FERTICULT MINERAL OIL / FERTICULT HIGH VISCOSITY OIL

CULTURE PERFORMANCE CHARACTERISTICS

The primary function of an oil overlay is to avoid fluctuations in the pH and temperature and to prevent the culture medium from evaporation, which would result in increased osmotic pressure.

The performance of an overlay with FertiCult Mineral Oil or FertiCult High Viscosity Oil in maintaining a stable pH, temperature and osmolality of an embryo culture medium is outlined in the experiments below:

1. EFFECT OF FERTICULT MINERAL OIL OR FERTICULT HIGH VISCOSITY OIL OVERLAY ON THE PH OF THE EMBRYO CULTURE MEDIUM

Test method:

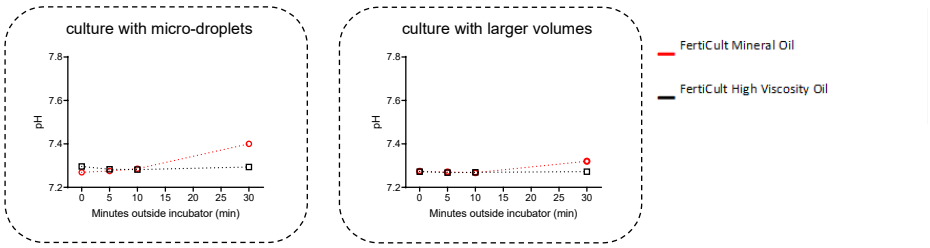
Petri dishes are prepared according to the following test set-up:

Culture with micro-doplets of culture medium	50 µL microdroplets of GAIN medium 4 mL FertiCult Mineral Oil
	50 µL microdroplets GAIN medium 4 mL FertiCult High Viscosity Oil
Culture with larger volumes of culture medium	1 mL GAIN medium 3 mL FertiCult Mineral Oil
	1 mL GAIN medium 3 mL FertiCult High Viscosity Oil

Prepared dishes were placed in a humified incubator at 37°C and with necessary CO_2 concentration to obtain an optimal pH of the culture medium after complete equilibration (i.e. 7.28). Dishes were taken out of the incubator and placed on a heated surface. Using an Epoc Blood Analyser, the pH of the culture medium was measured after 0, 5, 10 and 30 minutes out of the incubator.

Results:

Results are shown in the graphs below:



Conclusion:

The pH of embryo culture media (both micro-droplets/larger volumes) overlaid with FertiCult High Viscosity Oil does not increase and stays stable up to 30 minutes outside the incubator.
For FertiCult Mineral Oil, the pH of the culture medium slightly increased after 10 minutes outside the incubator and this was most pronounced when a micro-droplet culture was used. Nevertheless, the maximum pH value obtained (i.e. 7.4) was still within acceptable limits as described in literature.
Note that when no oil overlay was used, pH of the culture medium increased already from the moment dishes were taken outside the incubator! (> 8, data not shown)

2. EFFECT OF FERTICULT MINERAL OIL OR FERTICULT HIGH VISCOSITY OIL OVERLAY ON TEMPERATURE (RECOVERY) OF THE EMBRYO CULTURE MEDIUM

Test method:

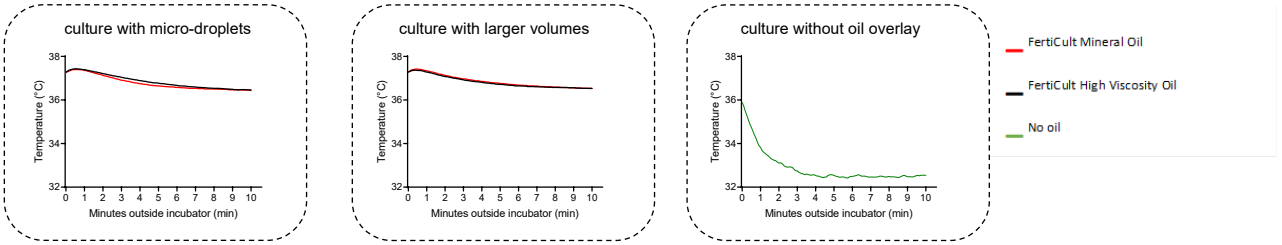
Petri dishes are prepared according to the following test set-up:

Culture with micro-doplets of culture medium	50 µL microdroplets of GAIN medium 4 mL FertiCult Mineral Oil
	50 µL microdroplets GAIN medium 4 mL FertiCult High Viscosity Oil
Culture with larger volumes of culture medium	1 mL GAIN medium 3 mL FertiCult Mineral Oil
	1 mL GAIN medium 3 mL FertiCult High Viscosity Oil
Culture without oil overlay	1 mL GAIN medium

