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ARTICLE

A pilot study to evaluate a device for the intravaginal culture of embryos

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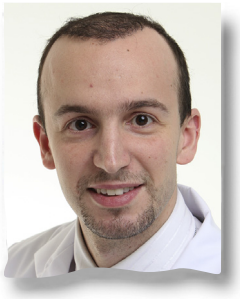

Dr Frederic Mitri completed his residency training in Obstetrics and Gynecology in Beirut, Lebanon. He is currently completing a clinical fellowship in Reproductive Endocrinology and Infertility at TCART, Toronto. His research interests include molecular and genetic biology, fertility preservation and poor responders.

Abstract The aim of this comparative randomized embryology trial was to determine if an intravaginal culture device (IVC) can provide acceptable embryo development compared with conventional IVF. Ten women between the ages of 27 and 37 years with an indication for IVF treatment were included in this study. After ovarian stimulation, oocytes were randomized to fertilization in the IVC device or using conventional IVF. Fertilization rates were higher in the IVF group compared with the IVC device (68.7% ± 36 % versus 40.7% ± 27%), respectively, whereas cleavage rates were similar (93% ± 1.5% versus 97% ± 6%) for both groups. A significantly lower number of embryos of suitable quality for transfer was obtained from the IVC device compared with conventional IVF (OR, 0.47; 95% CI, 0.26 to 0.87). The clinical pregnancy rate from transfer of IVC device embryos was 30%. Satisfaction questionnaires were also completed by all participants. Most women (70%) placed high importance on having had fertilization and embryo development occur while carrying the device. Overall, the IVC device produced reasonable pregnancy rates suggesting this technology may have a place under certain circumstances. Cost-benefit analysis, psychological factors and future studies must be considered.

KEYWORDS: culture system, embryo score, INVOcell, IVC device, IVF

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Introduction

During conventional IVF, oocytes and spermatozoa are incubated together in the laboratory, where fertilization and embryonic development are carefully monitored at several time points. Over the years, improvements in IVF methodology and culture media have been established with the aim of developing the most efficient incubator and providing optimum culture conditions for fertilization and embryo development (Gardner, 2008; Summers and Biggers, 2003). Twenty-four hour availability of laboratory personnel and equipment, sophisticated sequential culture media and highly trained embryologists are, however, unavailable in some areas. Furthermore, IVF is quite costly, and this may pose a barrier for some couples who are trying to conceive (Collins, 2001). On the basis of cost analysis, the embryology and laboratory services are the most expensive. The average cost of one standard IVF cycle in Canada can exceed $12,000 depending on the woman's age and quantity of fertility medication used; this figure may be higher in other areas. The cost per live birth can be several fold greater and may depend on other variables, including per cycle pregnancy rate and the number of embryos transferred (Bhatt and Baibergenova, 2008).

Intravaginal culture (IVC) technology was initially proposed and developed in 1988. Many improvements and modifications have been made to an earlier prototype device, which evolved into INVOcell™, a Health Canada approved IVC system, which has been ISO 10993 tested and received the European conformity (Frydman and Ranoux, 2008; Ranoux et al., 1988). With this technology, retrieved oocytes and processed spermatozoa are placed directly into the specially prepared gas permeable culture device. The device containing oocytes and spermatozoa is placed in the vagina, where fertilization and subsequent embryo development takes place. This procedure bypasses the requirement for sophisticated laboratory equipment or highly trained embryologists and reduces the cost per treatment cycle. In general, low ovarian stimulation protocols have been used in conjunction with this device, and have provided encouraging results globally (Bonaventura et al., 2006; Lucena et al., 2012; Wiegerinck et al., 1990). Out of 125 IVC cycles conducted, 40% of women were reported to have achieved clinical pregnancy, which compares favourably to conventional IVF programmes (Lucena et al., 2012). The objective of the present study was to evaluate fertilization rates and embryo development using the intravaginal culture system compared with conventional IVF in a comparative randomized embryology trial using sibling oocytes.

