In Vivo Embryo Culture Device

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Abstract

The INVO procedure using the INVOcell device is a simple technique of human assisted reproduction. The fertilization of the oocytes and the early development of the embryos occur in the INVOcell device placed into the vaginal cavity for an in vivo incubation. The vaginal cavity plays the role of the CO_2 incubator by supplying the temperature and gas environment needed for the embryo development. Capital equipments found in a complex IVF laboratory are not necessary as gametes and embryos are not stored in the laboratory. The INVO procedure can be performed in a medical office setting. INVO is a proven technology that showed clinical pregnancy rates equivalent to conventional IVF when using a prototype. This chapter presents the preliminary results obtained in combining mild ovarian stimulation with INVO using the new designed INVOcell device. The low cost of the INVO procedure and its large availability allow the treatment of a significant portion of infertile couples in the world who could not access any reproductive technology before. The more natural in vivo conception of the embryos with the participation of the patients has generated more interest in INVO from the infertile population and specialized physicians.

Keywords

INVO procedure • INVOcell device • Low cost • Vaginal incubation • Office setting procedure

The intravaginal culture (IVC) called the INVO procedure is a unique option for patients seeking treatment in infertility. The INVO procedure was created to simplify the process of in vitro fertilization (IVF) and early embryo development and reduce the sophisticated laboratory instrumentation required. Mild ovarian stimulation or natural cycle combined with the INVO procedure makes possible the treatment to infertile couples in a medical office setting such as the office of an infertile specialist [1], a satellite unit of a reproductive center, or in some areas an ob-gyn's office. The INVO procedure using the INVOcell device substitutes the CO_2 incubator used in conventional IVF with the vaginal cavity of the patient.

History of the Intravaginal Culture Also Called INVO

Since the birth of Louise Brown, the first baby born by IVF in 1978, several scientific milestones have been accomplished in assisted reproductive technologies (ART). Most of these scientific advances such as controlled ovarian hyperstimulation, embryo cryopreservation, and intracytoplasmic sperm injection (ICSI) have not only increased the embryo implantation rates but also created clinical, ethical, and legal issues and dramatically complicated the IVF procedure.

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Table 19.1 Initial results of INVO using the prototype device

Number of publications	Countries	Number of INVO cycles	Clinical pregnancy rate/cycle (%)
9	Austria, France, Germany, Japan, Netherland, UK, USA	815	19.6

These complicated technical advances have contributed to increased costs of IVF. The high cost of IVF has resulted in rejection of reimbursement by the insurance companies thus restricting IVF access to infertile couples who can afford to pay out of pocket. These technical advances have also contributed to the creation of governmental regulations for ART in many countries. All these factors have slowed the expansion of IVF technologies—a major factor that explains why, after more than 30 years of existence, so many infertile couples cannot and do not receive IVF treatment. The INVO procedure performed by a trained physician and embryologist in an office or satellite unit becomes accessible to many more insured and noninsured infertile couples.

Principle

The INVO procedure consists of utilizing the vaginal cavity environment for the oocyte fertilization and early embryo development [2]. The INVOcell device is specially designed for the INVO procedure [3]. The vaginal cavity provides the pCO_2, pO_2, and temperature for the culture of the gametes and the embryos [4]. The INVOcell device is permeable to gas and allows the equilibrium between the pCO_2 of the vagina and the pCO_2 of the culture medium. This system maintains the pH of the culture medium between 7.2 and 7.4 during the entire period of vaginal incubation. This in vivo fertilization and early embryo development involves the participation of the patient giving a more natural approach to the assisted conception.

Discovery of the Intravaginal Culture

The INVO technique was discovered around 1985. The laboratory IVF incubators at the time had CO_2 distribution controlled by a bead system. The rate of CO_2 mixed with the air was very imprecise and had peaks of low and high CO_2 concentrations that resulted in major variations in the pH of the culture medium. When cells or mouse gametes were placed in the same incubator in plastic tubes filled with culture medium and hermetically closed, the passage of CO_2 through the wall of the sealed plastic tubes altered the peaks of CO_2 and dramatically reduced the variations of pH in the culture medium. To simplify the process, the natural vaginal cavity providing CO_2 and O_2 was used. There was originally a concern of potential lesions of the uterine cervix from the prototype device during incubation that could interfere with the quality of the embryo transfer. This was eliminated by tests that demonstrated no lesions or interference. This was later confirmed by the results of the first INVO procedures showing comparable embryo implantations to conventional IVF.

