Chapter 6

SubEndometrial Embryo Delivery (SEED) with Egg Donation – Mechanical Embryo Implantation

Michael Kamrava and Mei Yin

Additional information is available at the end of the chapter

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1. Introduction

Egg quality at retrieval in IVF cycles is one of the prime prognostic factors of a successful outcome in IVF cycles. Thus egg donors provide a unique opportunity for assessing the feasibility of new protocols and techniques. In these situations, where the primary reason for resorting to IVF is peri/post menopausal state of the woman, using egg donors assures that at least the quality of the eggs are optimum, and most often the sperm quality, embryo quality at transfer, the recipient's uterus and endometrial condition are not adversely affected.

In patients undergoing *in vitro* fertilization (IVF) procedures one major set of hurdles, which often prevents healthy embryos from resulting in pregnancies, are problems associated with endometrial receptivity and implantation (1-4). From a clinical practice perspective in our new age of pre-implantation diagnosis and screening, the embryo transfer process may now be regarded as a rate limiting factor. Various techniques for embryo transfer (ET) have been advocated to increase pregnancy rates while reducing side effects from the procedure, such as lost embryos and ectopic pregnancies (5-7, 48). In addition, the advantages of using different catheters have been debated (8-11). These methods, however, use a "blind" technique of catheter introduction into the uterus. Since the embryo(s), having the zona pellucida at time of transfer, floats in the uterine cavity between one to three days from the time of transfer, the problems of "lost embryos" and the occurrence of ectopic pregnancies persist. We have hypothesized that the mechanical insertion of the blastocyst into the endometrium under direct visualization would increase the implantation and clinical pregnancy rate of IVF. The aim of this study was to re-investigate the potential of sub-endothelial ET, a procedure which originated from early mouse experiments (10) and in humans in the mid to late 1990's (12, 13) via trans-abdominal approaches. In contrast to these earlier investigations we propose to use hysteroscopy as a less invasive, visually confirmed, precise and reliable technique to direct and effect the implantation procedure.

2. Materials and methods

2.1. Patients

The study was approved by local review board at West Coast IVF Clinic, Inc. and a fully informed consent was obtained from all patients. There were 21 consecutive patients between 34-50 years of age with a diagnosis of peri/postmenopause or premature ovarian failure with or without tubal disease. They underwent 24 fresh IVF cycles in this study. Controlled ovarian hyperstimulation was initiated with follitropin β® (Follistim®, Organon Pharmaceuticals, Inc.). Premature surge of endogenous gonadotropins were controlled with ganirelix acetate (Antagon®, Organon Pharmaceuticals, Inc.). Oocyte retrieval was carried out in an office setting under local anesthesia and mild sedation. Embryo culturing was performed using sequential media (G1 and G2; Vitrolife, or Early Cleavage Medium® supplemented with SSS and Complete Multiblast Medium® with SSS; Irvine Scientific, USA) to day five or six. Up to 2 grade 1 expanded/hatching blastocysts were transferred (Fig 1A). Recipients were down regulated with long acting GnRH analog (Leuprolide acetate Depot, Abbott, USA). The endometrium was primed with Estradiol 2 mg tid until the day of donor egg retrieval, when it was continued or reduced to 1 mg tid. Luteal support was maintained with Progesterone in oil IM 50-100 mg/progesterone vaginal tablets (Endometrin®, Ferring, USA), 100 mg tid. until the day of Pregnancy test. If the test was positive progesterone was continued through the 8th week of pregnancy or sooner until a rise in serum progesterone was noted as the pregnancy progressed.

Serum human chorionic gonadotropin (hCG) was quantified on the tenth or eleventh day after SEED was performed on day six or five after retrieval, respectively. Although the assay sensitivity for detection of hCG was at 2 IU/ml a concentration of >5 IU/ml was used for confirmation of pregnancy.

