Autologous Endometrial Coculture in Patients with a Previous History of Poor Quality Embryos¹

Steven D. Spandorfer,^{2,3} Larry Barmat,² Jose Navarro,² Lynn Burmeister,² Lucinda Veeck,² Robert Clarke,² Hung-Ching Liu,² and Zev Rosenwaks²

Submitted December 21, 2001; accepted February 18, 2002

Purpose: To evaluate the effect of autologous endometrial coculture in patients (less than 36 years old) with a history of a single IVF failed cycle associated with poor quality embryos. **Methods:** *Design:* Controlled clinical study. *Setting:* University-based in vitro fertilization center. *Patients:* Twenty-six patients with a history of a single prior failed IVF-ET with poor preembryo quality. *Intervention(s):* Autologous endometrial coculture. *Main outcome measures:* Preembryo blastomere numbers and cytoplasmic fragmentation rates were compared between the treatment and previous cycle. Clinical pregnancy rates were analyzed.

Results: Twenty-six women with an average age of 32.8 ± 2.9 years underwent treatment. On Day 3 the overall mean number of blastomeres per preembryo on coculture compared to conventional medium in a previous cycle was 6.1 ± 1.8 vs. 5.1 ± 1.3 ($P = 0.01$; Wilcoxon test). The average percentage of cytoplasmic fragments on coculture compared to the conventional medium in a previous cycle was $14\% \pm 10$ vs. $22\% \pm 13$ ($P = 0.003$; Wilcoxon test). At transfer the mean number of blastomeres per preembryo on coculture was 7.4 ± 1.8 compared to 6.7 ± 1.5 on conventional medium in a previous cycle ($P = 0.02$; Wilcoxon test). The clinical pregnancy rate (positive fetal cardiac activity) per patient was 88.5%. The delivery rate was 73.1% (19/26).

Conclusions: There was an improvement in the preembryo quality for preembryos on autologous endometrial coculture compared to noncocultured preembryos from the same patient in a previous cycle. An excellent delivery rate was subsequently found.

KEY WORDS: endometrial; preembryo quality; coculture.

INTRODUCTION

The success after IVF depends on many factors. Maternal age is the most important variable that has been described. Another of the more critical factors involves the quality of the in vitro culture conditions. It is thought that in vitro development of preembryos is inferior to in vivo conditions (1–3). Various attempts at improving in vitro culture conditions by

modification of media contents have met with limited success (4,5).

We have developed a unique coculture system utilizing the patient's own endometrial cells and successfully applied this to our clinical IVF-ET program. In a previous randomized trial we have demonstrated the significant beneficial effects of autologous endometrial coculture for the growth of human preembryos. Preembryos grown in autologous endometrial coculture were of better quality (more blastomeres, less cytoplasmic fragmentation) than were those grown in conventional media (6).

In an attempt to remove the affect of age and to isolate the ability of autologous endometrial coculture to improve preembryo development, we attempted to

¹ Presented at the 55th meeting of American Society for Reproductive Medicine, Toronto, Canada, October 1999.

² The Center For Reproductive Medicine and Infertility, The New York Hospital, Cornell Medical Center, 505 East 70th Street HT340 New York, New York 10021.

³ To whom correspondence should be addressed.

study only young women (less than the age of 36 years) with normal ovarian reserve. We also restricted this study to patients that only received embryos grown in autologous endometrial coculture. In this study, we report our findings of human preembryo development on autologous endometrial coculture in patients under the age of 36 years with a single, previous failed IVF attempt associated with poor preembryo development.

MATERIALS AND METHODS

Twenty-six patients under the age of 36 years between January 1998 and June 1999 with a history of one previous failed IVF-ET attempt with poor preembryo quality at The Center for Reproductive Medicine and Infertility of The New York Hospital, Cornell Medical Center, were entered into this study. Poor preembryo quality was defined as preembryos <6 cells or preembryos <Grade 2 morphology at transfer on Day 3(7). The couples were treated with IVF-ET for a variety of infertility disorders. Approval for the clinical application of coculture was obtained from the New York Hospital, Cornell Medical Center's Institutional Review Board, and all patients had given their informed consent to participate in this study.