Prototype and Initial Results with the Prototype

Prototype

During the initial cases and publications utilizing the INVO procedure, a prototype which was a simple plastic tube was used [2]. The tube chosen showed the best sealed closure among several prototypes that were initially tested. The prototype device was filled with culture medium first to avoid air bubbles. Air bubbles could be caught by the viscous cumulus of the mature oocytes causing them to float up and decreasing the chances of fertilization. These air bubbles could modify the pH of the culture medium. With the prototype tube, oocytes could also be lost in the overflow during the closing of the prototype.

Initial Results with the Prototype

Several publications in international medical journals [1, 2, 4–10] were issued at this time reporting the results obtained by INVO using this prototype device (Table 19.1).

Some of these publications clearly indicated equivalent pregnancy rates between INVO using the prototype device and conventional IVF [2, 5, 9].

From the Prototype to the Improved INVOcell Device

The first practitioners using the INVO prototype device had many difficulties. The device could only be opened and closed once. There were observations of variations in pH of the culture medium, bacterial contamination, accidental openings of the prototype in the vaginal cavity, loss of embryos, and increased risk of vaginal bacteriosis. A new device, the INVOcell made of three parts, was designed to address all these identified technical problems [3].

Fig. 19.1 Inner chamber and open outer rigid shell of the INVOcell device

Fig. 19.2 Fully assembled INVOcell placed in the retention system and ready to be positioned in the vagina

The Inner Chamber

The inner chamber houses the culture medium and the gametes. A rotating valve allows several openings and closings of the inner chamber without introduction of air or contamination of the culture medium. The rotating valve has a small orifice at a bottom of a small well which prevents major variations in pH of the culture medium and loss of gametes due to possible overflow while filling. The volume of the chamber has been reduced to 1.08 mL from the initial 3 mL. At the bottom of the main chamber, a microchamber collects the embryos after incubation. This microchamber allows direct observation and selection of the embryos without transfer to a culture dish. Loading of the embryos can be done directly from the microchamber with the embryo transfer catheter.

The Outer Rigid Shell

The outer rigid shell protects the inner chamber from vaginal contaminations and keeps the inner chamber sterile (Fig. 19.1). It has a smooth external surface to prevent any lesions or irritations of the vagina and cervix during the 3 days of vaginal incubation. The wall is permeable to CO_2 and O_2. The rigid shell could be grasped if necessary with a forceps to remove it from the vagina. A locking position prevents any unexpected device opening during vaginal incubation.

The Retention System

The retention system has also been improved. Holes have been perforated in the membrane of the diaphragm for the elimination of the vaginal secretions during incubation (Fig. 19.2). Tests of comfort and retention, requested by regulatory agencies for approval of the device, were performed using the INVOcell and its retention system. The INVOcell device does not cause any discomfort or irritation of the vaginal cavity and does not increase incidence of bacterial vaginosis from the 3 days of incubation. No device expulsion was observed when the retention system was used. These results were confirmed by the first INVO procedures using the INVOcell.

The INVO Cycle

INVO Cycle

The indications for using an INVO cycle are similar to the indications using conventional IVF. INVO is not recommended in severe oligoasthenoteratozoospermia. All other indications can be treated by INVO.