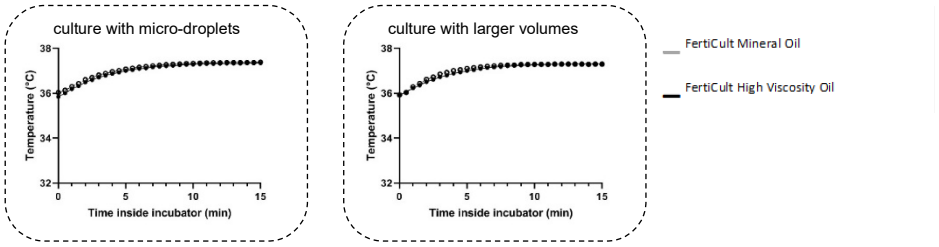
Prepared dishes were placed in a humified incubator at 37°C and with necessary CO_2 concentration to obtain an optimal pH of the culture medium after complete equilibration (i.e. 7.28). Dishes were taken out of the incubator and placed on a heated surface. A fine thermocouple was placed in the culture medium and temperature was measured every 5 seconds up to 10 minutes. Afterwards, dishes were placed back in the incubator and the temperature recovery was studied.

Results:

Results of temperature when dishes were taken out of the incubator are shown in the graphs below:



Results of temperature recovery when dishes were placed back in the incubator are shown in the graphs below:



Conclusion:

When culture media (both micro-droplets/larger volumes) overlaid with FertiCult Mineral Oil/High Viscosity Oil are placed on a heated surface outside the incubator for a short period of time, the decrease in temperature of the culture media is limited (± 1°C). Note that when no oil overlay was used, temperature of the culture medium decreased already more than 3°C after 5 minutes!

After manipulations on a heated surface, the temperature recovery of the culture media (both micro-droplets/larger volumes) overlaid with FertiCult Mineral Oil/High Viscosity Oil in the incubator takes about 10 minutes.

3. EFFECT OF FERTICULT MINERAL OIL OR FERTICULT HIGH VISCOSITY OIL OVERLAY ON THE OSMOLALITY OF THE EMBRYO CULTURE MEDIUM

Test method:

Petri dishes are prepared according to the following test set-up:

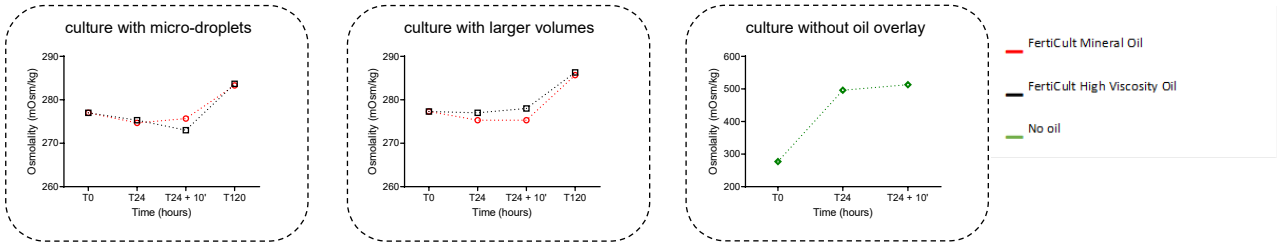
Culture with micro-doplets of culture medium	50 µL microdroplets of GAIN medium 4 mL FertiCult Mineral Oil
	50 µL microdroplets GAIN medium 4 mL FertiCult High Viscosity Oil
Culture with larger volumes of culture medium	1 mL GAIN medium 3 mL FertiCult Mineral Oil
	1 mL GAIN medium 3 mL FertiCult High Viscosity Oil
Culture without oil overlay	1 mL GAIN medium

Dishes were prepared and osmolality was determined ('T0' in the graphs below). Next, dishes were placed in a humified incubator at 37°C and with necessary CO_2 concentration to obtain an optimal pH of the culture medium after complete equilibration (i.e. 7.28). Osmolality values were measured in dishes after 24 hours in the incubator ('T24'). Dishes were subsequently taken out of the incubator and placed on a heated surface for 10 minutes after which osmolality was measured again ('T24+10'). Afterwards, dishes were placed in the incubator and osmolality was determined after an additional 120 hours ('T120').

The test method above mimics the standard procedure used during ART where embryo culture medium is first equilibrated overnight in the incubator, is taken out of the incubator to perform an ART handling procedure and is then placed in the incubator for further incubation.

Results:

Results are shown in the graphs below:



Conclusion:

Fluctuations of osmolality of embryo culture media (both micro-droplets/larger volumes), overlaid with FertiCult Mineral Oil or FertiCult High Viscosity Oil, are limited.

Note that when no oil overlay was used, the osmolality of the culture medium after 24h reaches values far above acceptable levels for gamete/embryo culture!