

**Effects of various embryo transfer techniques on pregnancy rates for day 3 and blastocyst embryo transfers.** D Kreiner, S Brenner, S Chen, J Moschella, M Kaufman and DJ Kenigsberg. Long Island IVF, Port Jefferson, NY.

**Objectives:** Various techniques may be employed especially when encountering a difficult transfer. The embryo transfer technique was studied to determine the effect of some of these various factors on the pregnancy rates. The factors reviewed in this study are; the use of tenaculum, the depth of placement of the tip of a transfer catheter in the uterine cavity and the use of a two step technique including a freshly loaded catheter threaded through a mock carrier sent in the uterus.

**Design:** Pregnancy rates were compared for all fresh and cryopreserved embryo transfers by a single physician from October 1998 to June 1999 with regard to the different transfer techniques studied.

**Materials and Methods:** Two hundred and forty-five consecutive embryo transfers including 76 blastocyst transfers and 169 day 3 embryo transfers (ET). Fifty-eight of these 245 transfers were from cryopreserved embryos. Placement of the tip of a Wallace catheter was determined during an ultrasound guidance of the embryo transfer using an ATL Ultramark 4 with an abdominal probe. The depth of placement of the catheter was measured using the cm markings on the carrier noting the distance the catheter is withdrawn from the top of the uterine cavity.

**Results:** Pregnancy rates (documented by ultrasound evidence of an intrauterine gestational sac) for all transfers using a tenaculum was 9.1% (3/33) vs. 44.1% (94/212) ( $p < 0.05$ ) without a tenaculum. The average embryos transferred was 3.10 for day 3 ET with and 2.95 without the use of a tenaculum and 1.91 for all blastocyst transferred either way. Higher pregnancy rates were obtained with placement of the tip of the Wallace catheter 1-2 cm from the top of the uterine cavity. Forty-nine% (48/98) of fresh embryo transfers 1-2 cm from the top resulted in a pregnancy vs. 32.3% (10/31) when transferred within 1 cm (2.00 blasts and 2.94 d3 embryos per transfer 1-2cm vs. 1.93 blasts and 2.77 embryos per transfer within 1cm,  $p = ns$ ). When the transfer was performed blindly

(inadequate ultrasound visualization) the pregnancy rate was 40.5% (17/42). The highest pregnancy rates were obtained when fresh embryo transfers were performed using the mock carrier in the canal to thread a freshly loaded catheter into the uterus. Sixty-five % of fresh transfers (13/20)(2.2 blastocysts and 2.90 d3 embryos per transfer) performed with this technique without a tenaculum conceived vs. 47.5% (76/160) without utilizing this technique (1.93 blasts and 2.95 d3 embryos per transfer).

**Conclusion:** 1) The use of a tenaculum at the time of embryo transfer is a significant detriment to successful implantation of human embryos. 2) Ultrasound guided transfer of day 3 and blastocyst embryos is optimally placed 1-2 cm from the top of the uterine cavity for higher pregnancy rates with IVF than embryos transferred higher or blindly. 3) Using a freshly loaded catheter threaded through a previously placed mock carrier in the uterine cavity to transfer embryos appears to improve pregnancy rates in IVF.

*The following research was presented November 2000 at the American Society for Reproductive Medicine. This was the first time it was demonstrated that one can improve the pregnancy rate with IVF by placing the embryo transfer catheter greater than 1cm from the top of the uterine cavity guided by an abdominal ultrasound.*



## ABSTRACT

**Pregnancy rates of a two step ultrasound guided embryo transfer 1-1<sup>1</sup>/<sub>2</sub>cm from the top of the uterine cavity.** D Kreiner, J Moschella, N Carlson, J Jelicks, M Bolkas, G Moodie, S Brenner, K Droesch, D Kenigsberg. Long Island IVF, Port Jefferson, NY.

**Objectives:** The embryo transfer is the weak link in the in vitro fertilization chain. Previous studies have demonstrated a variation of success relative to the experience of the operator, catheter placement and the negative impact of bloody catheters. We hypothesize that pregnancy rates may be improved by accurate and atraumatic placement of the embryos in the uterine cavity. The objective of this study was to evaluate the pregnancy rate with an embryo transfer technique developed to minimize trauma and optimize placement as suggested in a previous trial.