Natural Cycle or Mild Ovarian Stimulation Protocols

Current stimulation protocols use the association of gonadotropin-releasing hormone (GnRH) agonist and high doses of human menopausal gonadotropin (hGM) or follicle-stimulating hormone (FSH). These protocols recruit a lot of follicles and show complications such as severe ovarian hyperstimulation syndromes (OHSS) as well as multiple pregnancies with premature deliveries, birth defects, and maternal complications. The use of these protocols and their complications represent a very costly burden for society. The governments of several countries and specialized associations such as the American Society for Reproductive Medicine (ASRM) have developed regulations and guidance concerning the numbers of embryos to transfer. This has contributed to a returned interest in natural cycle and mild stimulation protocols that produce less embryos and are safer

for the female. The introduction of GnRH antagonists [11, 12] or indomethacin [13, 14] in preventing premature LH surges and ovulations has also contributed to the reintroduction of the natural cycle and mild ovarian stimulation protocols. Mild stimulations and natural cycle protocols with the INVO procedure contribute equally to the simplicity, low complication rates, and low cost of the INVO cycle.

Modified Natural Cycle

The monitoring of natural cycle is simple and inexpensive. Generally, an average of four rapid immunoenzymatic blood assays, when available, and two or three ultrasound exams starting at day 8 precede the retrieval. GnRH antagonist (0.25 mg daily) or indomethacin (50 mg 3× per day, as used in Dr. Lucina's study and discussed later) is started at day 8 or when the leading follicle reached 15 mm and is used to prevent premature ovulation. Triggering of ovulation is performed by the injection of 5,000 IU of human chorionic gonadotropin (hCG) when the size of the follicle reaches 18 mm and the estradiol 180 pg (pictogram)/mL, when dosage is available.

Clomiphene Citrate Protocol

The monitoring of the stimulation and the control of the premature ovulation are identical to the modified natural cycle. Induction of the ovulation is based on the same follicular size (18 mm) of the dominant follicle and is done using 10,000 IU of hCG. Clomiphene citrate (CC) is generally used at a dose of 100 mg/day from day 3 to day 7. In developing countries, an aromatase inhibitor, letrozole (2.5–7.5 mg/day), is used from day 3 to day 7 and is preferred to CC due to its lower antiestrogenic action and better embryo implantation, and it requires less exogenous gonadotropins [15] (letrozole is not allowed in the USA and Europe). Gonadotropin, hMG, or FSH, 75 units, may be added every day starting on day 3 or day 5 of the cycle depending of the number of follicles to recruit. Generally, two to seven oocytes are retrieved.

Luteal Phase Support

Luteal phase is usually supported by progesterone (200–600 mg a day) started after the follicle retrieval and continued until the 10th week of pregnancy when the placenta takes over the progesterone secretion. Estradiol support (4 mg a day) is also used in association with progesterone.

Follicle Retrieval

Transvaginal follicle aspiration using ultrasound vaginal probe is performed 36 h after hCG injection to get the best oocyte maturity from the dominant follicle(s). Mild ovarian stimulation protocols recruit few follicles, allowing a short retrieval time. The use of conscious sedation makes the retrieval procedure well tolerated by the patient without the need for general anesthesia [1, 2]. A pump with control of vacuum pressure (120 mm hg) is recommended for the follicle aspiration; if not available, a follicular aspiration using 10-mL syringes can be done [2].

INVO Procedure

In INVO, the vagina provides the proper incubation temperature and the correct CO_2 supplementation required for embryo development. The INVOcell device has been designed to maximize the transfer of CO_2 present in the vaginal cavity to the culture medium, maintain the pH of the culture medium during the period of incubation, and reduce the quantity of O_2 transfer to the medium. The INVOcell eliminates the need for a complex laboratory and simplifies all the steps of the assisted fertilization and early embryo development.

Sperm Preparation

In the INVO procedure, the sperm preparation takes place before oocyte retrieval, so the oocytes can be inseminated immediately after the retrieval procedure without major exposure to a detrimental environment.

Sperm collection: Collection is generally performed by masturbation; if the collection is done by intercourse, a non-toxic condom should be provided.

Sperm washing and selection: Currently, the "swim-up" and the density gradient separation are the techniques used for sperm preparation. These two different techniques have the same principles wash the sperm to eliminate the seminal fluid and components which may interfere on fertilization and select the most motile spermatozoa. Density gradients have demonstrated a better selection of motile spermatozoa in oligoasthenozoospermia.