Materials and methods

Settings and design

Patient cycle monitoring and follow-up was carried out at the Toronto Centre for Advanced Reproductive Technology (TCART, Toronto, Ontario, Canada), its affiliate satellite clinic in Kitchener, (Ontario, Canada) and Fertility Ontario (London, Ontario). Interventions and procedures, including oocyte retrieval, IVC device preparation and embryo transfer were all carried out at TCART. The study was a comparative embryology trial, and ethics approval was obtained from an external Research Ethics Board (Western Institutional Review Board; www.wirb.com) on January 29 2013, study number CP-004. Financial support was offered to women who participated in the study.

Study population

Women presenting to the clinic between the ages of 18 and 38 years, who had failed to conceive after 1 year of unprotected intercourse, had a normal recent pelvic sonogram and pap smear and in which IVF had been deemed to be the next step of treatment were invited to take part in the study. Inclusion criteria required a diagnosis of bilateral blocked tubes, early stage endometriosis (stage I or II) or unexplained infertility requiring IVF and a minimum of eight mature retrieved oocytes. Male partners were required to have a normal semen analysis. Willing individuals were eligible as long as they did not have any exclusion factors and understood and accepted the terms of the study. Exclusion criteria included obtaining fewer than eight mature oocytes based on morphology of cumulus cell expansion, a history of toxic shock syndrome, chronic illness, vaginal inflammation, infection, anatomic abnormalities or allergy to plastics, human proteins or gentamicin. Other exclusion factors included a history of pelvic surgery within the past 8 weeks, history of cervical infection with chlamydia or gonorrhoea in the past 12 months, pelvic inflammatory disease, severe endometriosis, body mass index 36 or over, use of donor spermatozoa or eggs, low ovarian reserve, polycystic ovaries, poor responders, prior history of ovarian hyperstimulation, inability to wear a diaphragm, smoking, drug or alcohol abuse and two or more previous failed IVF cycles. Women were diagnosed with low ovarian reserve based on anti-Müllerian hormone less than 1.1 ng/ml, FSH greater than 13 and antral follicle count less than five. Poor responders were identified on the basis of the Bologna criteria (Ferraretti et al., 2011). Between March and September 2013, a total of 15 women agreed to participate in this study. Five of these patients were excluded as they did not fulfill the eligibility criteria. In all of the stimulation cycles, sibling oocytes were randomized for both conventional IVF and for incubation with sperm in the IVC device for comparison. When more than 20 mature oocytes were retrieved, a maximum of 10 were placed in the IVC device and the remaining oocytes were used for conventional IVF.

Stimulation protocol

A long luteal phase gonadotrophin-releasing hormone (GnRH) agonist protocol was used with the GnRH agonist buserelin acetate (Suprefact; Sanofi-Aventis, Laval, Quebec) in daily doses of 200 μg subcutaneously starting one week before expected menses. After confirming ovarian quiescence by day 3 ultrasound and a normal baseline hormone profile (FSH <10 IU/l, oestradiol <200 pmol/l), two or more ampoules of 75 IU human menopausal gonadotropin (Menopur; Ferring, Toronto) were administered on day 3 of the cycle. Dose adjustments were made according to the rate of follicle growth with the aim of obtaining around 10 eggs. Follicle growth and hormone levels were serially monitored by ultrasound and blood tests until the dominant follicles reached an average

diameter of 18–20 mm. At that point, human chorionic gonadotrophin (HCG) (10,000 IU Pregnyl; Merck, Kirkland, Quebec) was administered subcutaneously to trigger ovulation. Thirty-six hours later, oocyte retrieval was carried out under transvaginal guided ultrasound and needle aspiration (Feichtinger and Kemeter, 1986).

Oocyte assessment

Mature oocytes were selected using magnification through the stereo microscope based on traditional morphological criteria (Mandelbaum, 2000). Cumulus, corona radiata and polar body were all assessed. Oocytes that conformed to the above criteria were randomly divided into two groups by the embryologist for conventional IVF in the incubator or for culture in the IVC device.

