pH Monitoring Practices and Benefits

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Summary

Controlled embryo culture conditions are critical for optimal success rates within the *in vitro fertilization* (IVF) lab. The daily quality control required to track culture conditions in real-time is labor intensive, costly, or misleading about true culture conditions. In this white paper, we discuss fundamentals behind the pH data point, the effects that laboratory staff and equipment have on media pH and discuss suggestions for pH value trending in embryo culture labs. pH trending data was collected using the SAFE Sens® TrakStation® pH monitoring product.

Introduction

A passive approach to embryo culture does not provide sufficient evidence of culture stability and is detrimental to embryo development. Treating pH as a constant value, solely measuring CO_2 gas percentage values, or taking the certificate of analysis pH value as dogma are not only inaccurate practices, but also increase risk for the IVF lab and for patient success. The reality of the IVF lab is that media pH will always be dynamic and in constant fluctuation- protein supplementation, temperature, humidity, and atmospheric pressure all affect medium pH and pH fluctuations as well as workflow patterns, dish prep protocol, and equipment quality. The IVF lab that asks what is the correct pH is asking the wrong question- the true question is how much pH fluctuation is acceptable, and what can we do in the lab to reduce harmful pH fluctuations?

pH Relevance to the IVF Lab

The effects of media pH on culture must first be discussed to understand pH from a quality control perspective. Non-optimal pH acts as a stressor on embryo development [1-6], with a media pH range of 7.0-7.4 best supporting embryo development [7-8]. pH plays a critical role in optimal development at multiple stages and can be affected by IVF procedures. Denuded mature oocytes lack robust mechanisms to regulate internal pH [9] and the meiotic spindle in mouse oocytes has been shown to be sensitive to culture media pH changes [10]. Cleavage stage embryos have a reduced ability to control internal pH compared to post-compaction embryos [11-12], and cryopreserved/warmed embryos have a reduced ability to regulate internal pH for several hours [13]. Brief periods of pH rising have been found to impact mouse blastocyst

development, hatching, and gene expression profiles [14]. Changes in the intercellular pH of embryos affect metabolic activity, and even influence fetal development [15-16].

The relationship between external media pH and internal embryo pH is further confounded by differences in media components [17-18]. The quantity of amino acids such as glycine, taurine, and glutamine affect media pH, as they act as zwitterions and buffer the internal pH in the embryo [11-12]. Monocarboxylic acid content (lactate and pyruvate) in culture media will also lower embryonic internal pH [19].

Subtle physiological changes in spermatozoa occur when pH is not adequately controlled. Spermatozoa cells function in a wide pH range with normal seminal fluid pH ranging from 7.2-8.0 but are still affected by pH fluctuation. Spermatozoa are immotile in low pH (<7) environments such as in the epididymis *in vivo* [21] and achieve optimal zona binding at a media pH near 7.5 [9]. Alkalization of the spermatozoa cytoplasm occurs during capacitation and triggers calcium influx, spermatozoa activation and hyperactivated motility [22].

Lab Workflow and pH

Equilibration time for external pH varies depending on embryo culture protocols, incubator settings, and dish prep materials used. For example, in microdrop embryo culture, the oil overlay acts as an initial barrier to gas diffusion extending the time required to reach equilibrium in culture. However, differences in dish prep techniques and oil types mean that each lab will have a unique pH equilibration time.

In microdrops of media, pH changes occur rapidly without an oil overlay (Figure 1) and quickly reach values outside of acceptable development ranges. Every lab should perform experiments using their equipment, oil volumes, and dish types to determine if sufficient steps are taken to maintain CO_2 content as well as prevent media evaporation in their protocols.

