



REAL-TIME ASSESSMENT OF PH STABILITY WITHIN AN IVF ISOLETTE CHAMBER

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ABSTRACT

Background: Maintaining stability of media pH during embryo handling within the IVF laboratory is critical to minimize environmental stress that may compromise viability [1]. Various methods can be used to stabilize pH of culture media. Validating these pH stabilizing approaches is important to verify that detrimental changes in pH are not occurring.

Objective: To assess stability of media pH over time within the confines of an IVF isolette chamber.

Materials and Methods: 100ul of bicarbonate buffered media with and without 35ul oil overlay were equilibrated overnight within a humidified incubator at 37°C with 7% CO₂ and pH measured to establish baseline pH over time. Media tubes were then moved to a warmed/humidified isolette (IVF-1 chamber) with 7% CO₂. pH was measured at 1min intervals over 20min, 2 and 8h using an optical pH sensor (SAFE Sens) to determine if isolette chambers maintained media pH. Regression lines were fitted to each data set and slopes, representing pH change or stability, were compared using Prism software.

Results: Average pH of bicarbonate buffered media with and without oil overlay in the incubator was 7.31± 0.001 and 7.32± 0.001, respectively. Upon transition to the isolette, average pH of media with and without oil over 20min was 7.29±0.002 and 7.31±0.001. After 20min, the slope, or pH increase, with and without oil overlay in the isolette were similar and did not differ from incubators. However, after a 2hr incubation in the isolette, while pH of oil covered media displayed stable pH/slope similar to incubators, media with no oil had a significantly increased slope, p<0.001 and a pH value >7.35, which was outside of acceptable laboratory range. Similar results were obtained following 8h incubation in the isolette, where the slope of the line and pH from media with no oil continued to increase (slope 0.01, pH>7.52) and were significantly higher than media with oil overlay, p<0.001. Visual observation indicated a lowered end volume in samples after 8hr with no oil overlay.

Conclusions: Despite not being air tight, an isolette can provide a stable pH for short term handling of gametes/embryos. Using oil overlay, over a 2h period, pH stability of bicarbonate buffered media in an isolette was similar to that of media in a traditional incubator. However, without oil overlay, pH increase occurs during incubation >2h. Despite a humidified environment in the isolette, this pH increase was likely due, in part, to media evaporation.

BACKGROUND

Reducing environmental stress imposed upon gametes and embryos while within the confines of the IVF laboratory is critical to optimize the culture system and subsequently maximize assisted reproductive outcomes.

pH of the culture medium is one environmental stressor that receives widespread attention when attempting to optimize the culture system. Culture medium pH is established primarily via the bicarbonate concentration in the medium and the CO₂ levels in the laboratory incubator. When culture dishes are removed from the incubator for routine observation and handling, detrimental pH deviations may occur

Fortunately, several approaches exist to help stabilize media pH. One approach includes use of a zwitterionic-buffered, like MOPS, to maintains pH in room atmosphere. Some have concern about buffer toxicity. Alternatively, oil overlay may be used to combat pH changes. Similarly, toxicity of oil may be a concern for some. Finally, portable isolette chambers with a CO₂ supply and internally mounted microscopes may also be used. Use of isolette chambers can reduce culture system variability by avoiding the used of specialized buffered media and can help avoid use of oil overlay. However,, these chambers aren't air tight, are expensive and can be cumbersome to use. Thus, their ability to maintain stable pH should be confirmed.

OBJECTIVE

To assess stability of media pH over time within the confines of an IVF isolette chamber.