Intravaginal culture system and procedure

The IVC device was provided disassembled, sterilized, prepackaged and labelled (Invocell™, Invaron Pharmaceuticals, Kelowna, BC). The apparatus consists of two main components, an inner chamber and a rigid outer sheath (Figure 1). The inner chamber was designed to be filled with culture medium and gametes such that it was air free and allowed for easy identification and removal of the embryos, which descend into the bottom of the chamber (microchamber). Following the product instructions, the inner chamber was filled with 1.08 ml of pre-equilibrated culture medium (G1, Vitrolife, Englewood, CO, USA) supplemented with 5% human serum albumin (HSA, Vitrolife, Englewood, CO, USA). The gametes were then loaded into the IVC device as previously described with a few modifications (Frydman and Ranoux, 2008). When loading the gametes, a special rotating valve helped to prevent air contamination and a narrow orifice prevented any sudden shift in pH.

A total of 35,000 washed motile spermatozoa were first placed into each device using a volume of less than 50 μl. On average, eight oocytes were added to the inner chamber of each device before the components were finally reassembled. The outer chamber, which is made of polystyrene with a large silicone O-ring, acted as a rigid shell for the inner chamber. The device was placed into the retention diaphragm, and both were then inserted into the vagina behind the cervix in the posterior vaginal fornix. A perforated flexible diaphragm was then inserted to help maintain the IVC in position. The device was carried in the vagina for three consecutive days during which it remained completely sealed. Participants were given instructions, including abstinence from sexual activity or vaginal douching. After three days, the device was removed from the vagina, disassembled and the embryos were carefully identified, washed and scored. No oocytes were lost or missing on opening the device. Under trans-abdominal ultrasound guidance, between one to three selected embryos from the IVC device were loaded and then transferred into the uterus using a standard embryo transfer catheter (Softpass, Cook Medical, Whitchurch-Stouffville, ON). The embryos were transferred in G1 culture medium (G1, Vitrolife, Englewood, CO, USA) supplemented with human serum albumin (HSA, Vitrolife, Englewood, CO, USA). Embryos from the IVC

