EMBRYO CULTURE USING SEQUENTIAL MEDIA

and Primo Vision time-lapse embryo monitoring system – Method 1

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 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 % CO_2 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

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

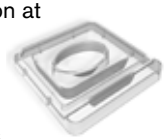
Day 1

1. Fertilisation assessment

For 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



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 % CO_2 overnight or for 2 days

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 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 % CO_2 ≥ 6 h

2. Move embryos to G-2™ PLUS

Check the Primo Vision culture dish for air bubbles and remove them if present. In the afternoon of day 3, wash the embryos in G-2 PLUS droplets and transfer the embryos to the new dish, one embryo per well. Culture at

37°C 6 % CO_2 2 days

Day 5

In the morning of day 5

Asses embryo cleavage using Primo Vision time-lapse system.

For blastocyst transfer, see separate Embryo transfer protocol

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