The Importance of pH Measurement Within the IVF Laboratory

by Jason E. Swain, PhD, HCLD, Assistant Professor, Department of Obstetrics & Gynecology, Scientific Director ART Laboratories, University of Michigan, Ann Arbor, MI, USA

You can contact Jason Swain at swainj@umich.edu

Jason E. Swain, PhD, HCLD

Optimizing growth conditions within the IVF lab is a common goal amongst embryologists. Paramount in this endeavor is minimizing detrimental environmental stressors, which is often achieved through setting thresholds or ranges for various environmental/lab parameters and measuring these variables on a regular basis. As ranges or thresholds are exceeded, corrective action is taken to return parameters to acceptable limits. This hopefully results in a consistent and stable laboratory environment suitable for embryo growth.

One example of an environmental variable that should be measured within the IVF lab is the pH of the culture medium (pHo). During routine handling and processing during common laboratory procedures, gametes and embryos can be especially sensitive to perturbations in pHo, which can lead to alteration in internal pH (pHi) and ultimately impact function and development (see review by Swain 2012). Thus, a proper and stable pHo is crucial.

pHo within the IVF laboratory is primarily the result of equilibrium reached between the CO_2 concentration within the incubator and the bicarbonate concentration of the culture medium, though other factors, such as protein, can also impact pHo. Furthermore, specialized media can be used to stabilize pHo outside the confines of the incubator. Because most laboratories purchase their media, pHo is adjusted most practically by altering incubator CO_2 concentration. This is an inverse relationship, with pHo decreasing as CO_2 increases.

While the optimum pHo is debatable (Swain 2012), and how regularly pHo should be measured can be disputed, most would agree that at some point the pHo should be measured. Simply relying on CO_2 measurements from the laboratory incubator is not prudent.

To begin to illustrate the need to measure pHo, two independent devices, Fyrite and an automated CO_2 infrared sensor (IR), were used for daily CO_2 measurements and compared against the infrared incubator CO_2 reading. Additionally, daily pHo measurements were recorded over 13 days. Importantly, Fryite (saturated KOH) was fresh, the IR device was new, and all devices/equipment were calibrated prior to use. Both Fyrite and IR readings were more variable than the incubator. The IR sensor readings differed significantly from both Fyrite and incubator readings, $p<0.05$ (Figure 1). Importantly, pHo was stable and within acceptable limits during the duration of the study. Thus, if CO_2 measurements alone were relied upon and incubators adjusted based on one of the independent measuring units, then pHo may have fallen out of range. Furthermore, daily CO_2 adjustment is tedious and likely unwise, as pHo remained stable. This demonstrates that equipment readings vary, that all equipment should be validated prior to implementation in the lab, and begins to show that simply setting a CO_2 value, especially trying to do so between labs that may use different equipment, is problematic. In this scenario, at a minimum, an initial pHo should be measured when an incubator is set up to determine which CO_2 reading from a particular device yields the desirable pHo. If there is confidence in the CO_2 reading, and it remains relatively stable, one may assume that the pHo is also stable. However, instrument drift over time and other variables likely dictate more routine pHo measuring.

As another example of the importance of pHo measurement, rather than simply relying on a CO_2 value, not all media contain the same concentration of bicarbonate. As a result, the same CO_2 concentration used to achieve the desired pHo of one medium may not be the same CO_2 concentration required for another. For example, some companies purposely alter bicarbonate concentrations to adjust pHo to permit use within the same incubator. This is often done to give a high-low-high pHo paradigm for use during fertilization-cleavage-blastocyst stages. This is a scenario where pHo should be different between media. However, without measuring pHo, it would be impossible to verify.

Additionally, another common scenario that results in differences in medium bicarbonate concentration and pHo is protein supplementation. When performed in the lab, this supplementation of liquid protein dilutes the concentrations of all media components, including bicarbonate. However, if purchasing media that is pre-supplemented with protein, depending on company policy, some manufacturers volumize after protein supplementation, thus resulting in a higher bicarbonate concentration than if adding protein within the lab. As a result, the pHo is higher in the pre-supplemented media, despite use of the exact same protein and exact same concentration (Figure 2).

Also important to consider is that the amount of protein

impacts pHo. Not only does adding more protein dilute the bicarbonate concentration, but protein supplements tend to be slightly acidic. Both mechanisms result in a lower pHo. A doubling of a protein concentration, from 5% to 10% v/v, often leads to a ~0.03-0.05 decrease in pHo (unpublished observations). This should again make it readily apparent that selecting a single CO_2 concentration and attempting to utilize it between laboratories using different media, protein or equipment is unwise.