A luteal phase endometrial biopsy was performed in a cycle prior to the patient's IVF procedure using a Pipelle Endometrial Suction Curette (Unimar, Wilton, CT). The sample was transferred to the laboratory in a sterile container filled with normal saline solution. The tissue was then minced into small pieces (1–2 mm³) and washed with Hank's Balanced Salt Solution (HBSS) (Gibco BRL, Grand Island, NY) to remove excess red blood cells and mucus. The HBSS was supplemented with 5,000 ug/100 mL penicillin-streptomycin (Gibco BRL, Grand Island, NY). A small portion of each endometrial biopsy was placed in 10% neutral buffered formalin solution for histologic assessment.

The detailed methodology used to digest the endometrial tissue has been previously described (8). Briefly, tissue dissociation was accomplished by four enzymatic digestion steps, using 0.2% collagenase Type 2 (SIGMA, St. Louis, MO). The separation of glands and stromal cells was accomplished by differential sedimentation at unit gravity. Tissue flasks were seeded and maintained at 37°C in 5% CO₂ air atmosphere, and the culture medium was changed every 2–3 days. After an approximate monolayer (85–95%)

was reached, the cells were released (Trypsin EDTA) and cryopreserved (glycerol and human serum in liquid nitrogen). The glandular and stromal cells were then thawed approximately the day before the administration of human chorionic gonadotropin during the patient's IVF-ET treatment cycle and seeded into a four-well tissue culture plate in preparation for preembryo placement.

Controlled ovarian stimulation protocols were implemented based on patient's previous response to ovarian stimulation, age, and Day 3 FSH (follicle-stimulating hormone) and estradiol hormonal status (8). Daily estradiol levels and transvaginal ultrasounds monitored follicular maturation. Human chorionic gonadotropin (5000–10,000 IU) was administered when at least two follicles were ≥ 17 mm followed by transvaginal oocyte retrieval 35 h later. Conventional in vitro insemination or intracytoplasmic sperm injection was performed based on appropriate indication (9). After 12–18 h of incubation, fertilization was confirmed by the identification of two pronuclei. Preembryos were then randomly allocated to growth in conventional media (Human Tubal Fluid plus 15% maternal serum) or on autologous endometrial coculture with Ham's F-10 (Gibco BRL, Grand Island, NY) supplemented with 15% maternal serum. All preembryos were maintained at 37°C in 5% CO₂ air atmosphere. Cleavage rates and morphologic appearance were assessed daily. Selective assisted hatching was performed as previously described (10). The morphologically best preembryos were transferred back to the patient 72 h after retrieval irrespective of culture system. In this study, only women undergoing an embryo transfer with embryos grown on autologous endometrial coculture were included. After preembryo transfer the coculture cells were fixed with 4% paraformaldehyde. Immunostaining of the coculture cells using a monoclonal antipancytokertin antibody (Sigma, St. Louis, MO) demonstrated approximately 25–50% glandular epithelial cells.

The Wilcoxon matched pairs test was used to compare differences between the cocultured and conventionally cultured preembryos. All values are reported as mean $\pm$ standard deviation. A *P* value of <0.05 was considered statistically significant. Implantation rate was defined as the number of intrauterine sacs with fetal cardiac activity per number of preembryos transferred. Clinical pregnancies included only those pregnancies with a fetal heartbeat documented on transvaginal ultrasound by Day 49.

Table I. Cycle Characteristics of the Study Patients

	Coculture cycle	Previous cycle with conventional medium	Wilcoxon test
Peak estradiol (pg/mL)	1649 $\pm$ 688	1622 $\pm$ 486	NS
Number of oocytes	12.9 $\pm$ 4.5	13.5 $\pm$ 3.4	NS
Fertilized oocytes (2PN)	8.8 $\pm$ 1.5	8.4 $\pm$ 2.1	NS
Preembryos for ET	3.4 $\pm$ 0.5	3.3 $\pm$ 0.7	NS

RESULTS

Twenty-six women aged 32.8 ($\pm$ 2.9) years underwent treatment. They each had failed a single IVF-ET attempt associated with poor preembryo development utilizing conventional treatment. The couples suffered from a variety of infertility disorders: idiopathic ($n = 4$), tubopertioneal ($n = 6$), anovulation ($n = 2$), endometriosis ($n = 2$), and male factor ($n = 12$).

Table I compares the cycle characteristics of the previous cycle as compared to the treatment cycle. No significant differences were found between the cycles in relation to stimulation or number of embryos transferred.

