Direct Gamete Uterine Transfer in Patients with Tubal Absence or Occlusion

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Purpose: Our aim was to examine the potential of the uterine cavity to affect fertilization and early embryo development.

Design: A prospective IRB-approved protocol for patients fulfilling study eligibility criteria was used.

Methods: Patients studied included those with primary or secondary infertility, aged less than 38 years, with no history of severe male-factor infertility, and with hysterosalpingogram- and laparoscopic-confirmed bilateral proximal tubal occlusion. Superovulation induction was accomplished with a combination of GnRH agonist and menotropins, with serum hormonal and sonographic monitoring. Within 24 hr prior to, and again at the time of, ovulatory hCG administration, progesterone (P4) was given. Sonographic-guided transvaginal retrieval was performed 35 hr after hCG. Between four and six oocytes were returned to the uterine cavity, admixed with sperm, immediately following retrieval. Luteal support consisted of daily P4 administration.

Results: Of the 20 patients recruited for the study, all completed the retrieval and transfer procedure. A total of four clinical pregnancies was achieved, with one early first-trimester loss, one late first-trimester loss (Trisomy 14), and two healthy term infants delivered. IVF of surplus oocytes demonstrated a 82.5% fertilization rate and 66.7% cleavage following cryopreservation.

Conclusions: Human fertilization can be achieved through direct uterine transfer of gametes. Furthermore, administration of P4 prior to the ovulatory dose of hCG is compatible with in vitro or in vivo fertilization and implantation.

KEY WORDS: gamete uterine transfer; preovulatory progesterone; tubal occlusion.

INTRODUCTION

With the sometimes prohibitive expense of ART procedures, it is important to assess modifications of IVF-ET approaches which may allow for the development of more cost-effective models and to gain insight into biological systems. In 1982, Craft *et al.* were the first to report on the direct transfer of sperm and oocytes into the endometrial cavity with a resulting pregnancy (1). This approach has certain advantages over in vitro fertilization, namely, avoidance of a 2-day procedure and simplification of laboratory involvement, as fertilization and culture are not required. Nevertheless, few reports have explored further direct gamete uterine transfer (GUT). For this reason, we attempted to modify the approach of direct gamete transfer into the uterine cavity and report our experience.

MATERIALS AND METHODS

All patients were recruited from the Division of Reproductive Endocrinology and Fertility to participate in this IRB-approved study. Patients selected were less than 38 years of age, had cycle day 3 FSH levels less than 20 IU/L, and had either surgically absent fallopian tubes or bilateral proximal tubal occlusion confirmed by hysterosalpingogram and laparoscopy. Additionally, semen analyses must have demonstrated a minimum of 20 million sperm/ml, 40% motility, and 40% normal morphology; donor semen recipients were excluded.

Once screened and informed consents had been signed, patients were begun on daily leuprolide acetate (Lupron; Tap Pharmaceuticals, Chicago, IL), 1.0 mg

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sc, in the midluteal phase of the cycle preceding the GUT procedure. On the third day of menses, patients had their serum 17 β estradiol (E_2) measured and a transvaginal ultrasound performed. Provided that E_2 levels were less than 180 pmol/L (conversion, 3.671 pmol/L = 1.000 pg/ml) and there were no ovarian cysts detected by ultrasound, the leuprolide dose was halved and human menopausal gonadotropins (hMG; Pergonal, Serono Laboratories, Randolph, MA) or urofollitropins (Metrodin; Serono Laboratories) were given by daily im injection. Cycle monitoring has been described previously (2). Briefly, serum measurements of E_2 were performed on alternate days. Gonadotropin dosing was adjusted to individual response. Once a linear rise of E_2 was noted on a semilog plot and E_2 was between 1100 and 2900 pmol/L, a transvaginal sonogram was performed to evaluate follicular size and number. With at least two follicles measuring 19 mm or larger, 10,000 IU human chorionic gonadotropin (hCG; Profasi; Serono Laboratories) was given to induce ovulation. Between 6 and 24 hr prior to hCG administration, 50 mg of im progesterone (P4) was given, and again at the time of hCG.

Thirty-five hours after administration of hCG, patients underwent transvaginal ultrasound-guided oocyte retrieval under iv sedation. Once both ovaries were harvested completely, four to six oocytes demonstrating the greatest maturity and quality were mixed with 500,000 sperm in a transfer volume of 80 μ l. Transfers were performed immediately via Tomcat catheter. Patients remained recumbent for 3 hr following the transfer and were discharged home to remain at bedrest for an additional 24 hr.