This importance of measuring pHo due to media formulation differences is also evidenced by examining zwitterionic buffered media containing HEPES. Depending on the formulation of a particular company, the same basal medium may have a dramatically different pHo (Figure 3). Again, without measuring the pHo of the medium, the lab would never know that they may be exposing their cells to conditions outside of a set "acceptable range".

The above scenarios suggest that measuring pHo is prudent for a variety of reasons. Measurement can validate functioning of the incubator and help determine reliability of CO_2 measurement, while providing insight into a particular acceptable range within a specific laboratory. Furthermore measuring pHo can help track variation in media formulation that may occur over time as recipes are adjusted and modified. These data would suggest that, at a minimum, pHo be measured when initially setting up equipment and installing a culture system. It should then likely be monitored following any major maintenance or media change, perhaps even with new media lots. Daily pHo measures may be performed, especially if this is used to replace daily CO_2 measurements required by some accrediting agencies. However, dailyadjustment of CO_2 should likely be avoided, as this could prove an exercise in futility. For simplicity, and cost effectiveness, weekly pHo measures may be more practical and are sufficient to detect pHo shifts that are trending out of range.

References

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

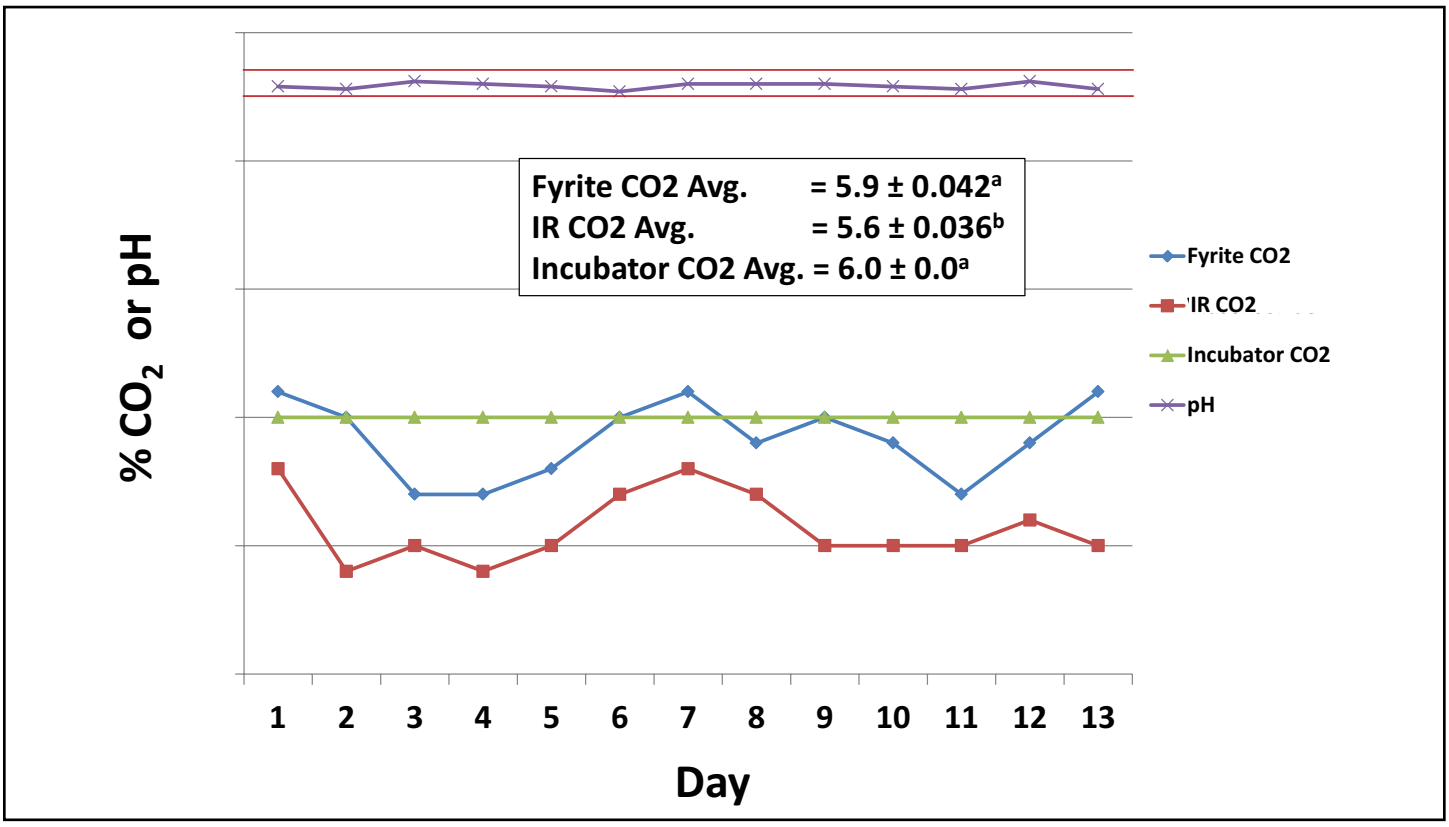


Figure 1. CO_2 readings were recorded over 13 days using a Fyrite and an automated IR measuring device. Readings were compared to IR incubator CO_2 ratings and also daily pH measurements. Fyrite and IR measurements differed significantly, despite both devices being new and calibrated. On average, the two independent devices differed by 0.3 units. IR also differed significantly from incubator CO_2 readings, $p<0.05$. Additionally, daily variation of both the IR and Fyrite devices was greater than incubator CO_2 readings. Importantly, daily pHo measurements were stable and within the laboratory set acceptable range of 7.27-7.32 (indicated by red lines). Thus, if CO_2 readings from an independent device alone are used to, pH values can differ dramatically depending on what device is utilized.

