# Embryo Culture

## 4-well dish

#### Day "-1":

Day "0":

Prepare a 4-well dish by filling each well with 400  $\mu$ l of GM501 Cult. Cover the wells completely with GM501 Mineral Oil and equilibrate overnight in a CO<sub>2</sub> atmosphere at 37 °C.

**ICSI** - Directly after microinjection add up to 6 oocytes per well for culture.

**IVF** - Add up to 6 oocytes per well for fertilisation and add the appropriate amount of prepared spermatozoa. Prepare an additional 4-well dish in the same fashion for the next day ("culture" dish).

## Day "1":

**ICSI** - No further intervention necessary. If desired, assess pronuclear status.

**IVF** - Carefully remove remaining cumulus and corona cells by pipetting and transfer the fertilised oocytes to the prepared "culture" dish.

# Microdrop dish

#### Day "-1": Prepare a microdrop dish with sufficient 80

- 100 µl drops (group culture) or 30 - 50 µl drops (single embryo

drops (single embryo culture) of GM501 Cult

and cover completely with GM501 Mineral Oil. Equilibrate overnight in a CO<sub>2</sub> atmosphere at 37 °C.

Dav "0":

**ICSI** - Directly after microinjection add the desired amount of oocytes to the respective drops (e.g. 3 oocytes per drop for group culture).

80 - 100 µl

GM501 Cult

(group culture)

**IVF** - Add 2-3 oocytes per drop for fertilisation and add the appropriate amount of prepared spermatozoa. Prepare an additional dish in the same fashion for the next day ("culture" dish).

# Day "1":

ICSI - No further intervention necessary.

If desired, assess pronuclear status.

**IVF** - Carefully remove remaining cumulus and corona cells by pipetting and transfer the fertilised oocytes to the prepared "culture" dish.

#### Simple "one-step" culture

Day -1	Day 0	Day 1	Day 2 or 3
Preparation of dishes for IVF or ICSI using GM501 Cult Media	Fertilization by IVF or ICSI and culture in GM501 Cult Media	Ongoing culture in GM501 Cult Media	Embryo transfer

#### Extended "one-step" culture

Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Preparation of dishes for IVF or ICSI using GM501 Cult Media	Fertilization by IVF or ICSI and culture in GM501 Cult Media	Ongoing culture in GM501 Cult Media	Ongoing culture in GM501 Cult Media. Preparation of new dishes for day 3 if wanted	Ongoing culture in new dishes with GM501 Cult Media	Ongoing culture in GM501 Cult Media	Embryo transfer

Gi129/V1

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30 - 50 ul

GM501 Cult

(sinale cell

culture)



