

## INTRODUCTION

Physician: For nearly three decades, Cook Medical has been engineering and manufacturing expertly crafted products to meet the exacting needs of infertility specialists across the world. On behalf of Cook, I'd like to welcome you to Cook ART Lab.

On today's tour, you'll learn about Cook's innovative, in vitro fertilization line – a total continuum of IVF devices for ovum aspiration, micro-manipulation, embryo culture and embryo transfer. This integrated solution of IVF devices can help optimize both laboratory and clinical performance.

A clinician and a laboratory representative will help describe the procedures and how to implement Cook's devices. The information provided on this tour should be used as a guide only. While the methods demonstrated are currently in practice in leading IVF centers, many alternatives and variations exist. Each laboratory should establish procedures and protocols that are optimized for the individual medical facility.

Let's get started.

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### DAY -1: Media Set-up & Preparation

Featured Products: MINC Benchtop Incubator, Culture Oil

Physician: The day before gamete collection, sometimes called Day -1, begins with culture media set-up and preparation.

Lab Rep: Cook has developed the STIC to help open media vials aseptically. With a sterilized STIC, remove the plastic cap using one of the hooks found at the end of each arm. Then use the hook to remove the aluminum seal.

Slide the stopper into the STIC's jaws and slowly rotate it to break the seal. Remove the stopper as shown.

Place the STIC on its side, making sure to contain the stopper in an aseptic area, preferably a laminar flow hood.

Physician: Now a lab representative will describe the techniques used to prepare dishes for all aspects of the in vitro fertilization process.

Lab Rep: Begin by airing the petri dishes in the laminar flow hood to allow any volatile contaminants to dissipate.

In the afternoon, set up the dishes. Fill the dishes with bicarbonate buffered medium and place them in Cook's MINC Benchtop Incubator overnight. This allows them to reach equilibrium in a mix of gases made up of six percent carbon dioxide, five percent oxygen and eighty-nine percent nitrogen.

Prepare the dishes one at a time to avoid osmotic stress caused by evaporation of the microdrop.

Physician: As an alternative, you can use the dishes you prefer. Prepare one dish at a time and cover with culture oil. If you prefer open culture, prepare each dish then return them to the MINC as soon as possible to avoid evaporation.

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## DAY 0: Ovum Collection Preparation

Featured Products: Follicle Flush Buffer, Test Tube Heater

Physician: The ovum aspiration process subjects the oocyte cumulus complex to a series of stressful events that can compromise its viability. Cook's products are designed to simplify the aspiration procedure, while maintaining optimal viability and minimizing trauma to the patient. The culture media used throughout this process are formulated to contribute the appropriate nutrients to match the gamete's and embryo's shifting metabolic requirements.

Lab Rep: Prior to ovum collection, an embryologist prepares the Falcon brand Test Tubes by filling each one with approximately 5 milliliters of flush buffer. The tubes are then taken to the procedure room and placed in the test tube heater. Cook's test tube heater is engineered to maintain the optimal temperature and minimize temperature-related damage to the oocyte. Next, a nurse performs a needle patency test. Then places the luer seal stopper in the test tube just before the aspiration needle enters the patient.

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## DAY 0: Ovum Collection

Featured Products: Ova-Stiff™ Ovum Aspiration Needle, Cook® Aspiration Unit™

Clinician: Local anesthetic has already been injected into the vaginal wall. Now a 16 or 17 gauge Ova Stiff Needle is advanced through the wall into the left ovary to begin aspirating the first follicle. We're moving into the next follicle, and you can see the fluid flowing into the test tube. It's normally clear during the aspiration of the first part of the follicle. As we get to the

end, you'll notice it turns red from a few spots of blood. It's important to watch the test tube and not let fluid run to the top. The stopper's design prevents fluid from traveling down the wrong line.

Now the needle is withdrawn and advanced into the next follicle. Once in the follicle, the needle is gently rotated to ensure the follicle wall doesn't become obstructed against the needle's bevel. Gently move the ultrasound probe to make sure the follicle is completely empty. Once again the needle is withdrawn and advanced into the next follicle. On the ultrasound monitor you can see that the follicle is decreasing in size. Rotating the needle and gently moving the probe helps ensure that the egg is retrieved – even in the very last milli-fluid.

Moving upwards and into the next follicle, we've retrieved the first egg. Again the needle is rotated to make sure the follicle is completely empty before moving to the next one. Now that the first ovary appears to be completely drained, the fluid can be washed through.