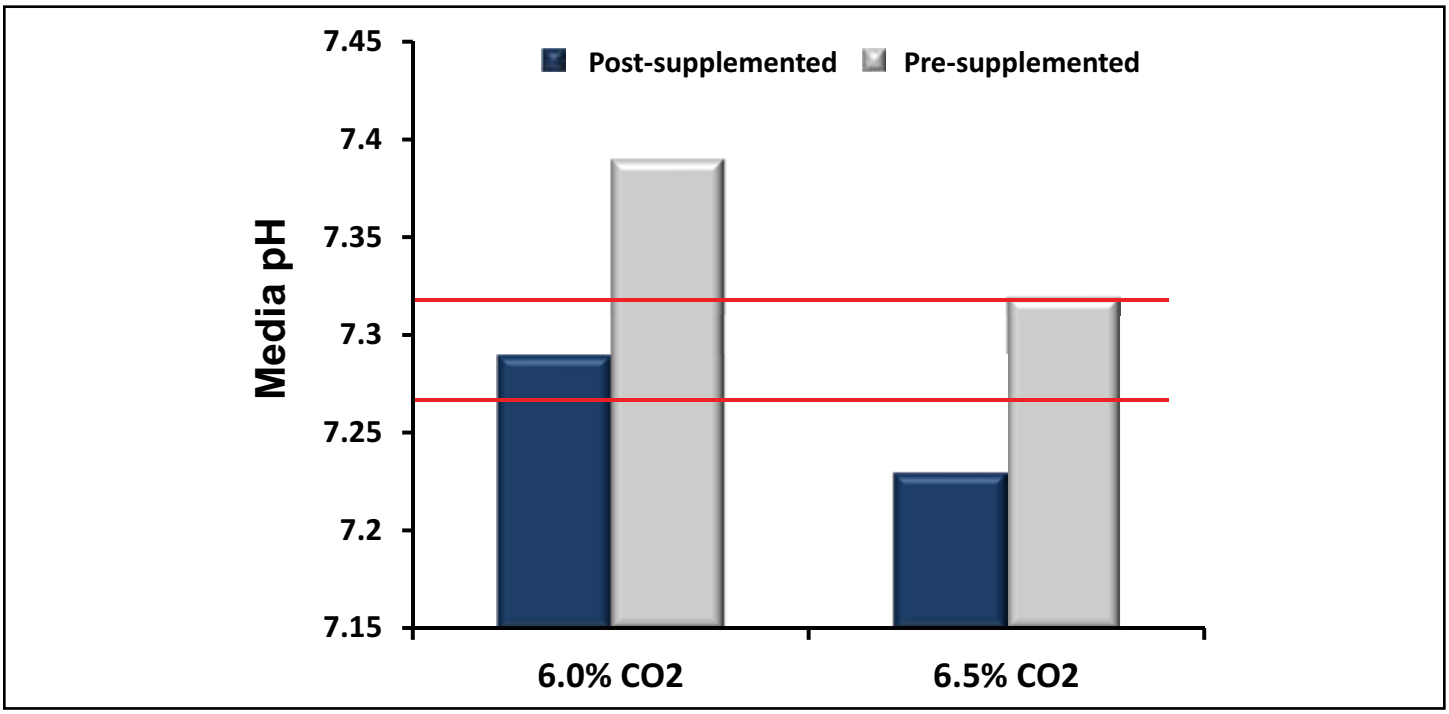


Figure 2. The required CO_2 concentration to achieve the desired pH can vary depending on how the media is prepared. Media pre-supplemented with protein by the manufacturer may have a higher bicarbonate concentration than media post-supplemented with protein within the IVF lab. Post-supplementation with liquid protein results in a dilution of media component