FERTILO

Engineered ovarian support cells for oocyte maturation

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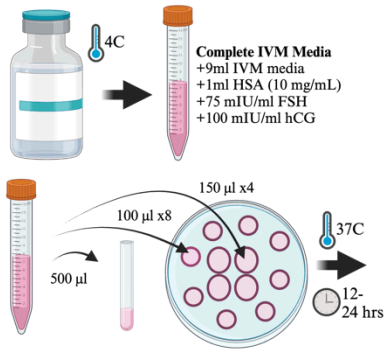
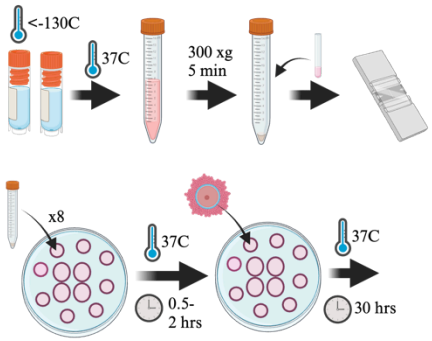
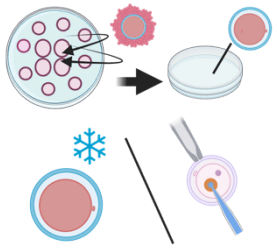
1. Day Before Oocyte Retrieval



2. Day of Oocyte Retrieval



3. Day After Oocyte Retrieval



Fertilo Instructions for Use

Intended Use
Fertilo is intended exclusively for use in the improvement of *in vitro* maturation of immature cumulus oocyte complexes.

Indicated Use
Fertilo is intended for the ex vivo maturation of immature oocytes in women undergoing in vitro fertilization.

Product Description
Fertilo consists of one vial containing ovarian support cells (OSC) cryopreserved in CryoStor™ CS10 Freezing Media. CryoStor CS10 is an animal component-free, defined cryopreservation medium containing 10% DMSO. Freezing media is not present in the co-culture system.

Precautions and Warnings
1. The clinical benefits of *in vitro* maturation in IVF cycles include the ability to harvest and utilize oocytes with little to no hormonal stimulation.
2. Often, fewer total oocytes are retrieved for IVF cycles after minimal hormonal stimulation compared to conventional ovarian stimulation protocols.
3. Minimal hormonal stimulation involves significantly lower risk of ovarian hyperstimulation syndrome compared to conventional ovarian stimulation.
4. Fertilo should never be administered to or into a patient

Do not use Fertilo if:
1. Packaging is damaged, compromised.
2. Product label is illegible.
3. Product has been subjected to improper temperature storage.

Caution
All cell products should be treated as potentially infectious. Fertilo is manufactured with xeno-free source materials. Fertilo was tested using a comprehensive screening panel and were found to be negative and/or non-reactive for adventitious agents including: HIV-1/HIV-2, HCV, and HBV.
Fertilo is used with human serum albumin, sold separately, as a media additive. No known test can offer assurances that products derived from human blood will not transmit infectious disease. Despite standard and effective measures to inactivate and remove viruses and pathogens, the possibility of transmitting infective agents from medicinal products prepared with human blood or plasma can not be totally excluded.

Caution
Thaw product vial carefully according to instruction to avoid risk of injury. Use of PPE is recommended.

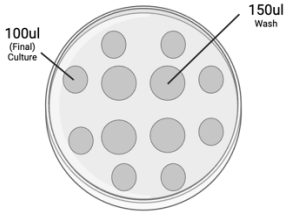
Quality Control Testing
Sterility, Mycoplasma and Adventitious Agent Tested
Endotoxin tested ≤0.25 EU/mL
MEA ≥ 80%
Purity ≤ 0.01% Residual Human Induced Pluripotent Stem Cells (hiPSC)
Note: Results of each batch are indicated on a Certificate of Analysis.

Storage and Stability
Fertilo is stored in the original container in liquid nitrogen.
Fertilo should be utilized immediately upon thaw.
Fertilo is provided in a vial intended for single-use only. Do not attempt to reuse.
Do not expose cells to temperatures that exceed 37°C.
Only IVF approved plasticware and consumables should be utilized when preparing Fertilo.

