Fertilo Clinical Use Recommendations

Introduction - What is Fertilo?

Fertilo is an *in vitro* maturation (IVM) product composed of ovarian support cells (OSCs) which are supplemented into IVM Medium, such as MediCult IVM Media. The OSCs utilized in Fertilo are produced from a single clinically compliant source of human induced pluripotent stem cells (hiPSCs). The OSCs, commonly known as granulosa cells, are a well studied ovarian cell type responsible for providing the required developmental niche for quality oocyte maturation. Fertilo is applied exclusively *in vitro*, with no aspect of the product being used beyond the oocyte maturation stage or for any *in vivo* use. Every lot of Fertilo OSCs is manufactured under strict manufacturing best practices, and comprehensively evaluated for conformance, OSC identity and purity, OSC performance and potency, and absence of advantageous human or animal pathogens including viruses, bacteria, and fungi. All Fertilo OSC batches are generated using reagents that are completely animal origin free (AOF) and meeting international fertility product standards for low endotoxin and mouse embryonic assay (MEA) safety. Each Fertilo batch is produced and delivered with a complete certificate of analysis (COA) to demonstrate product conformity according to Quality Management principles. Prior clinical and nonclinical studies of Fertilo have demonstrated it is a safe and effective approach to maturing oocytes and improving their developmental competence for high quality embryo formation.

Suggested Patient Population and Consenting

Recommended patient characteristics:

- Women who are candidates for IVF or egg freezing who are interested in or require low doses of hormonal stimulation regimens
- Women with high ovarian reserve, such as:
    - AMH $\geq$ 2 ng/mL and/or;
    - Norm-ovulatory patients with AFC $\geq$ 20 and/or;
    - PCO-like or PCOS patients
- Patients of any age may benefit from Fertilo; however, Fertilo has only been evaluated in clinical studies in patients 37 and younger

Informed Consent:

- Gameto does not require additional informed consent beyond the clinic's current Informed Consent. However, if the clinic wishes to offer patients an additional consent

form for the use of Fertilo or abbreviated hormonal stimulation, they may do so independently.

Patient minimal stimulation and retrieval

Fertilo has been utilized in two post market clinical studies and 7 independent embryology studies to date, encompassing over 200 patients. Based on these studies, we summarize basic recommendations for achieving optimal clinical outcomes with the product, which are detailed below.

Fertilo works for both contraceptive controlled cycles and without. However, our findings suggest that an OCP regimen of 10-15 days with a 5 day wash out period is advantageous for achieving more uniform patient response to stimulation, clear any atretic follicles, and to allow for timed retrieval programming.

Fertilo is compatible with a variety of minimal stimulation regimens, the goal of which is to reduce the gonadotropin administration prior to oocyte retrieval, avoid dominant follicle formation, and retrieve oocytes from follicles that are 12mm or smaller. In our recent post market clinical study, we determined that minimal stimulation utilizing clomid and recombinant FSH provided optimized outcomes in terms of oocyte yield, maturation, and high quality blastocyst formation. With Fertilo, it is recommended using no more than 4 injections of rFSH and no more than 5 days of clomid in total. An hCG trigger can be utilized or eliminated, depending on physician preference. Specific stimulation regimens are detailed below.

Recommended Minimal Stimulation Protocol:

The following is a suggested example of dosing and monitoring for a patient receiving combination of clomid and FSH:

1. OCPs 10-15 days
2. Stop OCPs and allow a 5 day washout
3. **Monitor Appoint 1:** Ultrasound at Baseline
    a. If all follicles 8 mm or less, proceed with 5 days 100 mg daily Clomid
    b. Any follicle 9 mm or greater at baseline -> Cancel and restart OCPs
4. Complete clomid regimen according to above
5. **Monitor Appointment 2:** Ultrasound and measure follicle sizes after completion of clomid
    a. Lead Follicles ≤ 6mm : 3 injections of 150 IU rFSH (Gonal-F)
    b. Lead Follicles > 6mm and ≤ 9 mm: 2 injections 150 IU rFSH
    c. Lead Follicles ≥ 10mm: 1 injection 150IU rFSH

6. Trigger 250µg r-hCG (Ovidrel) or 5000 IU hCG in the evening after the last rFSH injection.
    a. Retrieve oocytes 34 to 36 hours after trigger shot
    b. If no trigger is utilized, retrieve oocytes 42-46 hours after the last rFSH injection

More monitoring appointments may be utilized as necessary to optimize patient response and dosing, the above are suggested based on prior findings.