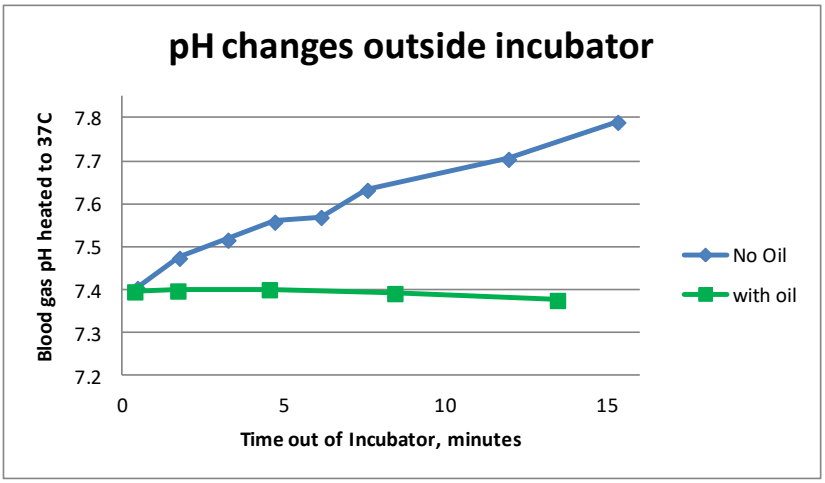

Figure 1 Samples Without Oil Increase In pH Quickly And Linearly, While Samples With Oil Maintain pH

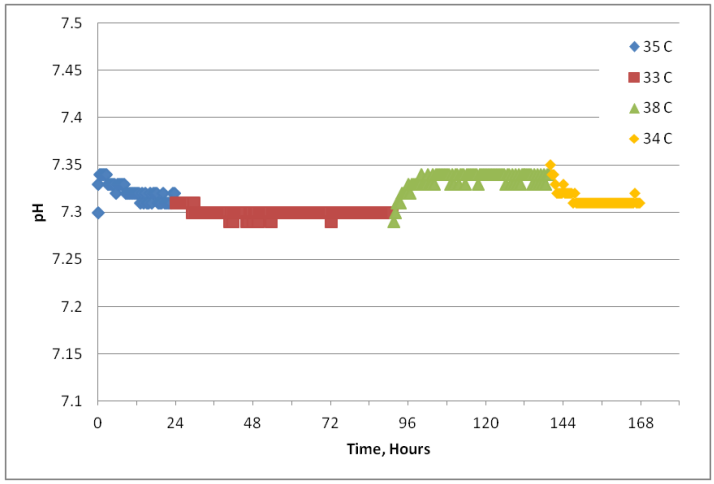

Figure 2 pH Trending In Relation To Benchtop Incubator Temperature Alterations

Temperature affects pH values (Figure 2) and in microdrop culture, the oil overlay acts as an insulator for media temperature. The lab should check direct surface temperatures in culture incubators as well as perform microdrop temperature testing to determine the true temperature of culture media. The heat exchange between disposable plastics, culture media, and oil type all affect the actual temperature of culture microdrops and subsequently, direct culture media pH values.

Frequent usage in large format incubators affects pH over the workday (Figure 3). Frequent usage leads to a cumulative effect on pH shifts in culture that are not immediately resolved. The workflow of the day and the efficiency of work have a direct effect on culture stability, even when using an oil overlay. A difference in incubator fluctuations can be significant enough that a re-evaluation of equipment maintenance, workflow patterns, or culture system disposable types is necessary.

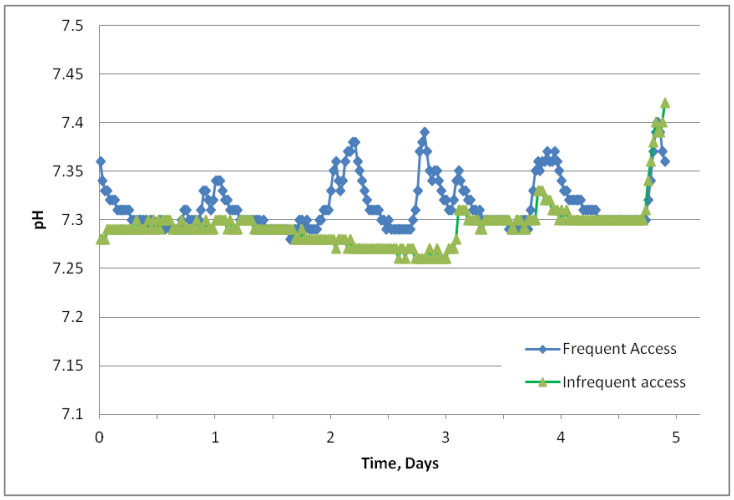

Figure 3 Morning pH Monitoring Spikes During Frequent Usage. Data Collected From An Incubator Equipped With A Thermal Conductivity Sensor

pH trends give Andrology and Embryology labs an effective tool to determine the effects of dish and media prep protocols on the culture system, or to observe how scheduling for clinical protocols such as embryo transfer, intrauterine insemination (IUI), and IVF sperm prep affect frequency of equipment usage.