**Design:** A prospective randomized study was performed from July 1999 to February 2000 of all individuals undergoing fresh IVF-ET on day 3. One operator performed the two step transfer technique during the entire study. This included specific placement of embryos 1-1½ cm from the top of the endometrial stripe. From July to December 1999, the other operators used a one step transfer with ultrasound confirmation of the placement of the catheter tip in the endometrial stripe. From January to February 2000, these operators also used the two step transfer technique.

**Materials and Methods:** A Wallace catheter was used for all embryo transfers. In the one step technique the internal catheter leads through the cervix. In the two step technique the external sheath is left in the cervix through which the internal catheter is inserted. Placement of the catheter tip was positioned by 2-D ultrasound guidance using an ATL Ultramark 4 with an abdominal probe at 1-1½ cm from the top of the endometrial stripe in the two step technique. The catheter tip was confirmed to be in the endometrial stripe when transferred with the one step technique. Statistical analysis was performed using the chi-square test.

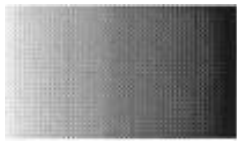
**Results:** From July to December 1999, the pregnancy rate using the two step technique, confirmed by ultrasound evidence of a gestational sac, of patients <40, was 81/150 (54.0%). Only 11/38 (28.9%) transferred by the one step transfer conceived,  $p < .05$ . Implantation rates were (134/464) 28.9% and (18/125) 14.4% respectively,  $p < .05$ . From January to February 2000, the pregnancy rate by rising HCG titers was 16/25 (64.0%) for the operator who had performed the two step transfer technique since July. The pregnancy rate for the other operators improved to 9/19 (47.3%) using the two step technique. The donor egg pregnancy rates were 11/14 (78.6%) with the two step and 1/5 with the one step technique.

**Conclusions:** The two step ultrasound guided embryo transfer with the catheter tip between 1-1½ cm from the top of the endometrial stripe provides superior pregnancy results to the one step transfer. These results suggest that the embryo transfer technique can be the significant pregnancy rate limiting step in the IVF process.



## OBJECTIVE

The embryo transfer is the weak link in the in vitro fertilization chain. Previous studies have demonstrated a variation of success relative to the experience of the operator, catheter placement and the negative impact of bloody catheters. We hypothesize that pregnancy rates may be improved by accurate and a traumatic placement of the embryos in the uterine cavity. The objective of this study was to evaluate the pregnancy rate with an embryo transfer technique developed to minimize trauma and optimize placement as suggested in a previous trial.



## DESIGN

A prospective randomized study was performed from July 1999 to August of 2000 of all individuals under-going fresh IVF-ET on day 3. One operator performed the two step transfer technique with specific placement of embryos 1-1½ cm from the top of the endometrial stripe during the course of the study. From July to December of 1999, the other operators used a one step transfer with ultrasound confirmation of placement of the catheter tip in the endometrial stripe. From January to August of 2000, all operators used the two step transfer technique.



# MATERIALS &

# METHODS

A Wallace catheter was used for all embryo transfers. In the one step technique the internal catheter leads through the cervix. In the two step technique the external sheath is left in the cervix through which the internal catheter is inserted. Placement of the catheter tip was positioned by 2-D ultrasound guidance using an ATL Ultramark 4 with an abdominal probe at 1-1½ cm from the top of the endometrial stripe in the two step technique. The catheter tip was confirmed to be in the endometrial stripe when transferred with the one step technique. Statistical analysis was performed using the chi-square test.



# RESULTS

From July to December of 1999, the new two step transfer technique pregnancy rate by gestational sac, age <40 was 81/150 (54.0%). During the same time period, 11/28 (28.9%) transferred by the one step transfer conceived,  $P < 0.05$ . All transfers by all physicians from January to August of 2000 were by the two step transfer method resulting in a 105/236 (44.50/0) pregnancy rate for patients <40. From October 1988 to August of 2000, the pregnancy rates for one step transfers was 87/198 (43.90/0) and for two step was 199/406 (49.00/0),  $P < 0.05$ .



