Technote

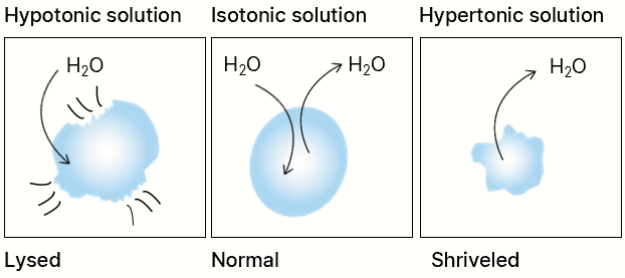
Safe culture in non-humidified EmbryoScope+

The EmbryoScope+ family of instruments offers a safe, non-humidified culture environment. Many factors contribute to creating a culture system which can maintain an osmolality that is within the accepted optimal range for mammalian embryos (255-300 mOsm/kg)[1], however the actual value for human embryos has not been defined, and may depend on culture media components including level of glycine [2]. The benefits of culture in a non-humidified incubator, are the drastically reduced risk of contamination, and reduction of incubator down-time for cleaning and sterilization. Although osmolality may increase during culture, it does not reach the threshold deemed critical for embryo development. We have tested osmolality increase in EmbryoSlide+ and the EmbryoSlide ic8 dish with continuous incubation for up to 8 days in both G-TL and Gx-TL culture media.

Osmolality and embryo culture

Osmolality is a measure of the number of osmotically active solute particles per kilogram of solvent and is commonly measured in (mOsm/kg). Osmosis is when water molecules travel across a membrane from a low solute concentration to a higher solute concentration. If culture media is hypotonic (lower concentration) with respect to the embryo, water will enter the cells and cause it to swell, while too high of an osmolality (hypertonic) will result in a loss of water from the cell, to the surrounding media, and the cells will shrivel. Culture media contains many components including salts, carbohydrates as well as amino acids, some of which can be used as organic osmolytes to regulate osmotic changes. One such important osmolyte used in modern culture media is glycine. Starting osmolality of culture media is also an important consideration when culturing undisturbed to blastocyst. G-TL has starting range is 265-275 mOsm/kg, while Gx-TL has a starting range of 260-270 mOsm/kg.

Other important considerations for how osmolality develops over time are surface area (SA) to media volume ratio of the media droplet, and height of oil covering the media[3]. Studies have shown that osmolality changes may differ depending on culture dish design[4], and culture media composition[5]. Droplet culture in a flat bottom dish has a larger SA/vol ratio than typical microwell culture. The EmbryoSlide+ and EmbryoSlide ic8 culture dish have both been tested and validated to ensure that osmolality when culturing to blastocyst do not exceed osmolality levels considered optimal for embryo development.



One of the steps that is crucial to all culture systems, is dish preparation. The following are recommendations to ensure a safe starting point for extended continuous embryo culture:

- Prepare only 1 dish at a time and use less than 2 minutes for the full process.
- Use cold media and prepare on a non-heated surface
- Use culture media with lower starting osmolality such as G-TL (265-275) or Gx-TL (260-270).
- If rinsing wells are not filled with media, an additional 0.1 mL of extra Ovoil or Ovoil heavy, should be used to compensate.
- In order to ensure that at least 1.6mL of oil is expelled from the pipette, it is advised to use 1.8mL oil, as some oil can adhere to the inside of the pipette tip[6].

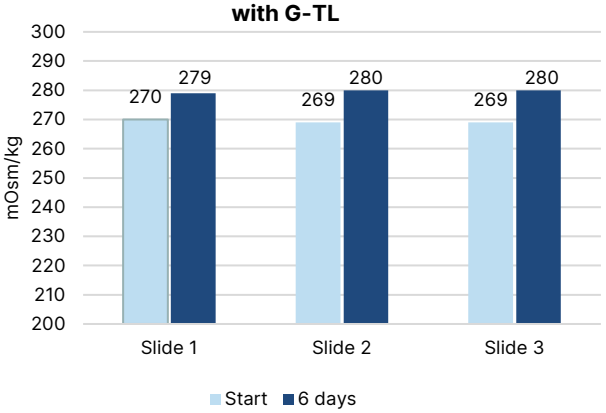
Visit our Academy Studio to watch videos about dish preparation.