Now for the second ovary. Once again the needle enters through the vaginal wall into the first follicle. Throughout the aspiration process, it's important to use a mild but constant vacuum pressure on the pump. A minus 100mm of mercury is being used. A low flow rate will take more time and decrease the efficiency of the collection process. It's also important not to have the pressure on the pump too high, which could lead to a higher risk of oocyte damage and particular-fractured zona.

If the flow is not correct, make sure the stopper is correctly positioned and that there are no cracks in the test tube.

Keep the aspiration pump running as you move from follicle to follicle to maintain constant pressure. Also routinely rotate the needle and move the probe. The motion will prevent the follicle wall from adhering to the needle.

There are times when a complete obstruction of the needle can occur. Sometimes epithelial cells from the vaginal wall, or intrafollicular wall cells can cause an obstruction. If that happens, use the higher pressure pump button on the Cook pump to help overcome any immediate blockages. If that doesn't work, it's generally safer to withdraw the needle completely and place it in a test tube containing buffer. Then try to re-aspirate the buffer through the line.

A complete obstruction can often be due to a clot from blood left in the line. If that occurs, remove the stopper – there's a luer setting at the top of the stopper – and use a syringe to inject buffer backwards through the stopper to clear the obstruction.

## DAY 0: Ovum Collection Needles

Featured Products: EchoTip® Cook® Double Lumen Aspiration Needle, Follicle Flush Buffer, Ova-Stiff™ Ovum Aspiration Needle—B Bevel, Small Gage Ova-Stiff™ Ovum Aspiration Needle

Physician: Cook offers several different families of needles for ovum collection – each with its own specific function. Let’s take a moment to review the other needles and how they can be used.

Each of the following ovum collection needles features the Cook patented EchoTip. The EchoTip markings improve the visibility of the needle tip on an ultrasound monitor. EchoTipped needles help achieve accurate placement of the needle for optimal ovum collection.

During ovum collection, there are instances when flushing the follicle is preferred. Cook has designed a family of needles specifically for this procedure. With the double lumen needle, the smaller lumen flushes the follicle and the larger lumen aspirates the contents. These procedures can be performed simultaneously or separately. The double lumen needle easily connects to a Luer-lock syringe for flushing with follicle flush buffer.

The buffer is protein-free to avoid foaming and formulated to minimize stress to the cumulus oocyte complexes. It’s HEPES buffered and must be warmed to 37 degrees Celsius before use.

The OPAA needle is a single lumen aspiration needle with a B bevel. The handle design assists follicular rotation during aspiration. Its thumb notch indicates bevel orientation. The Luer-lock proximal hub accepts syringes or aspiration lines.

Cook has also engineered a range of needles specifically for small follicles. Ranging from 18 to 21 gauge, these needles are all fitted with the smaller A bevel to ensure follicular contents are recovered.

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## DAY 0: Oocyte Cryopreservation

Featured Products: Oocyte Freeze Kit, Oocyte Thaw Kit

Physician: Cryopreservation of oocytes allows eggs to be stored indefinitely until they’re thawed and fertilized. This technology is especially important for women with fertility problems and women whose eggs may become destroyed or damaged from cancer or medical treatments.

Cook has an Oocyte Cryopreservation Kit and Oocyte Thawing Kit available. If you're interested in learning more about oocyte freezing, watch "Life from the Freezer."

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## DAY 0: Cumulus Oocyte Complex Identification

Featured Products: Fertilization Medium, MINC Benchtop Incubator

**Physician:** Using a laminar flow hood, either in the laboratory or in the procedure room, the embryologist then identifies the cumulus masses. Our lab representative will explain the Cumulus Oocyte Complex identification process.

**Lab Rep:** The embryologist searches for the Cumulus Oocyte Complex. Identified oocytes are washed clean of blood and cellular debris, then placed in dishes containing the previously-equilibrated fertilization medium. Generally, two or three Cumulus Oocyte Complexes are placed in each dish. Then they're returned to the Cook MINC Benchtop Incubator while the sperm is prepared for insemination or Intra Cytoplasmic Sperm Injection, commonly called ICSI.

**Physician:** The MINC is a benchtop incubator that Cook has engineered specifically for IVF. Its design initiates an automatic gas purge each time the lid is closed to re-establish the desired environment. As a result, the pH levels return to normal faster than in other incubators, resulting in less stress to the embryos.