# RESULT TABLES

**Table 1**

## **Pregnancy Rate Per Transfer Technique**

**Age < 40 July-December 1999**

	<b>Pregnant *</b>	<b>Non-Pregnant</b>
<b>One Step</b>	<b>11 (28.9%)</b>	<b>27</b>
<b>Two Step</b>	<b>81(54.0%)</b>	<b>69</b>

P <0.05

Chi-square analysis

\*Ultrasound evidence of gestational sac

**Table 2**

## **Pregnancy Rate Per Transfer Technique**

**Age < 40 October 1998-August 2000**

	<b>Pregnant*</b>	<b>Non-Pregnant</b>
<b>One Step</b>	<b>87(43.9%)</b>	<b>111</b>
<b>Two Step</b>	<b>199(49.0%)</b>	<b>207</b>

P <0.05

Chi-square Analysis

\*Ultrasound evidence of gestational sac



## CONCLUSIONS

The two step ultrasound guided embryo transfer with the catheter tip between 1-1½ cm from the top of the cavity appeared to have provided superior pregnancy results to the one step method. Subsequent pregnancy rates using the two-step transfer exclusively have not continued to be as great or higher than previous methods. These results suggest that the embryo transfer technique can be the significant pregnancy rate limiting steps in the IVF process. However, the entire IVF process can be affected at any point making evaluation of this transfer method difficult.

---

*This paper was written by us in 1987. Read the second paragraph in the discussion. I have been working on refining the embryo transfer technique for the past 17 years. This paper led to us performing salpingectomies and tubal ligations in women with hydrosalpinges.*

### **ABDOMINAL PREGNANCY AFTER IN VITRO FERTILIZATION AND EMBRYO TRANSFER**

Sergio Oehninger

David Kreiner

## Zev Rosenwaks

Jones Institute for Reproductive Medicine  
Eastern Virginia Medical School  
825 Fairfax Avenue  
Norfolk, VA 23507

CS/5-87

### **Introduction**

Ectopic pregnancy developing as a result of in vitro fertilization and embryo transfer (IVF/ET) occurred in the first clinical pregnancy of a patient of Steptoe and Edwards in 1976<sup>1</sup> and continues to be the major complication of their pioneering contribution to reproductive technology. A review of the world experience in IVF/ET to date reveals that approximately 5% of all clinical pregnancies result in ectopic pregnancies.<sup>2</sup> It has been suggested that these extrauterine pregnancies are related to the ET technique and are more likely to occur ectopically as a result of a high fundal transfer and with volumes of transfer medium > 50 ml.<sup>3</sup> Ectopic implantation has also been noted to occur with greatest frequency in patients with only one patent fallopian tube.<sup>4</sup>

However, to the best of our knowledge, no abdominal pregnancies and only one case of an ovarian pregnancy<sup>5</sup> have been reported in association with IVF/ET, although one abdominal pregnancy has been reported in association with the GIFT procedure.<sup>6</sup> Therefore, we present the case history of a patient who was treated at

the Jones Institute and subsequently developed an abdominal pregnancy after IVF/ET.

## **Material and Methods**

The patient was a 35-year-old married Caucasian female who conceived in her second treatment cycle. Her history included six years of primary infertility due to severe endometriosis. Following a prolonged complaint of dysmenorrhea, dyspareunia, menorrhagia, and medical suppressive therapy with danazol and birth control pills, she had two laparotomies. During the first one, obliteration of the cul-de-sac was found, and a right ovarian cystectomy (endometrioma), lysis of adhesions, and removal of endometrial implants were performed. During the second laparotomy the findings were severe pelvic adhesions, sigmoid colon adherent to the posterior wall of the uterus, fixed partial retroversion of the uterus, and bilateral hydrosalpinx. The right adnexa was completely buried in adhesions on the lateral pelvic wall. A left salpingectomy and left ovarian suspension were performed.

A subsequent hysterosalpingogram showed a normal uterine cavity, blocked left tube at the ampullary region, and a right hydrosalpinx with partial obstruction and delayed spillage of the dye to the abdominal cavity. Endometrial biopsy showed a secretory endometrium to be in phase. At that time the patient was referred to the Norfolk program for IVF/ET.

