Analysis of Embryo Culture Media pH Changes During Incubator Use and Media Evaporation Under Oil Using a Continuous pH Monitoring System

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BACKGROUND

Optimal embryo development relies on steady media pH and osmolality during IVF culture. A media pH range of 7.0-7.4 supports embryo development, with most current media ranging 7.2-7.4. Media pH is affected by multiple variables, including the percentage carbon dioxide gas, media equilibration time, temperature, and media components. Determining the pH of culture media in microdrops under oil is difficult using conventional pH meter equipment, and usually assumes a single point reading represents a pH culture system.

Osmolality is the temperature independent value of solute particles within a solution. Solute concentration is crucial in cell development, and osmotic stress can affect cell growth and volume. An osmolality range of 255-295 mOsm/kg supports embryo development, with current media favoring a lower range. Media may also include osmolytes to help regulate environmentally induced changes in osmolality.

Mineral oil is often used in microdrop culture systems to decrease evaporation rates, reduce fluctuations in temperature, and inhibit pH shifts inherent to *in vitro* culture. Embryo development rates are optimized with culture under oil. Washed mineral oil may eliminate water soluble contaminants and prevent nutrient (salt) extraction from culture media.

OBJECTIVE

To analyze the physical changes in pH, mass and osmolality that occur in embryo culture media during a prolonged incubation period. Analysis focuses on common laboratory procedures such as opening and closing the incubator lid and changing the humidity within the incubator.

DESIGN

Media pH data was collected using a real-time pH monitoring device: the SAFE (Sterile Automated Fluoroscopic Evaluation) Sens by Blood Cell Storage Inc. The media pH data was evaluated following scheduled benchtop incubator openings in a microdrop culture system. Exact replications of our culture dishes were also set up to evaluate any potential changes in mass as well as osmolality during our prolonged culture intervals.

MATERIALS AND METHODS

Media pH Analysis:

Different volumes of oil (35 µL or 50 µL) were used to cover 100 µL Life global® total® for Fertilization media in SAFE Sens sensor cups. After equilibration was established (t≥19 hours) and pH values stabilized, the benchtop incubator was opened and closed over 15 minute trials and the sensor was left to collect pH data for up to 7 days. Osmolality data was then collected from the media in the sensor cups and compared to osmolality data from microdrop culture dishes with differing oil overlay volumes.

Embryo Dish Culture Replication:

Two types of culture dishes were replicated: the Life Global® embryo GPS® Dish and the Nunc™ IVF Product Line 60 mm culture dish. 50 µL Life global® HP media drops were covered with differing volumes of washed mineral oil. Dishes were left in either a humidified or non-humidified incubator for 7 days. At the end of this period, dish mass and osmolality data was collected and compared to initial values.

LITERATURE CITED

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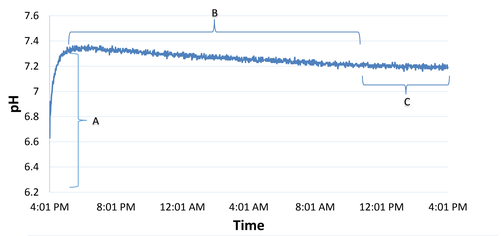
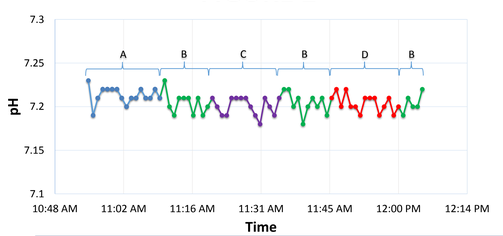
FIGURE 1



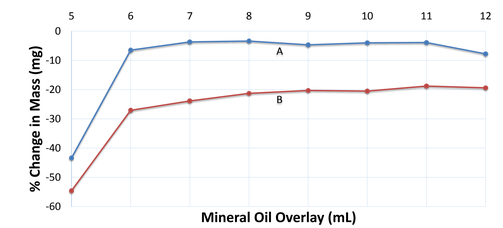
Safe Sens Data collected using 50 µL oil overlay. Data collected in 1 minute intervals.
A) Sensor Cup Wetting Stage
B) pH Equilibration
C) pH Stabilization (t ≥ 19 hours)

FIGURE 2



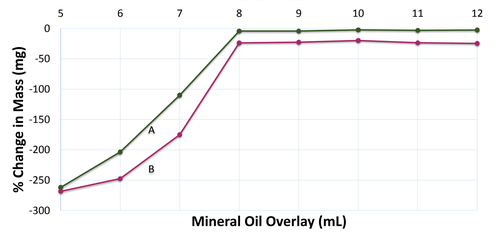
15 Minute Lid Open and Close Experiment
A) Open 30 seconds, close 30 seconds, open 30 seconds, close remainder
B) Lid remains closed
C) Open 1 minute, close 1 minute, open 1 minute, close remainder
D) Open 1 minute, close 30 seconds, open 1 minute, close remainder

FIGURE 3



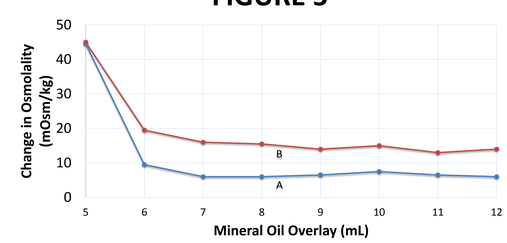
Change in Dish Mass Collected After 7 Days
A) Humidified GPS Dish Data
B) Non-Humidified GPS Dish Data