Equipment Function and pH

Equipment malfunction can affect pH values in embryo culture and reduce patient outcomes. Without proper incubator management, culture stability and embryo development will suffer without lab staff noticing.

CO_2 overshoot and water pan levels in large format incubators both affect true media pH values and fluctuations (Figure 4-5). Embryology and Andrology labs that use bicarbonate-based media should strive to maintain media pH as much as possible during regular cell manipulation workflow and stay observant of how their incubator functions and is maintained.

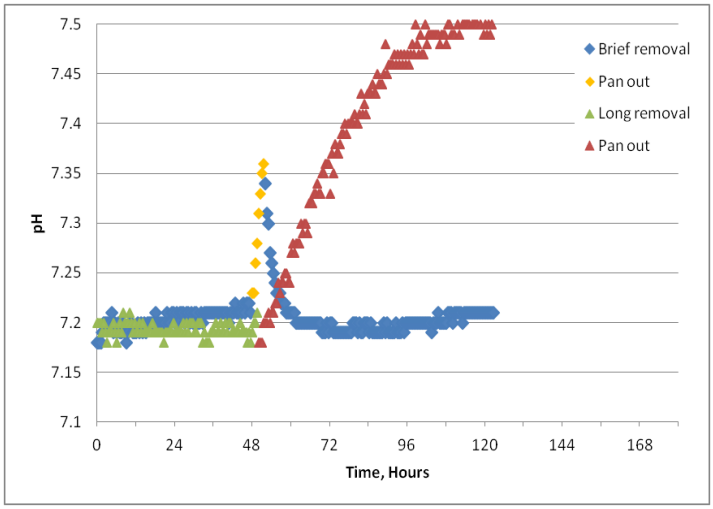

Figure 4 Consistency In Incubator Humidity Is Key To Reducing pH Fluctuation, particularly for incubators with Thermal Conductivity CO_2 sensors.

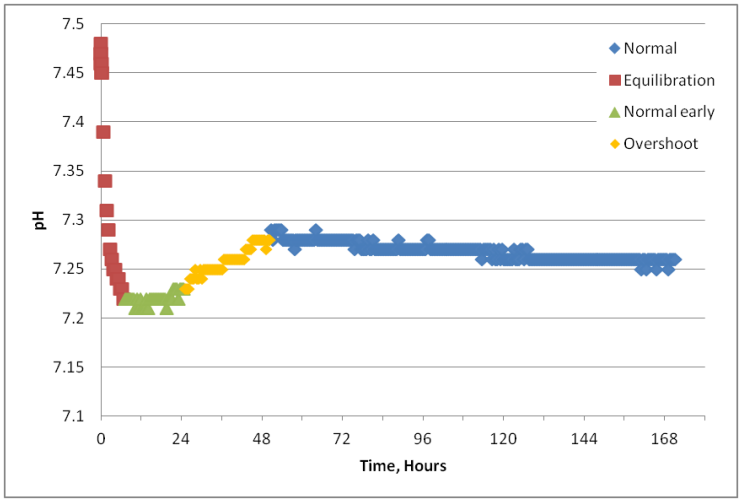

Figure 5 Moderate pH Increases From Gas Overshoot Within The Incubator.

Benchtop incubators show minimal changes in pH, primarily due to the optimization of the benchtop system. However, even benchtop incubators need consistent pH data to determine the true culture environment, and a CO_2 measurement may not be accurate. Equipment malfunction or external changes cause stressful pH values in embryo culture and reduce patient outcomes when out of ranges that support development (Figure 6).

Part of maintaining a sound culture system includes constant vigilance when it comes to details- the incubator cannot function effectively if it is not maintained correctly. Tracking pH allows the lab to receive trending data to catch inconsistencies and begin emergency procedures if things start to go wrong. Without pH tracking, the effects of compromised gas line tubing would not be determined until the next time pH was taken, during an investigation into poor embryo development, or until the tubing was manually fixed or replaced.

