EMBRYO CULTURE

Directions for supplementation of un-supplemented G-Series™ media can be found in the G-Series Manual on www.vitrolife.com. Once supplemented, the media should be used as the G-Series PLUS media described below.

Day 0


G-1™ PLUS


OVOIL™

Prepare micro-droplet culture dishes with 25 µL droplets of G-1 PLUS for washing and for culture. Cover with OVOIL and pre-equilibrate at

37°C 6 % CO_2 overnight


G-MOPS™ PLUS

Warm G-MOPS PLUS (for fertilisation assessment) in rinsed tightly capped tubes in a warming incubator **without CO_2*** at

37°C overnight

* An adequately calibrated warming block can be used for tubes instead of a warming incubator.

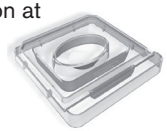
Ensure that the denudation and washing procedures are performed at 37°C

Day 1

1. Fertilisation assessment

For inseminated oocytes, transfer the oocytes to a centre well dish with pre-warmed G-MOPS PLUS. If denudation and fertilisation assessment can be performed within 2 minutes, G-IVF™ PLUS can be used instead of G-MOPS PLUS. Remove cumulus and corona cells from oocytes using a denudation pipette and assess fertilisation at

37°C



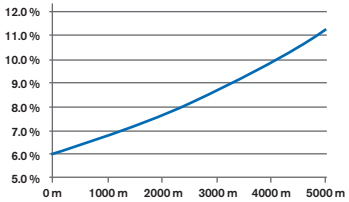
For ICSI oocytes, assess fertilisation in the G-1 PLUS micro-droplet culture dish.

2. Culture

Wash the zygotes extensively in the G-1 PLUS micro-droplet dishes prepared on Day 0 and transfer the zygotes to 25 µL G-1 PLUS culture droplets covered with OVOIL. Culture at

37°C 6 % CO_2 overnight or for 2 days

If your clinic is located at a higher altitude than sea level, CO_2 percentage should be increased, see graph below.



Day 2

Assessment

Assess embryo cleavage.

For embryo transfer day 2, see separate Embryo transfer protocol

Day 3

Assessment

Assess embryo cleavage.

For embryo transfer day 3, see separate Embryo transfer protocol

We recommend G-MOPS PLUS for washing and assessment of embryos before transfer to G-2 PLUS or EmbryoGlue®

Blastocyst culture

1. Prepare micro-well dishes for Blastocyst culture


G-2™ PLUS


OVOIL

In the morning of day 3, prepare micro droplet culture dishes with 25 µL droplets of G-2 PLUS for washing and for culture. Cover with OVOIL and pre-equilibrate at

37°C 6 % CO_2 ≥ 6 h

2. Move embryos to G-2™ PLUS

In the afternoon of day 3, wash the embryos extensively in equilibrated G-2 PLUS droplets and transfer the embryos to G-2 PLUS culture droplets, maximum 5 embryos per droplet. Culture at

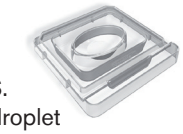
37°C 6 % CO_2 2 days

Day 4

Prepare micro-droplet culture dishes


G-2 PLUS


OVOIL

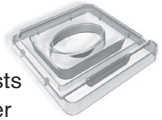


Prepare centre well dishes with fresh G-2 PLUS. Prepare micro-droplet dishes for prolonged culture if needed and pre-equilibrate at

37°C 6 % CO_2 overnight

Day 5

In the morning of day 5



Assess embryo cleavage, and move the blastocysts selected for transfer and cryo preservation to the equilibrated G-2 PLUS centre well dishes and leave at

37°C 6 % CO_2 until 10-30 min before transfer

For blastocyst transfer, see separate Embryo transfer protocol

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