

The impact of media/oil volumes and dish type on medium osmolality throughout embryo culture

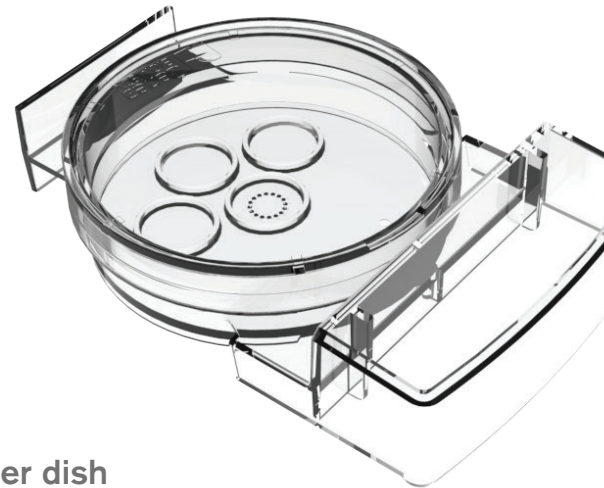
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Aims

The primary aim of this study was to investigate whether dish type and media/oil volume used during embryo culture impacted upon media evaporation and hence, on osmolality.

Introduction

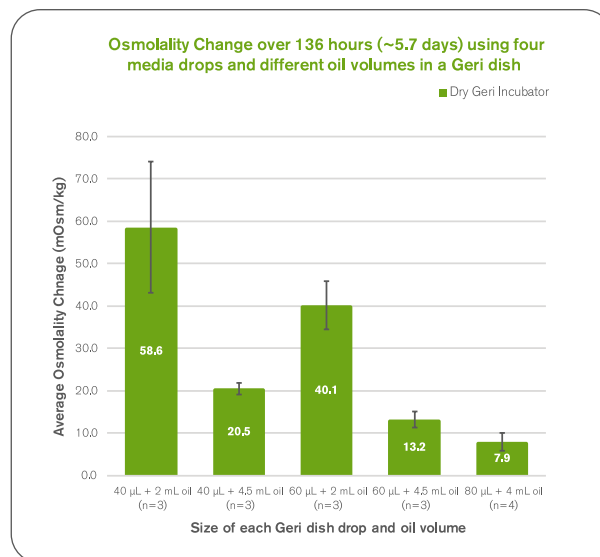
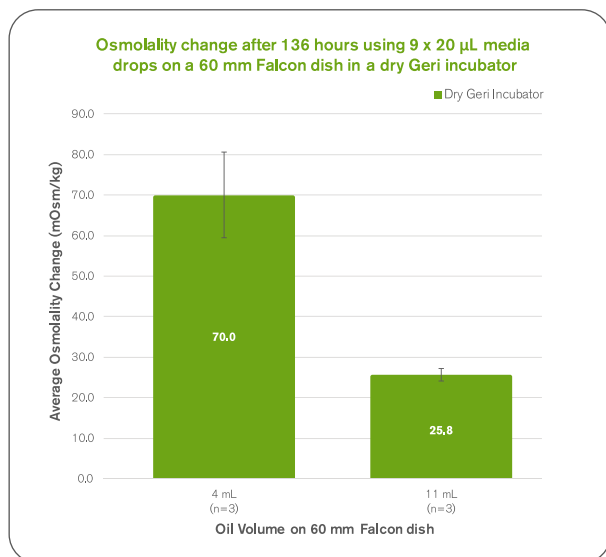
The importance of avoiding potential stressors on gametes and embryos while in vitro has been well documented¹. Osmolality is a crucial media parameter and thus, it is imperative to ensure it is adequately controlled to avoid compromising embryo development. The risk of significant osmolality increase is exacerbated in monophasic culture systems due to the time embryos spend in culture without medium replenishment. Mineral oil has been used as an overlay for culture medium for decades, the core function of which is to minimise medium evaporation and resultant osmolality changes. The volume of oil used to overlay medium droplets varies considerably between laboratories, as does the type of culture dish used.

A limited amount of work has been conducted to determine the optimal volume of mineral oil and the most suitable dish for embryo culture, and thus further studies are required to assist laboratories in making decisions on these key parameters, with the aim of providing the best possible environment for embryo development.

Methods

Experiments were conducted to monitor the level of evaporation of medium and consequently the change on osmolality in the Geri[®] dish using various configurations of medium and mineral oil for the intended period of culture in the Geri[®] incubator, used without humidification for the purposes of this study. Each dish configuration was tested a minimum of three times. Geri Medium and Sage Oil for Tissue Culture were used throughout.

Gravimetric measurements were performed using a Sartorius MSU324S analytical balance. To ensure results were in accordance with those obtained when using an



osmometer, method validation was conducted using an Advanced 3250 Osmometer. Weights were measured before and after the intended culture period, including standard equilibration time, of 5.7 days (136 hours). Various medium and oil configurations were used in 60 mm Falcon and Geri dishes, both commonly used in ART laboratories.

Results

Larger volumes of medium and oil resulted in decreased evaporation, consequently minimising medium osmolality changes over the 136-hour simulated culture period.

Specifically, the use of 40 μ L drops of medium with a 2 mL oil overlay in a Geri® dish led to considerable evaporation, equivalent to an average osmolality change of 58.6 mOsm/kg. An increase in the medium drop size presented some mitigation, with 60 μ L drops and 2 mL oil resulting in average osmolality change of 40.1 mOsm/kg. However, oil volume had a much more pronounced effect on curtailing osmolality increases, with a 4 mL overlay resulting in the minimal possible average osmolality change of 7.9 mOsm/kg being observed when using 80 μ L medium drops were used.

Furthermore, selection of a suitably designed dish for embryo culture proved to be imperative. The average osmolality change observed using a 60 mm Falcon dish (comprising nine, 20 μ L medium drops and 4 mL oil) was 70 mOsm/kg. With 11 mL oil overlay, the maximum possible without spillage, average osmolality change was 25.8 mOsm/kg, still presenting a potential risk to developing embryos. To that end, results highlighted that not only are medium and oil volume critical for minimising evaporation, but that selection of an optimised dish for embryo culture is equally important in the process of preventing osmotic shock.

Conclusions

We concluded that dish type and media/oil volumes are crucial to avoid osmolality changes during the embryo culture period. Therefore, the decision on those parameters should be considered in-depth to ensure optimal conditions for developing embryos, potentially leading to improvements in clinical outcomes.

¹ Swain, J.E., Cabrera, L., Xu, X., Smith, G.D. 2010. Brinster, R.L., 1965. Microdrop preparation factors influence culture-media osmolality, which can impair mouse embryo preimplantation development. Reprod Biomed Online. 24, 142-147.