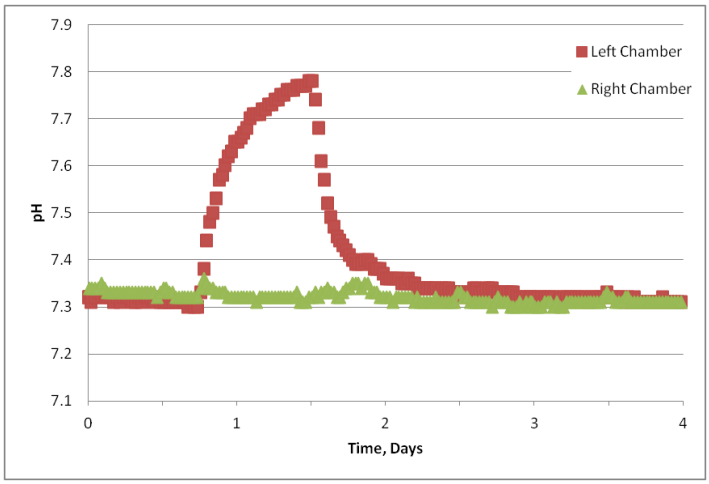

Figure 6 A Humidifying Bottle Gas Line Blockage In The Left Chamber Leads To A pH Spike From No Gas Flow

Media Contamination and pH Values

Monitoring pH from large batch lots of media acts as a first-alert to contamination in the culture environment. Bacteria is the most commonly found contaminate in cell culture and are detected through qualitative methods such as sudden pH drops, medium turbidity, and low-power microscope analysis. Some strains of bacteria, yeast and mold colonies exhibit stable pH values during inoculation, but over time with propagation a decrease in pH levels is observed.

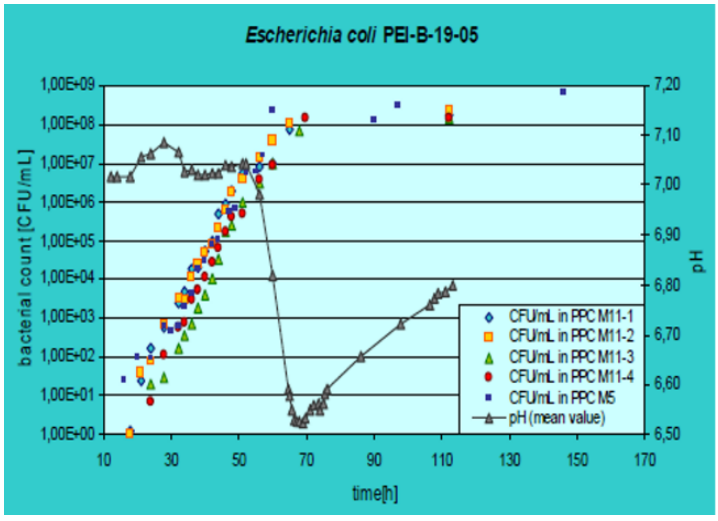

Figure 7 E. coli Contamination In Platelet Concentrates Leads To pH Drops With Cell Propagation. Data collected using the BCSI pH SAFE Reader.

Three common contaminants in the IVF lab include *Escherichia Coli* (*E. Coli*), *Candida Albicans* (*C. albicans*), and *Staphylococcus Aureus* (*S. Aureus*). Micro-organism contaminants commonly enter the culture system from skin flora, semen samples, follicular aspirates or from improper aseptic technique during the embryo transfer procedure.

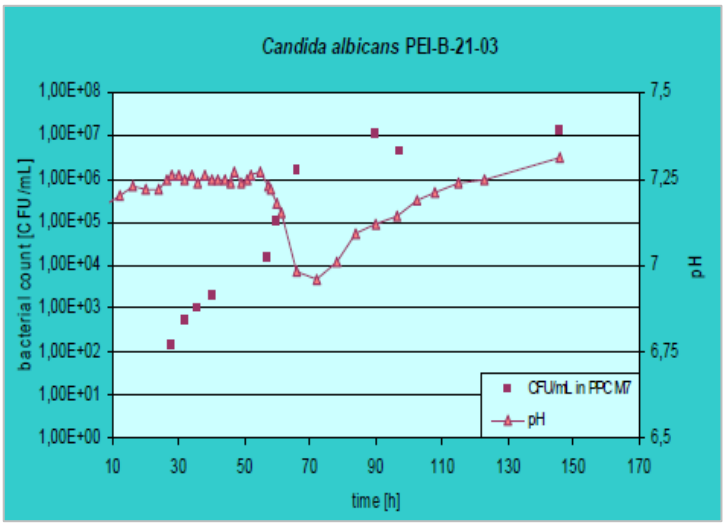

Figure 8 C. albicans Contamination In Platelet Concentrates Require A Longer Time For Cell Propagation To Affect pH Values. Data collected using the BCSI pH SAFE Reader.

