Media pH measurement in IVF: The Critical Success Factor

Proper setting, monitoring and stabilizing of pH levels during IVF laboratory procedures is crucial. pH levels control multiple intracellular processes that impact embryo and fetal development. If pH levels are not tightly monitored and controlled, the embryo's ability to grow, differentiate and develop is greatly reduced, potentially resulting in fewer live births per cycle.

What is pH and why is it important?

The pH of a liquid is a measure of its acidity. It is measured on scale of 1 to 14 that is proportional to the concentration of hydrogen (H^+) and hydroxide (OH^-) ions that are present in the liquid. Acidic pHs are closer to 1 and basic pHs are closer to 14. The pH is neutral at pH 7.0 when the concentrations of hydrogen and hydroxide ions are balanced. Multiple factors influence this proportion that can result in rapid changes in pH including humidity, temperature and altitude. The pH scale is logarithmic; this means the difference in ionic concentrations between pH 7 and pH 8 is 10-fold. What appears to be a small mathematical change in pH can actually be very significant.

The physiologic pH of human blood is generally near pH 7.4. Cells growing *in vivo* or in culture media *in vitro* have mechanisms to regulate their internal pH and different human and animal cell types have different internal pH optimums. In IVF, it is known that embryos lack the ability to regulate their internal pH until they reach the blastocyst stage. Research shows that embryos develop well in a pH range of 7.0 to 7.4 between fertilization and blastocyst. Furthermore conventional wisdom is that an optimal medium pH is slightly higher than the optimal intracellular pH of the embryos to offset the effects of metabolism by the embryo. IVF media manufacturers target their media to maintain pH within a narrow range, commonly between pH 7.2 and 7.4. Typically each medium is crafted to maintain a ±0.10 or ±0.05 pH unit range.

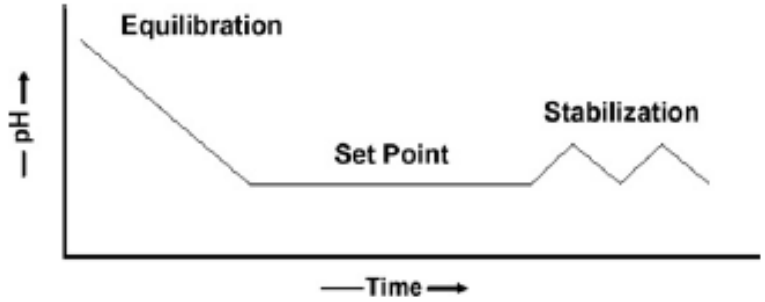
How does the IVF environment impact the pH of my media?

IVF culturing is typically done in conditions that attempt to mimic the conditions inside the female reproductive tract: body core temperature, elevated CO_2 and ideally reduced O_2. These conditions, and especially changes in these conditions, will impact the pH of a medium. Typically, medium are primarily buffered with sodium bicarbonate, and hence the equilibrium between CO_2 (its "partial pressure" or pCO_2) and the concentration of

bicarbonate ions are the prime determinants of the pH of the medium: the more CO_2 present in the atmosphere, the lower the resulting pH of the medium. A decrease in temperature will increase the amount of CO_2 that can dissolve in the medium and also lower the pH.

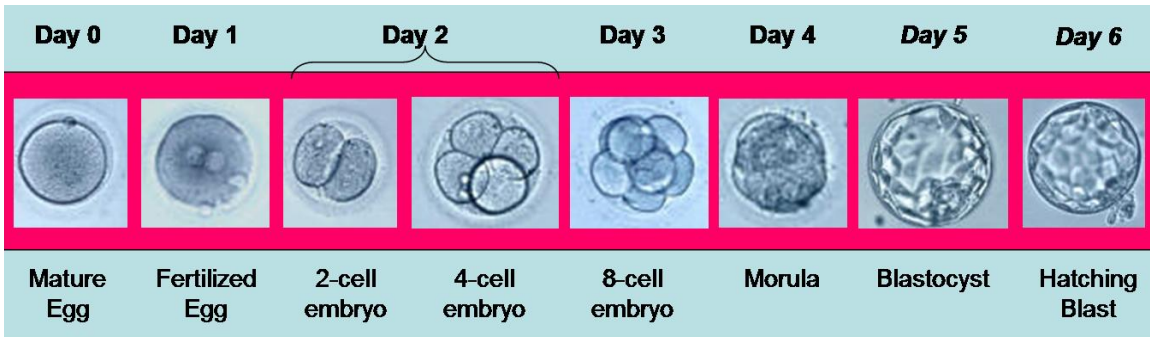
Because of the impact the environment has on medium pH, it is critical to control that environment within incubators to achieve the intended temperature and gas concentration levels, and they must be monitored routinely for proper operation. The target CO_2 level is often chosen with a specific medium pH target in mind. The CO_2 percentage can be based on the recommendation from a media manufacturer but the partial pressure of CO_2 is the important consideration for medium pH. As a result, the percentage of CO_2 may need to be adjusted for the altitude of the laboratory and weather conditions.

