mechanisms
 - Conveyed by cumulus cells
 - Activated ~6h after fertilization (Phillips et al. 1998, 2000, 2002)

Proper and stable pHo is therefore crucial

- We also know that cryopreserved/thawed embryos have reduced ability to regulate pHi
 - ~3h time frame for recovery (Lane et al. 2000)
- Sperm motility and binding to the zona pellucida is influenced by medium pHo (Hamamah et al 1996, Dale et al. 1998)

Embryo development can be influenced by medium pHo

Objectives – 1. Optimal pHo vs. pH_i

Other areas of pHo impact:

- pHo and Sperm Motility
- pH_i and Cellular Organization
- pH_i and Embryo Metabolism
- pH_i and Fetal Development



Objectives – 1. Optimal pHo vs. pH_i

What is the optimal pHo than:

- pHo higher than pH_i to combat acidification (~7.2)
 - Human embryo pH_i is ~7.1 (Phillips et al. 2000)
- <7.4 to avoid reduced development
- Optimum pHo likely varies from medium to medium
- Ingredients, like lactate and amino acids, can impact pH_i independently from pHo

Maintain a narrow and stable pHo

Objectives – 2. CO₂ vs. pH_o Measurements

Some other facts:

- Embryos utilize carbon from CO₂ 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)
 - pH_i regulation (Zhao & Baltz 1996, Edwards et al. 1998)

It is therefore difficult to isolate pH_o as a variable

So, what can we do?

Objectives – 2. CO₂ vs. pH_o Measurements

- Most researchers usually use 5 – 7% CO₂ in air, 4 – 10% CO₂ is common for most cell culture experiments.
- Each medium has a recommended CO₂ tension and bicarbonate concentration to achieve the correct pH_o and osmolality;
- Refer to the media manufacturer's instructions for more information.

It is therefore difficult to isolate pH_o as a variable

Objectives – 2. CO₂ vs. pH_o Measurements

Some “rhetorical” (but important) questions:

- But what about the CO₂ measurements?
- Do you trust your CO₂ incubator?
- How often do you measure the CO₂ level?
- How do you measure the CO₂ level?
- What is the “best” CO₂ analyzer?
- How often do you calibrate your CO₂ incubator concentration level?
- How often do you calibrate & validate your CO₂ analyzer?
- How do you calibrate your CO₂ analyzer?

It is therefore difficult to isolate pH_o as a variable

Objectives – 2. CO₂ vs. pH_o Measurements

Some other facts for a possible scenario:

- Not all culture media contains the same concentration of bicarbonate. This may affect the pH_o.
- Protein supplementation dilutes the concentration of media components.
- What do we do than?

It is therefore important to measure pH_o than simply relying on the CO₂ value

Objectives – 2. CO₂ vs. pH_o Measurements

Some other “disputable” facts:

- Zwitterionic buffered media containing HEPES
- Without measuring the pH_o of the medium a lab may never know if the cells are being exposed outside of a “acceptable range” and will therefore lack informations
- Viable data is essential

Important to measure pH_o due to media formulation differences

Objectives – 2. CO₂ vs. pHo Measurements

The above mentioned scenarios suggests that measuring pHo is wise & prudent for a variety of reasons.

- Measurement can validate functioning of the incubator and help determine reliability of CO₂ measurement
- Providing insight into a particular acceptable range within a specific laboratory
- Measuring pHo can help track variation in media formulation that may occur over time

References: Swain JE Is there an optimum pH for culture media used in clinical IVF? Hum Reprod Update 201218(3): 333-9