The MINCs heated chamber base plate and lid provide a stable thermal environment at 37° Celsius. And its 24-hour self-monitoring software tracks and reports temperature, gas flow and alarm events.

**Lab Rep:** The majority of the MINCs here are connected in series, but they're also placed next to where procedures are being performed to minimize exposure time to embryos outside the incubator. The MINC has become a critical part of the culture system. Larger traditional incubators can still be used for equilibrating media in test tubes.

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## DAY 0: Sperm Preparation

Featured Products: Sperm Gradient Kits, Sperm Medium, Sperm Cryopreservation Buffer

**Physician:** One of two methods is generally used to separate motile sperm from ejaculate: the density gradient centrifugation method or the sperm swim-up technique.

Lab Rep: For density gradient preparation of semen samples, a 40% / 80% density gradient mix to separate and purify the sperm sample is used. Prepare the sample in duplicate by loading the raw semen on top of two prepared gradients – taking care not to overload them.

Centrifuge the sample tubes for 20 minutes at 300g. Carefully remove the sperm pellet and wash it two more times by further centrifugation – 600g in 3 milliliters of gamete buffer – before finally resuspending the pellet in a small volume of sperm or fertilization medium. Then store the prepared sperm sample either for future insemination or ICSI.

Physician: An alternative method of sperm preparation is the swim-up technique. It can be carried out with either raw or washed semen.

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## DAY 0: ICIS Preparation

Featured Products: Hyaluronidase, Cook® Flexipets®, PVP (Polyvinylpyrrolidone)

Physician: Now we'll move on to discuss Intra Cytoplasmic Sperm Injection, commonly known as ICSI.

Lab Rep: To prepare for ICSI, remove the cumulus cells from the oocyte by incubating them in a diluted hyaluronidase solution for no longer than one minute. After incubation, move the Cumulus Oocyte Complexes into gamete buffer and denude them. Use Cook Flexipets to remove as many cells as possible from the zona pellucida of the oocyte.

Using a series of progressively narrower Cook Flexipets, continue to remove the coronal cells until all the zonae are free of cells. Place the tip of the Flexipet as close to the oocyte as possible and gently aspirate in and out of the Flexipet. To minimize movement at the tip, it helps to rest your wrist on the edge of the microscope stage or bench top. Once the oocytes are denuded, transfer them into pre-equilibrated injection dishes of Bicarbonate Buffered Medium and place them in the incubator.

If the ICSI procedure is expected to take longer than 10 minutes, prepare the injection dish with gamete buffer and warm to only 37 degrees Celsius before use.

Introduce the sperm into the side of the polyvinylpyrrolidone or PVP droplet. Overlay the center droplets with Culture Oil and allow the dish to equilibrate until it's needed. Then introduce the oocytes into the peripheral drops.

## DAY 0: ICSI

Featured Products: Cook® Precision Holding Pipettes, Cook® Precision Micro-injection Pipettes

**Physician:** The ICSI procedure is carried out using a holding pipette to secure the egg and an injecting pipette to introduce the sperm into the egg cytoplasm. The type of microscope and injecting device employed by individual laboratories may vary.

**Lab Rep:** Set up and align the holding and injecting pipettes according to laboratory procedures. Once the sperm for injection has been identified, strike the tail of the sperm as it swims perpendicular to the injection pipette.

Load the sperm tail first into the pipette, making sure the sperm can move freely inside the barrel.

Move the stage of the microscope so that a single oocyte is visualized in one of the culture drops. Fix the oocyte using negative pressure from the holding pipette. It's important that the oocyte is immobilized so that the polar body is either at the 12 or 6 o'clock position. Next, push the microinjection pipette through the zona pellucida and through the oolemma into the ooplasm at the 3 o'clock position.

Because of the extreme elasticity of the oolemma, the tip of the pipette may hit the inner part of the opposite zona pellucida without breaking the oolemma. As result, some cytoplasm must be aspirated into the injection pipette before the sperm is injected.

Once the oolemma is broken, deliver the sperm together with all of the cytoplasm back into the oocyte.

After the sperm has been deposited, gently withdraw the injection pipette. Releasing the negative suction will free the oocyte.

Repeat this process until all oocytes in the dish have been injected. After injection, wash and incubate the oocytes in pre-equilibrated cleavage medium for 16 to 18 hours.

