The Effects of Coculture with Autologous Cryopreserved Endometrial Cells on Human In Vitro Fertilization and Early Embryo Morphology: A Randomized Study

FELIX S. NIETO, WILLIAM B. WATKINS, ALEX LOPATA, H. W. GORDON BAKER, and DAVID H. EDGAR

Submitted: August 31, 1995
Accepted: November 9, 1995

Purpose: The aim of this study was to examine the influence of endometrial cells on the fertilization rate and early embryonic morphology following routine in vitro fertilization (IVF). Cryopreservation with subsequent thawing allowed the use of autologous somatic cells, thus minimizing the risk of transmission of infective agents. Interpatient variability was eliminated by randomizing oocytes from each cycle into the control or coculture group.

Results: Two hundred ninety-four oocytes from 24 IVF cycles (21 patients) were included in the study (145 coculture and 149 control). The normal fertilization rate of control oocytes (56.4%) was not significantly different from that of oocytes cocultured with endometrial cells (61.4%). The mean number of blastomeres in cocultured embryos (3.65) was not significantly different from the number in control embryos (3.46) 2 days after insemination, but the proportion of embryos with minimal or no fragmentation was significantly higher in the coculture group [34/84 (40.5%) vs. 17/80 (21.3%); P < 0.01].

Conclusions: The inclusion of cryopreserved autologous endometrial cells in routine clinical IVF procedures does not influence fertilization or the early cleavage rate but may reduce the extent of embryo fragmentation during the early cleavage divisions.

KEY WORDS: coculture; endometrial cells; in vitro fertilization; early embryo morphology.

INTRODUCTION
The failure of the majority of human in vitro fertilized (IVF) embryos to implant following intrauterine transfer may result from inadequacies in either the maternal or the embryonic components of the process. The relative contribution of impaired endometrial receptivity to failure of implantation is difficult to quantify since many of the critical features remain the subject of debate (1). Factors influencing embryo viability may be either intrinsic, e.g., chromosome abnormalities, or extrinsic, e.g., the culture environment. While it is obviously difficult to reverse the reduced viability resulting from the former, strategies aimed at reversing the effects of suboptimal culture conditions have been employed, the most notable being assisted embryo hatching (2) and coculture of embryos with somatic cells (3).

The concept that embryonic development and viability in vitro may be improved by mimicking the in vivo relationship between embryo and maternal environment has resulted in a number of reports of the use of somatic cell coculture in human IVF. Coculture of human zygotes on fetal bovine uterine fibroblasts has been reported to enhance the proportion of embryos with "good" morphology and the implantation rate in a randomized study (4), while early development was also found to be improved when Green monkey kidney (Vero) epithelial cells were cocultured with poor-quality human "spare" embryos (5). However, although it has been applied by a number of groups, the use of nonhuman somatic cells in clinical IVF is not universally acceptable and alternative approaches have therefore been adopted.

Coculture of embryos on epithelial cells derived from human fallopian tubal ampulla has been reported to improve human IVF outcome (6) and offers the theoretical advantage of closely resembling the in vivo situation. However, the routine use of homologous tissue in this context would not be practical, leaving the problem of possible contamination from heterologous
Endometrial Cells

Endometrial biopsy was carried out during the mid-luteal phase of an unstimulated cycle using an endometrial cell sampler (Gynoscann, Organon, Lane Cove, NSW, Australia). The tissue was washed in HEPES-buffered HTF medium (HTF-HEPES, Irvine Scientific, CA) containing 10% heat-inactivated patient serum before being minced into 1-mm³ fragments and incubated in 5 ml of a solution containing 300 IU of collagenase (Cl. histolyticum, Type I; GIBCO, Melbourne, Victoria, Australia) and 5 μg of deoxyribonuclease (Type I; Sigma, St. Louis, MO) per ml in Hank’s balanced salt solution (HBSS; GIBCO) for 60 min at 37°C with repeated gentle pipetting. The dissociation was terminated by the addition of 1 ml of cold patient serum and the mixture was passed through a 37-μm nylon mesh (Swiss Screens, Melbourne, Victoria, Australia). The retained epithelial tissue was washed, resuspended in the alpha modification of minimum essential medium (αMEM; Cytosystems, Castle Hill, NSW, Australia) containing 10% serum, and incubated at 37°C in a humidified atmosphere of 5% CO₂ in air in a 50-ml culture flask (Falcon, Becton Dickinson, Lincoln Park, NJ) until the resulting monolayer reached at least 50% confluence (between 24 and 72 hr following incubation). The monolayers consisted overwhelmingly of cells which exhibited an epithelial morphology, with negligible (<5%) contamination of cells with the characteristic morphology of fibroblasts.

Monolayer cultures were harvested by trypsinization (0.25%; Sigma), washed, counted in the presence of trypan blue, and diluted to a final concentration of 2 × 10⁶ viable cells/ml in Dulbecco’s phosphate-buffered saline (GIBCO) containing 10% dimethyl sulfoxide (DMSO; Sigma) and 20% serum before being dispensed in 1-ml aliquots into polypropylene vials (Nunc, Hamstrup, Denmark), wrapped in cotton wool, placed in a −80°C freezer for 2 hr, and finally, plunged into liquid nitrogen for storage.

Forty-eight hours prior to oocyte retrieval cells were thawed rapidly at 37°C, diluted with culture medium, centrifuged, subjected to one additional wash, seeded in 0.5 ml αMEM/10% serum in four-well dishes (Nunc), and allowed to reform a monolayer. The viability (by trypan blue staining) of thawed cells was always greater than 80% and the cells were seeded to ensure that monolayers were close to confluence at the initiation of coculture.

IVF Procedure

Ovarian stimulation was carried out as described previously (11). Following retrieval oocytes were allocated at random into wells containing washed monolayers of endometrial cells in 0.5 ml of αMEM/10% serum or noncoculture wells with the same medium. Monolayer cultures were always close to confluence at the start of the coculture period and the only cell division which took place during coculture was that which resulted in the transition from approximately 80% confluent to fully confluent. Following insemination with 100,000 motile sperm, oocytes were examined 15–20 hr later for the presence of pronuclei and transferred into fresh wells under similar conditions. The following day (day 2) the number of blastomeres

387