INVO*cell* DEVICE : INSTRUCTIONS FOR USE

INVO Bioscience

DEVICE DESCRIPTION :
The **INVO** Bioscience **INVO*cell***, device is a three-part assembly (see Figures 1 and 2) enclosed in two separate packages.

One package contains the inner chamber. The other package contains the top and bottom parts of the outer rigid shell.

The inner chamber holds gametes in cell culture medium and is placed in the vaginal cavity for incubation to allow embryo development , prior to the embryo(s) placement into the uterus .The outer rigid shell protects the inner chamber from contamination while in the vaginal cavity.

FIGURE 1 : INNER CHAMBER (Contents of Package One)

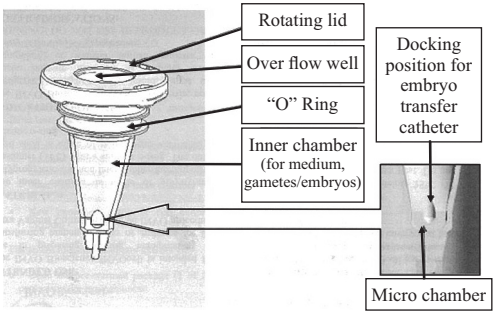
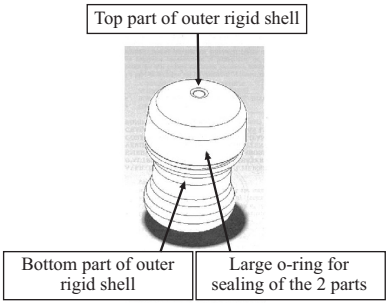


FIGURE 2 : OUTER RIGID SHELL FOR CELL CULTURE CONTAINER (Contents of Package Two)



INTENDED USE :
The **INVO** Bioscience **INVO*cell*** is intended for use in preparing, storing, manipulating or transferring human gametes or embryos for the Intra Vaginal Culture also called **INVO** procedure.

MATERIALS :
The inner chamber is made of polystyrene, polypropylene and non latex rubber/thermoplastic elastomer (TPE) with a silicone o-ring. The outer rigid shell is made of polystyrene with a large silicone o-ring.

INDICATIONS FOR USE:
The **INVO** Bioscience **INVO*cell*** is sterile, non-pyrogenic, embryo toxicity tested, single use plastic labware intended to prepare, store, manipulate, or transfer human gametes or embryos for the **INVO** procedure.

CONTRAINDICATIONS :
The **INVO** Bioscience **INVO*cell*** should not be used in patients with the following conditions

- Demonstrated hypersensitivity to the materials used in the **INVO** Bioscience **INVO*cell***
- Demonstrated vaginal infection, recent pelvic surgery or history of toxic shock syndrome
- Severe case of vaginismus
- Conditions other than those appropriate for treatment using the **INVO** procedure

STERILIZATION :
The **INVO** Bioscience **INVO*cell*** is provided sterile according to manufacturing procedures that have been validated to meet a sterility assurance level (SAL) of 10^{-6}. Do not re-sterilize.

NON-PYROGENIC :
The **INVO** Bioscience **INVO*cell*** is endotoxin tested by LAL yielding no more than 0.5EU/ml or 20 EU/vessel.

NON-EMBRYOTOXIC :
The **INVO** Bioscience **INVO*cell*** has been tested for embryotoxicity yielding at least 75% of both test and control 2-cell mouse embryos at hatched and / or blastocyst stage.

STORAGE :
Store at room temperature below 25°C (77°F), away from moisture and direct heat. Do not use after the expiration date.

WARNING: DO NOT BEGIN CLINICAL USE OF THE INVO BIOSCIENCE INVOCELL LABWARE WITHOUT ESTABLISHING COMPETENCY BY READING AND PRACTICING THESE INSTRUCTIONS FOR USE.

Micro beads (~90µm diameter) should be used to practice embryo aspiration from the cell culture container.

