Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen–thawed embryo transfer in normal responders

Bruce S. Shapiro, M.D., Ph.D.,[a,b] *Said T. Daneshmand, M.D.,*[a,b] *Forest C. Garner, M.Sc.,*[a,b] *Martha Aguirre, Ph.D.,*[a] *Cynthia Hudson, M.S.,*[a] *and Shyni Thomas, B.Sc.*[a]

[a] Fertility Center of Las Vegas; and [b] Department of Obstetrics and Gynecology, University of Nevada School of Medicine, Las Vegas, Nevada

Objective: To compare success rates between fresh ETs after ovarian stimulation and frozen–thawed ETs (FET) after artificial endometrial preparation, to compare endometrial receptivity.
Design: Randomized, controlled trial.
Setting: Private fertility center.
Patient(s): There were 53 patients completing fresh blastocyst transfer (fresh group) and 50 patients completing FET (cryopreservation group). All were first-time IVF patients aged <41 years, with cycle day 3 FSH <10 mIU/mL and 8–15 antral follicles.
Intervention(s): Randomized to fresh or thawed ET.
Main Outcome Measure(s): Clinical pregnancy rate per transfer.
Result(s): The clinical pregnancy rate per transfer was 84.0% in the cryopreservation group and 54.7% in the fresh group. The implantation rates were 70.8% and 38.9%, respectively. The ongoing pregnancy rates per transfer (at 10 weeks' gestation) were 78.0% and 50.9%, respectively. The attributable risk percentage of implantation failure due to reduced endometrial receptivity in the fresh group was 64.7%.
Conclusion(s): The clinical pregnancy rate per transfer was significantly greater in the cryopreservation group than in the fresh group. These results strongly suggest impaired endometrial receptivity in fresh ET cycles after ovarian stimulation, when compared with FET cycles with artificial endometrial preparation. Impaired endometrial receptivity apparently accounted for most implantation failures in the fresh group. ClinicalTrials.gov Identifier: NCT00963625. (Fertil Steril® 2011;96:344–8. ©2011 by American Society for Reproductive Medicine.)

Key Words: Embryo cryopreservation, IVF, blastocyst transfer, endometrium, endometrial receptivity, ovarian stimulation

Controlled ovarian stimulation (COS) with exogenous gonadotropins is associated with altered endometrial development that may impair endometrial receptivity in cycles of IVF (1–7). One possible mechanism of impairment is advancement of the receptive phase, resulting in embryo–endometrium asynchrony. Observations supporting this asynchrony hypothesis include histologic endometrial advancement after COS (7), a negative correlation between the degree of endometrial advancement and embryonic implantation (5), up-regulated P receptor expression after COS (6), and a negative correlation between implantation and premature P elevation (8, 9). There are also reports of a positive correlation between embryo developmental pace and implantation in fresh autologous IVF cycles but not in oocyte donation cycles and cycles using frozen–thawed embryos (10, 11).

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Reprint requests: Bruce S. Shapiro, M.D., Ph.D., Fertility Center of Las Vegas, 8851 W. Sahara Avenue, Las Vegas, NV 89117 (E-mail: bsshapiro@aol.com).

In frozen–thawed ET (FET) cycles, in which ovarian stimulation is not used, pregnancy rates have been reported to be greater than in fresh autologous cycles (12), particularly with slowly developing embryos (11, 13) or after early P elevation (14). However, this effect is not easily discerned in national or clinical averages (15), perhaps because typical FET cycles use "second best" embryos cryopreserved after their morphologically superior siblings were transferred fresh. Additionally, many freeze–thaw protocols use postthaw embryo culture of insufficient duration to confirm resumed embryo development. Postthaw survival alone is an inadequate measure of viability (14).