2.2. Description of hysteroscopic implantation

A lightweight flexible mini- hysteroscope (Storz™) was used for visualization of the endometrial cavity (Fig 1D). The scope incorporates a flexible distal end of 3mm in diameter with a straight through operating channel. In addition, the optic filter is directly connected to a light source, decreasing the weight of the scope. Nitrogen gas instead of CO2 is used for uterine distention. Nitrogen gas is inert and is used in the trimixture of Nitrogen, Oxygen and Carbon Dioxide utilized for embryo culture in an IVF laboratory. Gas pressure is set at max 70 mm mercury (HG). A maximum of 50 cc of gas is used

during the entire procedure. The transfer catheter is polycarbonate based with a tapered tip (to 500 μm), beveled to 45-60° (Initially made by Cook OB/GYN™, Spencer, Indiana, USA and subsequently made by Precision Reproduction, LLC Los Angeles, CA 90212 USA). The catheter is inserted to a distance of 0.5cm horizontally and to a depth of approximately 1mm below the surface of the endometrium, and 2 cm away from the junction of tuboendometrial border as observed hysteroscopically where the endometrium is thickest as seen through the

hysteroscope. The embryo(s) is deposited under direct hysteroscopic visualization (Fig 1D) using a 100 μl Hamilton syringe (Hamilton Company; Nevada, USA). No more than 2 embryos were implanted at any one site.