If you have questions regarding use of the **INVO** Bioscience **INVO*cell*** or the **INVO** procedure contact **INVO** Bioscience customer assistance by e-mail **INVO** Bioscience at : *clauderanoux@invobio.com*

REQUIRED ACCESSORIES :
The following equipment and supplies (or equivalent) are required to facilitate use of the **INVO*cell*** :

- Laminar flow workstation
- 37°C incubator
- Stereo microscope
- Bench centrifuge
- Adjustable volume pipettes covering 5-1000µl; and sterile pipette tips
- Complete P-1 medium with SSS -Irvine Scientific cat# 9926 or other medium approved to support embryo development for 3 days .
- Embryo transfer catheter (Wallace, Cooper Surgical, cat # ME1816, Cook cat # K-J-SPPE681700, Frydman, Fertility Technology Resources, cat #13070S0) and 1ml syringe.

WARNING: DO NOT USE OTHER EMBRYO CATHETER MODELS WITHOUT CHECKING THAT THEY FIT IN THE DOCKING POSITION APPROPRIATELY AND DO NOT TOUCH THE BOTTOM OF THE MICROCHAMBER]

- Powder-free gloves
- **INVO** Bioscience **INVO** holding block for the inner chamber (sold separately)

The specially designed holding block accessory acts as a passive thermal mass while physically stabilizing the inner chamber temperature during various steps of the process including microscopic visualization of embryos. This block is provided not sterile and should be cleaned to the standard of the microscope base and microscope per the laboratory procedure .

WARNING : IN THE INVO PROCEDURE, EMBRYO DEVELOPMENT IS FIRST EVALUATED AT THE END OF THE INCUBATION PERIOD WHICH IS 48-72 HOURS POST FERTILIZATION. THEREFORE ANY ABNORMALITIES THAT WOULD HAVE BEEN DETECTED AT AN EARLIER STAGE (PRO-NUCLEI STAGE) MAY NO LONGER BE APPARENT WHEN THE EMBRYOS ARE EVALUATED FOR TRANSFER. AS A RESULT THERE MAY BE INCREASED RISK THAT AN ABNORMAL (E.G.TRIPLOID) EMBRYO COULD BE TRANSFERRED TO THE UTERUS.

WARNING : THE INVO*cell* IS A SINGLE USE DEVICE. REUSE MAY RESULT IN CONTAMINATION.

WARNING : DO NOT RESTERILIZE. RESTERILIZATION MAY RESULT IN FAILURE OF THE INVO*cell*.

WARNING : UTILIZE A CULTURE MEDIUM THAT WILL SUPPORT CONTINUED EMBRYONIC DEVELOPMENT FOR UP TO 3 DAYS.

WARNING : <u>DO NOT INCUBATE BEYOND 3 DAYS</u>

WARNING : DO NOT USE IF PRODUCT OR PACKAGE APPEARS DAMAGED

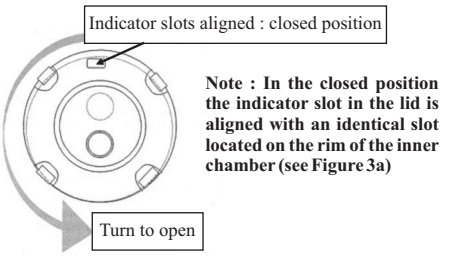
INSTRUCTIONS FOR USE :

1. Pre-warm the following in a 37°C incubator for at least 1 hour prior to use :
   - Unopened package (1 of 2) containing the inner chamber
   - Unopened package (2 of 2) containing outer rigid shell for the inner chamber
   - **INVO** Bioscience **INVO** holding block
   - New unopened bottle of cell culture medium

2. Placement of gametes (sperm and oocyte) :
   a. Open the pre-warmed package (1 of 2) containing the inner chamber.
   b. Place the closed inner chamber in the pre-warmed holding block and keep the outer rigid shell in its package at 37°C.

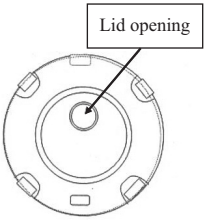
Note : The inner chamber is pouched in the closed condition (see Figure 3a).