concentrations, including bicarbonate. Thus, despite using the same basal media, the same protein and the same protein concentration, different CO_2 concentrations are required to achieve the desired pHo (7.27-7.32, indicated by the red lines). Measurement of pH is required to determine which CO_2 concentration is required.

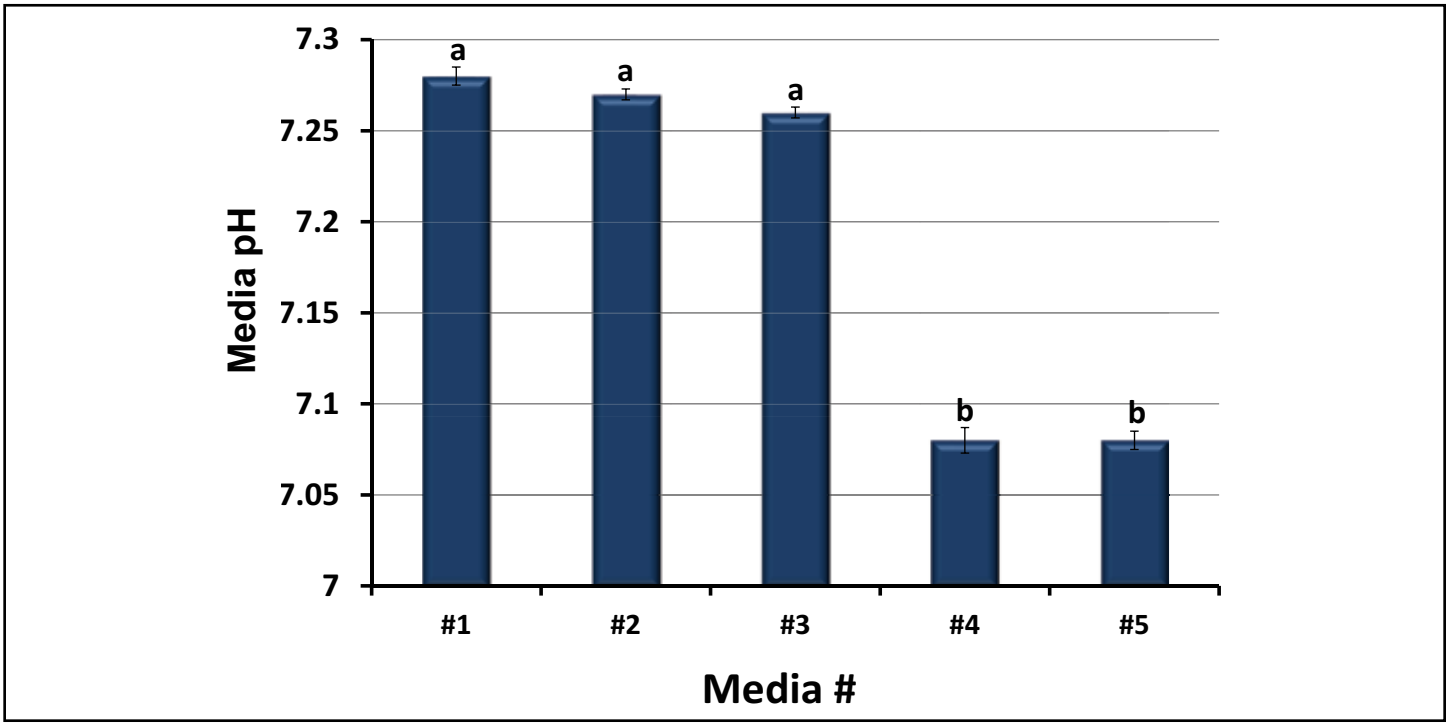
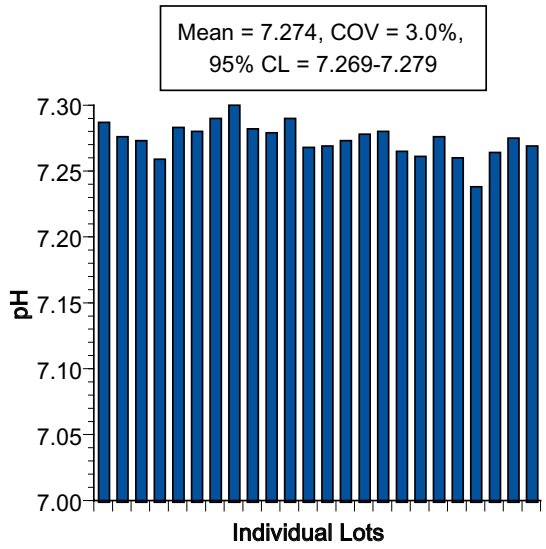