Oocyte Pick-up

Physicians should utilize the needle they are most comfortable with using for standard IVF practice. Based on our findings, a 17-gauge single lumen needle works well or alternatively a 19-gauge needle. All visualizable follicles should be punctured for extraction when possible and it is important that a high quality ultrasound with zoom capability is utilized. A reduced suction pressure of 70 mm Hg should be used for all retrievals with 17G needles, and no more than 100 mm Hg for 19G needles. Frequent needle rotation, known as curettage, must be performed to assist in small follicle extraction. Oocyte aspiration should be performed with Vitrolife ASP medium (or heparinized collection media) into warmed collection tubes. Due to potential clotting in the needle, frequent needle flushing should be performed. **No follicular flushing should be performed during aspiration.**

In general, it has been found that oocyte yield tends to be lower in small oocyte retrievals from abbreviated stimulations. However, it has also been found that as the number of retrievals performed by clinician increases, as does the number of oocytes retrieved. We therefore recommend if possible, the first few patients undergoing Fertilo protocol present with PCOS. This will give the clinician a larger cohort of cumulus oocyte complexes to attempt.

For more detailed information regarding the clinical procedures for small oocyte retrieval, please refer to our video abstract “Clinical Procedure for In Vitro Maturation (IVM) Treatment”.

Embryological Procedures

Oocyte Search During Pick Up

Immature COCs can be difficult to identify at first, as they are often darker and more compact than traditional IVF. Therefore, care should be taken during the search process. Based on our findings, we recommend the following embryology procedures to assist with the oocyte search and handling process for IVM.

Careful attention to plate, search media, and culture media handling should be followed, as immature COCs are highly sensitive to temperature, pH, and osmolality changes. As COCs

retrieved from abbreviated stimulations are highly compact and the follicular fluid more bloody, follicular fluid aspirated by the clinical team should be passed through a 70-micron cell filter. This filter method allows blood to pass through while trapping COCs and other cumulus cells, greatly improving the ease of the search. It is recommended that a 1000 µl pipette be used to transfer follicular aspirate through the filter to prevent shearing of the cumulus cells and ensure precision during filtration of COCs from blood.

The filter is then turned over atop a dish containing a search medium and washed briefly to allow release of COCs into the search medium. Clean media can be pushed through the back of the filter using a 1000 µl pipette if necessary to ensure the loosening of all collected cells. Once in the clean search dish, compact COCs are then identified under stereomicroscope and transferred to a holding droplet of the clinic's standard oocyte collection media until all COCs have been identified and isolated. These compact cumulus oocyte complexes may only have one to three layers of cumulus. Small oocyte retrievals also tend to produce string-like blood clots. It is advised that embryologists use needles to pull these apart as COCs may be attached. If staff allows, it is helpful for two embryologists to perform successive searches of fluid to ensure all compact COCs have been identified. As with all other embryological procedures, all searching should occur on a heated surface, with sufficient speed to ensure minimal disruption of COC quality. If search is not performed inside an isolette, HEPES buffered media should be employed.

Once all COCs have been identified, COCs with any excessive tissue should be cut with needles to ensure a more uniform cohort. The number of COCs collected should be recorded.

In Vitro Culture Preparation

As per the Fertilo Instructions for Use, A single culture plate is prepared the day before oocyte retrieval to allow for media and oil equilibration. GPS Universal dishes or BIRR 4+ 8 dishes are used for culturing, as these plates feature structured microwells that help retain Fertilo cells and COCs together during culture.

For the Fertilo co-culture, six 100 µL droplets of complete IVM medium are prepared. This medium consists of Medicult IVM base medium, 10 mg/mL human serum albumin (HSA), 75 mIU/mL rFSH, and 100 mIU/mL hCG. Additionally, four 150 µL wash droplets are prepared with the same complete IVM medium. All droplets are overlaid with IVF-grade oil. A separate 500 µL of complete IVM medium is placed into the incubator to equilibrate overnight for use in resuspending cells before co-culture. The plates are incubated overnight at 37°C, with a CO_2 concentration that maintains a pH of 7.2-7.4 and 5% O_2.