On the 3rd day after oocyte retrieval, the overall mean number of blastomeres per preembryo on coculture compared to conventional medium in a previous cycle was 6.1 ± 1.8 vs. 5.1 ± 1.3 ($P = 0.01$; Wilcoxon test). The average percentage of cytoplasmic fragments on coculture compared to the conventional medium in a previous cycle was $14\% \pm 10$ vs. $22\% \pm 13$ ($P = 0.003$; Wilcoxon test). At transfer, the mean number of blastomeres per preembryo on coculture was 7.4 ± 1.8 compared to 6.7 ± 1.5 on conventional medium in a previous cycle ($P = 0.02$; Wilcoxon test). In the women that had embryos grown in both autologous endometrial coculture and conventional medium, the embryos (prior to assisted hatching) grown in autologous endometrial coculture had more cells and less fragmentation (6.1 ± 1.8 vs. 4.9 ± 1.3 cells; $14\% \pm 10$ vs. $22\% \pm 15$).

The overall pregnancy rate was 92.3% (24/26) and the clinical pregnancy rate was 88.5% (23/26) for this group of patients. The delivery rate was 73.1% (19/26).

DISCUSSION

We have developed a unique autologous endometrial coculture system utilizing first passaged-cryopreserved stroma and glandular epithelial cells

that has demonstrated a significant improvement in preembryo quality (6,8). In this study, youthful patients (age <36 years) with a prior history of poor quality preembryos were found to have improved preembryo development and an excellent clinical pregnancy rate when utilizing autologous endometrial coculture. The preembryos placed on the patient's own endometrial cells had on average fewer cytoplasmic fragments and a greater number of blastomeres at embryo transfer in comparison to their previous failed IVF-ET attempt. It therefore appears that autologous endometrial coculture cells may provide a more suitable environment for preembryo growth than conventional medium in the patient with a prior history of poor preembryo development.

The optimal culture conditions for preembryo development is currently unknown. Studies have suggested that the ratio of sodium to potassium ions, the addition of glutamine, lack of glucose in the initial stages of cleavage, and the addition of EDTA may be beneficial in improving preembryo development (5,6,11). Unfortunately these medium are devoid of the growth factors secreted by reproductive tract cells and lack the potential paracrine involvement between the preembryo and reproductive tract cells. In other studies, we have demonstrated significant correlation with preembryo development and clinical pregnancy rate with the ability of autologous endometrial coculture to elaborate cytokines including GM-CSF, IL-1, and LIF (12–14). It appears that the autocrine and paracrine interactions between the preembryo and its culture environment may play a role enhancing preembryo quality and possibly improving pregnancy rates in IVF-ET.

A number of studies have evaluated the effect of various somatic cell lines on human preembryo development demonstrating improved preembryo development when utilizing coculture systems (15–18). Most of these studies have utilized nonhuman cell lines. In our previously reported controlled study, the patients' oocytes were randomly allocated to growth on autologous endometrial coculture or conventional medium. The fragmentation rate was significantly less for the preembryos in the coculture system compared to noncocultured preembryos. Also at the time of preembryo replacement, the mean number of blastomeres per preembryo was greater for the cocultured preembryos compared to the noncocultured preembryos. Furthermore, these results are consistent with our previous study where patients with multiple failures of IVF-ET demonstrated improvements in preembryo quality on autologous endometrial coculture

compared to their previous noncultured cycle (8). Although the literature predominately suggests improved preembryo development on feeder cell lines, there are some studies which have not demonstrated differences in mean cell numbers per preembryo or blastulation rates on coculture compared to control medium (17,19).

Importantly, we were able to demonstrate a clinical pregnancy rate of 88.5% per retrieval in this group of patients and a delivery rate of over 70%. We chose to study this group of patients, as they were more likely to produce enough oocytes that a lack of preembryos available for transfer was not thought to be a major reason for the failure of the previous attempt. The major limitation of this study is its lack of randomization for clinical pregnancy rates. Perhaps, what was learned from the previous IVF cycle may have benefited these patients and accounted for their improved success. A randomized controlled trial will be better able to clarify this issue.

This study suggests that in vitro culture conditions may be enhanced with the use of autologous endometrial coculture as reflected in improved preembryo quality. Further research is necessary to determine if this coculture system is best for patients with a single, previous failed IVF attempt with poor quality preembryos. A randomized trial is planned to address this issue.