Insemination Using the INVOcell

Insemination using the INVOcell is performed immediately after oocyte retrieval. This point is very important, especially when the facility performing the INVO procedure does not have a CO_2 incubator or any CO_2 supplementation. It minimizes the exposure of the oocytes to the ambient atmosphere that is low in CO_2 and rich in oxygen (O_2). To maintain the proper pH of the culture medium during the short exposures to the ambient atmosphere, it is recommended to use HEPES media.

INVOcell preparation: The INVOcell parts, the inner chamber, the rigid outer shell, and the retention system are prewarmed before use. The inner chamber is rinsed with culture medium and then filled with 1.08 mL of fresh culture medium. The inner chamber is closed and replaced in

the incubator until the placement of the gametes. Several culture media have been used successfully with the INVOcell; they have to be bicarbonate-buffered. The media have to support 3 days of culture. Media with the addition of small amounts of phenol red are recommended especially when no CO_2 incubator is available as the phenol red is a very sensitive pH indicator.

Placement of the gametes: The fraction of motile spermatozoa is introduced first into the inner chamber. A total number of *30,000 motile spermatozoa* are used for insemination regardless of the number of eggs placed in the device. In case of oligoasthenoteratozoospermia, the sperm number may be increased to 50,000 motile spermatozoa. The oocytes, immediately after retrieval, are placed in warmed culture medium with HEPES. When all the oocytes have been collected, they are rinsed in one drop of buffered bicarbonate culture medium to eliminate the HEPES and then are transferred in the inner chamber. The rotating valve is then closed. The inner chamber is placed into the bottom of the outer rigid shell. The outer rigid shell top is then closed in a locked position. The device is now ready for placement in the vagina (Fig. 19.2). If for any reason the patient is not ready for the placement of the device, put the device back into the incubator until it can be placed in the vaginal cavity. It is essential to transfer the device into the vaginal cavity as soon as possible after insemination; the vaginal cavity provides the correct gas environment.

In Vivo Embryo Culture

The fully assembled INVOcell device should be inserted into the vagina manually by the physician. The use of a speculum makes the process more difficult for the physician and very uncomfortable for the patient.

Vaginal incubation: The device is designed to be held in the fornix or in front of the cervix during the 2 or 3 days of incubation and maintained in place using the retention system. It is recommended that the couple have no intercourse during the period of incubation. Female patients can shower, but no bath, swimming, or vaginal douche is allowed due to the potential changes in vaginal temperature that could affect incubation. Normal daily activities can be performed during the 3 days of incubation. Instructions including recommendations are provided to the patient.

Embryo(s) Transfer

The embryo transfer is generally performed 3 days after the insemination at the reproductive unit.

Device removal: The device and retention system are removed manually by grasping the ring of the retention system and pulling them out. The device is rinsed with prewarmed saline solution to clean off the vaginal secretions.

Fig. 19.3 Fully assembled INVOcell in vertical position and inner chamber positioned in the holding block for observation

Embryo Settling

The laboratory has a CO_2 incubator: The outer rigid shell is opened and discarded. The inner chamber is placed in a vertical position in the holding block in the CO_2 incubator for 15 min. During this time, the embryo(s) settle at the bottom into the microchamber.

The laboratory has no CO_2 incubator: If no CO_2 gas is available, keep the inner chamber in the outer rigid shell. The layer of gas captured between the inner chamber and the outer rigid shell will help to maintain the pH and temperature of the medium during embryo sedimentation. Place the cleaned device in a plastic sterile container in a vertical position in the incubator for 15 min. Just before the embryo observation, discard the rigid shell and place the inner chamber in the holding block (Fig. 19.3).