Cohort cryopreservation at an early developmental stage followed by culturing embryos to an advanced stage after thaw allows confirmation of resumed and continuing development, preventing the transfer of embryos that arrest after the initial postthaw survival assessment. High implantation and pregnancy rates have been reported in FET cycles using entire cohorts of thawed bipronuclear (2PN) oocytes followed by postthaw extended culture (PTEC) to the blastocyst stage (14, 16). Autologous PTEC cycles have implantation and ongoing pregnancy rates per transfer similar to those of fresh oocyte donation cycles, indicating an absence of significant residual cryopreservation damage (17).

If blastocysts derived from PTEC are as viable as fresh blastocysts, then any difference in comparison between fresh autologous

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and PTEC blastocyst transfers should measure differences in endometrial receptivity. To achieve that goal, the present study used a prospective randomized trial to compare blastocyst transfer outcomes in autologous cycles of PTEC against fresh controls.

MATERIALS AND METHODS

An institutional review board approved this study before initiation. An independent monitor reviewed all study records. The registration number on clinicaltrials.gov was NCT00963625.

A two-stage, two-sided group sequential procedure with an overall type I error of .05 was used to test the primary hypothesis of a difference in the probabilities of clinical pregnancy for the two arms in this study, with a maximum sample size of 411 patients needed to achieve 80% power for detecting a difference of 15% in clinical pregnancy rate. The planned interim test specified a significance level of .03 after 100 blastocyst transfers were completed. The test statistic was a *Z* test based on the normal approximation to the binomial distribution (18).

The inclusion criteria were as follows: [1] the patient must be undergoing her first IVF cycle; [2] cycle day 3 FSH <10 IU/L; and [3] 8–15 antral follicles observed on baseline ultrasound scan. Genetic testing of embryos was excluded. First-time IVF patients were chosen because a history of failed fresh IVF cycles indicates poorer prognosis in repeated IVF cycles of blastocyst transfer (19).

Patients underwent COS with recombinant FSH (Follistim, Schering-Plough) and highly purified urinary FSH with "LH activity" from hCG (Menopur, Ferring Pharmaceuticals) in combination, and a GnRH antagonist (ganirelix acetate, Schering-Plough) was used for pituitary suppression. Final oocyte maturation was induced with hCG alone or, in those with greater ovarian response, with 4 mg leuprolide acetate concomitant with low-dose hCG (5–15 IU per pound body weight [11–33 IU/kg]) 34–36 hours before retrieval.

Immediately after retrieval, patients were randomized to either fresh blastocyst transfer (fresh group) or else blastocyst transfer after cryopreservation and PTEC (cryopreservation group) by drawing randomly among identical, opaque, unmarked sealed envelopes.

Patients in the cryopreservation arm had their 2PN oocytes frozen in 0.25 mL French straws with a CryoLogic FREEZE CONTROL CL-5500 using a slow-freezing technique. Oocytes were cooled to 0.0°C at −2°C/min, then to −2.0°C at −1°C/min, then to −7.0°C at −0.3°C/min. They were held at this temperature for 10 minutes. Halfway through this period, straws were seeded with cold forceps. Cooling resumed at −0.3°C/min until attaining a temperature of −32°C, after which all straws were plunged into liquid nitrogen. Embryo Freeze Media Kits were obtained from Irvine Scientific.

Entire cohorts of frozen 2PN oocytes were thawed at room temperature for 20 minutes in Embryo Thaw Media (Irvine Scientific) and subsequently cultured to the blastocyst stage.

Patients in the cryopreservation group were down-regulated with leuprolide acetate in a subsequent cycle and received oral E_2 (Estrace or equivalent, various manufacturers, 6.0 mg daily) and E_2 patches as needed starting 10–14 days before thaw to achieve a target endometrial thickness of at least 8 mm. Daily P injections (typically 100 mg) were started the day before thaw. Patients in the fresh group received similar E_2 and P supplements as in the cryopreservation group, but with P supplementation beginning 1 to 2 days after retrieval and E_2 initiated as needed. In both groups E_2 and P supplements were adjusted as needed to sustain serum levels of at least 200 pg/mL and 15 ng/mL, respectively, until increasing serum levels indicated placental production, typically at 9 to 10 weeks' gestation.