When medium is used it must first be equilibrated to the proper environment. Placing media in the incubator overnight allows enough time for the media to absorb the CO_2 and reach a set point. Then, while medium is used during embryo culture, environmental changes can cause smaller pH changes. These changes can include temperature fluctuations from opening the incubator door, atmospheric pressure changes with weather and even chemical changes due to cellular metabolism. It is vital to monitor pH continually because a single point-in-time sample is not sufficient to adequately control pH variations caused by embryo handling during culture.



What role does pH play during the IVF growth cycle?

In IVF, a mature egg and sperm are combined. The resulting embryo is then cultured for 3 to 5 days outside the body before it is transferred to the mother. Embryo Transfer is commonly done at either the 8-cell stage or the Blastocyst stage. Prior to reaching the blastocyst stage an embryo is not able to control its internal pH. If these pH levels are not tightly controlled it will negatively impact the cell metabolism. That decreases the embryo's ability to grow and develop.



Monitoring pH Today

The current technologies available for pH control in IVF are limited.

- It is possible to take a sample of media from the incubator and measure a point-in-time pH level with inexpensive electrode-based pH meters or with more expensive and more accurate blood gas analyzers (BGA). These methods only provide a snapshot of the pH level at that time and are subject to error during sampling. When a media sample is removed from an incubator it quickly outgases the CO_2 causing the pH level to increase. That pH level can change significantly in as little as 30 seconds. An oil layer can be used to slow the CO_2 outgassing but these samples cannot be easily read on imprecise electrode based pH meters. Both BGA and electrode based pH meters require additional calibration steps when they are used.
- Electrode pH meters have been made specifically for pH measurement in IVF. An electrode type meter is available to measure pH inside an incubator, but must be recalibrated each day as the proteins in media will, over time, interfere with accurate pH measurements. Calibration is required and takes about 5 minutes. Users are able to read the pH in a specific incubator each day with this technology. An average IVF laboratory will have 4 or more incubators they use simultaneously.
- Recent fluorescent based technologies offer pH monitoring inside certain types of incubators. The costs of these systems are about a half to three quarters of the cost of an incubator. This has largely prevented their widespread adoption.

An Ideal Solution

- SAFE Sens's new TrakStation enables *continuous* pH monitoring inside multiple incubators. A small sample of media with oil is placed in a disposable sensor cup inside the incubator. The sensor cup contains a fluorescent dye that is pH sensitive and can accurately detect changes in the media pH. Inside the incubator the sensor is placed on a fiber optic probe connected to the TrakPod which reads the fluorescent pH levels automatically. The TrakPod is attached to the TrakStation that continuously reports on and graphs the pH levels for 7 consecutive days before the disposable sensor cup needs to be changed. There is no user calibration required. Each TrakStation can continuously monitor pH in as many as eight (8) incubators simultaneously.

Summary

pH monitoring is critical for the successful development of transplantable embryos that can result in a live birth after just one IVF cycle. Continuous pH monitoring is the most effective way to ensure that an incubator environment is properly controlled and that pH levels are stable. SAFE Sens technology provides visual and logged pH data to clinicians and embryologists to improve their outcomes.

References for further information

The information presented in this document comes from these review papers of the state of the art in IVF and market experience of S Geelhood at BCSI:

1. Optimal Human Embryo Culture. JE Swain. Seminars in Reproductive Medicine 2015;33:103-117.
2. Decisions for the IVF laboratory: comparative analysis of embryo culture incubators. JE Swain. Reproductive BioMedicine Online 2014;28:535-547.

3. Optimizing the culture environment in the IVF laboratory: impact of pH and buffer capacity on gamete and embryo quality. JE Swain. Reproductive BioMedicine Online 2010;21:6-16.