Important to measure pHo due to media formulation differences

Objectives – 2. CO₂ vs. pH Measurements

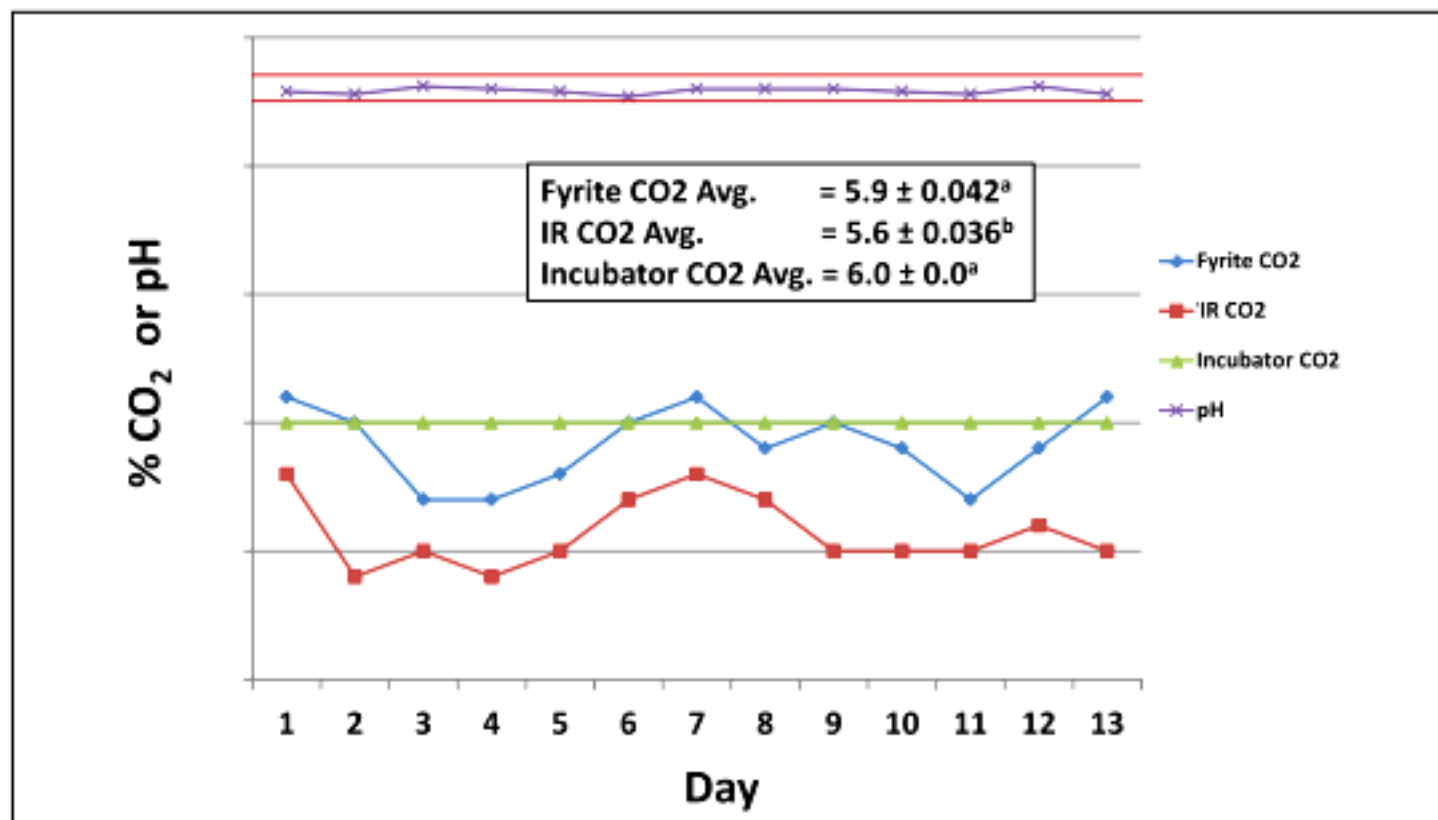
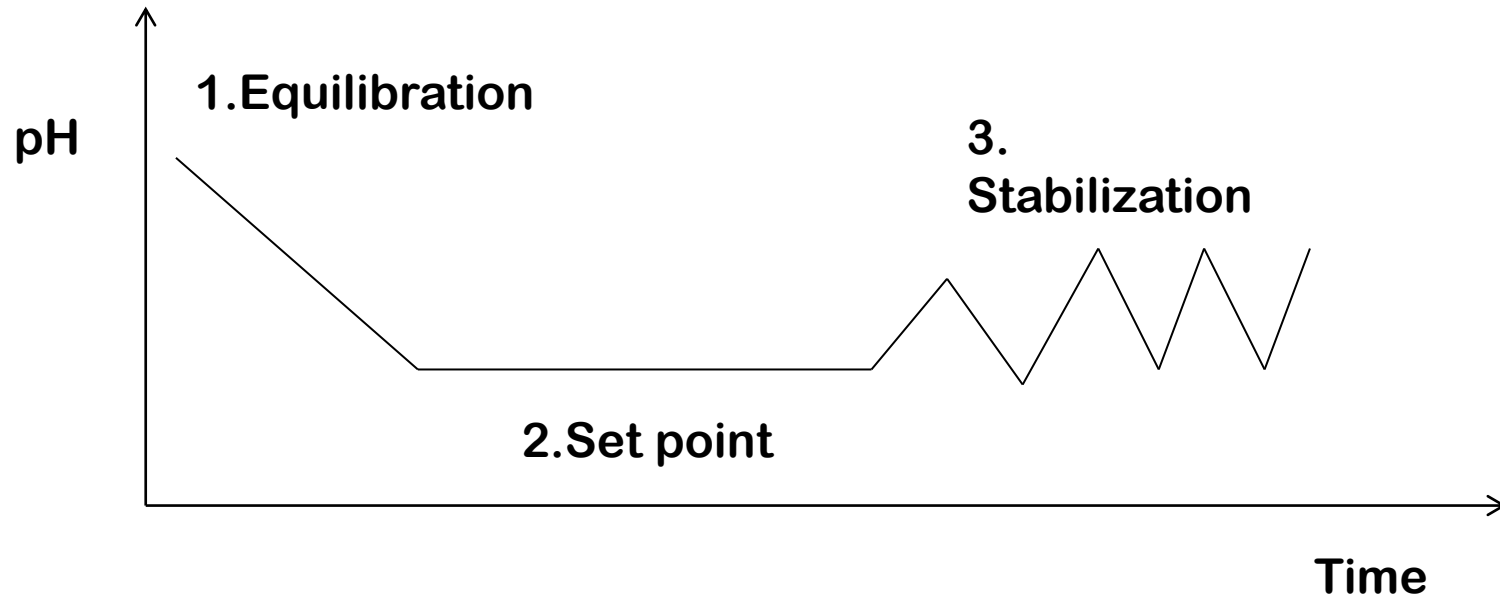