Instructions for Use
1. **Day Before Oocyte Retrieval (Day -1)**
Prepare Complete IVM (cIVM) Medium
1. Prepare 10 mL of complete IVM (cIVM) medium using a 15 mL conical tube as follows:
   a. 9 mL of IVM Media (MediCult IVM Media Component 2, or suitable alternative)
   b. 1 mL human serum albumin (10 mg/mL final concentration)
   c. 75 mIU/mL of follicle stimulating hormone (FSH, final concentration)
   d. 100 mIU/mL of human chorionic gonadotropin (hCG, final concentration)
2. Once media is prepared, use a micropipette to add 500 µL into a 5 mL vented top tube and place into an incubator overnight at 37°C and a CO_2 level that achieves 7.3 pH
   a. If a vented top is not available, alternatively use a 5 mL tube with the top loosely capped.
   b. The 500 µl aliquot of cIVM medium will be used the following day for Fertilo cell resuspension
   Note: This aliquot must be utilized within 24 hours of being placed in the incubator.
3. The tube containing the remaining cIVM medium must be labeled, tightly capped, stored at 4°C protected from light exposure and used within 1 week of preparation.
   a. This remaining volume of cIVM will be used in plate and cell preparation.

Prepare Culture Dish

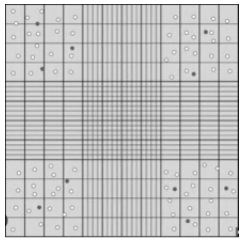
1. A 4+8-well IVF dish (BIRR catalog # 1134+8, or suitable alternative) should be used and prepared as follows:
   a. Using a micropipette, add 150 µL droplets of the prepared cIVM medium to the four large center wells of the dish. These wells will be utilized for washing.
   b. Using a micropipette, add 100 µL of cIVM medium to all eight small outer wells for culture.
   c. Each outer well may hold up to 5 cumulus oocyte complexes (COCs)
   d. Using a serological pipette, carefully overlay the dish with 8 mL of IVF grade oil and incubate overnight at 37°C, 5% O_2 and a CO_2 level that achieves 7.3 pH.
   e. The following image is an example dish:



2. **Day of Oocyte Retrieval (Day 0)**

Prepare Fertilo

1. Before Fertilo preparation, remove the prepared cIVM media from the refrigerator and allow the media to come to room temperature.
   a. **Do not exceed 2 hours at room temperature.**
2. At least one hour before oocyte co-culture, retrieve 2 vials of Fertilo from vapor phase liquid nitrogen storage.
3. Thaw 2 vials in a 37°C heated bead or water bath for 2-3 minutes. Check the vials periodically to ensure that all ice crystals have melted. Remove from the heated bath once no ice crystals remain and proceed with washing.
   a. **Do not exceed 3 minutes total thaw time** and proceed to the following step.
   b. Vials should not be thawed more than 2 hours before oocyte co-culture.
4. Using a micropipette, carefully add 250 µL of cIVM medium dropwise to each cryovial.
5. Using the micropipette, slowly mix cells drawing up and down twice, then transfer contents from both cryovials to a pre-labeled 15 mL centrifuge tube.
   a. **Ensure entire cell suspension is transferred**.
6. Using a serological pipette, slowly add 6 mL of cIVM medium to the centrifuge tube, carefully pipette up and down twice, then tightly cap the tube.
7. Centrifuge at 300 x g for 5 minutes to pellet the cells.
   a. **Recommendation: Use swinging bucket centrifuge**
8. Using a 1000 µL micropipette or 5 mL serological pipette, remove supernatant, taking care not to disturb the cell pellet.
   a. When approaching the exterior surface of the cell pellet a smaller micropipette may be used to remove the last volume of media without disrupting the pellet.
9. Retrieve the 5 mL vented tube with the 500 uL equilibrated cIVM medium aliquot prepared the day before, gently resuspend the cell pellet using this aliquot according to the dilution factor located in the certificate of analysis (CoA).
   a. Every lot of Fertilo will have an associated CoA.
   b. The dilution factor can be found in the top portion of CoA indicated by the designation “Dilution Factor”.
10. After resuspension of the cell pellet, perform a cell count as follows. The cells should be kept at room temperature or on a heated surface during counting.
   a. Using a micropipette, pipette a 15 µL drop of the cell pellet suspension onto a hemocytometer slide.
   b. Using a micropipette, pipette 15 µL of trypan blue solution into the previous cell suspension drop placed on the hemocytometer and mix well
   c. Pipette 10 µl of the solution into the hemocytometer chamber.
   d. Observe under the microscope immediately.
   e. Live cells will appear glowing white spheres with a greenish tint. Dead cells will appear as blue spheres.
   f. Using a cell counter, count all of the cells in each of the 4 corner quadrants. Count live and dead cells separately. The cell numbers must be counted in all 4 quadrants. The following figure is an example of live and dead cells on the hemocytometer cell:



   g. Use the total live cell count of the four quadrants (the sum of the 4 quadrants) and the following calculation to determine the required volume of Fertilo necessary to add to each 100 µl culture droplet.
   h. **Volume Required** =20,000/Total Live Cell Count
      i. Example Calculation:
      ii. Total Live cell count: 400 (70 quadrant 1 + 130 quadrant 2 + 100 quadrant 3 + 100 quadrant 4)
      iii. =20,000/400
      iv. Volume required = 50 µl
   i. To calculate the cell viability:
      I. Total # of live cells / (Total # of live cells + Total # of dead cells)
   j. Immediately after cell count is performed, retrieve the pre-prepared culture dish. Use a pipette to carefully remove the determined volume indicated by the calculation above from each well intended to be used in co-culture.
   k. Prepare as many wells as necessary according to number of projected COCs.
   l. Use a micropipette to add the determined volume of Fertilo to each well prepared in the previous step.
   m. At the end of the exchange, there should again be 100 µL in each co-culture drop.
   n. The dose of the Fertilo co-culture is 100,000 cells per 100 µL of culture medium in the application. In the formulation of each Fertilo vial no less than 310,000 viable cells are provided to allow for the preparation of three 100 µL droplets per vial, or six wells total.
   o. Check the droplets under a microscope to ensure presence of cells in suspension.
   p. Place the culture dish into the incubator to allow re-equilibration for a minimum of 30 minutes or maximum of 2 hours before IVM culture.

IVM Culture

1. At retrieval, identify immature COCs in the follicular aspirate and move to a holding dish for the remainder of retrieval.
   a. **Note:** Immature COCs are smaller and more compact than conventional COCs. It is recommended that a 70 µm filter be used to strain aspirate prior to oocyte search.
2. Immediately after retrieval, if using a 4+8-Well IVF dish, rinse COCs in the center 150 µL cIVM wash droplets then move to the culture droplets previously prepared with Fertilo.
   a. **Note**: No more than 5 COCs should be cultured per droplet of Fertilo.
3. Place the dish in the incubator at 37°C, 5% O_2 and a CO_2 level that achieves 7.3 pH for an optimal period of 30 hours (24-32 hours acceptable)

3. Day After Oocyte Retrieval (Day 1)

1. Remove the dish from the incubator after IVM culture.
2. Remove the COCs from the droplet and wash 3 times in at least two different wash droplets to remove any residual Fertilo OSCs in suspension using new wash droplets.
   a. To the extent feasible, ensure no residual Fertilo OSCs remain after washing by microscopic inspection.
3. COCs should then be transferred to a new dish and denuded through conventional hyaluronidase treatment according to site-specific procedures. This procedure will remove cumulus cells and any residual Fertilo OSCs remaining from previous step.
   a. All COCs treated with Fertilo must be fertilized through the use of intracytoplasmic sperm injection.
   b. For oocytes destined for egg freezing, proceed with the clinic's standard protocols.

Required Products Sold Separately

MediCult IVM® System
Human Serum Albumin
Pharmaceutical Grade Follicle Stimulating Hormone
Pharmaceutical Grade Human Chorionic Gonadotropin
70 µm Cell Strainer
Endotoxin Free 15ml Tube
Cell Counting Chamber (Hemocytometer)
4+8 Well IVF Dish