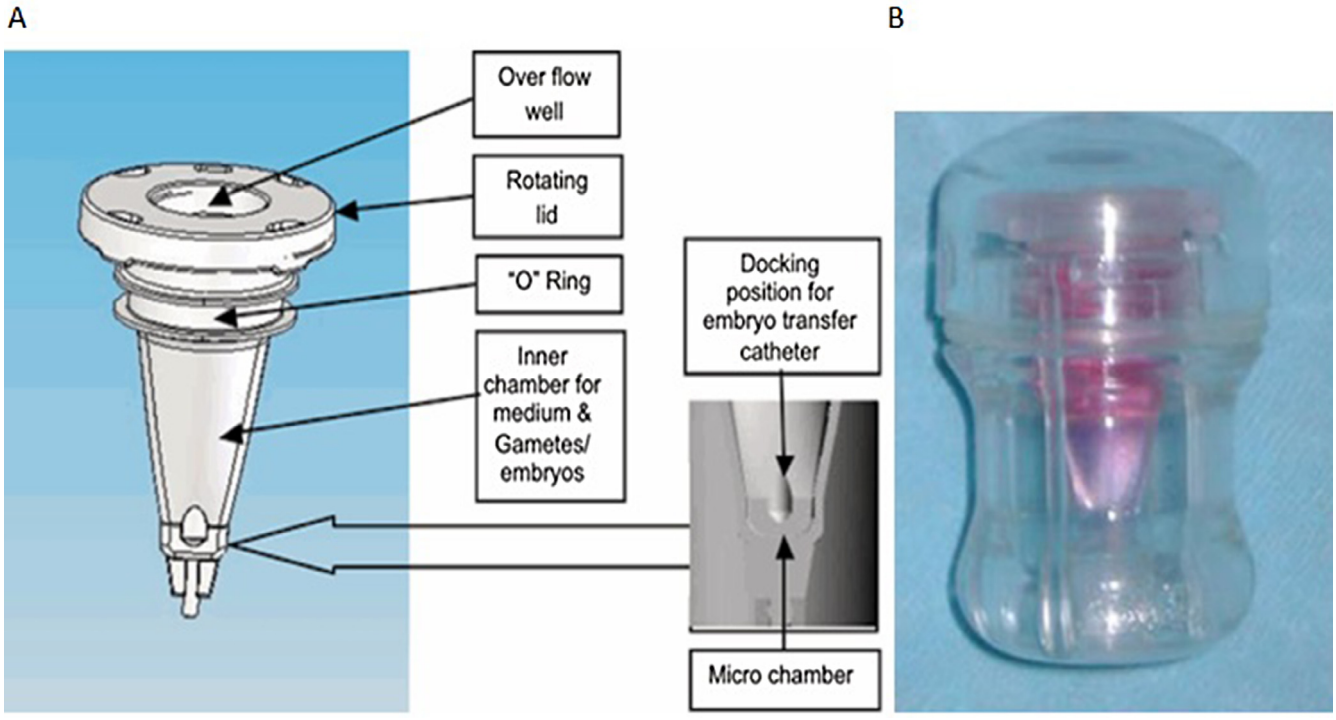

Figure 1 The INVOcell™ device: (A) the components of the inner chamber of the device; (B) rigid outer sheath encasing the inner chamber. Adapted from http://www.canadianfertilityinstitute.com (an introduction to INVOcellTM); http://www.canadianfertility.com/152-2/an-introduction-to-invocell/.

device were preferentially transferred; however, when none were available or of suitable quality, embryos from the IVF group were transferred. Transfers were only carried out using fresh embryos. The embryo transfer catheter was subsequently examined under the microscope after each embryo transfer to exclude the possibility of retained embryos. All supernumerary embryos with acceptable quality were cultured to blastocyst and vitrified.

Conventional insemination

A second group of sibling oocytes was fertilized in the laboratory using conventional IVF. The same culture medium was used with both the IVC device and conventional insemination. Groups of four to five mature eggs were incubated with 100,000 motile spermatozoa in a central-well dish (Central Well Dish [Falcon], Corning Inc., Durham, NC, USA) and fertilization was documented 12–20 h later by the presence of two pronuclei. Embryo development was assessed on a daily basis using the inverted microscope.

Blastocyst vitrification

Additional embryos of acceptable quality from either arm of the study, which had not been transferred, were further cultured to the blastocyst stage in the laboratory and then vitrified as previously described (Kuwayama, 2007).

Embryo scoring

All of the embryos that developed into either the cleavage or blastocyst stage were individually graded. Day 3 embryos were morphologically assessed according to the number of cells and level of fragmentation. The grade was determined based on the fragmentation pattern with Grade 1 corresponding to minimal fragmentation and grade 5 corresponding to extensive fragmentation (Veeck, 1986). Embryos with acceptable scores were considered suitable for transfer (4CGI, 4CGII, 5CGI, 5CGII, 6CGI, 6CGII, 6CGIII, 7CGI, 7CGII, 7CGIII, 8CGI, 8CGII, 8CGIII). Day 5 embryos were also graded adopting the scoring system used by Gardner et al. (2000) and Rehman et al. (2007). In brief, blastocyst expansion, trophectoderm and inner cell mass were each graded and given a numerical score (Rehman et al., 2007).

Luteal phase support

Progesterone supplementation using intravaginal suppositories was started the day of embryo transfer up until serum beta-HCG testing which was done 2 weeks later and if positive, repeated after two days. A total of 600 mg of daily intravaginal microinonized progesterone was administered (200 mg three times daily; Prometrium, Merck, Kirkland, Quebec). If pregnancy was confirmed after two weeks, patients were instructed to continue taking progesterone and serial serum beta-HCG levels were determined after two days and as needed thereafter. Five weeks after embryo transfer, transvaginal ultrasound was carried out in women who had a positive serum pregnancy test result in order to confirm clinical pregnancy. This was defined by the presence of a gestational sac detected by ultrasound.

Post-study questionnaire

A post-study questionnaire consisting of 12 questions was distributed to each participant. Scales quantifying the level of discomfort, significance of fertilization happening in the body rather than in the laboratory, and other questions relating to convenience, adverse effects and adherence to the instructions were all included in the questions (Table 1). All of the questionnaires were completed and analysed. The Pearson product-moment correlation coefficient was calculated for discomfort levels and number of eggs retrieved, in the questionnaire portion of this study.

Outcome measures and statistical analysis

Retrieved sibling oocytes were randomized between the IVC device and conventional IVF. Analysis of fertilization, cleavage rates and embryo quality comparisons between the two groups of oocytes, was carried out using the Mantel–Haenszel test. Mean values and standard deviations were computed and included in the analysis for the questionnaire portion of the study.

