Minimal evaporation using non-humidified incubation conditions

The EmbryoScope™ time-lapse incubator is a non-humid incubator which has the advantage of less risk of fungal contamination and easier incubator cleaning procedures. Oil overlay provides protection and stability of the microenvironment for the embryos.
An experiment was performed to estimate the evaporation of culture medium through the oil layer from EmbryoSlide® culture dishes incubated in the non-humid culture environment. Evaporation during 5 days of incubation in a non-humid environment at 37°C was determined in six dishes cultured with two different setups. The total evaporation of medium during 5 days was 4.50%, i.e. less than 1% medium evaporated per day.
Incubation of medium with an oil overlay in a non-humid incubation environment leads to minimal evaporation of media and has several other advantages for IVF laboratory routines and quality.

Advantages of a non-humid incubator with application of culture oil overlay

- ✓ Non-humidified incubators carry less risk for fungal contamination and are easier to clean and maintain
- ✓ Oil acts as a physical barrier, separating droplets of medium from the atmosphere and air borne particles or pathogens
- ✓ Oil delays gas diffusion, thereby keeping pH and osmolality of the equilibrated medium stable during culture, which protects the embryos from significant fluctuations in the microenvironment. However, if a change in pH occurs re-equilibration of the medium will also take longer.
- ✓ Vitrolife recommends using Ovoil™ culture oil which is produced under strictly controlled processes. Paraffin oil has been shown to give better conditions for embryo development[1].

Minimal evaporation of culture medium through the oil overlay

The aim of the experiment was to estimate evaporation of medium with an overlay of oil in culture dishes incubated in a non-humid incubation environment. Two different types of setup were incubated in EmbryoSlide culture dishes. In four dishes 25 µL medium in each culture well was overlaid with 1.5 mL of oil. To control for changes deriving from evaporation from the oil, medium was replaced by oil in two dishes giving a total 1.8 mL of oil in those dishes. An empty dish was included for standardization.
All dishes were incubated in the non-humidified environment of the EmbryoScope for seven days at 37°C and 6% CO2 without media exchange.
Weight loss after 3, 5, 6 and 7 days was measured using a Precisa XB 320M balance.

Weight loss during 5 days of incubation with medium and oil was on average 0.015g. When compensating for weight loss from oil, the average weight loss from media was 0.014g per EmbryoSlide culture dish. This corresponds to a 4.5 % weight loss per media volume over a 5 day culture period, i.e. less than 1% weight loss per day of culture. There was no weight loss from the empty dish.
The osmolality of the media after five days of incubation was estimated to be 282.2 mOsm/kg, still well within the osmolality range suitable for human embryo culture (255-295 mOsm/kg[2]).

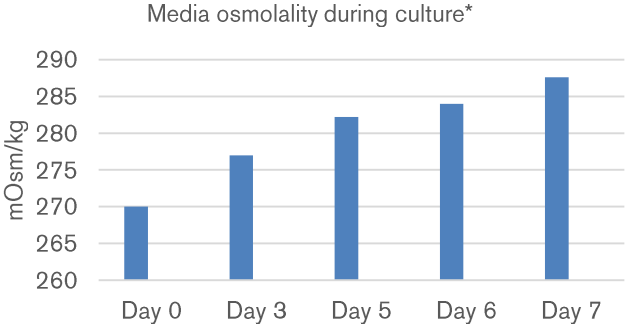

Figure 1: Osmolality of media during a seven day incubation period. After seven days osmolality was still within the range suitable for human embryo culture.
Culture beyond five days in EmbryoScope is not recommended as human embryo development under these conditions has not been tested.
The study was done in June 2016 and used Vitrolife G-TL medium and Ovoil.
**Media osmolality was estimated by weight loss and starting osmolality*
[1]Tae et al., JARG (2006): 23(3): 121-127
[2]Swain et al., RBMOnline(2012): 24(2): 142-147