MATERIALS & METHODS

100ul bicarb media ± 35ul oil overlay equilibrated overnight in a standard incubator to stabilize and obtain baseline pH

Media quickly moved to a gassed isolette (IVF-1 Chamber)

pH measured using real-time (SafeSens) at 1min intervals for 20min, 120min (2hrs) and 480min (8hrs)

Data graphed, regression lines fit and slopes (representing rate of pH change) compared using PRISM statistical software program. Averages compared using ANOVA and Tukey analysis

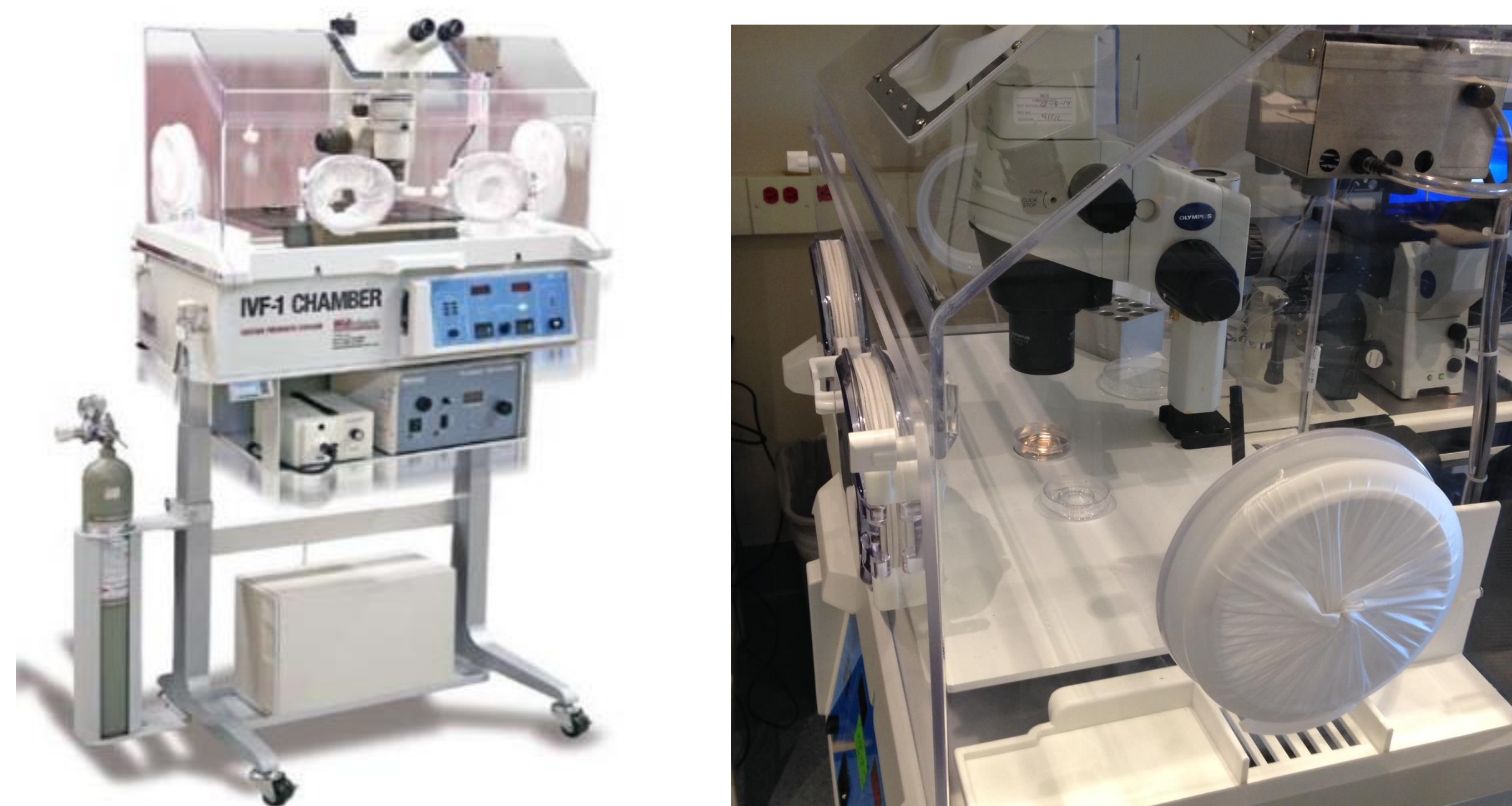


Figure 1. IVF-1 Chamber isolette. The chamber provides a warmed, humidified and gassed environment to handle and observe gametes/embryos.