Results

General results

Each of the 10 eligible participants between the ages of 27 and 37 years underwent one ovarian stimulation cycle. Out of 10 stimulation cycles, 164 oocytes were obtained (Table 2). A total of 81 oocytes was placed into the IVC device and 33 (40.7% ± 27%) oocytes fertilized resulting in 32 cleavage stage embryos (97% ± 6% cleavage rate). A total of 83 oocytes was inseminated in culture dishes and kept in the incubator (Table 2). Normal fertilization occurred in 57 IVF oocytes (68.7% ± 36%) resulting in 53 cleavage stage embryos (93% ± 1.5%). One patient (four eggs) from the IVF group and another patient (four eggs) from the IVC device group had total fertilization failure. A significantly smaller number of embryos that were suitable for transfer was obtained using the IVC device compared with IVF (odds ratio [OR] 0.47; 95% confidence interval [CI] 0.26 to 0.87). The proportion of embryos suitable for transfer per fertilized oocyte was similar in both groups. Seven out of 10 women received IVC embryo transfers (Figure 2). A total of 12 cleavage stage embryos was transferred into this group (average of 1.7 embryos per woman). Among the three patients who did not have embryo transfer, one had no fertilization using the IVC device. A second patient had one poor quality day three embryo, which arrested. The last patient was judged to be at high risk for developing ovarian hyperstimulation syndrome and therefore fresh embryo transfer was avoided. IVF embryos were transferred (fresh embryo transfer) in the first two patients

Table 1 Study questionnaire for acceptability and overall experience with the intravaginal culture device.

1. While wearing the device and retention system were you aware that they were in the vagina?
2. While wearing the device and retention system did you feel any discomfort? If yes, could you tell if it was related to the device or retention system? Please explain how you could tell.
   Try to quantify the discomfort: circle one of the numbers on the scale 0 being no discomfort and 10 being severe discomfort
3. While wearing the device and retention system did they become dislodged? Did you have to reposition them in the vagina? If yes, were you able to reposition them? Was the repositioning easy, somewhat challenging or difficult?
4. While wearing the device and retention system did they come out of the vagina? What caused them to come out of the vagina? On which day did they come out? Were you instructed by your physician to replace them? Was the replacement easy, somewhat challenging or difficult?
5. While wearing the device and retention system did you have intercourse? Bathe in a tub? Douche? Have a sauna? Go swimming?
6. While wearing the device and retention system did you perform any unusual or strenuous activity? If yes, explain briefly.
7. While wearing the device and retention system did you have to take them out of the vagina? If yes explain the reason.
8. While wearing the device and retention system did you have any unusual vaginal discharge? If yes, answer the following questions: Was the discharge abundant? Was the discharge odorous? What colour was the discharge? What was the consistency?
9. While wearing the device and retention system did you have any spotting?
10. While wearing the device and retention system did you have any vaginal itch?
11. How important was it to you to know that the fertilization was occurring in your body and not in a laboratory? (0 being not important at all, 10 being extremely important).
12. How important was it to you to know that the fertilization was occurring naturally? (0 being not important at all, and 10 being extremely important?).

Table 2 Data comparing the results between IVF and the intravaginal culture device among 10 women.

Variables	*IVF*	*IVC*	*Significance*
Number of mature oocytes (MII)	83	81	–
Number of 2PN	57	33	–
2PN/MII (%)	68.7	40.7	$P = 0.002$
Number of cleaved embryos (day 3)	53	32	–
Cleaved embryos/2PN (%)	93	97	NS
Number of usable embryos[a]	40	18	–
Usable embryos/total number of 2PN (%)	40/57 = 70%	18/33 = 55%	NS

IVC = intravaginal culture device; MII = second metaphase; NS = not statistically significant; 2PN = two pronuclei.
[a]Usable embryos has been defined as embryos that were either transferred or vitrified at the cleavage or blastocyst stage.

described above who failed to have any embryos developing from the IVC device. One patient received two cleavage stage embryos whereas two blastocysts were transferred to the second patient. Four positive beta-HCG results were obtained from the IVC device transfers, which resulted in three clinical pregnancies in this group. The biochemical pregnancy rate for the IVC group was 40% (per cycle) and 57% (per fresh embryo transfer procedure). The clinical pregnancy rate for the IVC group was 30% (per cycle) and 43% (per patient embryo transfer procedure). The beta-HCG results were positive in both women who had embryos transferred from the IVF group, and clinical pregnancies were subsequently detected by ultrasound.