REFERENCES

1. Erbach GT, Lawitts JA, Papaionnou VE, Biggers JD: Differential growth of mouse preimplantation embryo in chemically defined media. *Biol Reprod* 1994;50:1027-1033
2. Harlow GM, Quinn P: Development of mouse preimplantation embryos in vivo and in vitro. *Aust J Biol Sci* 1982;35:187-193
3. Jung T, Fischer B: Correlation between diameter and DNA or protein synthetic activity in rabbit blastocysts. *Biol Reprod* 1988;39:1111-1116
4. Telford N, Watson AJ, Schultz G: Transition from maternal to embryonic control in early mammalian development: A comparison of several species. *Mol Reprod Dev* 1990;26:90-100
5. Quinn P, Kerin JF, Warnes GM: Improved pregnancy rate in human in vitro fertilization with the use of a medium based on the composition of human tubal fluid. *Fertil Steril* 1985;44:493-498
6. Barmat LI, Liu HC, Spandorfer SD, Xu K, Veeck L, Damario M, Rosenwaks Z: Human preembryo development on autologous endometrial coculture versus conventional medium: A randomized trial. *Fertil Steril* 1998;70:1109-1113
7. Veeck LL: *Atlas of the Human Oocyte and Early Conceptus*. Baltimore, MD, Williams and Wilkins, 1991
8. Barmat LI, Liu H-C, Mele C, Xu K, Veeck L, Kowalik A, *et al.*: Autologous endometrial coculture in patients with repeated failures of implantation after IVF-ET. *J Assist Reprod Genet* 1999;16(3):121-127
9. Palermo GP, Cohen J, Alikani M, Adler A, Rosenwaks Z: Intracytoplasmic sperm injection: A novel treatment for all forms of male infertility. *Fertil Steril* 1995;63:1231-1240
10. Cohen J, Alikani M, Trowbridge J, Rosenwaks Z: Implantation enhancement by selective assisted hatching using zona drilling of embryos with poor prognosis. *Hum Reprod* 1992;7:685-691
11. Fitzgerald L, DiMattina M: Improved medium for long-term culture of human embryos overcomes the in vitro developmental block and increases blastocyst formation. *Fertil Steril* 1992;57:641-647
12. Spandorfer SD, Barmat LI, Liu H-C, Mele C, Veeck L, Rosenwaks Z: Granulocyte macrophage-colony stimulating factor production by autologous endometrial co-culture is associated with outcome for IVF patients with a history of multiple implantation failures. *Am J Reprod Immunol* 1998;40:377-381
13. Spandorfer SD, Clarke R, Bovis L, Liu H-C, Neuer A, Witkin SS, Rosenwaks Z: Interleukin-1 levels in the supernatant of conditioned media of embryos grown in autologous endometrial coculture: Correlation with embryonic development and outcome for patients with a history of multiple implantation failures after IVF. *Am J Reprod Immunol* 2000;43(1):6-11
14. Spandorfer SD, Levy D, Bagchi I, Liu H-C, Veeck L, Davis OK, Witkin SS, Rosenwaks Z: Autologous Endometrial Coculture (AECC) in Patients with IVF Failure: Correlations of Outcome with Leukemia Inhibiting Factor (LIF) and Calcitonin. *In American Society of Reproductive Medicine Annual Meeting*, October 1999
15. Thibodeaux J, Godke R: In vitro enhancement of early stage embryos with coculture. *Arch Pathol Lab Med* 1992;116:364-372
16. Wiemer KE, Hoffman DI, Maxson WS, Eager S, Muhlenberg B, Fiore I, Cuervo M: Embryonic morphology and rate of implantation of human embryos following coculture on bovine oviductal epithelial cells. *Hum Reprod* 1993;8:97-101
17. Tucker JM, Kort HI, Toledo AA, Morton PC, Wright G, Ingargiola PE, Sweitzer CL: Effect of coculture on subsequent survival and implantation of cryopreserved human embryos. *J Assist Reprod Genet* 1995;12:689-692
18. Quinn P, Margalit R: Beneficial effects of coculture with cumulus cells on blastocyst formation in a prospective trial with supernumerary human embryos. *J Assist Reprod Genet* 1996;13:9-14
19. Sakkas D, Jaquenoud N, Leppens G, *et al.*: Comparison of results after in vitro fertilized human embryos are cultured in routine medium and in coculture on Vero cells: A randomized study. *Fertil Steril* 1994;61:521-525