Embryo(s) observation and selection: The holding block containing the inner chamber is removed from the incubator and put on the microscope stage. The holding block has been designed not only to maintain the correct temperature and pH of the culture medium in the inner chamber but also to allow microscopic observation of the gametes and embryos directly through the wall of the inner chamber. In the holding block, the inner chamber is immersed in mineral oil which will eliminate irregularities of the device allowing clear viewing of the embryos directly from the microchamber. With the block in the vertical position, the oil is located internally in a reservoir (6.5 mL). During the microscopic observation, the block is flipped in a horizontal position. The microchamber is centered in the observation window and covered by the mineral oil coming from the reservoir of the block. When the embryo(s) have been located, the magnification is increased to grade and evaluate the stage of their development (two to eight cells). It is recommended that no more than two quality embryos be

Table 19.2 Results of the prelaunch clinical trial using the INVOcell

Groups	Cleavage rate (%)	Clinical pregnancy	
		Rate per cycle	Rate per transfer
Group 1 ≤10 Oocytes retrieved	52.20	31.80% (7/22)	38.90% (7/18)
Group 2 >10 Oocytes retrieved	49.30	11.70% (7/60)	13.50% (7/52)
Total	49.90	17.10% (14/82)	17.10% (14/82)

transferred to minimize the risk of multiple pregnancies. However, in some special circumstances, this number may be increased to three after discussing with the couple and after obtaining their agreement.

Embryo Transfer Catheter Loading

Embryo(s) can be loaded directly from the inner chamber: The embryo transfer catheter filled with culture medium is placed through the orifice of the open valve of the inner chamber positioned in the holding block. The embryo(s) can be visually selected and withdrawn from the microchamber into the transfer catheter by moving the syringe plunger attached to the transfer catheter up and down.

Embryo(s) loaded from a culture dish: A volume of 100 μL is aspirated from the microchamber under microscopic observation using a long pipette tip. This volume is transferred into a culture dish containing HEPES culture medium for observation and selection of the embryos for transfer. The selected embryos are then rinsed in fresh medium and loaded as classically into the embryo transfer catheter.

Embryo transfer into the uterus: The embryo transfer is performed using ultrasound guidance and an abdominal transducer to visualize the correct position of the catheter in the uterus. Any bleeding should be carefully avoided during the embryo transfer as it negatively impacts the prognosis of the procedure.

Preliminary Results

Prelaunch Trial

During the development of the new INVOcell device and its clearance by regulatory agencies, a lot of tests have been performed including a clinical trial. Results of this trial are shown in Table 19.2. A high number of oocytes, over ten per retrieval, were obtained in almost three-fourths of the cases (group 2) as all the stimulation protocols used GnRH agonist with high doses of gonadotropins. By agency request, no more than ten eggs could be placed in the INVOcell. Therefore, the embryologist had to select ten oocytes with the best maturity among the 20–30 retrieved oocytes. This factor certainly explains the low pregnancy rate obtained in group 2. In group 1, patients received the same regimen of drugs but developed only ten or less than ten oocytes. In this group of low responders with the highest pregnancy rate, all the oocytes were placed in INVOcell without preselection by the embryologist.

Only the two best embryos were transferred, resulting in 14 clinical pregnancies with 12 births of normal babies and only one set of twin.

Table 19.3 Results of the first postlaunch INVO procedures using INVOcell

Countries	Number of cycles	Clinical pregnancies (rate)
Austria, Bolivia, Brazil, Ecuador, India, Mexico, Nicarragua, Pakistan, Panama, Peru, Spain, Turkey, Venezuela	457	128 (28%)

Table 19.4 Results of the clinical trial performed at CECOLFES

Number of cycles	125
Number of embryos per transfer	2.4
Number of single pregnancies	40
Number of multiple pregnancies	10
Total number of pregnancies	50
Clinical pregnancy rate/cycle	40%
Clinical pregnancy rate/transfer	43.9%[a]

[a] Eleven embryo transfers were not performed, nine due to poor or no fertilization. In the two last cases, the husbands were not available at the embryo transfer, and embryos were cryopreserved by vitrification

Postlaunch INVO Cycles

Since the launch of the product at the end of 2008, several hundred procedures have been done with the INVO procedure using the INVOcell. Only results of one fraction of these procedures are reported in Table 19.3, the ones for which data have been obtained and confirmed. These procedures were performed in different countries in reproductive centers as well as in newly created INVO units.