Of the remaining 20 IVC cleavage embryos, 16 were further cultured. Successful blastocyst stage development was found in six of these embryos (37.5 % ± 28%), which were subsequently vitrified, whereas the remaining 10 embryos arrested at the cellular stage and were discarded. Forty-five IVF cleavage stage embryos were cultured for a further 2–3 days and 32 embryos developed to the blastocyst stage (71% ± 16% blastocyst rate). Thirty blastocysts were vitrified and two were directly transferred (fresh transfer). The remaining embryos arrested at the cellular stage and were therefore discarded.

Questionnaire

Questionnaires about the acceptability and overall experience with the IVC system were completed and received before removing the device on the day of embryo transfer (Table 1). Most of the participants were satisfied with the device, and most women reported mild discomfort usually in the form of pressure. On a scale of 1 to 10, the mean discomfort score reported while carrying the device was 3.2 ± 2.4. Number of eggs retrieved did not correlate with the level of discomfort reported and it is difficult to determine whether part of the symptoms could have been related to the preceding oocyte retrieval procedure. The device remained in the vagina but

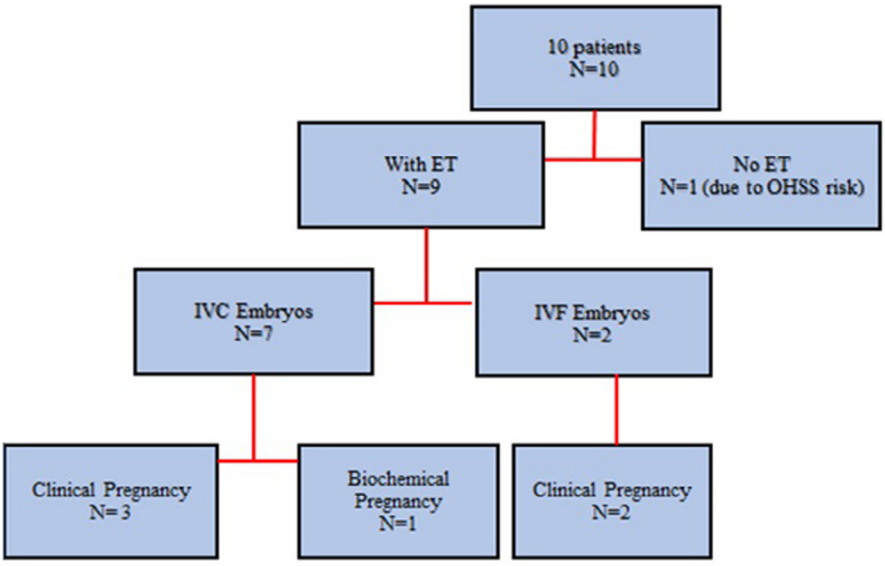

Figure 2 Embryo transfer and pregnancy among 10 patients.

was dislodged in two cases and these women managed to easily reposition the dislodged device by themselves without removing it. Four women had some form of vaginal spotting which could have been related to the oocyte retrieval procedure and four women reported whitish odourless discharge, which they attributed to the vaginal progesterone. No adverse events occurred. The mean score for appreciation that fertilization was happening in a device within the body rather than in the laboratory was 7.1 ± 1.6.