Surplus oocytes underwent in vitro fertilization and cryopreservation if the couple consented. Cryopreservation was performed at the pronuclear cell stage. Subsequent thaw and embryo transfer were performed following confirmation of cell division.

Luteal support consisted of continued daily P4 injections. All patients obtained a quantitative β -hCG 13 days following the procedure and again 2 days later. If positive, the β -hCG determination was repeated within 2 to 4 days, and with an appropriate rise, a transvaginal sonogram was scheduled 5 to 6 weeks following the GUT. If the rise was less than 100% or if the patient experienced vaginal bleeding or cramping, β -hCG determinations were repeated more frequently and an earlier ultrasound was performed. Pregnancies were followed until the outcome was known.

Cycle performance parameters included determination of the peak E_2 , defined as the serum E_2 measured

within 24 hr of hCG administration, and the number of dominant follicles, defined sonographically as those with mean diameters greater than or equal to 16 mm 24 hr prior to hCG administration. Data regarding surplus oocyte fertilization rates following IVF and thaw-survival are also presented.

Results are presented as means $\pm$ standard deviation.

RESULTS

A total of 20 patients was recruited for the study. The mean age of the patients enrolled was 32.1 ± 3.4 years, the mean parity was 0.15 ± 0.36 , and the mean cycle day 3 FSH was 10.8 ± 3.3 IU/L. Cycle performance parameters included the following: peak E_2 , 3869 ± 1193 pmol/L; number of dominant follicles, 8.2 ± 3.9 ; and number of oocytes recovered, 9.8 ± 4.2 . A mean number of 5.2 ± 0.7 oocytes was transferred per case.

Four clinical pregnancies were achieved among the 20 patients completing the study. One ended in early first-trimester loss, one in late first-trimester abortion (Trisomy 14), and two pregnancies were delivered at term as healthy singletons. There were no multiple gestations.

Of the 20 study patients, two couples declined cryopreservation and five couples did not have surplus oocytes for IVF. Among the remaining 13 couples, a total of 80 oocytes was sent for IVF; 66 (82.5%) demonstrated fertilization, of which 6 (9.1%) were polyspermic. The 60 remaining cells were cryopreserved at the pronuclear stage. To date, a total of 45 embryos has been thawed for transfer and 30 (66.7%) have demonstrated cleavage. Those embryos which did not survive the freeze-thaw process demonstrated broken zonae in four cases, nonviable cytoplasm in eight cases, degeneration in culture in one case, and failure to cleave in two cases. Subsequent frozen embryo transfers have yielded no pregnancies.

DISCUSSION

While human fertilization normally occurs in the ampullary portion of the fallopian tube, intrauterine conception has been shown possible. The Estes procedure, which described implantation of the cut surface of a single ovary into the resected stump of an oviduct and uterine horn, allowed ovulation to occur directly into the uterus and, thereby, achieve pregnancy (3).

Pregnancy rates following this procedure were disappointingly low and leave its mention for historic interest only.

The current study confirms that the endometrial cavity has the capacity to allow sperm and oocytes to fertilize and develop into a viable pregnancy. Previous experience with direct gamete uterine transfer has been very limited (1, 4–9). Because not all authors selected patients with confirmed bilateral tubal occlusion, fallopian tube contribution to pregnancy was not excluded in all reports (1, 6–9). Our study limited patient participation to those with confirmed bilateral tubal occlusion to avoid the question of possible retrograde gamete transfer into the fallopian tube, where fertilization might occur, or ovulation of a nonaspirated follicle and its subsequent insemination resulting in pregnancy.

The literature numbers a total of 155 cases completed, which have resulted in 24 clinical pregnancies [pregnancy rate (PR), 0.155], with 5 ending as first-trimester losses (0.208 spontaneous abortion rate), 1 ectopic gestation (0.042 ectopic rate), and 18 designated as ongoing or delivered (0.116 per cycle) (1, 4–9).

