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Assisted Implantation: Direct Intraendometrial Embryo Transfer

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Abstract

We report herein a technique for direct intraendometrial transfer (DIET) of human embryos. In this study we evaluated whether 2-day embryos injected into the endometrial stroma can develop normally into viable pregnancies. After in vitro fertilization the embryos were injected into the endometrium of 14 women under direct visualization using a CO_2-pulsed flexible hysteroscope. A total of 44 embryos were transferred, resulting in 2 clinical (14.3% per cycle) and 2 chemical pregnancies. In 1 patient, amniocentesis revealed monosomy X and the pregnancy was terminated at 18 weeks. The 2nd patient had an uneventful pregnancy and delivered a healthy baby. The results from the present study, demonstrating a low implantation rate after DIET of 2-day embryos, suggest that the endometrial stroma does not provide an optimal environment for early embryonic development. The acidifying effect of CO_2 used for insufflation may also explain the low pregnancy rate after DIET. We conclude that it is possible to achieve pregnancy by DIET in humans, but presently this procedure can be considered only in cases where the implantation site needs to be precisely determined.

Key Words

Human embryo
Implantation
Intraendometrial embryo transfer

Introduction

Embryo transfer (ET) is a critical step affecting the success rate after in vitro fertilization (IVF). However, despite marked progress that has been made in various aspects of IVF such as controlled ovarian stimulation, gamete and embryo culture and assisted fertilization, the technique of ET has remained largely unchanged since the birth of the first IVF baby [1].

Cleaved embryos are routinely transferred transcervically into the uterine cavity 42–72 h after insemination. There is no way to ascertain that the embryos will stay in the uterine cavity, adhere to and eventually invade the endometrial mucosa. Indeed, expulsion of the embryos into the cervical canal [2, 3], or implantation in the fallopian tube are not uncommon after their transfer to the uterine cavity.

To prevent expulsion of transferred embryos, attempts have been made to immobilize the embryos with fibrin sealant [4], but this method did not improve the pregnancy rate. Kato et al. [5] have reported high pregnancy rates after ultrasound-guided transmyometrial embryo transfer. It is unclear whether the embryos were placed in the uterine cavity or within the endometrium. It is also not known whether an embryo transferred under luminal epithelium is able to develop normally and whether it is possible to increase pregnancy rate by assisted implantation. In the mouse, pregnancies were achieved by transferring blastocysts into the endometrial stroma [6]. By using this technique it was possible to increase pregnancy and embryonic viability rates in asynchronous embryo transfer in the mouse [7].

Implantation of human embryos involves integrated series of processes including adhesion to the endometrial

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mucosa and invasion of the blastocyst through the luminal epithelium into the endometrial stroma [8]. Bulletti et al. [9] injected human blastocysts into the endometrium of human ex vivo perfused uteri and demonstrated that blastocysts can successfully undergo implantation and trophoblastic invasion during 52 h of extracorporeal perfusion.

We describe here a method for direct intraendometrial transfer (DIET) of early cleaved embryos in humans and report our preliminary results using this technique.

Materials and Methods

Patients and Protocols

Fourteen women with only one or with no previous IVF/ET attempt were included in the study. The mean (± SD) age was 29.3 (± SD) years. The indications for IVF were male factor infertility (n = 11), unexplained infertility (n = 2) and mechanical infertility (n = 1). All patients were informed of the procedure and gave written consent in accord with out Institutional Ethics Review Board.

For ovarian stimulation, patients were injected with two ampules of follicle-stimulating hormone (Metrodin, Teva, Petah Tiqva, Israel) and two ampules of human menopausal gonadotrophin (hMG, Pergonal, Teva) on days 3 and 4 of the cycle. This was followed by injection of two to three ampules of hMG on subsequent days. In 3 patients, gonadotrophin therapy was preceded by mid-luteal injection of gonadotrophin-releasing hormone (GnRH) analogue (*D*-Trp-6-LHRH, Decapeptyl 3.75 mg; Ferring, Melmo, Sweden) to obtain pituitary suppression (as measured by serum estradiol levels <140 pmol/l).

Human chorionic gonadotrophin (hCG, Chorigon, Teva) 10,000 IU was given when at least one of the follicles had reached an average diameter of 16–18 mm. In 2 patients with excessive response to gonadotrophin stimulation, GnRH analogue (Decapeptyl 0.2 mg, s.c.) was administered instead of hCG, to induce a preovulatory LH surge and to prevent the ovarian hyperstimulation syndrome [10]. Oocytes were retrieved transvaginally 36 h after hCG or GnRH analogue injection. Routine IVF procedures were used in 3 cases with mechanical or unexplained infertility, and intracytoplasmic sperm injection [11] was employed in 11 cases in which male factor was the cause of infertility.

Human tubal fluid medium (HTF, Irvine Scientific, Irvine, Calif., USA) plus 10 and 15% synthetic serum substitute (Irvine Scientific) were used for oocyte and embryo culture, respectively. Cleaved embryos were transferred into the endometrium 42–48 h after insemination. Excess embryos were frozen and kept in liquid nitrogen for later use.

The luteal phase was supported with hCG 2,500 IU administered on days 2, 5 and 8 after follicle aspiration. In 2 high responder patients who received GnRH analogue instead of hCG, the luteal phase was supported with daily injection of progesterone in oil 50–100 mg (Gestone, Paines & Byrne, Greenford, UK) and oral estradiol valerate 2 mg (Progyluton, Schering AG, Berlin, Germany) beginning 1 day after ET.