A screening laparoscopy was performed to establish ovarian availability and to determine the best technique for oocyte retrieval. A frozen pelvis was found, with no visualization of either adnexal area. For this reason a percutaneous transabdominal/transvesical ultrasonically-guided puncture was the method selected for oocyte retrieval.

During the first IVF/ET treatment cycle, follicular development was stimulated with a gonadotropin combination protocol. Two ampules of FSH (Metrodin, Serono) were administered on days 3 and 4 of the cycle, and two ampules of hMG (pergonal, Serono) were administered on days 3 to 8. This was followed by hCG administration (10,000 IU) on day 9. Three mature oocytes were

aspirated 36 hours later, and two concepti (one four-cell and one six-cell) were transferred 40 hours post-insemination. These techniques have previously been described. 7-9

Unfortunately, pregnancy failed to ensue, and eight months later a second treatment cycle of IVF was undertaken, with the same gonadotropin combination protocol (2 FSH/2 hMG/ hCG). Two ampules of FSH were administered on days 3 and 4, and two ampules of hMG were given on days 3 to 6. A long coasting period (hMG/hCG interval) was used, however, and hCG was administered on day 8. Oocyte recovery was undertaken 36 hours later when the estradiol (E2) level was 1252 pg/ml. A biological shift (vaginal maturation index and cervical score) had occurred on day 7, when ultrasound examination demonstrated three follicles > 12 mm in diameter, in addition to several smaller follicles seen on the day before oocyte retrieval. At retrieval four preovulatory oocytes (three Metaphase I, one Metaphase II) were obtained, and all fertilized successfully. One four-cell conceptus at 44 hours, one four-cell conceptus at 42 hours, one conceptus questionably fragmented at 42 hours, and one two-pronuclear conceptus at 42 hours were transferred for a total of four concepti. The transfer was uneventful, devoid of bleeding or regurgitation.

The embryo transfer technique used in Norfolk has been previously described.<sup>10,11</sup> Briefly, the Norfolk program uses its own device, which consists of a Teflon catheter 46 cm in length with an inner diameter of 1.2 mm. The tip is solid and round, with a notch on one side, through which the concepti are aspirated and ejected. The catheter is carried within a metal cannula that serves as a guide. A tuberculin syringe is attached to the proximal end. The catheter is loaded as follows: A tuberculin syringe attached to the proximal end of the catheter is used to aspirate the concepti into the catheter. Aspirated into the catheter in succession are 0.02 ml of growth medium, 0.01 ml of air, 0.03 ml of growth medium containing the concepti, 0.01 ml of air, and 0.02 ml of growth medium. During transfer the patient is in the knee-chest position, and the cervix is cleansed and grasped with a single toothed tenaculum. Previously, the size of the uterus has been determined to avoid trauma during the transfer procedure. The tip of the cannula is introduced

approximately 1-2 cm into the endocervical canal, and the catheter is then advanced to the fundus. When the top of the fundus is reached, the catheter is withdrawn slightly, and the pre-embryos are propelled by a single stroke of air (0.02 ml) provided by the syringe. Following transfer, the patient is confined to bed rest in the prone position for four hours, then released from the hospital with instructions to rest in bed until the next morning, when she may resume normal activities. All patients are given progesterone (P) supplement (25 mg in oil, intramuscularly) beginning on the day before transfer.<sup>12</sup>

In the patient under study, a beta-hCG pregnancy test was positive on day 10 post-transfer, with serum E2 levels of 865 pg/ml and serum P levels of 90.3 ng/ml. Thereafter, the patient was referred for obstetrical care.

