CONTINUOUS SINGLE CULTURE® COMPLETE RECOMMENDED MICRODROP PROTOCOL

CONTINUOUS SINGLE CULTURE® COMPLETE (CSCM-C) IS OPTIMIZED TO BE USED IN A UNINTERRUPTED CULTURE SYSTEM WITHOUT MEDIUM RENEWAL OR DISH CHANGE ON DAY 3.

MEDIUM PREPARATION
CSCM-C is a complete, ready to use medium containing Human Serum Albumin (HSA) as the protein component. It is not necessary to add any other protein before use.

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 15 mm):** On the day before oocyte retrieval (Day -1) prepare dishes with eight 0.1 mL (100 uL) volume drops of pre-supplemented CSCM-C along the perimeter of the dish with an 11 mL volume oil overlay.

**Embryo Culture Dishes (60 x 15 mm):** On the day of oocyte collection (Day 0), prepare micro drop dishes by aseptically dispensing at least six 0.05-.1 mL (50 uL-100 uL) drops of pre-supplemented CSCM-C medium into the dish. Four micro drops should be placed at the 3, 6, 9 and 12 o’clock position and the two remaining drops should be placed in the center of the dish to be used as wash drops. 11 mL’s of Oil for Embryo Culture should be used to completely cover CSCM-C micro drops. To avoid evaporation and changes in osmolality do not prepare multiple dishes at the same time. Immediately place the dish in a CO₂ incubator.

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

**NOTE:** To ensure proper equilibration of medium and oil, all dishes prepared on Day -1 and Day 0 should be pre-warmed to 37°C and equilibrated to the desired pH overnight in a 5-6% CO₂ 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.

CO₂ AND PH
We recommend using reduced oxygen when possible; however, CSCM-C can be used in ambient oxygen incubators. Following CO₂ equilibration, the pH of CSCM-C medium should be within the acceptable pH range of 7.25-7.4 with a targeted pH of ~7.32-7.35.

**Tri-gas Incubator:** Ideally, we recommend 6% CO₂, 6% O₂ and 88% N₂ for optimal fertilization and embryo growth at or near sea level.

**CO₂ Incubator:** Alternatively, we recommend a 5-6% CO₂ for sufficient fertilization and embryo growth at or near sea level.

**NOTE:** 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 CO₂ pressure to achieve the optimal pH for CSCM-C (7.32-7.35). To adjust pH, increase CO₂ levels to lower pH and decrease CO₂ 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 CSCM-C droplets in the pre-equilibrated insemination dish (prepared on Day -1). It is recommend to aseptically dispense 75,000 – 175,000/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-supplemented CSCM-C in the pre-equilibrated insemination dish (prepared on Day -1), and return the 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 2-5 2PN zygotes into each of the four pre-equilibrated 50 ul-100 ul microdrops in the culture dish previously prepared on Day 0. We recommend embryos to be group cultured (maximum 5 embryos per micro drop) in an uninterrupted culture system (without dish change or medium renewal), until the desire stage of embryo development is achieved.

NOTE: 5 is the maximum number of embryos that we recommend being cultured in each 50 uL-100 uL microdrop.

1. 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.