Real Time pH Monitoring Shows Important Information About Embryo Culture Conditions

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BACKGROUND

The pH of media used for embryo culture is important to control and record. Historically, CO_2 set-points in incubators are adjusted to attempt to create the ideal media pH environment for any development stage of embryos. CO_2 adjustments are generally done by spot checking the pH of equilibrated media once a month or once a week.

In addition to obtaining the ideal pH, maintaining a stable pH environment is important for embryo development.[1] It has been observed that: denuded mature oocytes lack robust mechanisms to regulate internal pH[2], cryopreserved/warmed embryos have a reduced ability to regulate internal pH for several hours[3], and cleavage stage embryos have reduced ability to regulate internal pH compared to post-compaction embryos.[4] Additionally, changes in intercellular pH of embryos impacts metabolic activity[3], can impact organelle localization[5], and can even influence resulting fetal development.[6] Thus, maintaining a stable and appropriate external pH (media pH) is critical.

A new technology, SAFE Sens TrakStation, enables real time non-invasive pH monitoring within an incubator environment. Use of this technology can reveal incubator conditions which are not ideal for maintaining ideal pH levels.

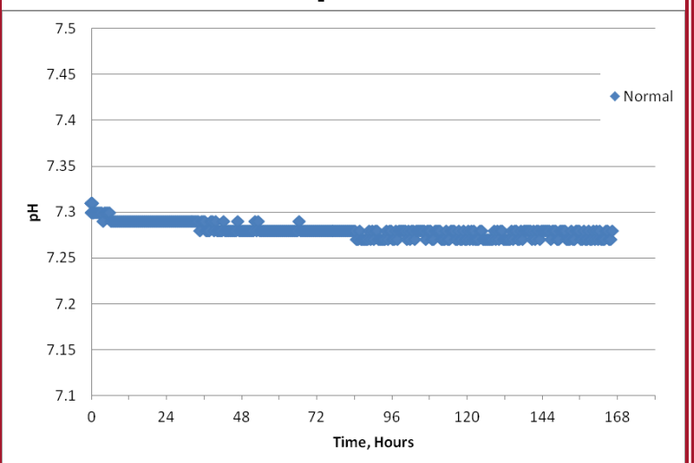
METHODS

SAFE Sens TrakStation and TrakPods were placed in various incubator types including benchtop, a cabinet with thermal conductivity CO_2 sensor, and a cabinet with infrared CO_2 sensor. pH monitoring was performed using an IVF media. Various external stimuli were applied to the incubator environment. The resulting pH measurements from those impacts were recorded and trended. pH values were also spot checked against a blood gas analyzer at various points in time.

RESULTS

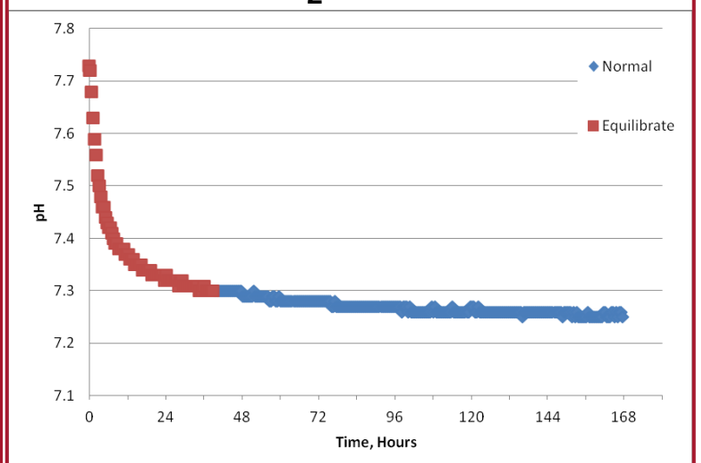
CO_2 adsorption into media is slow due to the use of an oil overlay in normal culturing conditions. Sufficient time for the initial equilibration of media in a CO_2 environment is critical to achieve the desired pH. In many cases, at least 24 hours is needed for pH to reach steady state levels. Benchtop incubators do a very good job of maintaining pH during a 7 day period of time. Lid openings and temperature fluctuations in benchtop incubators do have an impact on the pH monitored.
Cabinet style incubators have some pH variability due to fluctuations in CO_2 levels. Those incubators which use thermal conductivity CO_2 sensors to maintain CO_2 levels show much greater pH changes when humidity and/or temperature change. Door openings cause pH changes in the media.

NORMAL pH PROFILE



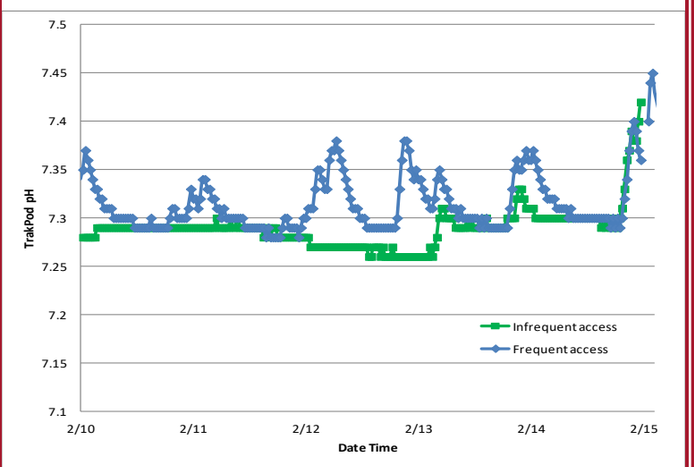
•Results in a sealed incubator reflect steady pH for a full 7 day monitoring period

