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INVO: a simple, low cost effective assisted reproductive technology

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INVO procedure is a simple and effective infertility treatment that uses a new device, the INVOcell. INVO can be performed in a physician's office or in a satellite facility of an IVF center. The INVO procedure consists of fertilization of oocyte(s) and early embryo development in the INVOcell placed into the maternal vaginal cavity for incubation. The vaginal cavity replaces the complex *in vitro* fertilization (IVF) laboratory. This study presents the specially designed device, INVOcell that has received CE Certification. INVOcell overcomes the disadvantages of the previously used prototype and makes the procedure simpler and reproducible. INVO is a proven procedure that has demonstrated comparable results to conventional IVF when comparative studies were performed. Over 800 cycles have been published worldwide that showed a clinical pregnancy rate of 19.6%. The INVO technology can be performed in an office setting with minor capital equipment. INVO is a simple low-cost procedure that can be available almost everywhere. INVO allows the treatment of a new population of infertile couples who could not benefit from IVF due to cost and availability. The participation of the patient in the process of fertilization and early embryo development is a psychological benefit that creates a high level of acceptance of INVO.

Keywords: INVOcell device; intravaginal culture; *in vitro* fertilization; office setting procedure; low cost

Introduction

In vitro fertilization (IVF) is now an established treatment for infertility. IVF and variations such as intra cytoplasmic sperm injection (ICSI) requires complex laboratory equipment and very experienced laboratory personnel. Consequently, these treatments are expensive and are only available for a small fraction of the infertile population. The INVO technique, IVC (Ranoux *et al*., 1988), is a simple alternative to IVF, where the vaginal cavity of the patient substitutes the complex IVF laboratory. This study will describe a new device, the INVOcell, specially designed for the vaginal incubation. The INVOcell prevents the technical problems that were described during the use of a prototype. INVO could transform the treatment of infertility.

Materials and Methods

The principle of the INVO technique consists of oocyte fertilization and early embryo development in an air-free plastic device permeable to gas. The device placed into the maternal vaginal cavity utilizes the pCO_2 of the vagina to equilibrate the pCO_2 of the culture medium and maintain the pH of the medium during the period of incubation (Fukuda *et al*., 1996). INVO has been used with mild ovarian stimulation or no stimulation and allows the physician to perform an IVF-like procedure in an office setting or in a satellite facility of an IVF center.

Mild ovarian stimulation or no stimulation

Several protocols have been used successfully:

(i) FSH/hMG with or without GnRH antagonist, with ovulation induction triggered by hCG: Patients are injected with 1–2 ampoules of follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG) every day starting at Day 3 of the cycle. Gonadotropin-releasing hormone (GnRH) antagonist is given late in the follicular phase (Days 8–10). Five to ten thousand international units (IU) of human chorionic gonadotropin (hCG) are used to trigger ovulation. This protocol allows recruiting an average of 10 follicles.

(ii) CC alone, CC and FSH/hMG with ovulation induction triggered by hCG: Clomiphene citrate (CC, 50 or 100 mg) is given from Day 3 to Day 7, FSH, 75 IU is injected every day or 150 IU every two days from Day 6. This protocol allows recruiting an average of 5–6 follicles.

(iii) Natural cycle, natural cycle with GnRH antagonist with ovulation induction triggered by hCG: Cycles without ovarian induction have been performed, but premature ovulation and onsets of luteinizing hormone (LH) surge (Taymor *et al*., 1992) have complicated the protocol and lowered the pregnancy rate (10% per cycle, see Table I). The use of antagonist

Table I. Summary of the results obtained in publications concerning INVO.