The pregnancy rate of DOST/GUT (direct oocyte-sperm transfer/gamete uterine transfer) has been low yet fairly consistent among studies. Some groups have directed their attempts to improve pregnancy rates by brief periods of coincubation of sperm and oocyte prior to transfer to allow for gamete binding (1, 4, 7–9). There appears to be no significant advantage, however, to coincubate gametes prior to transfer. Other modifications have included stripping the cumulus away to expose the zona and presumably facilitate sperm binding (1, 8, 9). This, too, has not had a significant impact on success. Our study did not utilize a coincubation period but, rather, transferred gametes immediately following retrieval. The presumed benefit is a reduction in extracorporeal manipulation of the gametes.

Our approach focused on attempts to influence endometrial receptivity by administering P4 prior to hCG administration. Several authors have examined P4's apparent positive effects on implantation. Ben-Nun and colleagues described the use of 50 mg P4 10 hr before the ovulatory dose of hCG in patients undergoing IVF-ET (10). The clinical pregnancy rate among those receiving P4 was 41.2% (21/62), while a pregnancy rate of 23.3% (14/74) was observed among those without preovulatory P4 administration. Importantly, these two groups did not differ in all other parameters including ovarian steroid production, oocyte maturation, fertilization, and cleavage rates (10).

Howles *et al.* administered 25 mg P4 to patients 6 hr prior to ovulatory hCG in their group of IVF-ET patients (11). These authors were unable to demonstrate a significant difference in clinical pregnancy rates between treated and untreated patients (22.5 vs 18.5%, respectively). Interestingly, two biochemical pregnancies were reported in their treated group, which led the authors to speculate that P4 administration may have permitted less "fit" embryos to implant by creating either a more receptive endometrium or production of poorer-quality oocytes (11). It is noteworthy that, unlike Ben-Nun and co-workers' induction protocol, Howles *et al.* used clomiphene citrate along with menotropin. Clomiphene's antiestrogen action has been reported to exert deleterious effects on the endometrium, and this may have contributed to their findings (12). Howles *et al.* also used a lower dose of P4 closer to the time of ovulation and some pharmacologic or temporal threshold may exist before any beneficial effect from P4 is observable. Endometrial biopsy has confirmed that glands and stroma mature at different rates and that glandular secretory changes may require longer exposure to P4 to synchronize this histologic dissociation (10, 13).

Preovulatory elevation in P4 has demonstrable effects upon the endometrium, yet its impact directly on the oocyte is less clear. Rising P4, either derived endogenously from the preovulatory follicle or administered exogenously; can elicit an endogenous LH surge (11, 14). This surge, however, does not reliably occur in all patients (11) or necessarily occur with great enough intensity to allow for both luteinization and ovulation. Inhibin-like substances also produced during superovulation (15) or individual sensitivity of the hypothalamopituitary axis to progesterone may explain differences in individual gonadotropin surge occurrence.

Progesterone can affect the oocyte membrane, as is the case with certain amphibians, and actively participate in resumption of meiosis by induction of protein synthesis (16). Edwards observed an increase in dispermic and fragmented embryos coincident with high levels of progesterone (17), though others have not supported this finding (18). Indeed, Silverberg *et al.* have reported no demonstrable adverse affect in quality of oocytes or embryos among patients with elevated P4 on the day of hCG (19). Comparing the success of frozen ETs between high- and low-P4 patients, the authors reported equal pregnancy rates and were unable to attribute the poor pregnancy rates observed during the initial IVG/ET high-P4 cycles to hormonal action on the oocyte (19). The quality of oocytes recov-

ered during our study was comparable to that of oocytes recovered during our simultaneously running IVF-ET program. Our study indicates that pre-hCG presence of P4 did not prohibit successful fertilization and implantation. Further studies will be needed to assess to what degree (if any) pre-hCG P4 administration may enhance successful fertilization.

CONCLUSIONS

In conclusion, we have demonstrated that pregnancies can be achieved by direct uterine transfer of gametes. Furthermore, a P4 rise prior to hCG does not exclude fertilization or implantation. Additionally, because both retrieval and transfer are performed during a single procedure, GUT offers greater convenience to patients over IVF-ET. Immediate gamete transfer also minimizes demands on laboratory and clinical staff, thus allowing GUT to be offered at a significant cost reduction compared to standard IVF-ET. In our facility, though similar induction protocols are used and monitoring is identical, GUT is almost 30% less expensive. Minimization of extracorporeal manipulation of the gametes also provides a treatment for couples with tubal-factor infertility, yet strong religious or ethical concerns against standard IVF. We recommend further studies to examine the role of endometrial modification and its impact on pregnancy using this model.