Figure 3a : Top view of lid in closed position



Note : In the closed position the indicator slot in the lid is aligned with an identical slot located on the rim of the inner chamber (see Figure 3a)

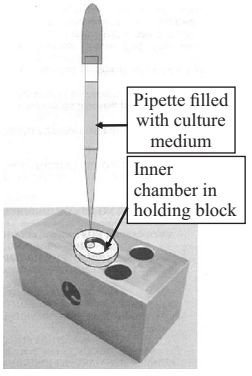
Figure 3b : Top view of lid in open position



c. Turn the lid counter clock-wise to open (see figure 3). Rinse the inner chamber with pre-warmed culture medium using an adjustable volume pipette to add and remove medium through the circular opening in the lid (see Figure 4)

d. the volume of the inner chamber is 1.08 ml. Using a sterile glass pipette or a pipette with a long thin plastic tip , fill the inner chamber with pre-warmed culture medium.

FIGURE 4 : FILLING THE INNER CHAMBER



e. Close the inner chamber and aspirate the culture medium in excess in the overflow well. Turn the inner chamber upside down to verify the absence of major air bubbles. If necessary eliminate any trapped air bubbles filling the micro-chamber .
f. Reopen the chamber
g. Using an adjustable volume pipette with a 0-200 ml tip, transfer 30,000 motile sperm into the inner chamber. The added volume is generally under 50 µl.
h. Using a glass pipette or an adjustable volume pipette with a 250-1000 µl tip, transfer oocytes into the inner chamber. The added volume should not exceed 50 µl.

WARNING : DO NOT USE A 0-200 µL TIP TO ADD OOCYTES SINCE THEY MAY STICK IN THE TIP AND /OR BECOME DAMAGED .

I Close the inner chamber by rotating the lid clock-wise and remove any cell culture medium remaining in the overflow well area above the lid with a pipette .

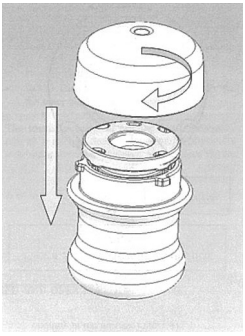
3. Outer rigid shell installation

a. Open the pre-warmed package (2 of 2) containing the outer rigid shell top and bottom parts.

CAUTION : Do not touch the inner surfaces of the outer rigid shell top part during installation .

b. Carefully introduce the inner chamber into the outer rigid bottom part. Verify that the small tip at the lower extremity of the inner chamber is positioned in the small cylinder feature located inside the bottom of the outer rigid shell. Put pressure on the top of the lid to engage the two containers.

FIGURE 5 : CLOSING THE OUTER RIGID SHELL

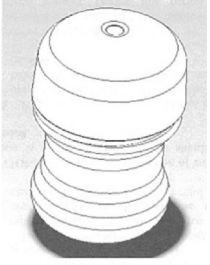


c. Close the outer rigid shell by pushing the top part down on the large o-ring ,with three tabs of the bottom part engaged in the corresponding holes of the top part . Turn the top part clockwise to lock the outer rigid shell closed (see figure 5)

CAUTION : Verify that the outer rigid shell is correctly locked before placement of the INVO*cell* in the vaginal cavity.

d. If necessary, place the loaded, fully assembled **INVO*cell*** (see figure 6) in the 37°C incubator until ready for placement in the patient .

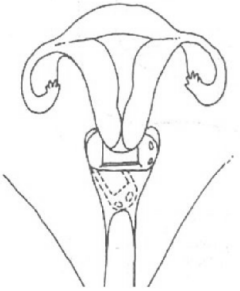
FIGURE 6 : FULLY ASSEMBLED INVO*cell*



4. Intra -vaginal placement :

Rinse the loaded ,fully -assembled **INVO*cell*** with water or saline solution, pre-warmed to 37°C and insert it longitudinally in the vagina. Push deep into the vaginal channel and then rotate 90 degrees in the horizontal plane to position in the posterior formix behind the cervix (see figure 7).