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## DAY 0: Insemination

**Physician:** In classical IVF, the Cumulus Oocyte Complexes are inseminated by adding a controlled concentration of sperm cells, generally about 100,000/ml. Ideally, insemination should be carried out in a controlled

environment chamber. If one is not available, then a heated stage on a dissecting microscope should be used.

**Lab Rep:** Add a controlled concentration of sperm in the smallest possible volume to each well containing two to three Cumulus Oocyte Complexes. View the wells on a dissecting microscope under dark field to ensure that motile sperm are present. Then return the dish to the incubator until the fertilization check 16-18 hours post insemination. If a short insemination is used, the sperm and oocytes are incubated for two hours before removing the oocytes and placing them in fresh culture medium.

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## DAY 1: Pronuclei Observation

**Physician:** 18 hours after insemination the pronuclei are checked to see if the remaining cumulus cells have been removed from the zygote.

In the case of ICSI zygotes, the pronuclei are also observed. Since they have already been denuded, there is no need for cumulus cell removal.

**Lab Rep:** Check the IVF inseminated embryos for fertilization at 16 to 20 hours post insemination. Using a Cook Flexipet, strip off all cumulus remnants.

You can observe fertilization in ICSI zygotes 14 to 18 hours post injection. Using the Flexipet, roll the zygote under maximum magnification on the dissecting microscope (~40x). Assess the number of pronuclei, polar bodies and general morphology of the zygote. Count only those oocytes with two pronuclei as being fertilized. Score all zygotes and group them together for culture in Equilibrated Cleavage Medium, making sure they are fully washed before incubation.

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## DAY 2-5: Embryo Observation

Featured Products: Cryopreservation Kit, Cook® Flexipets®, Blastocyst Medium

**Physician:** Different laboratories will transfer embryos on different days post oocyte collection – typically on Day 2, Day 3 or Day 5.

In the case of embryo observation on Day 2 – approximately 24 hours after the fertilization check or 40 to 48 hours post insemination – the embryos are assessed for either embryo transfer, cryopreservation or extended culture. These Day 2 embryos are generally between the 2 to 4 cell stage.

- Lab Rep: Using a Flexipet, roll the embryo, viewing it under maximum magnification on the dissecting microscope. Assess the embryos according to the number and regularity of blastomeres, the degree of cytoplasmic fragmentation and the cleavage rate.
- Physician: For Day 3, 66 to 74 hours post insemination, the embryos can be assessed for embryo transfer, cryopreservation, or extended culture to Day 5 or 6.
- Lab Rep: To assess the Day 3 embryos, place the dish containing the embryos onto the warming stage of a dissecting microscope and check the embryos for further cleavage. Using a Cook Flexipet, roll the embryo while viewing it under maximum magnification. Once again assess the embryos according to the number and regularity of blastomeres, the degree of cytoplasmic fragmentation and the cleavage rate. The embryos should be at the 6 to 8 cell stage of development. On the morning of Day 3, move the embryos for extended culture into equilibrated blastocyst medium.
- Physician: For extended culture after 48 hours, the embryos in blastocyst medium remain in the incubator.
- Lab Rep: Select blastocysts containing numerous cells and a well-defined inner cell mass for transfer. While maintaining physiological pH and temperature, score the blastocysts on an inverted microscope.

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## DAY 2-5: Embryo Transfer Catheters

Featured Products: Sydney IVF Embryo Transfer Catheter Set – With Microvol® Technology, Soft-Pass™ Embryo Transfer Catheter Sets, Cleavage Medium, Blastocyst Medium

- Physician: Scientists and clinicians often struggle with the discrepancy between embryonic development and pregnancy rates. Placing the embryos in the correct position in the mother's uterus with minimal trauma is essential. Unfortunately, a well-formed embryo might never become successfully implanted in the mother's womb. One reason may be the embryo transfer techniques. Problems such as uterine contractions and blood and mucous on the tip of the transfer catheter can make placement of the embryos in the uterus difficult – and in the worst case, result in failure.

Cook has a family of precision-crafted embryo transfer catheters to ensure that this critical procedure is simple, atraumatic and repeatable.

First, let's look at the Cook Sydney IVF catheter. Its gliding coaxial catheter with a pre-curved, bulb tip helps ease the catheter's passage through the cervix. It's also soft and flexible to help prevent any possible harm to the endometrium. Like other Cook transfer catheters, the Sydney IVF catheter incorporates Microvol technology, which allows the embryo to be transferred in a minimal amount of medium. Less fluid reduces the potential that the embryo will move away from where it's placed during transfer.