Transfer Technique

Patients received diazepam 5 mg i.v. (Teva, Petah Tikva, Israel) for sedation prior to embryo transfer. The procedure was performed with a CO_2-pulsed 3.6-mm flexible hysteroscope (Olympus, Hamburg, Germany). The hysteroscope was first introduced into the uterine cavity without cervical dilatation to the level of the isthmus. The uterine cavity was expanded and visualized, the tip of the hysteroscope was advanced to the site of endometrial injection and the insufflation gas was turned off. At that time, the transfer catheter (90 cm in length and 0.8 mm in diameter; Vygon, Ecouen, France) was first connected to a 1-cm^3 syringe and filled with culture medium (HTF plus 15% synthetic serum substitute). The embryos were loaded into the tip of the catheter. No air bubbles separated the embryos from the column of culture medium. To enable an easier penetration of the catheter into the endometrium, its tip was beveled to 45°.

The insufflation gas was turned on and the transfer catheter was introduced into the common operating channel of the hysteroscope. The tip of the catheter was inserted beneath and in parallel to the endometrial mucosa to a distance of 5–15 mm. The culture medium containing the embryos was slowly injected into the endometrium, creating a clearly identified small elevation at the site of injection. The catheter was slowly withdrawn and the site of endometrial penetration was observed for approximately 10 s. The injection site was carefully observed for any backflow during injection and immediately thereafter. The insufflation gas was turned off and the hysteroscope was removed. The catheter was carefully checked for any remaining embryos. The patient was observed for 3 h and then discharged.

Results

Intraendometrial injection of the embroys was successfully accomplished in all patients. No cervical dilatation was required to introduce to hysteroscope into the uterine cavity. Except for some discomfort and mild lower abdominal pain, the procedure was well tolerated by the patients. In 13 cases the embryos were injected into the posterior wall and in 1 patient with retroverted uterus, into the anterior wall of the uterus at the fundal region. The volume of culture medium injected was 50–80 µl in the first 6 patients and 30 µl in the remaining 8 patients. Some fluid leak from the site of injection was noted in 2 patients from the first group and in 1 patient with 30 µl culture medium injection. None of them conceived.

The mean (± SD) number of embryos transferred was 3.14 ± 0.36 embryos per patient. Three embryos were transferred in 12 patients and 4 in 2 patients. A total of 44 embryos were injected into the endometrium, resulting in 2 clinical pregnancies (14.3% per cycle) and 2 chemical pregnancies. The overall implantation rate (number of sacs/number of embryos) was 4.5%.

In 1 patient, amniocentesis and chromosome analysis revealed monosomy X and the pregnancy was terminated at 18 weeks. The 2nd woman had an uneventful pregnan-

cy and delivered spontaneously at term a 3,065-gram healthy baby girl (October 1995). In both cases, the embryos were injected into the endometrium underlying the posterior wall of the uterus and the placenta was later located in this region.

Discussion

In this study, we have demonstrated that DIET can be accomplished successfully, resulting in pregnancy, but the implantation rate following DIET of 2-day embryos is low, less than half of the expected implantation rate in our IVF/ET program. Among possible causes contributing to the lack of implantation of a potentially viable embryo after a conventional ET are (1) failure of the embryo to adhere to the endometrial mucosa, resulting in expulsion of the embryo from the uterus or its implantation at an ectopic site (e.g. tubal pregnancy), and (2) failure of the hatched embryo to lyse the endometrial epithelium and invade the endometrial stroma. The transplantation of embryos under the uterine epithelium can theoretically increase implantation rate by preventing the expulsion of the transferred embryos from the uterus and also by eliminating the possibility of implantation at ectopic sites. It may also assist the embryo in the process of implantation because it bypasses the first steps of normal implantation, including adhesion of the hatched embryo to the endometrial lining and lysis and invasion of the endometrial epithelium. DIET may allow selection of the optimal site for embryo implantation and placentation, thereby avoiding less favorable sites such as endometrium underlying submucous myoma, scar tissue, uterine septum, or lower uterine segment. Choosing the optimal site for implantation may theoretically not only increase implantation rate and reduce abortion rate, but may also decrease the incidence of placenta previa and abnormal placentation (e.g. placenta accreta).

In our study, 2-day embroys were transferred into the endometrial stroma. In their new environment the embryos developed to the blastocyst stage while residing in the stroma instead of in the uterine cavity. It is possible that the uterine stroma does not provide an optimal milieu for early embryonic development, accounting for the low pregnancy rate (14.3%) and implantation rate (4.5%) observed in our study. In their study in the mouse, Nakayama et al. [7] have transplanted blastocysts intraendometrially, resulting in normal living offspring at a similar rate to conventional transfer. It remains to be determined whether DIET of blastocyst-stage human embryos will result in higher implantation rate than 2-day-old embryos.

Another possible explanation for the low implantation rate after DIET in the present study is the acidifying effect of CO_2 used for insufflation on endometrium and transfer media. The use of N_2 instead of CO_2 to expand and visualize the uterine cavity may circumvent this problem.

In summary, this study suggests that embryo adhesion and lysis of the endometrial epithelium are not essential for establishing pregnancy in humans. Further research is necessary to determine the cause(s) of the low implantation rates after DIET of early-stage human embryos. Presently, DIET can be considered only in cases where the implantation site needs to be precisely determined.

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