Clinical Trial at CECOLFES (Colombia)

In addition to these INVO procedures, a clinical trial has been performed by one of the first users of INVO, the Dr. Elkin Lucena at CECOLFES in Colombia. Dr. Lucena gave us the permission to report the results of the first 125 INVO procedures that he will published in a peer review journal (Table 19.4) [16]. These procedures were done in

Table 19.5 Preliminary results using the INVOcell for oocyte maturation and embryo development after ICSI

Procedure	Countries	Number of cycles	Number of clinical pregnancies (rate)
ICSI	Austria, Brazil, Colombia, Peru, Turkey	190	66
IVM, ICSI	Colombia, Venezuela	7	2
Total		197	68 (34.5%)

female population including 40 years old patients and older with an average age of 33.8. These 120 infertile couples should have been treated with a conventional IVF. Severe male factors needing ICSI were excluded. Mild ovarian stimulation was only used with indomethacin started at day 8 to block the ovulation. An average number of 6.5 oocytes were obtained per punction of which an average of 4.2 was inseminated in INVO. The excess oocytes (2.3 per punction) if matures were cryopreserved for future use.

Potential Disadvantages/Advantages

Potential Disadvantages

Products of degradation from dead cells and metabolism of the live cells are known to be detrimental to fertilization and embryo development. In the INVO procedure, the concentration of motile sperm has been deliberately reduced to 30,000–35,000 motile sperm per milliliter. This concentration represents less than a third of the sperm concentration generally used in conventional IVF. It has been demonstrated that this sperm concentration gives the best fertilization rates, with the lowest production of degradation products and rate of polyspermic embryos (unpublished study). The absence of embryo checking at 16–20 h postinsemination could fail to identify polyspermic embryos. Polyspermic embryos were frequently observed at the beginning of IVF due to an immaturity of the retrieved oocytes and a very high motile sperm concentration used for insemination. The formation of pronuclei is a dynamic process; it seems irrational to try eliminating polyspermic embryos by a few seconds of observation. Eliminate the main causes for polyspermic embryo formation by a better oocyte maturity and by decreasing the sperm concentration has seemed to us a more logical approach.

Advantages

1. INVO dramatically reduces and simplifies gametes and embryos handling and manipulations.
2. An embryologist with little experiences in reproductive technologies can be trained quickly and obtain excellent results.
3. The fixed laboratory equipment is low cost and not complex allowing creation of INVO units requiring little equipment maintenance and quality controls.
4. The frequent electric breakdowns observed in developing countries that affect the results of IVF/ICSI, even when battery backup or generators are available, did not impact the result of INVO.
5. The gametes and embryos are not stored in the laboratory during the 3 days of incubation. Therefore, gas supplementation, battery backup, positive pressure and air filtration, alarm system, and embryologist on call are not necessary. All these factors have contributed to the decreased cost of INVO.
6. The risk of mixing up gametes or embryos has been reduced, and INVO gives the patient a new sense of participation with in vivo conception.

Other Applications of the INVOcell

Gamete and embryo transportation in INVOcell have been reported [9] and are still performed by users of INVO. Reproductive centers have incubators overloaded with eggs and embryos requiring frequent openings of the doors and creating major variations in CO_2 and temperature. The INVOcell has been used successfully to vaginally incubate embryos inseminated through ICSI. The INVOcell has also been used to mature immature oocytes intravaginally and later to incubate the embryos inseminated by ICSI as shown in Table 19.5.

What Did We Learn from INVO?

Oocyte In Vitro Maturation After Retrieval Is Not Required

From the beginning, the oocytes that have being retrieved after hCG induction were immediately inseminated. This was possible by using 36 h between the hCG injection and the oocyte retrieval.

Low Sperm Concentrations Are Used Successfully for Insemination

INVO has always been used with very low sperm concentrations for oocyte insemination. A concentration of 30,000 motile spermatozoa/mL is generally used and gives comparable fertilization rate and pregnancy rates to the conventional IVF. Concentrations as low as 5,000 motile spermatozoa/mL were also used but abandoned due to inconsistency in fertilization (nonpublished).

Embryo Development in Low Oxygen Concentration (≈ 5%)

The INVOcell device is permeable to gas and equilibrates the pCO_2 and pO_2 of the culture medium of the inner chamber with the pCO_2 and pO_2 of the vagina. In this system, the vagina works as a CO_2 generator and an O_2 reducer. The INVO process was the first fertilization system using low oxygen concentration in air.