Figure 1. CO₂ readings were recorded over 13 days using a Fyrite and an automated IR measuring device. Readings were compared to IR incubator CO₂ ratings and also daily pH measurements.

Fertility Magazine • Volume 15 • www.FertMag.com

Objectives – 3. The 3 phases of culture media pH

Important to focus on the 3 phases of media pH



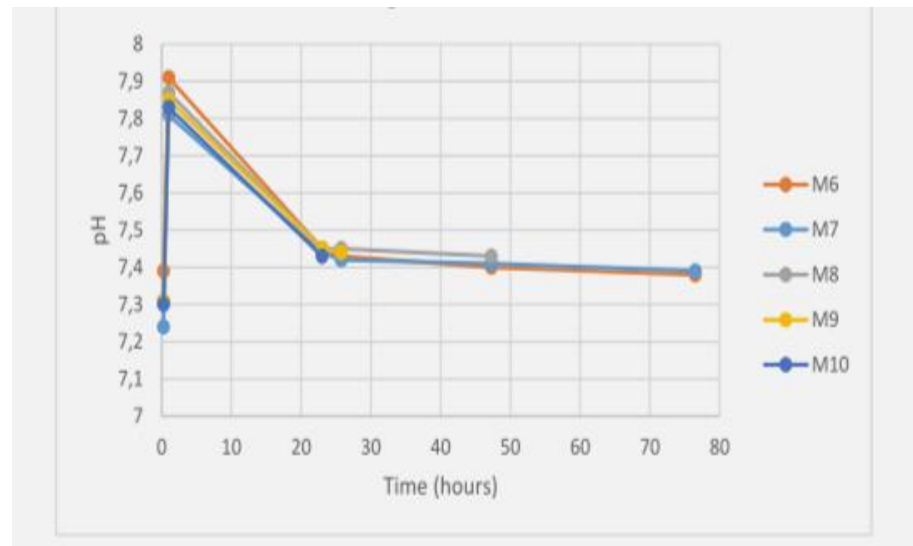
Objectives – 3. The 3 phases of culture media pH