Physician: Cook manufactures transfer catheters to match individual clinicians' and laboratories' preferences for access and embryo placement. Options include curved or straight tips, EchoTipping, malleable or stiff obturators, distal or proximal markings, with or without Microvol technology, and Teflon or Polyethelene guide catheters.

Physician: The Soft-Pass Embryo Transfer Catheter, like the Sydney IVF, is also soft and flexible in design, but it features a straight tip and incorporates Microvol technology. The Soft-Pass has the additional advantage of EchoTipping for ultrasound-guided transfer. During the transfer procedure, a metal band incorporated into the tip of the Soft Pass Catheter highlights the tip under ultrasound, helping the clinician visualize correct placement in the uterine cavity. The Soft-Pass also has an optional stainless steel support cannula to further aid catheter positioning,

Regardless of the catheter used, the embryo transfer procedure is essentially the same for all stages of embryo development. The only variant is the embryo transfer medium used. Day 2 and Day 3 stage embryos are transferred in cleavage medium. Day 5 embryos are transferred in blastocyst medium.

DAY 2-5: Embryo Transfer  
 Featured Products: Ova-Stiff™ Ovum Aspiration Needle, Cook®  
 Aspiration Unit™

Lab Rep: Embryos are selected for transfer based on their morphology and development rate. The embryo's culture medium is the most suitable for transfer. Rinse the Cook two-piece non-toxic 1.0 mL syringe and fill.

Attach the syringe to the transfer catheter. Expel all but 50 microliters of the medium through the catheter. Working in a controlled environment chamber, draw in 2 microliters of air, then 2 microliters of medium, immediately followed by the embryos in an additional 5 microliters of medium. Finally, draw in another 2 microliters of air. Withdraw the inner

catheter into the outer catheter and pass it to the clinician to traverse the cervix.

**Clinician:** Extend the inner catheter through the outer catheter into the uterine cavity. Gently expel the embryos by depressing the plunger 10 microliters. Then carefully remove the catheter. Flush the inner catheter and examine it to ensure that the embryos were released.

Ultrasound can be used to ascertain the position of the uterus before transfer and can also be used afterwards to determine the position of the embryos between the two air bubbles. For difficult passage through the cervical canal, we use an obturator to assist in getting the bulb tip through the internal Os.

## DAY 2-5: Embryo & Blastocyst Cryopreservation

Featured Products: Cryopreservation Kit, Blastocyst Cryopreservation Kit, Thawing Kit, Blastocyst Thawing Kit, Blastocyst Vitrification Kit, Blastocyst Warming Kit

**Physician:** Embryos and blastocysts can be frozen using cryopreservation kits at the cleavage stage or blastocyst stage of development. The kits differ only in the type of cryoprotectant used to successfully dehydrate the cells. In the case of cleavage stage embryos, propanediol/sucrose are the cryoprotectants of choice. For blastocysts, Cook offers a glycerol/sucrose solution.

If and when the patient returns for a frozen embryo replacement cycle, the embryos or blastocysts can be thawed on the day of transfer. For patients who want culture-thawed embryos, thaw the units needed a day ahead to assess their developmental potential. If embryos are to be cultured to Day 3, they can be left in the same medium or transferred into fresh, equilibrated cleavage medium for overnight incubation.

**Physician:** To be successful, any cryopreservation strategy must minimize the impact of ice crystal formation, solution effects, and osmotic shock on the embryo. Currently, there are two common methods for cryopreserving excess embryos. The traditional method is to slowly cool the embryos and the surrounding solution to storage temperature while deliberately initiating ice crystal formation remotely from the embryo.

A more recent method of embryo cryopreservation is vitrification.

**Lab Rep:** Vitrification uses high concentrations of cryoprotectants – in this case DMSO, ethylene glycol and trehalose – which allow for a very fast cooling

rate. The rapid cooling transforms the solution into a glass-like amorphous solid free from any crystalline ice structure. As a result, no ice crystals form either inside or around the embryo.

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## CONCLUSION

Physician: We hope you've enjoyed learning about Cook's integrated assisted reproduction system. Please contact us if you have questions about any of our devices. And if you haven't already, take some time to explore the rest of this website. You can access resources in our Knowledge Center, learn about the latest news and events, participate in forums and live chats, or explore Cook's full women's health online catalog. And feel free to revisit any sections of the tour.

Thank you.