Publication	Location	No. Cycles	Clinical pregnancy rate per cycle
Ranoux *et al*. (1988)	France	100	20%
Sterzik *et al*. (1989)	Germany	22	22.7%
Ranoux and Seibel (1990)	France	100	22%
Wiegerinck *et al*. (1990)	The Netherlands	43	14%
Freude *et al*. (1990)	Germany	15	20%
Taymor *et al*. (1992)	USA	51	10%
			Natural cycle
Sharma and Hewitt (1993)	UK	334	20.7%
Fukuda *et al*. (1996)	Japan	58	20.7%
Batres *et al*. (1997)	USA	92	19.6%
Total		815	19.6%

at Day 8 to Day 10 prevents the LH surge and dramatically increases the pregnancy rates (Rongières-Bertrand *et al*., 1999; Mendez-Lozano *et al*., 2008). Generally 1 to 2 follicles are recruited. These mild stimulation protocols allow to retrieve few follicles and to minimize the discomfort of the patient during the follicle retrieval.

Follicle retrieval

Thirty-four to thirty-six hours after the hCG injection the follicle aspiration is performed transvaginally using ultrasound vaginal probe guidance. A light sedation, with or without local vaginal anesthesia, replaces the general anesthesia and allows the retrieval to be performed in an office setting.

INVO procedure

The INVO procedure is a major simplification for fertilization and embryo development. The principle is simple. The vagina of the future mother replaces the complex laboratory as the site of incubation.

(i) The procedure: The sperm preparation is generally performed 1 h prior to the oocyte retrieval to allow the biologist to perform the insemination immediately after oocyte retrieval. Gradients of density are used to wash the sperm and select the most motile spermatozoa. The device should be filled with medium without interposition of air. Air bubbles could be trapped in the cumulus of the mature oocytes and bring them to the surface and therefore prevent fertilization by spermatozoa. A small fraction of the motile spermatozoa (30 000) is used to inseminate the oocytes in the device. After follicle aspiration, oocyte(s) are identified in the follicular fluid. As no pre-incubation of the oocytes is necessary, oocytes are immediately placed into the device, the fraction of motile sperm is added before or after transfer of the oocytes. The device is closed, placed into a protective outer rigid shell and then positioned into the vaginal cavity for 2 or 3 days. A retention system is used to secure the device in the vagina during incubation. No activity restriction is required for the patient, but baths are not recommended as they may modify the temperature of incubation. After incubation the retention system and the device are removed from the vagina in the physician's office. The outer rigid shell is removed, the device is opened and the contents observed under microscope to find the embryos. The two best ones are loaded into a catheter and transferred immediately into the uterine cavity. Ultrasound guidance may be used in order to improve the quality of the transfer.

(ii) The Device:
  (a) Prototype. A prototype was used during the first development and in the publications concerning the INVO procedure. The prototype was chosen because it showed the best sealed closure among the prototypes that were initially tested. To avoid air bubbles the corpus and the plug of the prototype tube were filled with culture medium, then the gametes were introduced and the tube was closed. Several disadvantages were described such as:
    (1) only one sterile opening and closing were possible.
    (2) During the closing of the prototype oocytes could be lost in the overflow of medium and the culture medium could be easily contaminated by errors in manipulation.
    (3) A large opening of the tube could rapidly modify the pH of the medium.
    (4) A plastic envelope was sealed thermally around the prototype to protect the contents of the tube from the vaginal secretions. During the sealing of the envelope with heat, the increase of temperature could affect the gametes.
    (5) Openings of prototypes have been reported during its placement or removal from the vaginal cavity.
    (6) To prevent expulsion of the device, a diaphragm was used to maintain the device in the vagina during the period of incubation. A risk of bacterial vaginosis was increased as vaginal secretions were retained behind the membrane of the diaphragm.
    (7) One major advantage of the INVO technique is that corona cells surrounding the zona pellucida and the cumulus cells are spontaneously removed after 2 or 3 days of vaginal incubation. The disadvantage of these spontaneously denuded embryos was the difficulty to find them rapidly under microscope in the large volume of medium even for a trained embryologist (chamber volume of the prototype equals 3 ml).
  (b) INVOcell. The new device has been specially designed for the INVO procedure which addresses the disadvantages described above (Figs 1–3).