Equilibration

To be considered:

- Media volume
- Oil volume
- Dish and lid opening
- Door openings
- pH measure start/stop
after min. 8 hours

IVF medium Equilibration in a 6 chamber benchtop incubator



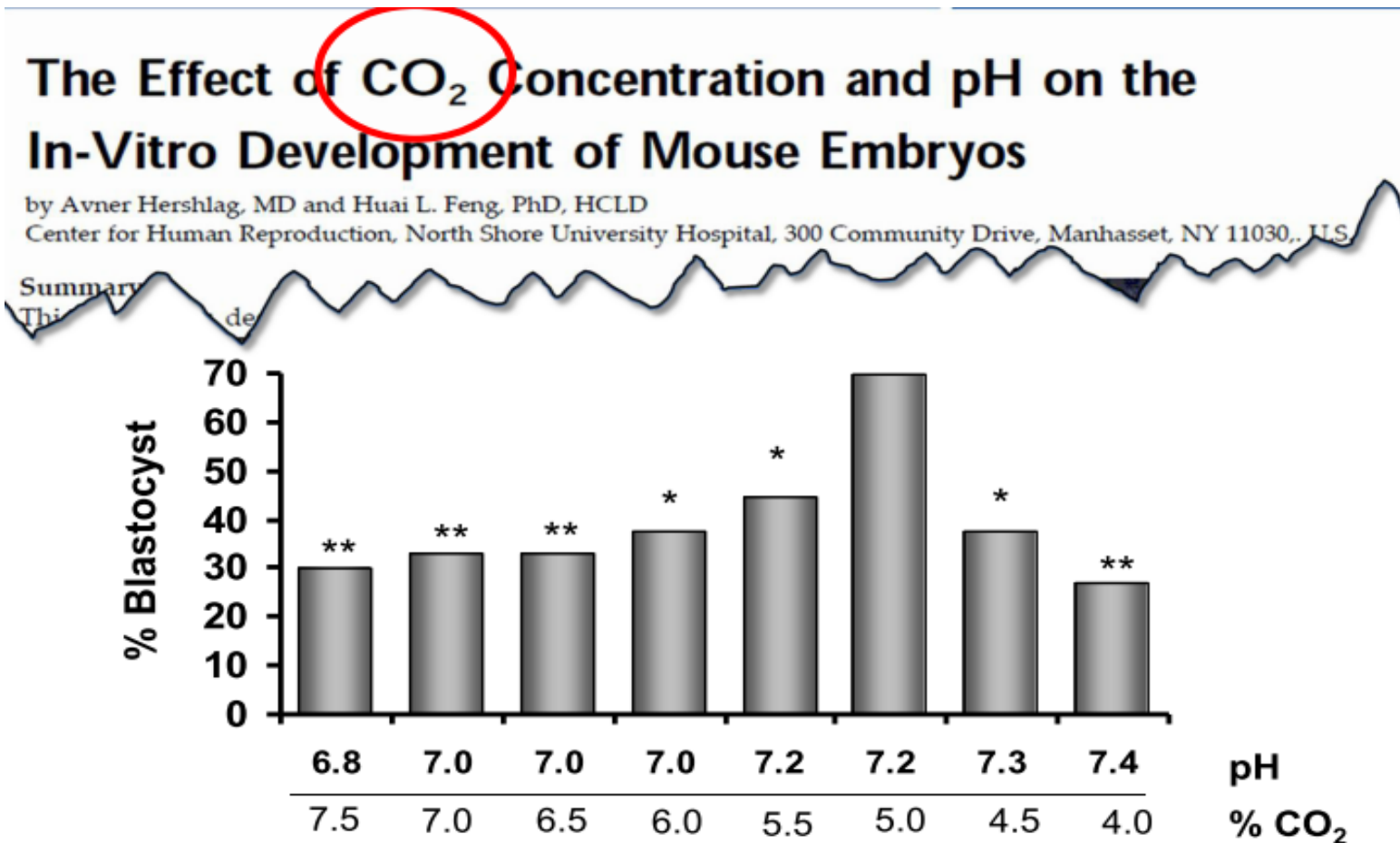
SAFE Sens measurement of IVF Medium over 3 days

