EMBRYO CULTURE USING SEQUENTIAL MEDIA
and Primo Vision time-lapse embryo monitoring system – Method 2

Day 0

G-1™ PLUS
OVOIL™

Prepare the Primo Vision culture dish by priming each well with G-1 PLUS medium. Load each well separately and then add 80 microlitres of G-1 PLUS to cover all the wells. Note that the medium drop should be as flat as possible and that air bubbles must be avoided. Add two wash droplets of G-1 PLUS and cover with OVOIL and equilibrate at 37°C 6 % CO₂ overnight.

Day 1

1. Fertilisation assessment
For standard IVF, if denudation and fertilisation assessment can be performed within 2 minutes, this can be done in the insemination dish. Otherwise, transfer the oocytes to a centre well dish with pre-warmed G-MOPS PLUS before starting the denudation procedure.

G-MOPS™ PLUS
Warm G-MOPS PLUS (for fertilisation assessment) in rinsed tightly capped tubes in a warming incubator without CO₂* 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.

Remove cumulus and corona cells from the oocytes using a denudation pipette and assess fertilisation at 37°C.

For ICSI oocytes, assess fertilisation in the medium the oocytes were incubated in after ICSI.

2. Culture
Check the Primo Vision culture dish for air bubbles and remove them if present.
Wash the zygotes in G-1 PLUS wash droplets in the Primo Vision culture dish prepared on Day 0 and transfer the zygotes to the wells, one zygote per well. Culture at 37°C 6 % CO₂ overnight or for 2 days.

Day 2 and Day 3

Assessment
Assess embryo quality using Primo Vision time-lapse system.

For transfer day 2 or day 3, see separate Embryo transfer protocol.

Blastocyst culture
1. Prepare G-2 PLUS for medium exchange
G-2™ PLUS
OVOIL

In the afternoon of day 2, alternatively 6 hours prior to use, prepare a culture dish with 200 microlitres G-2 PLUS covered with OVOIL and equilibrate at 37°C 6 % CO₂ ≥ 6 h.

2. Exchange the medium for blastocyst culture
In the afternoon of day 3, carefully aspirate the G-1 PLUS medium, leaving 10-15 microlitres and then add 80 microlitres of G-2 PLUS from the separate equilibrated culture dish. To minimize dilution of G-2 repeat the exchange.

Day 5

In the morning of day 5
Assess embryo cleavage using Primo Vision time-lapse system.

For blastocyst transfer, see separate Embryo transfer protocol.