EMBRYO CULTURE USING SEQUENTIAL MEDIA
and Primo Vision time-lapse embryo monitoring system – Method 1

**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.

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.

   **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.

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

   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.

   * 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 2 and Day 3**
   - **Assessment**
     - Asses embryo quality using Primo Vision time-lapse system.

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

   **Blastocyst culture**
   1. **Prepare the Primo Vision culture dish for blastocyst culture.**

   **G-2™ PLUS**
   - OVOIL

   In the afternoon of day 2 or in the morning of day 3, prepare a new Primo Vision culture dish with G-2 PLUS for washing and for culture. Load each well separately and then add 80 microlitres of G-2 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-2 PLUS. Cover with OVOIL and equilibrate at 37°C 6 % CO₂ ≥ 6 h.

   **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.