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Does a dry or humid incubation environment affect continuous and undisturbed media protocols?

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Study question

Can continuous embryo culture medium maintain a safe and effective osmolality in dry and humidified incubators under both light viscosity and heavy viscosity oil overlay?

Summary answer

Undisturbed embryo culture is possible in 25uL and 100uL media volumes with both light and heavy oil and in both dry and humidified environments.

Study design, size, duration

Osmolality, pH, and mass were measured in humidified and dry incubators over 8 days of continuous embryo culture conditions. Four dishes each of 25uL and 100uL drops of embryo culture medium (CSCM-NXC) were analyzed per incubator type (Non-humidified – Miri, Humidified – Astec). Additionally, each micro-volume was analyzed under both Light Oil for Embryo Culture and Heavy Oil for Embryo Culture (FUJIFILM Irvine Scientific). In total, 2352 data points for osmolality, pH, and mass were examined.

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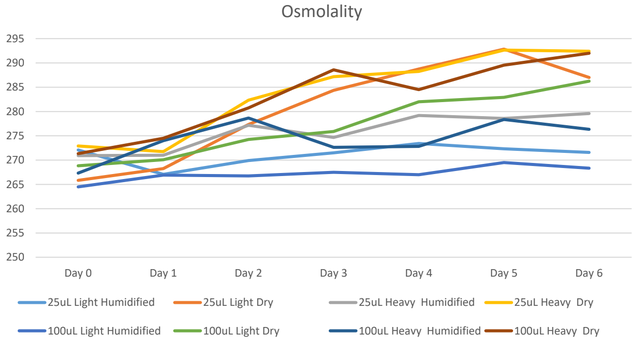
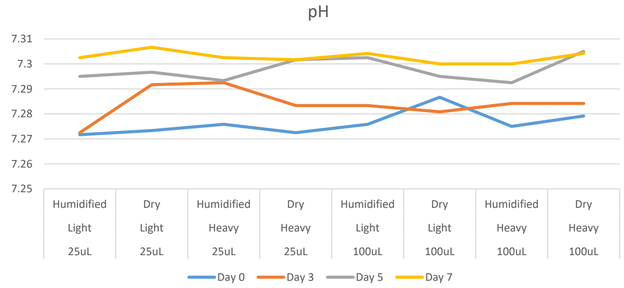


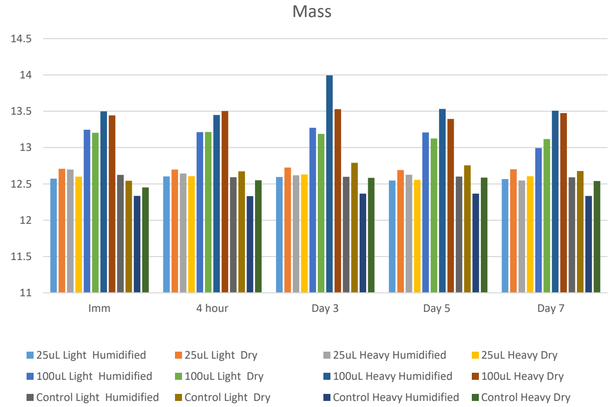
Participants/materials, setting, methods:

A private IVF laboratory conducted the testing with no patient samples introduced to the embryo culture media. To mitigate early evaporative loss, dish preparation was strictly time and temperature controlled. Osmolality was measured in triplicate on days 0-7 using Advanced Micro-Osmometer Model 330 (Advanced Instruments). Mass measures were taken in triplicate on days 0-7 using the Torbal AGZN120 Analytical Balance. pH was measured in triplicate using the i-STAT 300 (Abbott) on days 0,3,5,7.



Main results and the role of chance:

The pH in 25uL and 100uL drops under light oil and heavy oil remained well within suitable ranges and stable when compared day to day, from day 0 (M=7.276, σ=0.011) through day 7 (M=7.303, σ=0.011). The average aggregate pH overall was 7.290 (σ=0.012). Aggregate osmolality, measured as milliosmoles per kilogram (mOSM/kg), over the course of incubation in all conditions also remained well within acceptable safety range (M=277mOsm/kg, σ=5). Dry incubator incubation exhibits a 7% increase from day 0 to day 6 (M=270mOsm/kg, σ=4 and M=289mOsm/kg, σ=5) compared to a 2% increase in humidified incubation (M=269mOsm/kg, σ=5 and M=274mOsm/kg, σ=5) regardless of oil type or microdrop size, both remaining within accepted limits. Additionally, when monitoring mass in grams (g) in 25uL as a function of evaporative loss, humidified incubation reduces from 12.6346g to 12.5566g (day 0 to day 7). In dry incubation, there is negligible change day 0 to day 7 (12.6543g and 12.6538g). Similarly, in 100uL, the change in humidified incubators is 13.37222g to 13.25025g (day 0 to day 7) and dry incubation reduces from 13.3236g to 13.2963g. The changes in mass of prepared culture dishes maintains consistency with the study findings for osmolality.



Limitations, reasons for caution:

As CSCM-NXC is for use through day 6 of embryo culture, the extended simulated embryo culture length is for data collection purposes only. There is no developmental data to include in this study. Additional studies must be conducted in order to explore any differences when embryos are present in culture.

Wider implications of the findings:

The ability to culture embryos continuously in dry incubator environments with micro-volumes of culture medium is widely debated. These many data point study has shown that safe and efficacious embryo culture can be undertaken undisturbed, therefore relaxing some of the burdensome and labor-intensive processes in laboratories employing this equipment.