P-268 Assessing the effect of media, oil and culture dishes on media osmolality and its dynamics in the culture system

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Study question: Can lab-related variables (media type, oil viscosity, micro-droplet volume and culture dish design) modulate media evaporation and improve its stability during culture?

Summary answer: Using dishes with pre-defined wells, big volume micro-droplets and high-viscosity mineral oil can help to reduce media evaporation and improve osmolality stability during embryo culture.

What is known already: Osmolality measures the number of solute particles present in a solution and is an important variable of a human embryo culture system. High ambient temperature and low humidity may induce evaporation in culture media, increasing its osmolality. In addition, recent tendencies in IVF laboratories, such as extending the embryo culture uninterruptedly until day 6/7 or the use of dry benchtop incubators, may intensify evaporation. Surpassing a 300mOsm/kg threshold can result deleterious for embryo development and impair clinical results. Different strategies (e.g. oil type/volume, dish type, micro-drop volume) have been proposed to reduce evaporation and stabilize osmolality during culture.

Study design, size, duration: Four variables were analyzed in their capacity to reduce media evaporation: type of culture medium, micro-droplet volume, oil viscosity and type of culture dish. Dishes were prepared with 5ml of oil and 50µl microdroplets (25µl were used for the comparison of micro-droplet volumes). Dishes were cultured in parallel in a dry benchtop incubator (AD-3100, Astec), and osmolality measured daily for seven days with a freezing point depression osmometer (Osmo1®, Advanced Instruments, accuracy ≤2mOsm/kg).

Participants/materials, setting, methods: The following comparison groups were analyzed: 1) Seven commercial single-step media with three differing initial osmolalities (approximately 260, 280 and >290mOsm/kg); 2) oil with high, medium or low viscosity; 3) 50 vs. 25µl microdroplets; 4) 35mm flat Petri dish vs. 35mm dish with defined wells. Temperature in the incubator was monitored continuously (T+Button, BrightSentinel), as well as room temperature and humidity (Octax Log&Guard, Vitrolife). All were stable at 37.3±0.05oC, 22.1±0.6 oC and 67.4±7.4%, respectively.

Main results and the role of chance: Evaporation occurred in all the studied groups, but its rate was modulated by various parameters. Culture dishes designed with pre-defined wells reduced evaporation when compared to regular Petri dishes (Increase 11.3mOsm/kg and increase 12.5mOsm/kg, respectively from day 0 to 7 (P=0.007)). Similarly, oil viscosity had an impact in osmolality stability during culture, with an increase of 14.7mOsm/kg, 16.3mOsm/kg and 19.2mOsm/kg observed when using mineral oil with high, medium and low viscosity, respectively (P=0.009). Finally, reducing the volume of the medium microdroplets from 50 to 25µl derived in higher evaporation rates, but without significant differences (Increase 14.7mOsm/kg and increase 15.8mOsm/kg, respectively (P=0.325)).

Different evaporation rates were observed between the seven studied culture media attending their three-differing initial osmolalities. Significant differences

were observed for a media respect another three media with differing initial osmolality (P= 0.001, P=0.01 and P=0.015). Their initial osmolality had a direct correlation with the maximum osmolality reached at the end of culture. Thus, media with a high initial osmolality (>290mOsm/kg) resulted in hyperosmotic media above the recommended 300mOsm/kg threshold by the end of culture and, by contrast, the studied media with lower initial values were able to maintain osmolality below 300mOsm/kg for the whole duration of the culture.

Limitations, reasons for caution: While a clear effect was observed by the studied variables, other parameters, such as oil volume or dish preparation techniques, could also play a role in osmolality maintenance and could be studied in the future. Additionally, these findings could vary between different centers and should be validated in each laboratory.

Wider implications of the findings: Osmolality has been shown to have a direct impact on embryo development, embryo quality and clinical outcomes. Carefully defining the consumables and methodologies used in the IVF laboratory will improve the stability of the culture system and, consequently, reduce the stress imparted to the embryos and gametes under culture.

Trial registration number: Not applicable