Cumulus Cells Perform as a Homologous Coculture System

The INVO procedure was the first coculture system using cumulus cells. This coculture system is simple, non-labor intensive, and safest for the patient [17, 18]. These cumulus cells filter and absorb the toxins and nitrogenous residues produced by the dead cells and the metabolism of the live.

Embryo Cumulus Cells Are Removed Naturally During Incubation

Spontaneous removal of embryo cumulus and corona cells has always been observed after 2 or 3 days of incubation in the INVO device. This natural and complete denudation, a sign of quality culture conditions, is the result of the enzymatic action of the spermatozoa as well as a mechanical action related to the patient mobility during the period of incubation. In the INVO procedure, culture media change is not performed so the presence of infection or toxins cannot be tolerated. The oocytes are trapped in a cumulus mass dense and viscous when any toxicity is present during the 2 or 3 days of incubation.

Positioning of INVO Among the Current Reproductive Techniques

The INVO procedure is a new infertility treatment positioned between intrauterine insemination (IUI)—simple, inexpensive, but not very successful (less than 10% of birth per cycle) [19]—and conventional IVF—complex, very expensive, but very successful (28–32% birth per cycle have been reported by several international registries for patient under 40 years old). INVO when associated with mild stimulation is a simple and successful procedure. INVO presents the advantage to treat more indications of infertility than IUI (85 vs. 50%) and to offer a diagnostic value (fertilization or not) that IUI does not.

Potential Role of INVO in Developed Countries

Governmental regulations are in place in many of the developed countries restricting the use of infertility treatments to reproductive centers. These centers have already invested in complex equipment reducing the cost saving of the INVO cycle. However, the simplicity of INVO allows performing three to four INVO cycles for one cycle of IVF or ICSI. Reproductive centers can treat a lot more patient without overloading incubators or investing in new equipment and increasing the number of embryologists. INVO may also be used in satellite units to extend the geographic influence of the reproductive center.

Role of INVO in Developing Countries

It seems irrational to treat infertility in developing countries that are frequently overpopulated, have difficulties in feeding their existing population, and have other medical priorities than to treat their infertile population. However, infertility has major socioeconomical consequences in developing countries [20, 21]. Couples who do not have children are rejected socially and do not provide offspring that economically support the elderly population. It is estimated that by 2050 most of the developing countries will not be able to renew their population [21]. The main cause of infertility is blocked tubes due to infectious diseases. Unfortunately, IVF or ICSI, the most effective treatments, is not available due to the cost to build a reproductive center and the cost of a treatment cycle. The low cost of equipment used in an INVO unit, the low maintenance of this equipment, and the rapid training of an embryologist with little experience are factors which will contribute to the expansion of the role of INVO in the treatment in infertility in developing countries.

Future Implications and Developments of the INVO Technology

Only a low percentage of infertile couples in the world can benefit from the treatment of assisted reproduction techniques due to their cost and availability.

If the cost per pregnancy using INVO confirms to be lower than the cost per pregnancy using IUI, INVO should replace IUI as the first treatment option for infertility. The diagnostic value of INVO and its ability to treat a larger percentage of the infertile population than IUI are other contributing factors for using INVO as the first-line treatment of an infertile couple.

In developing countries or regions of the world where IVF/ICSI is not available or is not affordable for a large majority of infertile couples, INVO combined with natural cycle or mild stimulation and performed in INVO satellite units represents an attractive infertility treatment option. These INVO units can be built very rapidly and do not need a large financial investment in fixed equipment. The maintenance does not require a lot of expenses and need little quality controls. The units can work even when gas supplies and

electric power are erratic. The trainings of the clinician and the embryologist can be completed rapidly. The INVO procedure is so simple that the results are highly reproducible even with an embryologist with little experience. INVO involves the participation of the patient in the process of fertilization and early embryo development. INVO is an in vivo conception with a high level of acceptance among the patients who benefited from the procedure especially patients with religious convictions and ethical concerns for conventional IVF.

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