Figure 3. Not all media are formulated to give the same pHo under the same conditions. This includes IVF handling media buffered with HEPES. Despite all being an "HTF" based medium with HEPES, 5 different media were sampled and yielded significantly different pHo values. When measured at 37°C, media #4 and #5 yielded a pHo significantly lower than media #1, #2 and #3, $p<0.01$. This demonstrates the need to measure pH of all media utilized under the specific conditions used within a particular laboratory.

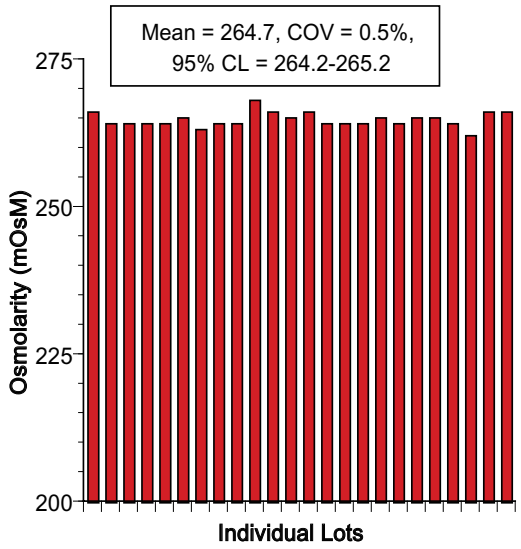
The Consistency of global® Medium

Don Rieger, PhD
May 15, 2013

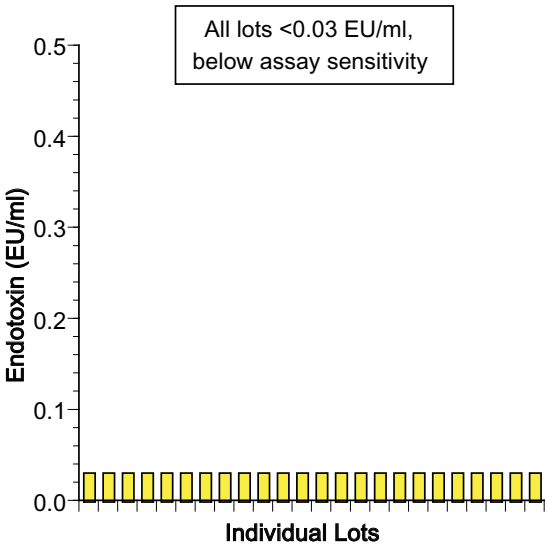
The following graphs show the results of the quality control measurements of the same 24 sequential lots of **global®** medium manufactured between May, 2012 and April, 2013



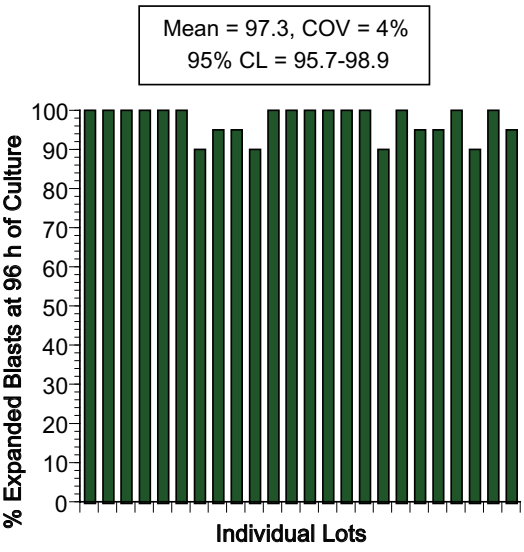
Third-party measurements of the pH of 24 sequential lots of **global®**. With 10 mg/ml protein, at 37°C and 5.5% CO_2



The osmolarity of 24 sequential lots of **global®** measured by freezing-point depression



Third-party measurements of the endotoxin content of 24 sequential lots of **global®**



The results of third-party 1-cell mouse embryo assays of 24 sequential lots of **global®**

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