EMBRYO CULTURE USING G-TL
and Primo Vision time-lapse embryo monitoring system

Day 0

- **G-TL™**
- **OVOIL™**

Prepare the Primo Vision culture dish by priming each well with G-TL medium. Load each well separately and then add 80 microlitres of G-TL 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-TL and cover with OVOIL and equilibrate at 37°C 6% CO₂ overnight.

The rim around the matrix of micro wells does not mark the edge of the proposed droplet of culture medium. The role of the rim is to stabilize the movement of the medium so that the embryos stay in the wells.

When preparing a droplet, spread it out over the edge of the rim and try to achieve a flat dome.

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.

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

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

**2. Culture**
Check the Primo Vision culture dish for air bubbles and remove them if present.
Wash the zygotes in G-TL 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.

Day 2 - 5

**Assessment**
Assess embryo development using Primo Vision time-lapse system.

For embryo transfer, see separate Embryo transfer protocol.