INITIAL CO_2 ABSORPTION



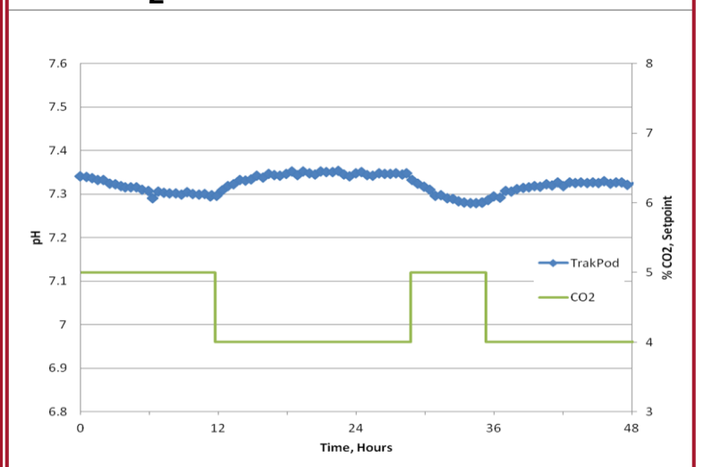
•Increasing CO_2 = decreasing pH
•pH decreases in first 24 hours as media absorbs CO_2

INCUBATOR DOOR OPENINGS



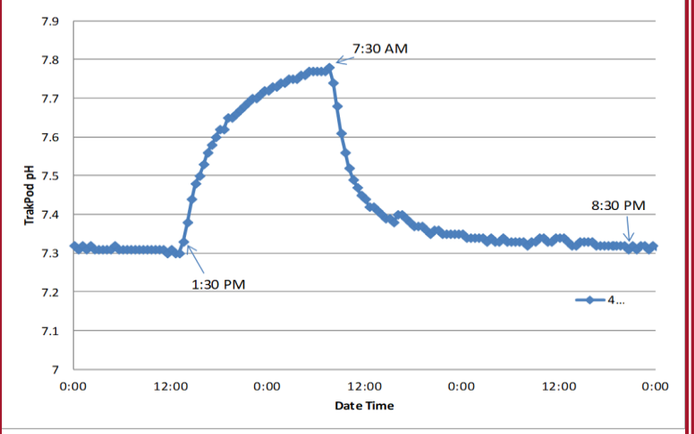
•Opening the incubator door results in CO_2 loss
•pH spikes due to door openings or incubator shutdown for cleaning

CO_2 SETPOINT CHANGES



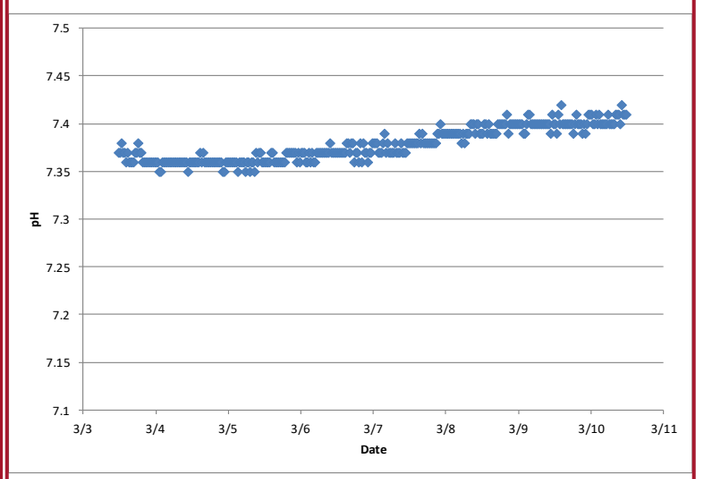
•1% CO_2 pH change = pH change 5-6 hours later
•Theoretical pH change is 0.09 pH units

GAS LINE BLOCKAGE



•Overnight pH spike due to no gas flow in benchtop chamber
•Similar spike will occur if gas runs out

MEDIA EVAPORATION



•pH rises as media evaporates
•Using more oil overlay in dry culture incubators can prevent this pH increase

DISCUSSION

A single point pH reading protocol at 24 hours provides initial pH information for culture conditions, but does not reflect what happens in culture media as it stays in that environment. pH fluctuates due to CO_2 changes, humidity changes, temperature changes and media evaporation. It is helpful to monitor potential fluctuations caused by outside variables using non-invasive continuous pH monitoring.
These studies educate us about fundamental mechanisms during embryo culture. The culturing environment is well known to be an important variable in embryo development. The impact on pH from these incubator variables studied can now be understood and improvements to culture environments can be made by any lab.
Furthermore, when pH is monitored continuously throughout the embryo culturing process, it is possible to ensure that the temperature, CO_2, humidity and other environmental variables create the ideal media pH environment for embryo growth. When deviations in pH are observed, real time prevention and adjustments to the culture conditions potentially improve outcomes for cell growth.
Limitations: The technology looks only at a surrogate media sample that is similar to the media that embryos are cultured in during normal practice. There were no embryos cultured in these tests to show embryo development outcomes.

CONCLUSIONS

These studies educate us about fundamental mechanisms during embryo culture. The culturing environment is well known to be an important variable in embryo development. The impact on pH from these incubator variables studied can now be understood and improvements to culture environments can be made by any lab.

The SAFE Sens TrakStation enables a new ability to track pH in real-time within an incubator. The system reduces staff time to use and offers new insights into improvements in incubator handling practices. By controlling the variables in the laboratory that will directly impact the pH of the media in real time, it is possible to provide an ideal set of media conditions for cell growth. Since pH of media is very important in embryo development this is likely to improve the outcome of embryo culturing procedures and ultimately increase the pregnancy success rates for clinics utilizing the technology.

References

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