Figure 2. SAFE Sens real-time pH meter. A) optical sensor B) control unit C) sample tube.

RESULTS

Table 1. Average pH of media with and without oil overlay following culture overnight in an incubator (baseline) and then for 20, 120 or 480 minutes inside an IVF isolette. Different superscripts represent statistically significant differences in pH between time-points within the same treatment (oil vs. no oil), p<0.01.

	Incubator	IVF Isolette Chamber			
	Overnight pH	20min Avg pH	120min Avg pH	480min Avg pH	
Oil Overlay	7.31 ± 0.001	7.29 ± 0.001	7.29 ± 0.002	7.31 ± 0.02	
No Oil Overlay	7.32 ± 0.001 ^a	7.31 ± 0.001 ^a	7.35 ± 0.003 ^{ab}	7.41 ± 0.03 ^b	

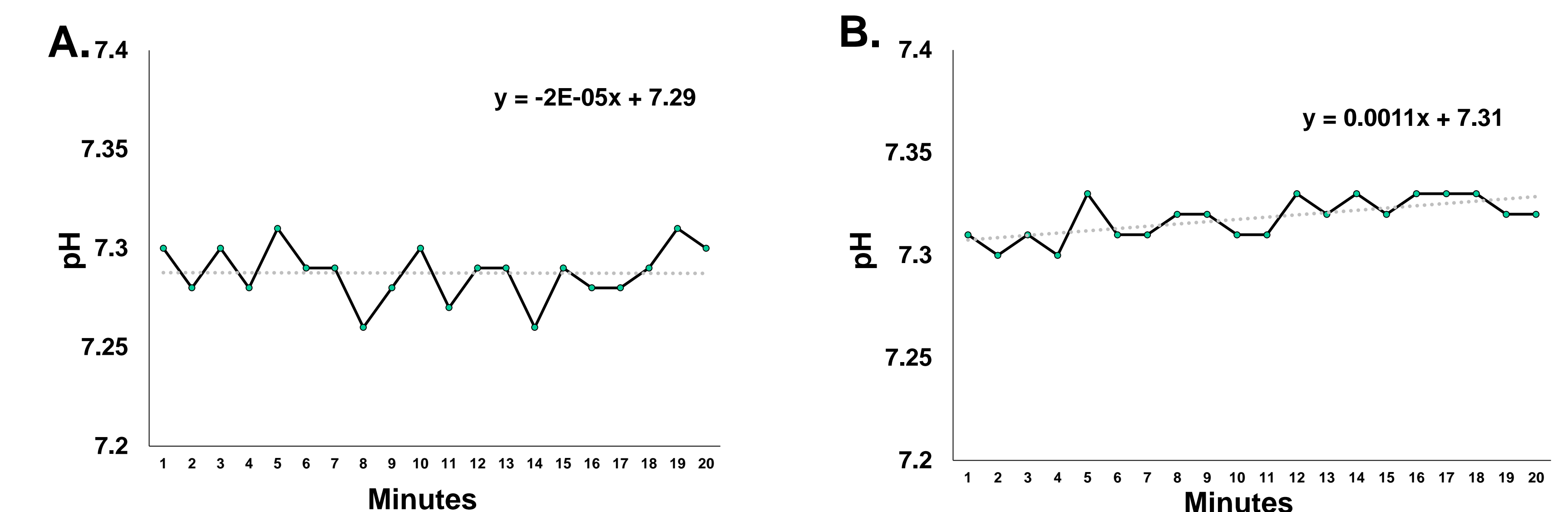


Figure 3. Comparison of pH change (slope) of media A) with oil overlay and B) without oil overlay in an IVF isolette over 20 minutes following removal from a lab incubator. No statistical differences in pH change were observed.