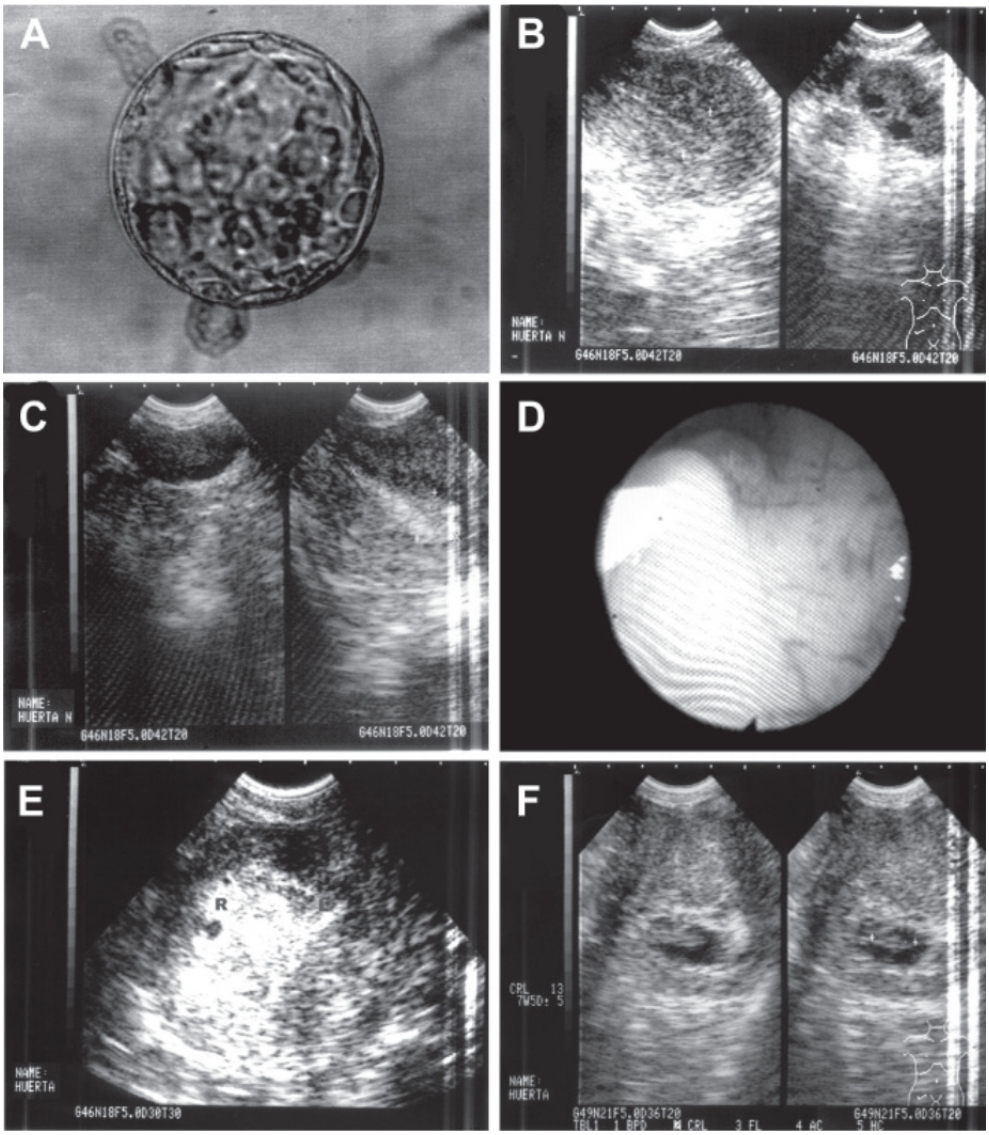

Figure 1. Stages of subendometrial embryo transfer. Expanded hatching blastocyst (A); estrogenic endometrium (B); progestational endometrium (C); subendometrial embryo transfer (D); early gestational sac at 5 weeks (E); fetus at 6 weeks (F).

3. Results

In this series, 24 IVF cycles in 21 patients were completed. Endometrial thicknesses varied between 7 and 16mm by transvaginal ultrasound. There were sixteen positive βhCG's at levels greater than 5 IU/ml. There were five biochemical pregnancies, and eleven clinical pregnancies as evidenced by the presence of a gestational sac (Fig 1E) visualized by ultrasound examination at five weeks of gestation and heart beat at six weeks of gestation (Fig 1F). There were 5 spotaneous abortions. Healthy babies were delivered by seven patients. No ectopic pregnancies (tubal, placenta previa, cervical, or heterotopic) were seen (Table 1). There were 4 twins from day five and none from day 6 implantations.

	Day 5 Implantation	Day 6 Implantation	Combined D5 and D6
Patient starts	14	10	24
Total Pregnancy/Start	8(57%)	8(80%)	16(67%)
Biochemical Pregnancy	2	2	4
Ectopic Pregnancy	**0**	**0**	**0**
Spotaneous Abortions	2	3	5
Multiple Pregnancy	4	0	4
Live/Start	4 (29%)	3 (30%)	**7 (29%)**

Table 1.

4. Discussion

Various techniques and technologies for ET have been proposed since the introduction of IVF. This list includes ultrasound-controlled transcervical intrauterine transfer or transmyometrial transfer and more invasive procedures, often referred to as surgical ET, which include: gamete intra-fallopian transfer (GIFT), zygote intra-fallopian transfer (ZIFT), pronuclear stage transfer and embryo intrafallopian transfer (EIFT) (14-17). Although ultrasound guided ET was desired to improve successful pregnancy outcomes and reduce side effects, it has been received with mixed results (18-32). It also requires simultaneous coordination of two professionals, the physician who performs the transfer and the ultrasonographer (29). Furthermore, all transcervical and transmyometrial techniques involve "blind" introduction of the embryo(s) via transfer catheters with no real time flexibility of the tip of the transfer catheter and subsequent release of embryo(s) onto the surface of the endometrium. As a result if the embryo fails to adhere, due to some luteal phase defect or other, undefined "implantation window" problem, there is a significant risk that the embryo might be washed out of the cervix or become lodged in the fallopian tubes. In part, to compensate for this potential conceptus loss, physicians have adopted the practice of transferring higher numbers of embryos back to the uterus. Here we re-investigate the potential of surgical implantation of embryos developed to the blastocyst stage *in vitro* by day 5 or 6 post insemination. It does appear that this procedure may enable

circumvention of those problems associated with the maternal receptivity aspect of the so called "window of implantation"(4). Under normal, non-assisted, circumstances, implantation begins six to seven days post ovulation. It involves multiple steps which can be summarized as pre-attachment, attachment-invasion, and decidualization - early placentation (33, 34). The reader is referred to a recent paper by Dominguez et al. (2) for a comprehensive review. Thus far, mechanisms for repairing defects in this process or clinically relevant markers of uterine receptivity have proven elusive. Similarly to the now well-accepted procedure of ICSI (35), where a single sperm is mechanically injected into an oocyte, with the development of this project we aim to develop an instrument and procedure whereby "mechanical" implantation of the embryo is achieved.