Development and integration of SAFE Sens pH sensor in to a bench top incubator for culturing of human embryonic cells . Danish Technological Institute January 2015

Remember: pH is a measure of time

Objectives – 3. The 3 phases of culture media pH

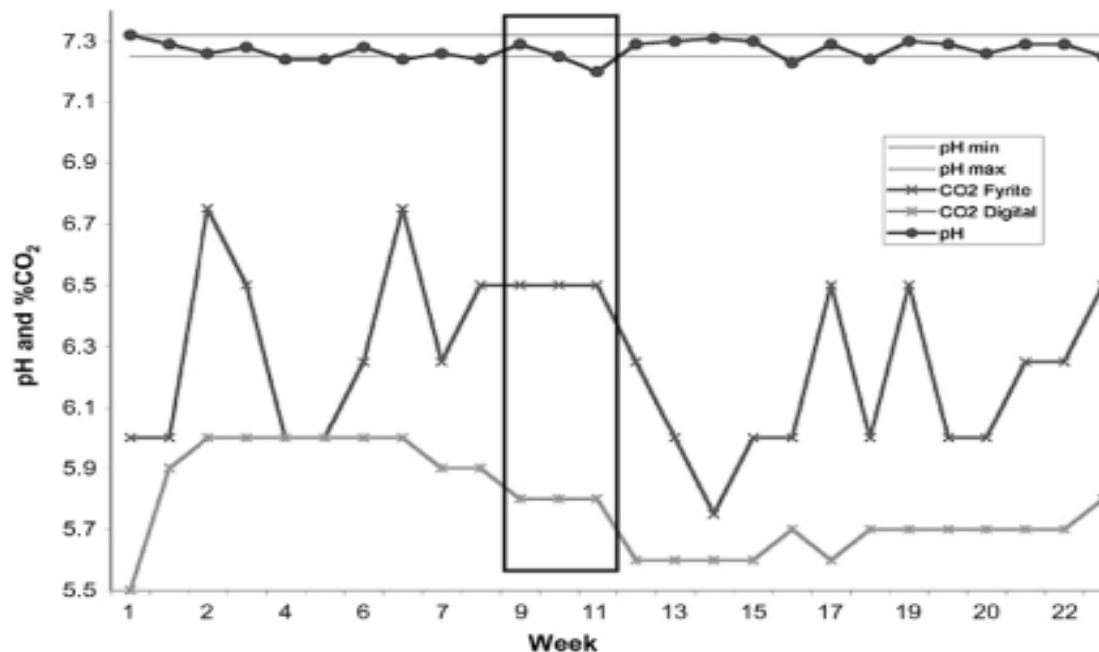
CO₂ Set Point



Fertil Mag 2005

Objectives – 3. The 3 phases of culture media pH

CO₂ Set Point



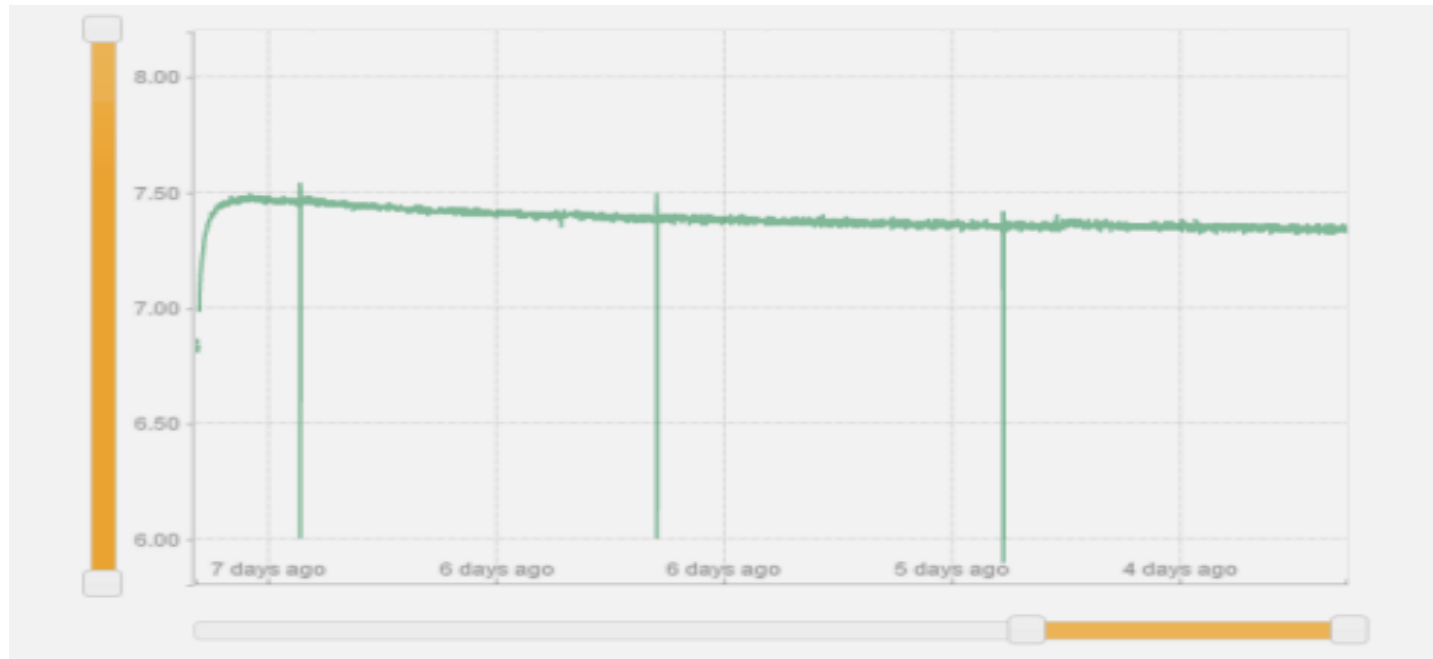
Demonstration of the fluctuation and inaccuracy of fyrite as an indicator of pH (adapted from Pool (2004)).

Remember: It matters how and what technology you use to measure pH and CO₂

Objectives – 3. The 3 phases of culture media pH

Stabilization

Continuous logging with 1 minutes interval of pH using SAFE Sens technology



Development and integration of SAFE Sens pH sensor in to a bench top incubator for culturing of human embryonic cells . Danish Technological Institute January 2015

Remember: It matters how and what technology you use to measure pH and CO₂

Objectives – 4. Finding the “best” pH measuring technique

Finding the “best” pH measuring technology

Does it matter?

- Inside or outside of the incubator?
- Invasive or non-invasive?
- **Sampling or time-measurements?**
- What do we use as a reference?
- How often does it require calibration?
- Glass electrode, ISFET, Optical?
- Is it going to interfere with my daily routine, process?
- Would it make my life easier in the lab?

How to decide?



Objectives – 4. Finding the “best” pH measuring technique

Finding the “best” pH measuring technology

What do we expect from the winner?

- Accuracy
- Reliability
- Can be validated for cell culture
- Improving the cell culture outcome
- Data logging
- Making my lab life easier
- Price competitive
- ...

SURE THING BOSS



Objectives – 4. Finding the “best” pH measuring technique

Finding the “best” pH measuring technology



Ion selective sensors



Glass sensors

What about Optical Fluorescence?



ISFET technology



pH test strips

Objectives – Finding the “best” pH measuring technique

I find the optical fluorescence technology to be the best because it offers:

- Proven Accuracy
- Proven Reliability
- Can be validated
- Will improve cell culture outcome
- Data logging available
- Non-invasive
- Makes the lab life easier
- Price competitive
- ...



And the winner is...

Remember: It matters how and what technology you use to measure pH

Objectives – Finding the “best” pH measuring technique

I find the optical fluorescence technology to be the best:



pH Trackstation



pH Sensor interface



SV² pH sensor



And the winner is...

Remember: It matters how and what technology you use to measure pH

Objectives accomplished?

*"I hear, I know.
I see, I remember.
I do, I understand."*

(Confucius, 551BC – 479BC)

Thank you for your attention!

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