During her follow-up, weekly b-hCG determinations were carried out, and the rise was consistent with an intrauterine pregnancy. Approximately 34 days post-transfer (4 weeks after transfer or 8.5 weeks after the last menstrual period), she presented with vaginal bleeding. An ultrasound showed a gestational sac presumed to be within the uterus. One week later, ultrasound showed an ectopic pregnancy in the cul-de-sac. A laparotomy was performed after all options had been discussed with the patient. Accordingly, it was elected to do a right cornual tubal ligation at the time of surgery to prevent the occurrence of a tubal or abdominal pregnancy in subsequent IVF/ET attempts. The surgical procedure was extremely difficult because of the frozen pelvis. Omentum, sigmoid colon, and small bowel adhesions to the uterus and left cornual region were carefully dissected. The gestational sac was seen to be free of the fallopian tube and deep within the cul-de-sac, covered by filmy adhesions. These adhesions were lysed, and the pregnancy was removed. The abdominal pregnancy had implanted over the mesentery of the sigmoid and rectosigmoid. Neither ovary could be visualized nor palpated because of the dense adhesions. The pathologic study of the cul-de-sac material revealed fetal and placental tissue, confirming the diagnosis. The patient had a normal postoperative course and is planning to return for another IVF-ET attempt.

## Discussion

It is no surprise that an abdominal pregnancy occurred as a result of IVF/ET, since it has clearly been demonstrated that ectopic pregnancy is a real risk of this procedure. 2 It is estimated that there have been approximately 2500 pregnancies through IVF so that one abdominal pregnancy and one ovarian pregnancy out of the 2500 clinical pregnancies is about 1.5 to 4 times the incidence seen naturally in the population.<sup>13,14</sup> The proportion of ectopic pregnancies that are ovarian or abdominal would be 1/125 each, which is not significantly different from that found naturally in the population.<sup>13,14</sup>

Considering the high risk nature of IVF patients and the fact that they would be unable to become pregnant by any other means, we should still be thankful for this advance in reproductive technology. However, with refinement in the technology with particular attention to the transfer technique, whether with respect to catheter position, medium volume, or occluding the tubal ostia, we hope that soon this complication risk may be minimized. Furthermore, it is imperative that the physician who is caring for pregnant IVF patients be aware of these risks.

## References

1. Steptoe PC, Edwards R: Reimplantation of a human embryo with subsequent pregnancy. *Lancet* 1:880, 1976.
2. Cohen J, Mayaux MJ: IVF pregnancies: results of an international survey. Presented at the Fifth World Congress on IVF/ET, Norfolk, VA, April 1987 .
3. Yovich JL, Turner SR, Murphy AJ: Embryo transfer technique as a cause of ectopic pregnancies in in vitro fertilization. *Fertil Steril* 44:318, 1985.
4. Saunders DM: The Australian register. Presented at the Fifth World Congress on IVF/ET, Norfolk, VA, April 1987.
5. Carter JE, Jacobson A: Reimplantation of a human embryo with subsequent

ovarian pregnancy. *Am J Obstet Gyn* 155:282, 1986.

6. Abdalla HI, Ahuja KK, Morris N, Lynn J: Combined intra-abdominal and intrauterine pregnancies after gamete intrafallopian transfer. *Lancet* 2:1153, 1986.

7. Jones HWJr, Acosta AA, Andrews MC, Garcia JE, Jones GS, Mayer J, McDowell JS, Rosenwaks z., Sandow BA, Veeck LL, Wilkes CA: Three years of in vitro fertilization at Norfolk. *Fertil Steril* 42:826, 1984.

8. Rosenwaks Z, Muasher SJ, Acosta AA: Use of hMG and/or FSH for multiple follicle development. *Clin Obstet Gyn* 29:148, 1986.

9. Veeck LL: Extracorporeal maturation: Norfolk, 1984. *Ann NY Acad Sci* 442:357, 1985.

10. Jones HWJr, Acosta AA, Garcia J, Sandow B, Veeck L: On the transfer of conceptuses from oocytes fertilized in vitro. *Fertil Steril* 39:241, 1983.

11. Garcia JE: Conceptus transfer. In: Jones HWJr et al, eds: *In Vitro Fertilization-Norfolk*. Baltimore: Williams & Wilkins, 1986, p 215.

12. Jones GS: The role of luteal support in a program for IVF. In: Edwards RG et al, eds: *Implantation of the Human Embryo*. London: Academic Press, 1985, P 285.

13. Hallatt JG, Grove JA: Abdominal pregnancy: a study of twenty-one consecutive cases. *Am J Obstet Gyn* 152:444, 1985.

14. Atrash HK, Friede A, Hogue CJR: Abdominal pregnancy in the United States: frequency and maternal mortality. *Obstet Gyn* 69:333, 1987.