Discussion

Out of 164 oocytes that were randomly distributed between the IVC device and the regular IVF incubator, fertilization rates were 1.7 fold higher for the conventional IVF group (68.7% versus 40.7%; $P = 0.002$) whereas cleavage rates were similar, resulting in a significantly larger number of embryos available for embryo transfer (53 embryos as compared with 32). On average, 25,000 spermatozoa were incubated per oocyte in the IVF group compared with around 4000 spermatozoa per oocyte in the IVC group. It is possible that the lower sperm concentrations may have negatively affected the fertilization rates. New data suggest that sperm and cumulative oocyte complexes could be incubated for a short period of time *in vitro* to ensure sperm binding to the zona pellucida followed by delayed (2 h) placement of the device (Kevin Doody, personal communication). In the present study, cleavage occurred in 32 out of 33 (97%) of the fertilized embryos with the IVC device compared with 53 out of 57 (93%) of the embryos that developed using IVF. Although a similar proportion of embryos suitable for transfer was noted in both groups, a larger number of embryos suitable for transfer was obtained using IVF because more embryos were available as a result of the higher fertilization rate. Comparison between blastocyst development rates cannot be made as a significant proportion (71%) of IVF cleavage embryos developed into blastocysts, whereas almost one-half of the embryos from the IVC device were transferred directly at the cleavage stage. Even if we assume that all of the embryos that were transferred from the IVC device could have developed to the blastocyst stage, however, the total blastocyst development rate would be 18 out of 32 (56%), which is still lower than that noted using conventional IVF. The clinical pregnancy rate achieved with the IVC device in our study was 30% per initiated cycle and 43% per patient with an embryo transfer. Overall, 33% of the transferred embryos implanted. These results compare favourably with the 2012 CDC National Data, and suggest that the success rates using this device are reasonable. A recent clinical trial using the IVC device reported a pregnancy rate of 40%, which is higher than that noted in this study (Lucena et al., 2012). When top-quality embryos were available, only one embryo was transferred per patient. When lower quality embryos were available, two to three embryos were transferred, particularly for patients above the age of 35 years. All of the pregnancies among the IVC group were singletons consistent with the low rate of multiple gestations previously reported (Lucena et al., 2012).

It is relevant that most women placed a high importance on carrying the device within their body rather than having fertilization and embryo development occur in a laboratory incubator, and this was reflected by the questionnaire scores. These women felt that fertilization was more 'natural', as a result of feeling closer to their embryos while carrying the device. This further highlights the psychological aspects and additional stress factors involved during conventional IVF, which often may be neglected. This study does provide some interesting data about the outcomes obtained using the IVC device in a homogeneous population of women. Compared with IVC, IVF seemed to be superior in fertilization and embryo developmental rate; the IVC device, however, was associated with good patient satisfaction levels.

Some women may benefit from using this IVC technology over conventional IVF. A large category of women, however, do not have access to cost-effective fertility treatment, and the IVC device may be an acceptable option. Specialized laboratory air and gas systems optimize O_2, CO_2, and temperature fluctuations and require daily inspection and continuous

power usage, which may be unavailable in certain areas. In 2004, around 180 million couples faced infertility and many of these couples were both financially underprivileged and did not have access to assisted reproduction techniques within their area of residence (Ombelet, 2011). In a less sophisticated setting, minimal stimulation, oocyte retrieval and embryo transfer could be done by a single fertility specialist and sperm preparation, cumulus-oocyte identification and placing the sperm and oocytes into the IVC device could be done by a single embryologist. In this situation, minimal personnel and laboratory equipment would be required and cost savings would be substantial. It would be reasonable to assume that women living in remote areas will benefit the most from this technology as it is more likely for this population to desire childbearing at a younger age compared with women living in more cosmopolitan urbanized areas where childbearing is often delayed (Yi and Vaupel, 1989). These good-prognosis young women respond well to ovarian stimulation and are good candidates for low cost mild stimulation and perhaps even clomiphene or letrozole in conjunction with the IVC, lowering medication expenses. Individualized cost-benefit analysis needs to be determined for the IVC technology compared with routine IVF. There may also be a favourable psychological component with many women feeling more comfortable carrying their own developing embryos.

The number of women who participated in this study was relatively small, which could affect the accuracy of our results, although the total paired oocyte pool was large enough for robust statistical analysis. The currently utilized IVC device is relatively new having evolved from earlier technology. With further understanding and refinement, fertilization rates and embryo numbers may be enhanced. Additional studies using a larger number of participants are required to replicate these results and possibly provide more insight into the overall advantages and disadvantages of using the IVC device. In addition, further research is needed to determine if the IVC device, which uses a single culture medium, could be successful in culturing embryos to the blastocyst stage.

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