FIGURE 7 : PLACEMENT OF THE VESSEL IN THE FORNIX



5. A diaphragm with holes in the membrane is a retention device and should ell also be placed in the vagina to maintain the **INVO*cell*** device in the vaginal cavity.

Note : It is easier to position the INVO*cell* and the retention device at the same time in vaginal cavity by placing the INVO*cell* in the membrane of retention device .

CAUTION : Use of the retention device may result in discomfort.

CAUTION : In the event the patient is intolerant of the retention device, the INVO*cell* device may be placed without the retention device. In this case, the patient should be informed of the need to take special precaution to prevent expulsion during activities such as urination and bowel movement (see patient instructions).

6. Period of Vaginal Incubation :

Incubate the **INVO*cell*** in the patient's vagina for 2 or 3 days .

WARNING : <u>DO NOT INCUBATE BEYOND 3 DAYS</u>

CAUTION : Advise the patient to avoid the following activities during INVO with the INVO Bioscience **INVO*cell*: swimming, bathing in a tub (a shower is permissible), sauna, sexual activity, strenuous activity of any type.**

CAUTION : **Instruct the patient to contact the physician if any discomfort is felt.**

CAUTION : **Instruct the patient not to remove the INVO*cell* from the vaginal cavity and to avoid manipulation of the INVO *cell.***

CAUTION : **Provide the patient with instructions for replacement of the INVO*cell* in the event it moves from its original position.**

7. Removal of the INVO Bioscience **INVO*cell* :**

a . After incubation, pull with your finger on the rigid external ring of the retention device, the **INVO*cell*** will automatically follow and remove them from the vagina longitudinally .

b. Rinse the **INVO*cell*** with pre-warmed saline solution and wipe with gauze to remove excess vaginal secretions .

c. Holding the vessel upright ,open the outer rigid shell by turning the top part counter clockwise. Remove the inner chamber. Then discard the outer rigid shell.

CAUTION : If obvious contamination of culture medium is observed, the embryos should be discarded .

d. Place the inner chamber (vertically, in the upright position) in the pre-warmed holding block. Place the holding block in the incubator.

8. Embryo Sedimentation :

Leave the inner chamber in the incubator for fifteen minutes. During this period, the embryo (s) will settle into the micro chamber located at the bottom of the inner chamber.

9. Embryo location loading of the embryo transfer catheter.

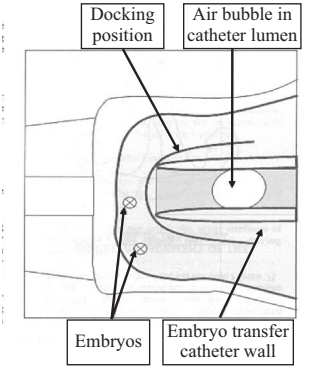
a. Set up the catheter for loading the embryos .

CAUTION : Observe and record the starting time of step 9b. If loading the embryo transfer catheter takes more than 10 minutes, re-warm the holding block and the cell culture chamber in the incubator for 20 minutes.

b. Place the closed inner chamber horizontally in the microscope viewing port (side hole) of the holding block with the aligned indicator slots uppermost .

c. Locate the embryo (s) settled in the bottom of the micro-chamber with the microscope (see figure 8). The number of embryos resulting from incubation should be compared to the number of oocytes placed in the i n n e r chamber and differences noted.

FIGURE 8: MAGNIFIED VIEW OF MICROCHAMBER AS OBSERVED DURING LOADING OF THE EMBRYO TRANSFER CATHETER



d. Gently open the lid of the inner chamber by turning it counter clockwise .

e. Introduce the catheter through the opening in the lid into the docking position adjacent to the micro chamber see (figure 8). pull gently on the syringe plunger to aspirate the embryo (s) .

Note : Generally a small air bubble(≈ 5 µl) is used to separate the column of medium in the catheter from the loaded volume containing embryo(s).

f. Withdraw the catheter slowly from the inner chamber and proceed with embryo transfer .

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Federal law (USA) restricts this device to sale only by or on the order of a physician.