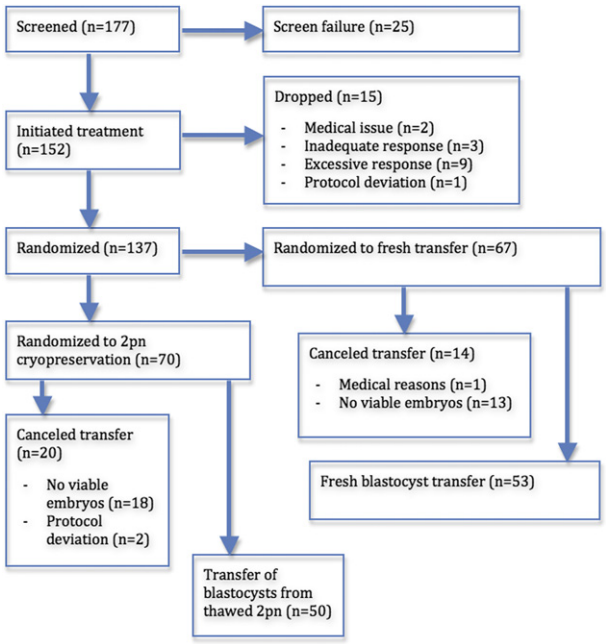
Quinn's Advantage Protein Plus Cleavage Media and Quinn's Advantage Protein Plus Blastocyst Media (Sage) were used to culture all embryos. The morphologically best one or two blastocysts were transferred on the first day on which at least one good expanded blastocyst appeared. Supernumerary expanded blastocysts of high quality were cryopreserved.

Cancellation was defined by the failure to have blastocysts to transfer. Pregnancy was defined by the observation of increasing serum hCG titers within 10 days after blastocyst transfer. Clinical pregnancy was established by the observation of intrauterine fetal heart motion by 7 weeks' gestation. Ongoing pregnancy was defined as intrauterine fetal heart motion at 10 weeks' gestation. The implantation rate was the ratio of the number of observed fetal hearts to the number of transferred blastocysts. Early pregnancy losses included any pregnancies that did not become ongoing pregnancies.

The main outcome measure was clinical pregnancy rate, which has been shown to be comparable to live birth rate as a measure of efficacy (20). The main outcome measure was compared with a *Z* test at this interim test point. Other measures were compared using Fisher's exact test or Wilcoxon's test, as appropriate. Logistic regression was used to assess the effects of multiple nominal independent variables on nominal dependent variables. Statistical analyses were performed with JMP version 7 (SAS Institute). A *P* value of <.03 was considered significant for the interim test of the primary outcome, whereas a *P* value of <.05 was considered significant for other measures. All tests were two-sided.

FIGURE 1

Disposition of enrolled patients. 2pn = bipronuclear.



Shapiro. Impaired endometrial receptivity. Fertil Steril 2011.

RESULTS

A breakdown of the 177 enrolled patients is shown in Figure 1. Of those, 103 had blastocyst transfer.

Potential confounders are compared in Table 1. The two groups were similar in age, diagnosis, baseline (cycle day 3) serum FSH level, antral follicle count, duration of stimulation, total FSH dosage, serum E_2 and P concentrations on the day of trigger in their stimulated cycle, number of follicles on the day of trigger, number of retrieved oocytes, and number of mature oocytes. The fresh group had significantly more 2PN oocytes when compared with their counterparts in the cryopreservation group. The two study groups did not differ significantly in number of transferred blastocysts or endometrial thickness on the day of trigger (measured 2 days before thaw in the cryopreservation group).

Table 2 compares outcomes of blastocyst transfers in the two groups. The cryopreservation group was associated with significantly greater rates of clinical pregnancy per transfer, ongoing pregnancy per transfer, and implantation.