Ectopic Pregnancies after IVF specially for tubal disease account for approximately 8-10% of pregnancies (7, 48). Hysteroscopic SEED minimizes the chances of "losing" the embryo, and virtually eliminates ectopic pregnancies (tubal, placenta previa, cervical, or heterotopic) from embryo transfer, as the embryo(s) is embedded into the endometrium and not floating in the uterus. Using the flexible mini-hysteroscope affords an objective and accurate confirmation of the placement of the embryo that should make the procedure replicable, and thus more reliable with more consistent and improved results. Allowing the embryos to reach the blastocyst stage prior to transfer is gaining more acceptance (37-39). It allows both for more normal embryos to be naturally selected and for a more accurate selection of more viable, healthier embryo(s) (40-42). Thus a less number of embryos can be selected for transfer with more certainty for a successful singleton pregnancy (43, 44). This is congruent with the results in this study where there were no multiple pregnancies from day 6 implantations (Table 1).

A previous report on the use of SEED technique documented a promising set of results in patients with a variety of reasons for IVF (36). In this report we wanted to focus on a specific group of patients to better define the role of SEED technique. An overall pregnancy rate of 67% with a live birth rate of 29% was achieved. This is consistent with treating a better prognostic group of patients, i.e. egg donors in contrast with a non selective group of patients (36).

A possible drawback with the transcervical hysteroscopic embryo implantation (SEED) is the potential to scratch the endometrium and trigger some deleterious effect. Yet this is a potential hazard of "blind" procedures as well. The risk of disruption of the uterine lining, however is postulated to be less than "blind" and ultrasound guided transfers due to the advantage of direct visualization of the uterine lining and not requiring movement of the catheter to facilitate identification during ultrasound (32). As opposed to rigid endoscopes which may cause trauma to the uterus, the hysteroscope used in this study is a mini-hysteroscope with a 3mm diameter and flexible tip that allows one to easily follow the curvature of the uterus. With this protocol, though, the physician may then choose a non-scratched portion of the endometrium for implantation. Having said that, a growing number of literature suggests that mild inflammation may very well facilitate, if not be required for implantation and placentation (45-47).

Likewise, visualizing implantation allows for the physician to avoid losing embryos due to intrinsic uterine contractions or those brought on by the transfer, enabling the physician to defer the procedure until the enhanced activity has subsided. Furthermore, visualization allows one to place the embryo at a different location if trauma ensues. Also, the catheter used is semi-rigid to prevent kinking as it passes through the endoscope yet with enough flexibility to bend with the endoscope however bend and become kinked to prevent inadvertent passage into the myometrium. In addition, the uterine cavity is allowed to be distended during introduction of the hysteroscope into the uterus by slow passage through the endocervical canal. This would allow the hysteroscope to move in a gaseous space and not in direct contact with the endometrium as is the case with the blind procedure. In our study, no disruption to the uterine lining or uterine bleeding occurred. Increased cost is another drawback, however utilizing a hysteroscope with an objective replicable procedure that improves results will decrease the costs from multiple failed IVF-ET attempts and improve patient satisfaction.

5. Conclusion

We suggest that using a hysteroscopic subendometrial embryo delivery (SEED) for transferring advanced blastocyst(s) is a reasonable and effective method of embryo transfer. It will virtually eliminate ectopic pregnancies of all locations, i.e. tubal pregnancies as well as placenta previa, cervical, and heterotopic pregnancies, from IVF. Furthermore, it would allow for a targeted objective, reliable, safe and replicable method for single embryo transfer, as new and improved techniques along with modified media for handling, culture, and selection of embryos are introduced. This would greatly alleviate the anxiety, and cost to the patient as it decreases the number of attempts at using IVF in achieving a successful singleton pregnancy.

Author details

Michael Kamrava and Mei Yin
West Coast IVF Clinic, Inc., Beverly Hills, California, USA

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