REFERENCES

1. Craft I, McLeod F, Green S, Djahanbakhch O, Bernard A, Twigg H: Human pregnancy following oocyte and sperm transfer to the uterus. *Lancet* 1982;1:1031-1033
2. Bohrer M, Kemmann E: Risk factors for spontaneous abortion in menotropin-treated women. *Fertil Steril* 1987;48:571-575
3. Estes WL Jr: Ovarian implantation. The preservation of ovarian function after operation for disease of the pelvic viscera. *Surg Gynecol Obstet* 1924;38:394-398
4. Veersema S, Bernardus RE, Veersema D, Vermeiden JPW, Schoemaker J: Pregnancy following transcervical transfer of oocyte and sperm. *N Engl J Med* 1989;320:1499
5. Sterzik K, Rosenbusch B, Grab D, Lauritzen C: Pregnancies following direct oocyte-sperm transfer (DOST): A simple alternative to conventional in vitro fertilization (IVF). *J Vitro Fert Embryo Transfer* 1991;8:241-242 (Letter to the Editor)
6. Kubik C, Guzick DS, Berga SL, Anthony AJ: Establishment of pregnancies in humans after transcervical transfer of gametes immediately after oocyte retrieval. *Fertil Steril* 1990;54:1174-1176
7. Campagnoli C, Brigante C, Busacca M, Di Sario MM, Doldi N, Peris C, Vigano P, Vignali M: Pregnancies following gamete intrauterine transfer (GIUT) in patients with tubal infertility. *Gynecol Endocrinol* 1991;5 (Suppl 1):40
8. Rizk B, Bekir JS, Avery S, Smith S, Edwards RG: Intrauterine replacement of oocytes with bound sperms: A pilot study. *Hum Reprod* 1990;5 (Suppl 1):5
9. Quinn P, Steinberg J, Schein S, Greenberg P: Direct ovum-sperm transfer (DOST) to the uterus as a mode of treatment following failed IUI but preceding IVF or GIFT. 44th Annual Meeting of the Pacific Coast Fertility Society, April 17-21, 1996. *Fertil Steril* (Suppl) A26.
10. Ben-Nun I, Ghetler Y, Jaffe R, Siegal A, Kaneti H, Fejgin M: Effect of preovulatory progesterone administration on the endometrial maturation and implantation rate after in vitro fertilization and embryo transfer. *Fertil Steril* 1990;53:276-281
11. Howles CM, Macnamee MC, Edwards RG: The effect of progesterone supplementation prior to the induction of ovulation in women treated for in vitro fertilization. *Hum Reprod* 1987;2:91-94
12. Gonen Y, Casper RF: Sonographic determination of a possible adverse effect of clomiphene citrate on endometrial growth. *Hum Reprod* 1990;5:670-674
13. Garcia JE, Acosta AA, Hsiu J-G, Jones HW: Advanced endometrial maturation after ovulation induction with human menopausal gonadotropin/human chorionic gonadotropin for in vitro fertilization. *Fertil Steril* 1984;41:31-35
14. Lui JH, Yen SSC: Induction of midcycle gonadotropin surge by ovarian steroids in women: A critical evaluation. *J Clin Endocrinol Metab* 1983;57:797-802
15. Nader S, Berkowitz AS, Maklad N, Wolf DP, Held B: Characteristics of patients with and without gonadotropin surges during follicular recruitment in an in vitro fertilization/embryo transfer program. *Fertil Steril* 1986;45:75-78
16. Schorderet-Slatkline S, Baulieu EE: Induction by progesterone and a "maturation promoting factor" of soluble protein in *Xenopus laevis* oocyte in vitro. *Ann NY Acad Sci* 1977;286:421-433
17. Edwards RG: In vitro fertilization and embryo replacement. *Ann NY Acad Sci* 1985;442:1-24
18. Whitman GF, Luciano AA, Maler DB, Peluso JJ: Human chorionic gonadotropin localization and morphometric characterization of human granulosa-luteal cells obtained during in vitro fertilization cycles. *Fertil Steril* 1989;51:475-479
19. Silverberg K, Martin M, Olive DL, Burns WN, Schenken RS: Elevated serum progesterone levels on the day of human chorionic gonadotropin administration in in vitro fertilization cycles do not adversely affect embryo quality. *Fertil Steril* 1994;61:508-513