(iii) Disposable equipment: other disposable equipment than the INVOcell device are necessary to perform the INVO procedure such as:
  (a) Needle for oocyte retrieval
  (b) Cover for the vaginal probe
  (c) Petri dishes, tubes, pipettes, container to collect the sperm, slides and gloves used to prepare the gametes
  (d) Embryo transfer catheter
  (e) Culture media: saline solution to wash the vaginal cavity, medium to flush the follicle if used, gradient density for sperm preparation, culture medium to wash the sperm and used in the INVOcell.

(iv) Capital equipment:
  (a) Rapid hormonal assay instrument. Hormonal assays are important to evaluate the follicle growth and the timing of ovulation. Instrumentation for rapid hormonal assays is generally available in clinical laboratories. Small instruments with individual disposable test units are also available. Leasing of the instrument is generally included in the cost of the disposable test units.
  (b) Ultrasound machine with vaginal probe and vacuum pump. Ultrasound is also valuable in the monitoring of ovulation and is necessary for the follicle retrieval. The ultrasound machine with vaginal probe is generally available in the

Figure 1: INVOcell fully assembled device, before positioning in the vagina.
This device is made of two parts, the inner chamber that contains the culture medium [Complete P1 SSS (Synthetic Serum Substitute) from Irvine Scientific, Irvine, CA, USA, was used successfully in a clinical trial] and the gametes and the outer rigid shell that keeps the orifice of the valve sterile and protects the inner chamber from the vaginal contaminations.

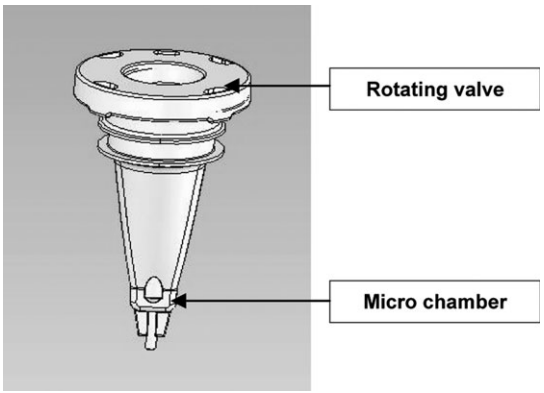

Figure 2: Inner chamber.
A rotating valve allows several openings and closings of the inner chamber without interposition of air or contamination of the culture medium. A small orifice prevents major variations in pH of the culture medium and looses the gametes due to overflow. The volume of the chamber has been reduced (1.1 ml). A micro-chamber collects the embryos after incubation, by placing the inner chamber in a vertical position during 15 min. Embryos can be observed in the micro-chamber under microscope without transferring them to a culture dish and therefore can be loaded in a catheter for their immediate transfer to the uterus of the patient.

office of a physician who treats infertility. The investment to get the ultrasound machine with the vaginal probe, its needle guides and the vacuum pump to aspirate the follicular fluid may vary from $7000 to $30 000, depending of the age of the instrument and where it is purchased.

(c) Laminar flow including warm bench, small incubator and stereomicroscope with video system and bench centrifuge. This capital equipment is desired to perform the biologic steps of the INVO procedure. Identification of oocytes in the follicular fluid, sperm preparation and embryo observation can be performed successfully without clean environment. However, we recommend the use of a laminar flow hood for the INVO procedure to decrease the risk of contamination and major variations in temperature of the culture media and gametes. The investment varies from $7000 to $30 000.