FIGURE 4



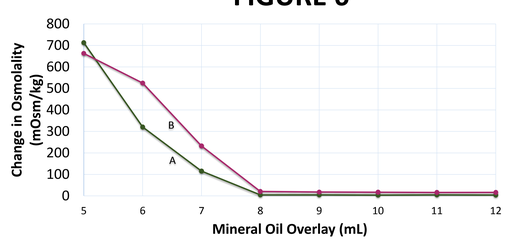
Change in Dish Mass Collected After 7 Days
A) Humidified 60 mm Culture Dish Data
B) Non-Humidified 60 mm Culture Dish Data

FIGURE 5



Change in Dish Osmolality Collected After 7 Days
A) Humidified GPS Dish Data
B) Non-Humidified GPS Dish Data

FIGURE 6



Change in Dish Osmolality Collected After 7 Days
A) Humidified 60 mm Culture Dish Data
B) Non-Humidified 60 mm Culture Dish Data

TABLE 1

Mineral Oil Overlay Volume (mL)	5	6	7	8	9	10	11	12
% Change in Mass With Humidity (mg)	-43.4	-6.5	-3.7	-3.4	-4.7	-4	-3.9	-7.8
Osmolality With Humidity (mOsm/kg)	313	278	274	274	275	276	275	274
% Change in Mass No Humidity (mg)	-54.6	-27.1	-23.9	-21.3	-20.3	-20.5	-18.8	-19.4
Osmolality No Humidity (mOsm/kg)	312	287	283	283	281	282	280	281

GPS Dish Values after 7 day incubation period

TABLE 2

Mineral Oil Overlay Volume (mL)	5	6	7	8	9	10	11	12
% Change in Mass With Humidity (mg)	-262	-203.5	-110	-4.3	-4.3	-2.4	-3.2	-2.7
Osmolality With Humidity (mOsm/kg)	985	593	388	277	277	276	277	276
% Change in Mass No Humidity (mg)	-268.4	-247.5	-175.1	-23.9	-22.7	-20	-23.6	-24.8
Osmolality No Humidity (mOsm/kg)	930	792	500	288	285	284	283	284

60 mm Culture Dish Values after 7 day incubation period

RESULTS

The equilibrated pH using the SAFE Sens (Figure 1) was 7.25±0.03 (n=11). This is compared to the control testing pH (7.22± 0.01) using a handheld pH testing device (n=50) with a standard error of difference of 0.009.

During controlled openings (Figure 2), the pH values measured using the SAFE Sens were within 0.05±0.01 of their equilibrated averages (n=22). This is similar (0.06±0.01) to the pH range seen in unopened incubator conditions. Media under a 35 µL oil overlay in the SAFE Sens sensor cup (n=5) had a statistically significant final pH value of 7.29 when compared to equilibrated averages (P≤0.001). Media under a 50 µL overlay cup (n=5) had a final pH value of 7.20, a statistically non-significant change in pH compared to equilibrated averages (P≤0.11). Media under a 35 µL oil overlay increased in osmolality by 98±2.43 mOsm/kg (n=4), compared to 3.5±1.1 mOsm/kg (n=5) with a 50 µL oil overlay.

Mass of culture drops decreased across all oil volumes in all culture dishes (Figure 3, Figure 4). The changes in mass values (Table 1, Table 2) are compared to the average change in mass found in a set of control mineral oil experiments with GPS culture dishes (0.34 ±0.40 mg, n=12) and 60 mm culture dishes (0.15 ± 0.24 mg, n=12) over a 7 day period. The mass difference between humidified and non-humidified environments for the GPS dish was statistically relevant, (P≤0.05) while the difference in mass was not statistically relevant in to 60 mm culture dish (P≥0.05).

Osmolality increased across all oil volumes in all culture dishes (Figure 5, Figure 6) compared to an initial average osmolality value of 268±1.52 (n=26). GPS dish osmolality values (Table 1) were within published acceptable ranges for embryo culture when using a mineral oil overlay of 6 mL or greater. However, the osmolality was outside of the certificate of analysis values for all oil overlay volumes. The difference in osmolality between humidified and non-humidified conditions was found to be statistically relevant (P≤0.05). The 60 mm culture dish osmolality values (Table 2) were within a range of acceptable values for embryo culture using a 8 mL mineral oil overlay or greater. These dishes also had an osmolality outside of the certificate of analysis values for all oil overlay volumes. The difference in osmolality between humidified and non-humidified conditions was not found to be statistically relevant (P≥0.11).

CONCLUSIONS

Real-time data collected by SAFE Sens demonstrates that the pH of media is stable during incubator condition changes. However, microdrop pH and osmolality change over time due to media evaporation. A sufficient oil overlay for drop culture protects against drastic changes by slowing evaporation, and a pH sensor unaffected by oil overlay (the SAFE Sens) makes prolonged quality control analysis of protected embryo culture media possible. An oil volume overlay of 35 µL is sufficient for short term pH monitoring, while an oil volume overlay of 50 µL prevents significant changes in media contained in SAFE Sens sensor cups for weeklong testing.

While a sufficient volume of mineral oil protects media from detrimental changes caused by evaporation, changes will still occur over a prolonged incubation period. This trend is supported by the culture drop results from the GPS dishes and the 60 mm culture dishes. Humidity in the benchtop incubator decreases changes in mass and osmolality for microculture, but media evaporation is present even when the oil overlay is significant. These factors should be accounted for in laboratory QC while preparing dishes.

These results can help determine an ideal media to oil overlay system that allows a realistic time for pH stabilization, and protects media from pH shifts and other detrimental effects of media culture evaporation.

ACKNOWLEDGMENTS

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