Optimising osmolality in Geri® dish during culture

Inadequate control of media osmolality may have a serious impact on embryo development. The risk of significant osmolality increase is exacerbated in 1-step culture systems due to the time embryos spend in culture without media replenishment. We have demonstrated that media and oil volumes and the type of culture dish have a far greater impact on media evaporation and hence osmolality increase, than a humidified culture environment, as previously described by Mori et al (2010)[1]. Specifically, osmolality increases over 5 days in a dry Geri incubator were 7.9 mOsm/kg when the suggested volumes were used, compared with 25.8 mOsm/kg when the maximal oil volume (11 mL) was used in a 60mm Falcon dish. It is clear that by controlling these factors, it is possible to control osmolality and provide optimal culture conditions for embryos.

Objective

Experiments were conducted to monitor the level of evaporation of medium in various configurations of medium and mineral oil volumes during the course of the intended incubation periods, in both humidified and dry incubator environments.

Methods

To improve accuracy in osmolality values obtained for each of the media and oil configurations tested, measurements were taken gravimetrically. This method was validated by comparing values obtained from direct osmolality measurement using an Advanced 3250 Osmometer with those calculated via weight change, measured using a Sartorius MSU324S balance. To control for potential evaporation from oil and plastic, both capable of retaining moisture, a control dish for each condition was prepared containing the same volume of oil but no media. The evaporation calculation for the media-containing dish was then adjusted to account for the weight change observed in the control dish containing only oil.

Weights of several dish configurations were measured before and after 136 hours (~5.7 days) of incubation, representing the maximum intended period of culture (including equilibration). Chambers remained closed for the entirety of the simulated culture period. To ensure measurement accuracy in each experiment, all four wells (three wash wells and the central well) were filled with the defined volume of media.

Evaporation Assessment using Geri® Dishes

Contrary to popular opinion, media and oil volumes had a far greater impact on evaporation from dishes than the simple introduction of humidification. Thus, humidified incubation should not be considered a guaranteed method of preventing osmolality increases, whereas media and oil volume should be carefully controlled. Figures 1 and 2 demonstrate that if greater volumes were used, osmolality increases during culture were reduced. Specifically, less than 4.5 mL of oil and/or 60 µL drops of media caused osmolality to rise by at least 15 mOsm/kg, which could cause osmotic stress to embryos. By contrast, if 80 µL drops of media were used and overlaid with 4 mL of oil, the increase in osmolality was limited to <10 mOsm/kg, regardless of whether the system was humidified.

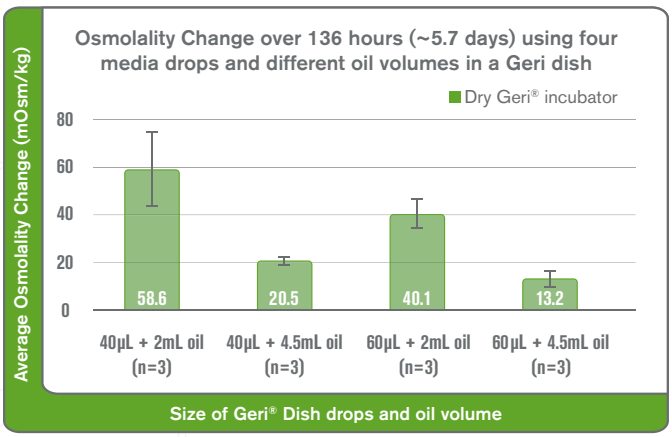

Figure 1 - Osmolality of media in Geri® dishes after 136 hours of culture in the dry Geri® incubator (error bars indicate standard error)

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To determine if the oil volume could be reduced following the introduction of humidity, various oil volumes were overlaid onto 80 µL media drops in the humidified Geri® incubator. As shown in Figure 2, although a 3.5 mL oil overlay has a similar impact to 4 mL in a humidified setting, the advised overlay volume remained unchanged at 4 mL. This allows for unavoidable variability in dish preparation between users and laboratories, and also provides a safety net in the event of loss of humidity.