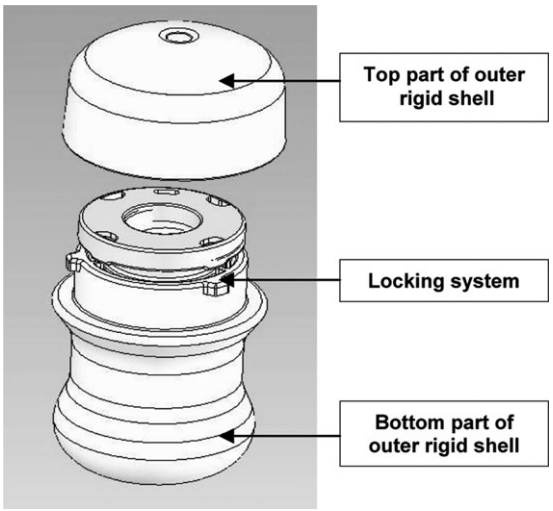

Figure 3: Outer rigid shell.
The external surface is smooth to prevent any lesion or irritation of the vaginal or the cervical epithelium during the 3 days of vaginal incubation. The wall of the INVOcell is permeable to CO_2. The rigid wall protects the inner chamber but allows the physician to grasp the device with a forceps to place it or remove it from the vagina. A locking system prevents any unexpected opening of the outer rigid shell. The retention system has been improved. Holes have been perforated in the membrane of the diaphragm for elimination of the vaginal secretions during the incubation.

Results

The INVOcell device has been ISO 10993 tested (and mouse embryos tested) to assess toxicity and biocompatibility. Clinical tests have been performed to evaluate the comfort and retention of INVOcell within the vagina. Preliminary analysis of a clinical trial performed in an infertile population has shown the INVOcell to be a well accepted, non-traumatic, effective device with births of normal babies. Complete results from the clinical trial will be published in the future. This clinical trial also demonstrated that Complete P1 SSS could be used for 3 days of incubation without medium change. Over 800 cases of IVC performed by infertility groups around the world (France, Germany, Netherlands, England, USA, Japan) have been documented in peer-reviewed journals, demonstrating success rates (average clinical pregnancy rate of 19.6% per cycle) which were comparable to success rates of conventional IVF reported by the user of INVO at this time (Table I).

The clinical pregnancy rate per cycle that went to oocyte retrieval was reported in most of the publications on IVC or could be easily calculated. We used this clinical pregnancy rate to allow pooling the results of all these different publications and calculate the average clinical pregnancy rate per

cycle (19.6%) presented in Table I. When comparative studies were performed, pregnancy rates obtained by IVC were comparable to those observed in IVF (Sterzik *et al*., 1989; Ranoux and Seibel, 1990). Another publication on IVC by Costoya *et al*. (1991) is not reported in Table I. This publication concerned 23 cycles of which 18 proceeded to retrieval but could not be used, as only a fraction of the retrieved oocytes (78 on 102) were inseminated by IVC. The method for selecting these 78 oocytes was not defined. Costoya *et al*. (1991) achieved only an average 10.2% fertilization rate as other publications generally reported an average fertilization rates between 50.3 and 69.5% for IVC. This abnormally low fertilization rate suggests a toxic factor, methodological problem or a wrong utilization of the prototype as suggested by three fertilized oocytes blocked at the pronuclei stage and two diagnosed contaminations of the culture medium by *Candida albicans*. On the five cleaved embryos transferred one birth was achieved, demonstrating the efficacy of the INVO technique when performed correctly.

Discussion

The results from over 800 clinical cases of IVC and preliminary studies of the INVO procedure using the INVOcell device indicate that INVO provides a simple and effective alternative to conventional IVF.

Disadvantages and advantages of a cycle using an INVO procedure are discussed below.

Potential disadvantages of the INVO procedure

Two major criticisms have been made to the INVO technique:

(i) The culture medium is not changed during the 2 or 3 days of incubation. Therefore, the oocytes are in contact with dead spermatozoa and the products of degradation related to the metabolism of the cells.
(ii) The absence of the embryo check at 16–20 h post-insemination could omit polyspermic embryos that could develop normally and be transferred to the uterus.

The low sperm concentration used in IVC or INVO reduces the products of degradation in the medium as well as the rate of polyspermic embryos without any decrease of the fertilization rates. Improvements in stimulation protocols with a better maturity of the retrieved oocytes have also contributed to a decrease in the rate of polyspermic embryo. In addition, higher miscarriage rates were not observed by the users of the INVO procedure.

