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IrvineScientific

Uninterrupted Culture Media Recommended Protocol

Continuous Single Culture -NX (CSCM-NX) and Continuous Single Culture (CSCM)

4/5-Well Dish

CSCM-NX and CSCM are optimized to be used in an uninterrupted culture system without medium renewal when using 500 µL of uninterrupted culture Medium for embryo culture.

MEDIUM PREPARATION

CSCM-NX and CSCM do not contain protein components. General laboratory practice includes protein supplementation when using this medium[1].

PROTEIN SUPPLEMENTATION

It is recommended to supplement CSCM-NX or CSCM with 10% Serum Substitute Supplement (SSS) for optimal fertilization and embryo culture. To prepare supplemented medium mix CSCM with protein in a sterile falcon tube:

- 9 mL of the uninterrupted culture medium with 1 mL of SSS to obtain a 10% v/v protein supplemented culture medium.
- Human Serum Albumin (HSA): Mix 9.5 mL of the uninterrupted culture medium with 0.5 mL of HSA (5% v/v), for a final total protein concentration of 5 mg/mL.

DISH PREPARATION

A sufficient volume of protein supplemented medium is required so that oocyte collection, insemination and embryo culture dishes can be prepared.

Oocyte Collection and Insemination Dishes (60 x 15mm): On the day before oocyte retrieval (Day -1) prepare dishes with eight 100 µL volume drops of pre-supplemented uninterrupted culture medium along the perimeter of the dish with an 11 mL volume oil overlay.

Embryo Culture Dishes (4/5-well culture dish): On the day of oocyte collection (Day 0), prepare 4/5-well dishes by aseptically dispensing 500 µL volume of pre-supplemented uninterrupted culture medium and 400 µL volume oil overlay in each well. If using a 5-well dish, the medium in the center well is used for rinsing the embryos prior to placing them into the remaining 4-wells for extended culture.

Time-lapse Surveillance System: CSCM-NX and CSCM works effectively in all time-lapse surveillance systems and should be used accordingly to the individual system's protocol.

To ensure proper temperature and pH of medium and oil, all dishes should be pre-equilibrated overnight to 37°C in a 5–6% C_2 incubator on the day before oocyte collection (Day -1) and on the day of oocyte collection (Day 0). Minimum incubation time should be no less than 6 hours.

CO_2 AND PH

CSCM-NX and CSCM can be used in ambient oxygen incubators but we recommend using reduced oxygen when possible. Following CO_2 equilibration, the pH of the uninterrupted culture medium supplemented with SSS should be within the acceptable pH range of 7.25–7.40 with a targeted pH of ~7.26–7.35.

Tri-gas Incubator: Ideally, we recommend 6% CO_2, 6% O_2 and 88% N_2 for optimal fertilization and embryo growth.

CO_2 Incubator: Alternatively, we recommend a 5–6% CO_2 for sufficient fertilization and embryo growth.

pH levels are affected by the altitude above sea level (elevation), as the elevation increases the gas pressure over the culture medium decreases requiring a higher CO_2 pressure to achieve the optimal pH for the uninterrupted culture medium (7.26–7.35). To adjust pH, increase CO_2 levels to lower pH and decrease CO_2 levels to raise pH to fall within the specified range. pH should always be verified with pH measurements using a pH meter.

OOCYTE RECOVERY

Immediately upon oocyte collection and identification, it is recommended that oocytes be placed into the pre-equilibrated oocyte collection dish (prepared on Day -1). Following the trimming of the cumulus coronal complex from each oocyte, place 1-3 oocytes into a fresh drop of the oocyte collection dish and return to the controlled gas incubator at 37°C for the desired recovery period. Oocytes should be incubated for a minimum of 1 hour prior to insemination or denuding if ICSI is to be performed. Oocytes should remain in the collection dish for up to 4 hours prior to conventional IVF or insemination by Intracytoplasmic Sperm Injection (ICSI).

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INSEMINATION OF OOCYTES FOR CONVENTIONAL IVF

Upon oocyte recovery, trimming of cumulus complex and incubation for up to 4 hours (minimum 1 hour), remove the oocytes from the collection dish and transfer the oocytes to the supplemented uninterrupted culture medium droplets in the pre-equilibrate insemination dish (prepared on Day -1). It is recommend to aseptically dispense 50,000–100,000/mL motile sperm per micro droplet containing 1–3 oocytes. Return the insemination dish to the incubator and check for normal fertilization 16–20 hours post insemination.

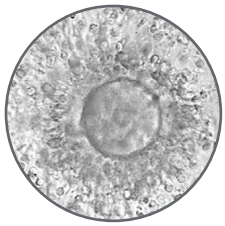
ICSI

Following >1 hour post oocyte denuding, remove denuded oocytes from incubator and inseminate with sperm as per standard ICSI protocol. Immediately following insemination, place 1–3 inseminated oocytes into a fresh drop of the pre-equilibrated insemination dish (prepared on Day -1), and return dish to the incubator and check for normal fertilization 16–20 hours post insemination.

EMBRYO CULTURE POST FERTILIZATION

Following fertilization assessments with the identification of the presence of normal fertilization (two pronuclei and two polar bodies), transfer 2PN zygotes into the pre-equilibrated 4/5-well CSCM-NX or CSCM culture dish previously prepared on Day 0. We recommend embryos to be group cultured (maximum of 5 embryos per well) in a static, uninterrupted culture system, without dish change or medium renewal on Day 3, until the desired stage of embryo development is achieved.

DAY 1
Oocyte



DAY 2
2PN

DAY 3
2-4 Cells

DAY 4
8 Cells

DAY 5
Compacting-Cavitating Cells

DAY 6
Blastocyst Stage

1.FUJIFILM Irvine Scientific has not validated these procedures and each laboratory should consult its own laboratory procedures and protocols which have been specifically developed and optimized for your individual medical program.

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