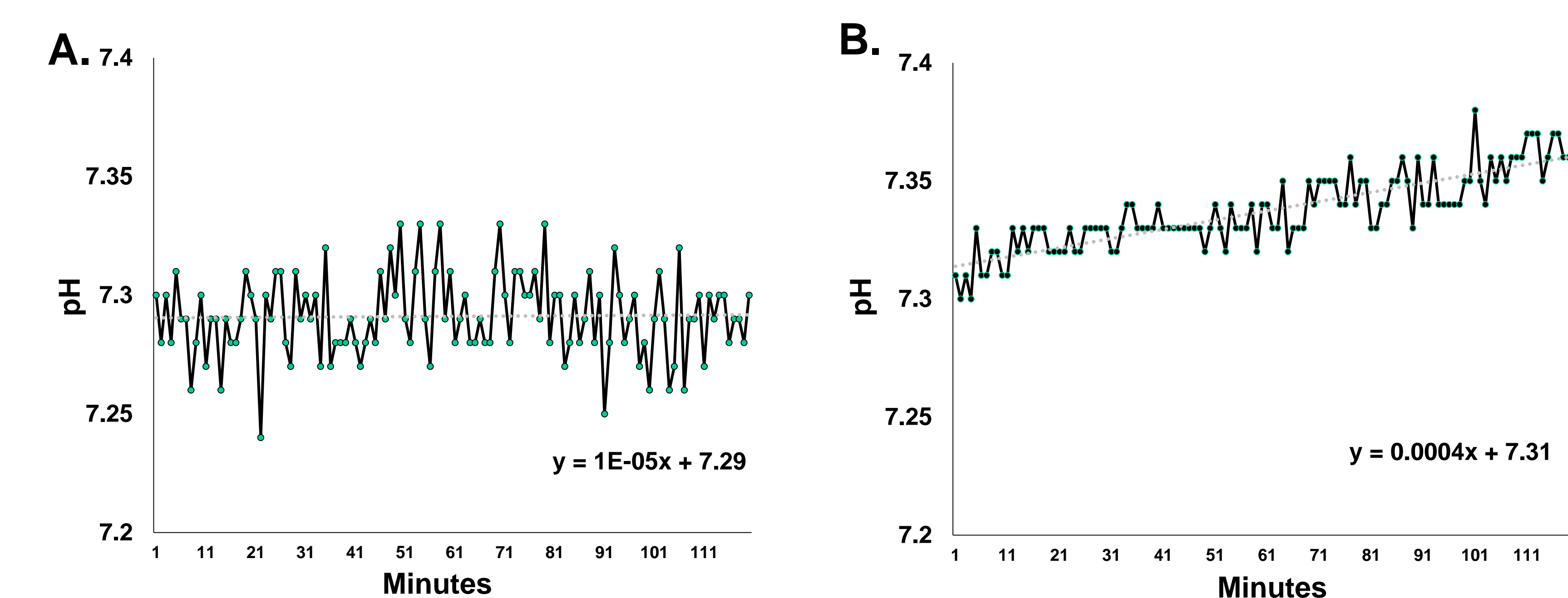


Figure 4. Comparison of pH change (slope) of media A) with oil overlay and B) without oil overlay in an IVF isolette over 120min (2hrs) following removal from a lab incubator. Statistically significant differences in pH change (slope) were observed, p<0.001. After 120min, pH of the media with no oil overlay increased to a value outside the acceptable laboratory range (7.35). This was more pronounced after 480min.

CONCLUSIONS

- Despite not being air tight, an isolette can provide a stable pH for short term handling of gametes/embryos.
- Using oil overlay, over a 2h period, pH stability of bicarbonate buffered media in an isolette was similar to that of media in a traditional incubator.
- Without oil overlay, pH increase occurs during incubation >2h.
- Despite a humidified environment in the isolette, pH increase over time was likely due, in part, to media evaporation.