The use of mild stimulation protocols presents several advantages

(i) It eliminates the side effects related to the high estrogen levels of an ovarian hyperstimulation such as hot flushes, discomfort, abdominal pain or complications such as an ovarian syndrome of severe hyperstimulation that could lead to the hospitalization of the patient in intensive care or even to her death.
(ii) It reduces the monitoring of the stimulation.
(iii) It decreases the time and discomfort of the follicle retrieval due to the small number of follicles retrieved. It allows the physician to perform the retrieval under light sedation in the office.
(iv) It prevents using embryo cryopreservation. Generally two embryos with the best quality will be transferred.

If more embryos are obtained, embryos of lesser quality will be discarded. This policy will help to decrease the rate of multiple pregnancies that creates a huge financial burden for the society.

The use of INVO procedure adds other advantages

(i) The INVO procedure does not need major capital equipment. This capital equipment can be easily stored in the office of a physician (12 square feet or 1.14 m^2) and does not need extensive maintenance and quality controls.
(ii) In INVO, embryos are not stored in the office therefore sophisticated incubator with CO_2, air filtration system, alarm system and embryologist on call are not necessary.

In a busy IVF center, sperm preparation, oocytes retrieval, oocyte insemination, change of culture medium, embryo denudation, check for fertilization and embryo cleavage are spread over several hours and even days. For evident reasons of organization and efficiency, a technician will prepare sperm samples of different patients at the same time, perform retrieval, IVF/ICSI fertilization, medium change or check fertilization for several different couples in a row. Very rarely the same embryologist will be able to perform all these steps for the same patient. This complex organization requires a strict patient identification but increases the risk of errors in labeling or handling of the gametes and embryos.

The simplicity of the INVO procedure allows a single technician to carry out all the tasks of INVO in the same period of time (60–90 min) without any assistance. It allows the technician to complete the treatment of one couple before starting the treatment of another couple and dramatically decreases the risk for mislabeling and mishandling.

(i) In an IVF center, incubation of gametes and embryos of several different couples in the same incubator may create the opportunity for errors. In the INVO procedure, the incubation of the gametes and embryos in the maternal vagina and the transfer of the embryo(s) immediately after INVOcell removal eliminate any risk of errors.

The INVO procedure can be performed in an office setting, which is a familiar environment, at a lower cost. INVO can be available almost everywhere with less travel expenses for the infertile couple. One limitation for a more generalized application of the technique in Europe is the actual EU guidelines for the manipulation of gametes and embryos, which favor certified and legally controlled IVF centers. These guidelines do not allow the manipulation of gametes and embryos outside these protected environments. The creation of INVO low-cost satellite units in physician offices that are trained, affiliated and controlled by IVF centers is one of the most economical solution to answer the increasing demand of the infertile population and to reverse the falling birth rates of European countries (Rand Research Brief, 2005).

Down-sized capital equipment, mild stimulation protocols and simplifications brought by INVO which requires less

expertise and time consuming from the operators contribute to lower the overall cost of an INVO cycle. INVO procedures have been performed for 1/3 to 1/2 of the cost of a conventional IVF.

Conclusion

IVC is a well researched and simple alternative to conventional IVF. The INVO procedure and INVOcell device represent significant advancements of IVC. BioXcell has received ISO 13485 certification and a CE Certification for the INVOcell device. The INVOcell is specifically designed to address the challenges faced by clinicians using earlier prototype devices. For example, the INVOcell uses a precisely designed rotating valve to eliminate the risk of gametes loss and medium degradation. In addition, the design of the inner chamber, which contains gametes and medium, provides for easy identification and transfer of the embryos to the mother. Combined with the mild or no stimulation protocol, the INVO procedure is an attractive infertility treatment option for patients who cannot afford conventional IVF or who live too far away from a clinic that offers IVF. Further, in developing countries, where infertility rates are high and access to cost-effective infertility treatment is low, INVO provides an excellent treatment option.

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