https://www.vitrolife.com/academy/educational-material/time-lapse/

Osmolality changes with culture in the EmbryoScope+

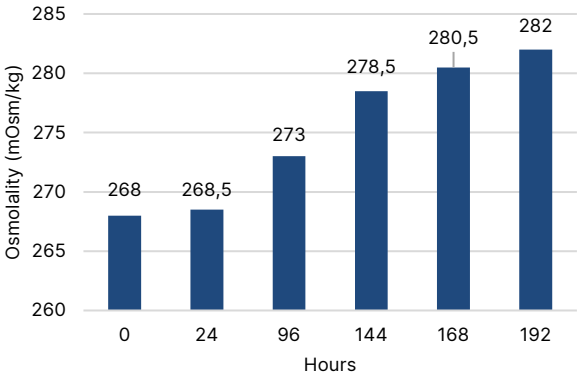
Our time-lapse culture dishes and our EmbryoScope+ family of instruments must pass a strict range of tests, including a MEA test of all instruments prior to release for installation. We have measured osmolality changes in both EmbryoSlide+ and EmbryoSlide ic8 Culture Dishes with G-TL and Gx-TL media under Ovoil Heavy. With growing trends of extended culture, we have measured up to 8 days of incubation (7 days of culture plus overnight equilibration) in order to test beyond the limit of expected continuous culture period conditions.

Osmolality in EmbryoSlide+ Culture Dish with G-TL



Data on file 2021: Osmolality increase was measured in triplicate in EmbryoSlide+ with 180µl G-TL og 1.6mL overlay of Ovoil. Heavy. Osmolality was measured from the bottle and after 6 days (144 hours) of incubation

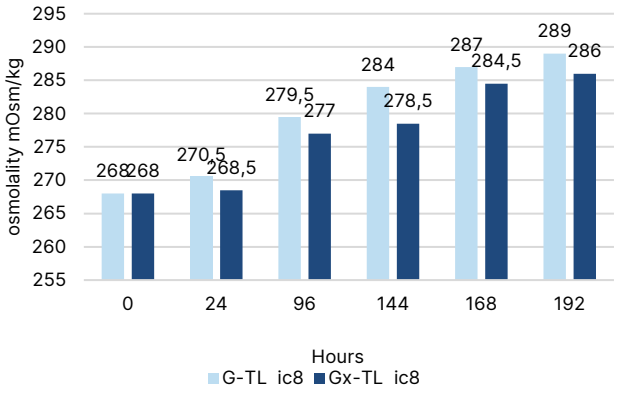
Osmolality in EmbryoSlide+ with Gx-TL*



*Data on file 2024: Osmolality increase was measured in duplicate using an Osmo1 single sample micro osmometer. Samples were taken from EmbryoSlide+ Culture dish sampling 20ul of Gx-TL covered with 1.6mL Ovoil Heavy or in EmbryoSlide ic8 with a pooled sample from 4 wells with 20ul G-TL or Gx-TL per well. After 7 days of culture plus overnight equilibration (8 days of incubation), osmolality remained well below critical levels.

NOTE: results were gathered for 8 days of incubation to simulate extreme limits, however this is not a recommendation to culture embryos continuously past days recommendations listed by culture media suppliers.

Osmolality in EmbryoSlide ic8 Dish with G-TL and Gx-TL*



Osmolality and embryo development

Studies in animal models have demonstrated that even with a rise in osmolality, embryo development was not affected if remaining below the range accepted for optimal development [7,8]. There are numerous studies validating that excellent embryo development is achieved in the EmbryoScope+ time-lapse incubator with continuous culture to blastocyst[9-13]. In conclusion, non-humidified culture in the EmbryoScope+ time-lapse system in EmbryoSlide+ or EmbryoSlide ic8 Culture Dish, with G-TL or Gx-TL media and Ovoil or Ovoil heavy, supports excellent embryo development. Non-humidified culture has the additional benefit that contamination risk is greatly reduced, downtime for sterilization/cleaning cycles is eliminated and reduces unnecessary added running cost of humidification flasks.

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