Some forms of culture media contamination are undetectable without direct testing. For example, bacterial mycoplasma contamination shows no outward physical signs such as turbidity or pH changes. While the use of gentamycin in media combats mycoplasma growth, contamination can still occur in media that has been supplemented with antibiotics.

Tracking pH values in inoculated blood platelet bags has led to interesting graphical representations of how specific contaminants alter pH in a closed culture system. *E. Coli* (Figure 7) and *C. albicans* (Figure 8) propagation triggers metabolic consumption and directly decreases pH once the bacteria has reached a count of between 10 Million to 100 Million CFU per mL [22]. *S. aureus* (Figure 9) propagation causes steep and rapid acidification of media pH due to the bacteria consumption of glucose and production of lactic acid [23]. The speed of pH reduction also correlates to the growth properties of the given microorganism. pH monitoring provides a warning signal to contamination when alternate measurement tools will not sound alarms in the lab until contaminant propagation is significant enough to be seen qualitatively.

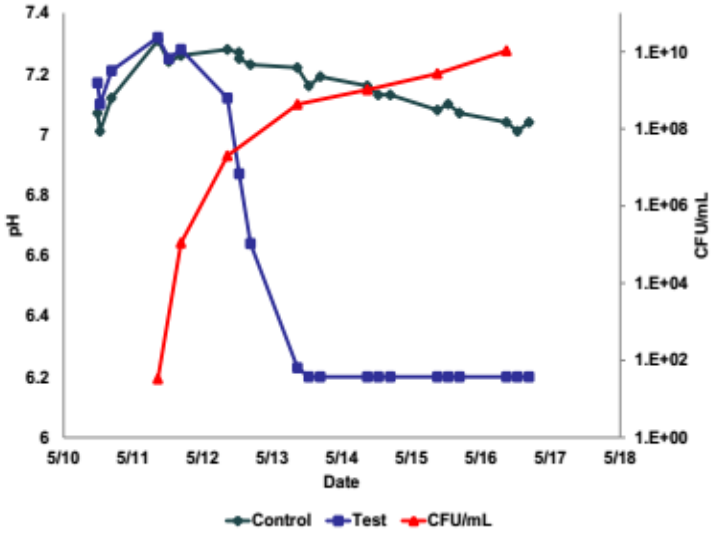

Figure 9 S. Aureus Propagation, Consumption Of Glucose, And Production Of Lactic Acid In Platelet Concentrates Lead to pH Value Decreases. Data collected using the BCSI pH SAFE Reader.

Expected Variation in pH Values and Media Prep Suggestions

Slight value variation should be expected during external media pH documentation- do not become beholden to a specific pH number, but instead observe laboratory values under media company suggestions and use quantitative values to determine a custom pH range. If pH values fall out of the expected range by 0.01 or 0.02 pH units, this may be due to probe/meter variation – do not adjust CO_2 levels to reach an ideal pH reading. Rather, analyze pH trends so that the lab has knowledge of the constant ebb and flow of its specific culture system, and if needed, take further action quickly. The limitless combinations of factors that affects pH mean that no lab can compare their pH culture conditions directly with another lab.

Equipment tracking is critical for all incubators used for culture, dish prep, and sperm prep. Even if embryo culture is performed in benchtop incubators rather than in big box incubators, proper pH is critical for any working media. With pH tracking, the lab can answer many unanswered questions. What is happening to the media that will be used for the embryo transfer in 20 minutes? Are the dishes ready and fully equilibrated for clinical patient usage? What is the shortest time the lab can make a dish to be optimal for embryo development? Are the sperm preps functioning physiologically the way they are supposed to? Is the incubator behaving as expected, or is equipment function affecting the pH of every media that went inside of it? These sorts of questions show why it's so critical to understand the functionality of the incubator you use with pH-sensitive media, including in big box incubators.

Final Thoughts

pH monitoring using the SAFE Sens TrakStation creates a continuous story of the pH changes in the lab over time and is critical to optimize the development of transplantable embryos. pH profiles identify practices that may be yielding suboptimal culture conditions and enable the lab to take appropriate corrective actions. Real and lasting changes to lab protocols are possible by taking advantage of the data observed through continuous pH monitoring. The confidence achieved from optimizing culture and the ease of mind that comes with tracking incubator quality are beneficial to the lab, the IVF staff, and the patients.

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