Fertilo Ovarian Support Cell (OSC) Preparation

See the detailed Fertilo Instructions for Use for a comprehensive description of the preparation protocol. Briefly, 30 minutes to 2 hours before oocyte culture, Fertilo ovarian support cells (OSCs) are prepared. Two Fertilo cryovials are thawed for about 2-3 minutes at 37°C using a heated bead or water bath. Cells are gently resuspended with 250 µL pre-warmed complete IVM medium, added dropwise, and transferred to a 15 mL conical tube. Then, 6 mL of complete IVM medium is added, and the tube is centrifuged at 300xg for 5 minutes. It is recommended to use a swing bucket centrifuge, but fixed rotator centrifuges work as well. The supernatant is carefully aspirated, and the cell pellet is resuspended using the previously equilibrated medium, based on the lot-specific concentration provided in the Certificate of Analysis (COA). A cell count utilizing a hemocytometer is employed to determine the exact cell concentration. In Fertilo, an exact dose of 100,000 OSCs per 100 µl droplet is employed. In the Fertilo plate, the necessary volume of complete IVM medium is removed from each droplet and replaced with an equal volume of the Fertilo cell suspension based on the cell concentration. Prepare as many culture droplets as the concentration allows, minimizing pipetting to avoid disrupting the droplet equilibration. The Fertilo droplets should be allowed to equilibrate for 30 minutes to 2 hours before COC culturing.

In Vitro Maturation Culture

After oocyte retrieval and isolation, all COCs are placed directly into the Fertilo IVM culture droplets. No more than 5 COCs should be cultured per droplet. COCs are first transferred through the wash droplets, followed by placement into the Fertilo droplets, ensuring minimal transfer of media. The COCs are cultured in Fertilo for 24 to 32 hours with an optimal period of 30 hours, with the plates incubated at 37°C, CO2 levels adjusted to achieve a pH of 7.2-7.4, and 5% O2.

Denudation and Maturity Assessment

After IVM culture, COCs are briefly denuded using hyaluronidase and then mechanically stripped to remove all cumulus cells and any residual Fertilo cells using the clinic's standard denudation protocols. The denuded oocytes are held in the wash droplets until all oocytes are fully denuded. The oocytes are then washed and assessed for maturity. The embryologist should perform a final visual check to ensure no residual Fertilo cells are transferred with the denuded oocytes. Following this, the patient cycle proceeds as clinically indicated for embryo formation via ICSI or oocyte cryopreservation.

Alternative Use Formats of Fertilo

Utilization of Fertilo for Rescue IVM

Fertilo has been demonstrated as effective for use in rescue IVM applications, in which a patient undergoes conventional stimulation and immature oocytes identified at retrieval are cultured with

Fertilo to “rescue” their maturation. In this context, the oocytes are procured in the form of metaphase I (MI) or germinal vesicle (GV) oocytes devoid of cumulus enclosure (i.e. denuded). Fertilo is applied for rescue IVM in an identical manner as discussed above for minimal stimulation IVM treatment. For optimal outcomes, co-culturing with Fertilo should occur for 24 hours in a rescue IVM setting. Rescue IVM is well known to utilize oocytes of inferior quality to minimal stimulation IVM, but may be beneficial for patients with few or no mature oocytes after conventional stimulation.

Utilization of Fertilo with conventional insemination

Fertilo has been shown to be compatible with conventional insemination in mice, bovine and human non-clinical studies. The preferred method and clinically tested protocol for use with Fertilo is intracytoplasmic sperm injection (ICSI) but conventional insemination may likewise be utilized when needed or preferred. For use with conventional insemination, extra washes should be utilized of the COCs post-IVM to ensure no residual Fertilo OSCs remain present. If the COCs appear visually compact after IVM, ICSI should be utilized instead of conventional insemination for improved outcomes.

Utilization of Fertilo with alternative IVM media or supplements

Fertilo has been shown to be compatible with MediCult IVM media and SAGE IVM media in human and mice non-clinical models. The preferred and clinically tested IVM media for Fertilo is MediCult IVM. Fertilo has been demonstrated to be incompatible with conventional embryo culture media such as Global Total media, due to metabolic imbalance that does not support cumulus cell growth and function. Careful testing and clinical caution should be utilized if alternative media is considered for use with Fertilo. Additional supplements beyond FSH and hCG, including androstenedione, estradiol, EGF, and AREG have been utilized successfully with Fertilo in both non-clinical human and animal models as well as human clinical testing. Fertilo has not been evaluated for compatibility with biphasic IVM media and supplements. Careful testing and clinical caution should be utilized if alternative supplements are considered for use with Fertilo.