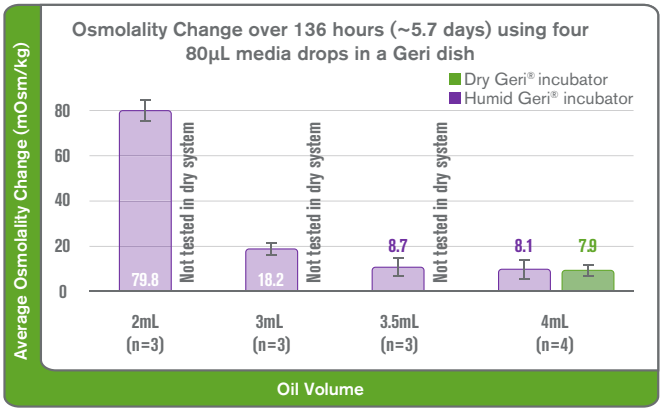

***Figure 2** - Osmolality of media in Geri® dishes after 136 hours of culture in the Geri® incubator (error bars indicate standard error)*

Evaporation Assessment using 60mm Dishes

Along with the tests conducted using the Geri® dish, further experiments were performed with 60mm Falcon dishes. The methods followed were the same as those described in relation to testing with Geri® dishes. All 60mm dishes were prepared with nine 20 µL droplets of media.

The results observed led to the discovery of a key finding; in the conditions tested, the 60mm Falcon dishes were more susceptible to osmotic changes in a dry incubator than the Geri® dish. As shown in Figure 3, the osmolality after 136 hours increased by >20 mOsm/kg in 60mm Falcon dishes when 20 µL drops were overlaid with 11 mL of oil (the maximum safely possible in a 60mm dish). By comparison, the osmolality increases in the Geri® dish with advised media and oil volumes equated to <10 mOsm/kg. Additionally, there were no notable differences between the evaporation in the Geri® incubator and MINC® incubators in the absence of humidification, when the same dish configuration was used.

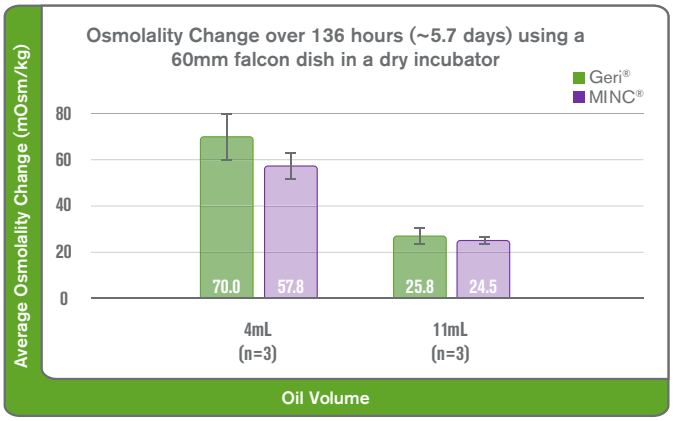

***Figure 3** - Osmolality of media in 60mm Falcon dishes after 136 hours of culture in a dry incubator (error bars indicate standard error)*

The optimal performance of the Geri® dish is likely due to its design. As shown in Figure 4, the Geri® dish consists of 16 micro-wells within the central well, with one embryo being cultured per micro-well. The unique design means that the surface area of media in contact with oil in each well (and thus subject to evaporation) is reduced in comparison to other dishes. To this end, when prepared with advised media and oil configuration, the Geri® dish has proven to be optimal in terms of minimising evaporation and consequential increases in osmolality, regardless of whether dry or humidified incubators are used.

This work was conducted in 2017 and used Gems® Geri Medium and Sage IVF Oil.

[1]*Mori C et al. (2010) Fertil Steril, 94 (4) (Suppl.): S151*

Conclusion

To provide optimal culture conditions for embryos, we recommend that 4 mL of oil is overlaid onto four 80 µL drops of media in a Geri® dish. The use of 60mm dishes is not advised because even with the maximal oil overlay volume, osmolality cannot be controlled to the extent where no risk is presented to embryos in culture.

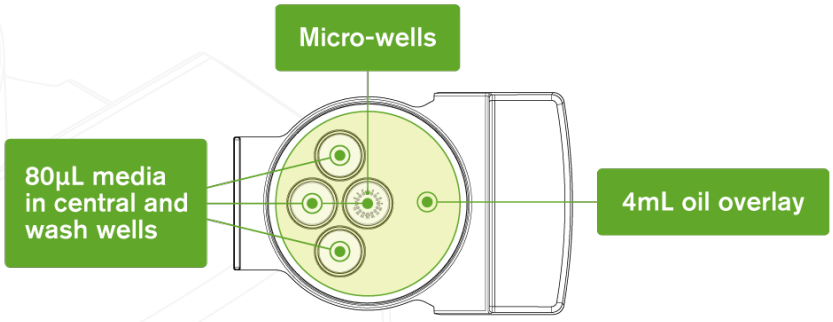

Figure 4 - *Schematic diagram of a Geri® dish*

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